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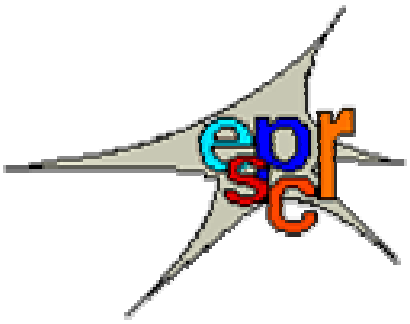

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH *BULLETIN*

N° 50 -Dec 2004

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HAPPY NEW YEAR 2005

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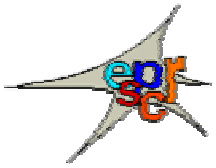

LETTER TO THE EDITOR
DISCUSSION, REVIEW,
SHORT COMMUNICATION, ...

A LETTER FROM THE PRESIDENT

CHALLENGES AND GOALS FOR 2005

Dear friends,

It was a pleasure to meet so many of you in Paris, where the high quality of the scientific program and presentations demonstrated the vitality and dynamism of our Society. The report published in this issue of our Bulletin will give those who could not attend the Meeting an overview of the main scientific aspects covered by the different speakers. I thank all the contributors for their work, and particularly Jean Marie Naeyaert who has coordinated the preparation of this report.

As the first year of the term of the Officers has elapsed, I would like to share with you some thoughts on the priorities for the near future. One important issue will be to adapt our status to the new regulations of the European Community, and this may call for slight changes in our Constitution. Another key aspect will be to improve our financial situation, which cannot be done without the help and continuous support of the membership, and may require an extra effort from the Meeting Organizers.

But the main point that I wanted to raise in this letter is the need show a strong and clear support to Pigment Cell Research. Since it was created in 1987, with Joe Bagnara as Founding Editor, the Journal has increased its quality and impact factor (2.919 in year 2003), and has established itself as an invaluable tool for the pigment cell community. Under the leadership of Vince Hearing, the Journal jumped to number 61 of 156 journals in the highly competitive Cell Biology section of the ISI. With its current impact factor, it would have ranked number 4 out of 38 in the Dermatology category, which is indeed a demonstration of the success of Vince Hearing's work as Editor, and of the good health of the Journal. Now, Colin Goding took over in handling the Journal, and for the first time, Pigment Cell Research has a European Editor-in-Chief.

Colin has already implemented a number of changes to make the Journal even more attractive, and these will start to be apparent from the first issue in 2005. I have no doubt that Colin will be a successful Editor. However, it is also clear that it will not be an easy task to maintain the strong upwards trend of the Journal and to increase further not only its impact factor, but more importantly its contribution to research in melanocytes and other pigment cells. A strong support from the ESPCR, and a committed input from its membership will be necessary, and will certainly result in mutual benefits.

Therefore, I think that during 2005 the ESPCR must show very explicitly its full support to Pigment Cell Research and the new Editor-in-Chief. One obvious way to do so is to increase the number of subscriptions. Indeed, the current situation is not too good. Subscription to Pigment Cell Research is mandatory for JSPCR full members, and about two thirds of the PASPCR members are subscribers. On the other hand, the maximal number of subscriptions from the ESPCR was a modest 37 in 2003 (less than 25% of the listed members), and this number went even down to 27 in 2004. Clearly, there is room for improvement.

In order to make the commitment of the Society to support the Journal very visible, the ESPCR Council has decided to put the subscription to Pigment Cell Research as the default option on the subscription forms for 2005 that you will soon receive. This does not mean that the subscription is mandatory, and you can check a box not to get the subscription, if you wish. But for this policy to be successful, members must appreciate the benefits of receiving and reading Pigment Cell Research, take advantage of the very attractive price offered through the ESPCR, and subscribe.

However, to increase the number of personal subscriptions is only one way to support the Journal. Citing the authoritative reviews and the many excellent papers published in recent issues is another. But the most important one is, obviously, to submit your work for publication in Pigment Cell Research, with the added value that the visibility to the overall pigment cell community will probably be higher than in any other Journal.

Pigment Cell Research has become an essential part of the life of the pigment cell Societies, and in a sense it is our window to the rest of the scientific community. Just as Colin Goding said most pertinently in his final speech in Paris, the Society and the Journal are tied, and the strength of each one of the parties is beneficial to the other. Therefore, I think that increasing the input of the ESPCR membership in terms of subscriptions and scientific contributions is one of our most important challenges for the next years.
This is an excellent opportunity to send all of you my warmest seasonal greetings, and to wish you a happy and successful New Year, on behalf of ESPCR and its Council.

Looking forward to seeing you in Reston

José Carlos García-Borrón

A message from the President of the International Federation of Pigment Cell Societies, to members of the ESPCR, JSPCR and PASPCR

<www.ifpcs.org>

Dear Friends and Colleagues,

Once again it is a pleasure to send seasonal greetings to all of you around the world, and profound wishes for a more peaceful and harmonious year in 2005 than in 2004. The constant news of wars and terrorism could give us a pessimistic view of how humanity is developing, but surely the great majority of us prefer peace and friendship, even if these people and their feelings do not feature much on the television. This message is to pass on to you some news from the IFPCS, the Federation that comprises the three Pigment-Cell Societies listed above. It seems a good thing that we have some international organizations like this, that aim to promote communication and excellent relations among those from many countries. This will be my last letter to you as IFPCS President, yet it seems hardly any time since the first in 2002. It has been a real pleasure to serve the scientific community in this way.

Our field of pigment-cell research seems to have been growing for some years, with ever more high-quality science and exciting progress. This year saw radical changes, with the official inauguration of two entire new scientific societies in the field. These are the Asian Society for Pigment Cell Research (ASPCR), and the Society for Melanoma Research (SMR). The ASPCR will hold its first meeting in New Delhi in February 2005 (<http://www.aspcr.org/>), while the SMR, a worldwide organization, held its second meeting in Phoenix, Arizona in November 2004. (SMR: <http://www.societymelanomaresearch.org/>) It will meet next in Holland in 2006. Both societies have some informal links with the IFPCS, and these may develop further in the future. Among the IFPCS societies, regional meetings for 2004 were expertly organized for the ESPCR in Paris by Lionel Larue and colleagues, for JSPCR in Kumamoto by Toshiro Kageshita, Tomomichi Ono and colleagues, and for the PASPCR in Newport Beach, California by Frank Meyskens and colleagues. Those attending all three meetings were treated to a combination of excellent science and pleasant social occasions (which can be at least as scientifically productive as the sessions). As usual, anyone unable to attend can find the abstracts published as supplements to recent issues of the journal *Pigment Cell Research*.

You can keep up with IFPCS events on the IFPCS web site (<www.ifpcs.org>), and many thanks as ever to Bill Oetting for maintaining that site. One member retired from the IFPCS Council in late 2003 (too late for the ESPCR Newsletter), namely Prof Masako Mizoguchi. She has been a keen and energetic Council member since joining in 1996 - warmest appreciation on behalf of IFPCS for her long and valuable service. Prof Kowichi Jimbow replaces her for JSPCR. Next year, as most of you probably know, we have a major IFPCS event, namely the three-yearly IPCC (International Pigment Cell Congress), September 18-23, 2005, to be chaired by Vince Hearing in Reston, Virginia, USA, near Washington DC. All the member societies meet together at the IPCC, in place of their annual meetings. Please note the date. All of you are warmly invited to attend this important conference, the planning for which is now almost complete. You can find the superb scientific program at the web site, <ipcc.info>, where you can register starting from January 2005. Note the low registration rate for (paid-up) IFPCS society members, so this is an excellent time to join or rejoin your regional Society. In general I urge you to continue to support the Societies and the Federation by remaining a member and by encouraging colleagues to join. Without their members the Societies would not exist and none of these conferences would happen. What are the dues for? - they go to good causes like meetings and travel fellowships. SMR members will also attend the IPCC, with melanoma plenary sessions later in the IPCC, followed by a one-day melanoma workshop organized by the SMR.

Another notable event, due on January 1, 2005, is the change of Editor-in-Chief of the IFPCS journal *Pigment Cell Research.* We are enormously grateful to the outgoing editor Vince Hearing for his unflagging commitment and outstanding achievements for the journal over the last five years. The Impact Factor has now risen for 5 successive years, leaping to over 2.9 for 2003, and the number of submitted articles has nearly doubled since 2000. The steady flow of excellent review papers, the increased numbers of colour pages and the rapid turnover of manuscripts are some more of the good reasons to submit work to *PCR*, and to recommend your library and your colleagues to subscribe to the journal. Your support is of great importance for its continued success, so please consider sending in a paper soon. That is the ideal way to support the journal and its new Editor-in-Chief, Dr Colin Goding, whom IFPCS welcomes and wishes him every success. He has already introduced a range of further ideas for the journal, including a new look for the cover and layout as from early 2005, and free online access to highly-cited reviews. See <www.blackwellpublishing.com/pcr>. We continue to be indebted to *PCR*'s generous corporate sponsors: Johnson & Johnson, L'Oréal, Shiseido and Unilever.

The IFPCS Special Interest Groups or SIGs have had another good year. All are welcome to join these interest groups by contacting the appropriate chair - see <www.ifpcs.org>. The IFPCS Pigment Cell Development Group chaired by Bill Pavan held a successful workshop at the NIH in April 2004, and provisionally plans another one to incorporate Genetics as well as Development, at the 2005 IPCC. The Vitiligo group has been re-formed under its new chairs Alain Taïeb and Mauro Picardo, and will also organize an IPCC satellite meeting. This seems a timely development, with the growing interest in this topic. The Melanoma interest group under Meenhard Herlyn has of course grown into a full society, the SMR, so it no longer exists as a SIG. If anyone feels there is scope for a new interest group in another field of pigment cell research, please do not hesitate to contact me (dbennett@sghms.ac.uk). For that matter please contact me with any ideas or thoughts for the IFPCS.

In conclusion, thanks to everyone for their continued enthusiasm for the field of pigment-cell and melanoma research, and for the Societies. A special welcome to those researchers who are new to the topic; no doubt you too will soon fall in love with the melanocyte as an outstanding model system, and will come to appreciate the remarkable level of friendliness and co-operation that prevails in this research field. And best of luck to the new IFPCS President, whoever it is, who will be writing this message in another year's time.

Best wishes to everyone for success and happiness in 2005.

Dot Bennett
[President, IFPCS]

MEETING REPORT
ESPCR Meeting Paris, September 22-25, 2004

Plenary Symposium I: Developmental Biology of Pigment Cell
Chairs: Dr WJ Pavan, Dr JP Thiery
Contributed by Dr W Pavan

The first plenary session included talks describing experimental approaches to understanding mechanisms involved in the development of pigment cell lineages from the neural crest. Dr. Nishikawa from the RIKEN Center for Developmental Biology in Kobe, Japan started the session by describing the expression patterns of several genes during progression of the melanocyte stem cells through its various stages of self renewal and differentiation in various microenvironments of the skin. Dr. Delmas from the CNRS-Institut Curie in Orsay, France next described the essential roles of ß-catenin in melanocyte development by specifical overexpressing or deleting b catenin in melanocytes during development. Dr. Kelsh of the University of Bath in UK spoke of the identification of the molecular defect in the iridophore deficient zebrafish mutant *shady* as being alterations in *Anaplastic lymphoma kinase* function. Dr. Kos of Florida International Institute from Miami, Florida, USA described experiments in which she manipulated the timing and levels of Endothelin 3 and Endothlin Receptor B and examined its effects on melanocyte development and function. Dr. Paulhe of Centree Medical Universitaire in Geneva, Switzerland presented findings examining the motif needed for proper intracellular transport of stem cell factor. Dr. Pavan of the National Institutes of Health in Bethesda, MD, USA described the use of retroviruses to express WNT signaling genes in specific neural crest cells and examine their effects of melanocyte cell fate. Finally Dr. Morrison of McDAniel College in Westminster, MD, USA described the basis of the variations observed in the pigment patterning of the panther chameleon.

Plenary Symposium II: Molecular aspects of adhesion and migration
Chair: Dr L Larue, Dr B Wehrle-Haller
Not available

Plenary Symposium III: Melanoma and Naevi
Chair: Dr H Arnheiter
Contributed by Dr M Paggi

The third plenary session was focused on several molecular aspects regarding nevi and melanoma. Dr. Bennett, St. George Medical School, London, UK, illustrated a number of molecular features concerning cell senescence; this phenomenon, considered as a barrier to melanocyte transformation to melanoma, can be anyway overcome by specific molecular changes regarding key factors involved in cell growth control. Dr. Sarasin, Institut Gustave-Roussy, Villejuif, France, illustrated the cDNA microarray analysis of 117 human melanomas, all correlated with clinical follow-up, which drove to the identification of 50 genes whose expression is associated with clinical outcome. Dr. Paggi, Regina Elena Cancer Institute, Rome, Italy, after cDNA array identification of few genes differentially expressed in human metastatic melanoma, described the functional role of two of these (L-ferritin and HtrA1) in the development and maintenance of the metastatic phenotype. Dr. Javelaud, Hôpital Saint-Louis, Paris, France, described the inhibitory function of Smad-7 overexpression on the behavior in vitro of invasive melanoma cells. Dr. Sviderskaya, St. George Medical School, London, UK, illustrated the role of the INK4A-ARF tumor suppressor locus in mouse melanocytes senescence. Dr Tavitian, Service Hospitalier Frédéric Joliot, Orsay, France, showed the Libechov Minipigs animal model for melanoma studies, described as effective in correlating FDG-PET data with staging, histology and clinical outcome, describing results strongly in accordance with those described for human melanoma. Finally, Dr. Yazdi, Ludwig Maximilians University, Munich, Germany, analyzed

tumor-infiltrating lymphocytes in a large panel of melanoma lesions, showing the presence of several focal clones with different rearrangements within a single malignant melanoma.

Plenary Symposium IV: Pigmentary disorders, including vitiligo
Chairs: Dr I Jackson, Dr E Coudrier
Contributed by Dr S De Schepper

Dr Alain Taïeb presented "A melanocytorrhagic hypothesis in vitiligo: in vivo and in vitro studies". The group of Dr Taïeb compared epidermal reconstructs made with normal melanocytes and keratinocytes to reconstructs of normal keratinocytes and melanocytes derived from non-lesional sites of non-segmental vitiligo (NSV). The latter showed a decreased number of melanocytes, suggesting an intrinsic abnormality in these melanocytes. When the "normal" reconstructs were treated with decomplemented serum of NSV patients, an abnormal motility of the melanocytes was seen. Several studies point to a role of the c-kit receptor herein.

Dr Sofie De Schepper gave an overview of pigment cell-related manifestations in Neurofibromatosis Type 1 (NF1). NF1 is a neurocristopathy, which means its affects neural crest-derived cells such as melanocytes. Lisch nodules, intertriginous freckles and café-au-lait spots are the most important melanocyte-derived NF1 symptoms. Lisch nodules mainly consist of 3 cell types: pigmented cells which contain melanosomes but also have some features of Schwann cells, fibroblast-like cells and mast cells. This resembles the cell population that we also find in neurofibromas. Freckling in NF1 is found typically in areas where skin meets skin such as the axillae, the inguinal region,…which suggests a physical factor (temperature, sweat, friction,…) in their origin. NF1 freckles display an increased number of melanocytes in comparison to sun-induced lentigines. Sometimes the skin overlying a neurofibroma (especially plexiform neurofibroma) can become hyperpigmented. This can indicate tumor aggressiveness or spinal cord involvement of the tumor. The etiopathogenic mechanism is still unclear.
Café-au-lait spots are by far the best known NF1 symptom. Some in vitro studies on NF1 café-au-lait melanocytes were performed and recent studies focus more and more on a possible role of growth factors such as stem cell factor (SCF) and hepatocyte growth factor (HGF). An analogous growth factor-related mechanism has recently also been described in neurofibromas.
For further study, biopsies were taken from café-au-lait spots and healthy skin of NF1 patients and normal healthy controls. Immunohistochemical staining showed an increased number of melanocytes (almost two-fold increase) in NF1 café-au-lait spots compared to healthy control skin (normal or café-au-lait). The number of mast cells however was increased in NF1 café-au-lait skin and normal skin and in healthy control café-au-lait skin compared to normal skin of healthy controls. This could indeed point to a role of SCF and mast cells in NF1 pathogenesis.
Up till now no loss of heterozygosity (LOH) has been detected in NF1 café-au-lait melanocytes, keratinocytes and fibroblasts. This means that, in contrast to neurofibroma formation, loss of the second healthy NF1 allele can not be the genetic trigger for café-au-lait spot formation.
In addition, a newly detected direct interaction between neurofibromin and the Alzheimer protein, amyloid precursor protein, could reveal a new function of this protein complex in melanosome transport.
Dr Magnaldo presented "An attempt at cutaneous gene therapy in Xeroderma Pigmentosum". In xeroderma pigmentosum (XP) the repair mechanism for UV-induced DNA damage is deficient. With genetic correction using retroviral vectors the group of Dr Magnaldo attempted to restore the repair capacity in XP-C keratinocytes (XP-C is one of the 7 classical XP groups linked to the corresponding XPC gene). They reconstructed XP-C skin in vitro from XP-C keratinocytes and fibroblasts. By using retroviruses, expressing the XPC DNA repair gene, the abnormal differentiation and proliferation of the XP-C keratinocytes could be reversed and the cells showed normal DNA repair and survival.

Unfortunately the presentation by Dr Jimbow was cancelled.

Dr Moretti presented her work on " Epidermal apoptosis in vitiligo"
Immunohistochemistry and in situ hybridization showed increased TNF-α expression in lesional vitiligo skin compared to perilesional skin. Normal control skin did not express TNF-α. A TUNEL assay showed positive cells in lesional and perilesional vitiligo skin (indicating apoptosis), while no apoptosis was found in normal control skin. This suggests that in vitiligo skin apoptosis of epidermal cells may be induced by TNF-α.

Dr Eves talked about "The influence of media and cell-cell interactions on the development of plasma polymer surfaces for the treatment of patients with vitiligo". Their aim was to develop a chemically defined substrate that was suitable for the coculture and subsequent grafting of melanocytes and keratinocytes to vitiligo patients. Four culture media were tested: two for keratinocyte culture (Green's and keratinocyte-defined media) and two for melanocyte culture (MCDB 153 and M2).
They concluded that a combination of a chemically defined substrate, produced by plasma polymerization of acrylic acid, allylamine or a mixture of the two, with M2 media is a promising approach for coculture of melanocytes and keratinocytes for vitiligo grafting.
Questions remain however about the clinical use of M2 media on vitiligo patients.

Plenary symposium V: Genetics of Pigmentation
Chairs: Dr D Bennett, Dr F Beermann
Contributed by Dr F Beermann

The plenary symposium V had 3 invited lectures and 4 selected presentations, which were chosen from submitted abstracts. The symposium was opened by a lecture of **Heinz Arnheiter (NIH)** who reported on mice carrying a genetically engineered mutant of MITF. MITF (microphthalmia transcription factor) is one of the key proteins for pigment cell development and maintenance, and is implicated in gene regulation of pigmentation genes. Activity of MITF is regulated both transcriptionally and post-translationally. In vivo, phosphorylation of MITF leads to increase in transcriptional activation, which is then followed by an increased degradation of the protein. Since in vitro data have shown that the serine residues are crucial for this regulation, it remained an open question as to the in vivo relevance. Thus, a mutation of one of the serines (Ser73Ala) was introduced into the *Mitf* gene locus of the mouse using homologous recombination in ES cells. Resulting mice (which either retained the neo cassette or lacked the neo cassette (de"floxed")) had no pigmentation phenotype, but showed white feet and a belly spot. Using RT-PCR, it then became clear, that one of the exons (exon 2b) is preferentially spliced out in these *Mitf*-mutant mice. **Jean-Jacques Panthier (France)** reported on new data on the *patchwork (pwk)* mouse, which is characterized by a "salt & pepper" phenotype, with either black or white hairs, but no grey hairs. It soon became clear, that melanoblasts are affected in these mice, and that the effect of a *pwk* gene must be non-cell autonomous. Mapping efforts localized the mutation on mouse chromosome 10, with a possible candidate gene, called *strawberry notch* (*mSno*), which is a related member to the Notch gene family. *mSno* was found to be overexpressed in homozygous *pwk/pwk* mutant embryos. In addition, overexpression of *mSno* in melanocytes of transgenic mice using the *Dct* promoter recapitulated the phenotype of the patchwork mutant mouse, thus suggesting that *mSno* is "the" gene affected in *pwk*. Moreover, this highlights the possible importance of the Notch signalling pathway in melanocyte biology and development. **Ian Jackson (MRC, Edinburgh)** then gave a talk on regulated expression of the melanocortin 1 receptor (MC1R) reporting on differences between mouse and human MC1R. Signalling through MC1R is antagonized by its agonist, Agouti signalling protein. Transgenic rescue experiments of mutant mice showed that a BAC (bacterial artifical chromosome) carrying a wildtype mouse MC1R was able to rescue the mutant phenotype. Surprisingly, replacement of mouse MC1R by a human MC1R gene within the BAC led to animals which were nearly black on an agouti background, thus suppressing the pheomelanic contribution in the agouti hair. This suggests, that the human MC1R is more potent then its mouse counterpart. In addition, a difference could be due to cis-acting elements, since a human MC1R gene in context of a

human MC1R BAC yields normally pigmented agouti mice. To understand regulation of MC1R better, MC1R::lacZ transgenic mice were produced (using BAC transgenesis). In these mice, lacZ expression is visible already in early melanoblasts, which is surprising, since MC1R was thought to exclusively function during melanogenesis.
These 3 invited lectures were followed by 2 selected presentations on the MC1R and 2 presentations on regulation of mouse tyrosinase gene expression. **Samantha Robinson (Southampton, UK)** reported on the cutaneous response to UV in dependence of MC1R genotype. No major difference was detected in terms of melanization (after 24h), of eryhrema, p53 expression and cyclobutane pyrimidine dimers. **Eugene Healy (Southampton, UK)** addressed the intracellular localization of MC1R. Even though commonly accepted that MC1R is found at the cell surface, experiments using GFP-tagged MC1R (as C-terminal fusion) revealed that MC1R is also localized at the melanosome. Purification of melanosomes revealed MC1R-EGFP fusion proteins, and immunofluorescence showed co-localization with gp100 and TYRP1, thus suggesting a yet unknown role for MC1R in the melanosome. **Victoria Tovar (Madrid)** reported on studies to understand the mouse distal regulatory region (also called locus control region, LCR). By carefully dissecting the core enhancer, she showed that mutating the 2 known elements "A" and "B box" in vivo (in YAC transgenics) leads to reduced and variegated pigmentation. Moreover, she reported on the strategy to remove the LCR region in vivo in transgenic mice. To do so, the 3.7 kb region containing the LCR was flanked by loxP sites using homologous recombination in ES cells. This enables the removal of the LCR by Cre-loxP-mediated recombination. The resulting phenotype was not reported, and awaits final breeding of the mice. **Fabien Muriser (ISREC, Switzerland)** reported on the strategy to unveil important regulatory elements in the tyrosinase gene. Using bioinformatics, several stretches of homologous sequence were identified in noncoding sequences of the tyrosinase gene. To identify their importance, he uses modified tyrosinase BACs with a lacZ as reporter gene. lacZ expression from a nonmodified Tyr::lacZ BAC is found in eye and skin melanocytes, thus reproducing endogenous expression of tyrosinase.

Plenary Symposium VI: Signalling pathways and transcription in Pigment Cells
Chairs: Dr R Kelsh, Dr R Marais
Contributed by Dr S De Schepper

Dr Colin Goding presented a lecture on " A transcription factor cascade regulating melanocyte proliferation".
Two pathways are implicated in melanocyte development and melanoma: B-Raf (constitutively active in 70% of melanomas downstream of KIT) and β-catenin. Both regulate Brn-2 expression, which in its turn blocks MITF, a transcription factor required for melanoblast and melanocyte survival and differentiation. MITF can directly activate p21, inhibiting proliferation and can also regulate T-box transcription factor Tbx-2. Tbx-2 suppresses an INK 4a/ARF independent anti-senescence pathway (dominant negative Tbx-2 induces senescence in human melanoma cells) and B-Raf mutations may thus be advantageous.

Dr Simon Saule spoke about "Transcription factors and pigment cell differentiation in the neuroretina".
MITF encodes a basic helix-loop helix transcription factor which plays an essential role in the differentiation of retinal pigment epithelium (RPE). The presumptive RPE is characterized by early expression of MITF and Otx. Deficiency of MITF or Otx impairs RPE development, possibly suggesting a cooperation or feedback loop of Otx to MITF for the control of RPE specific gene expression. The group of Dr Saule found Otx-1, Otx-2 and Otx-5/crx to be functionally equal for the specification of the RPE.

Dr Galibert presented "Dual regulation of melanocyte specific genes by two key BHLH-LZ transcription factors: MITF and USF-1".

MITF and USF-1 transcription factors both belong to the basic helix loop helix-leucine zipper transcription factors. Usf-1 is required for UV-induced melanocyte specific gene expressions, while MITF regulates constitutive gene expressions. This suggests a dual regulation model of pigmentation genes depending on environmental stress.

Dr Nico Smit presented his "Study of calcineurin function in epidermal and melanoma cell cultures".
Calcineurin is a calcium/calmodulin-dependent protein phosphatase that can be inhibited by drugs such as cyclosporine or tacrolimus. Calcineurin can activate T-cells by dephosphorylation of the nuclear factor of activated T-cells (NFAT) and the importance of calcium/NFAT signaling via calcineurin in melanocyte, melanoma and other skin cultures was investigated.

Dr Susan McNulty presented her work on "The effects of CK2 inhibition on apoptosis in human metastatic melanoma".
The group previously showed that NFκB binding by RelA is constitutively elevated in human metastatic melanoma cultures relative to normal human melanocytes. In addition immunohistochemistry showed elevated RelA in melanocytes of human naevi and melanoma relative to normal skin but expression of its inhibitor IκB-α was significantly lower in metastatic melanomas than in intradermal naevi. CRel showed a progressive increase in staining from naevi to melanoma and IκB-ε, which primarily inhibits the nuclear localization of cRel, was nearly exclusively cytoplasmic in melanoma biopsies, while RelA and phosphoserine-529 RelA showed elevated nuclear expression. CK2 (casein kinase II) phosphorylates IκB-α at serine 293 and RelA at serine 529. Thus a contributory role for CK2 in deregulation of NFκB activity in some melanomas can be hypothesized. Using several techniques the effects of RelA and CK2 inhibition on the expression and localization of NFκB, its inhibitors, apoptotic regulators and cell death were examined.

Dr Markus Böhm talked about "Evidence for expression of multiple members of the family of suppressors of cytokine signaling in normal and transformed human melanocytes".
His group investigated the expression of different members of the SOCS (suppressors of cytokine signaling) family in normal human melanocytes and melanoma cell lines. CIS, SOCS-5 and 7 were similarly expressed in melanoma cell lines and normal human melanocytes. SOCS-6 RNA was higher in normal human melanocytes compared to melanoma cell lines. SOCS-3 expression was silenced in normal human melanocytes and variable in melanoma cell lines. There was no correlation between IFN-α sensitivity and SOCS-3 expression but the latter does correlate with IL-6 sensitivity in melanoma cell lines in vitro. Recent data show differences between SOCS-1 mRNA and protein expression in normal human melanocytes and melanoma cell lines. SOCS-1 protein is suppressed in melanoma cells but SOCS-1 mRNA is present. In addition there seems to be no relation between IL-6 and IFN-α sensitivity and endogenous SOCS-1 in vitro.

Dr Robert Ballotti presented "A role of MITF in death and survival of melanocytes and melanomas".
MITF has a dual function in melanocytes. On the one hand it controls melanin synthesis and melanocyte differentiation via TYR,TRP1 and DCT, on the other hand it influences melanocyte survival and proliferation via BCL2, TXB2 and p21.
MITF expression is diminished during TRAIL-induced apoptosis in melanocytes and melanomas but inhibition of MITF expression is not sufficient to induce apoptosis. MITF is a substrate of caspases and can be cleaved by caspases after aspartate 345. Mutation of MITF at the caspase cleavage site impairs apoptosis. MITF C-terminal fragment induces melanoma cell death and MITF can be converted to a death inducing peptide upon caspase processing.
HIF1α is a new target for MITF:

- MITF binds to and activates the HIF1α promoter and mediates the effects of cAMP on the HIF1α promoter
- MITF could, through HIF1α regulation, participate in melanoma neovascularisation and favor glucose metabolisation in melanoma.

These results could make MITF a potential target for new anti-melanoma therapy.

Plenary Symposium VII: Melanogenesis
Chairs: Dr C Goding, Dr S Pavel
Contributed by Dr C Goding

Zalfa Abdel Malek spoke of the effects a-MSH and endothelin-1 (ET-1) that act as UV-induced keratinocyte-derived factors to stimulate melanogenesis and proliferation of primary human melanocytes. MSH and ET-1 were shown to act synergistically, with ET-1-mediated up-regulation of the MSH receptor MC1R possibly acting to potentiate the synergistic effects. In addition, MSH and ET-1 can also antagonise UV-stimulated apoptosis of melanocytes in culture, a result underpinned by the observation that no enhanced survival was observed in cells in which either the MC1R was mutated or in which signalling from the ET-1 receptor was blocked. The mechanism underlying the pro-survival effects of MSH and ET-1 were also investigated with the observation that these growth factors triggered activation of the anti-apoptotic Akt pathway and could also stimulate the expression and phosphorylation of the Mitf transcription factor as well as inhibit UV-mediated down-regulation of the pro-survival factor Bcl2.
Signalling via the MC1R receptor was also the subject of a presentation by Jose Carlos Garcia-Borron. Previously it had been thought that G-protein coupled receptors such as MC1R functioned as monomers to activate the associated heterotrimeric G-proteins. More recent evidence (see the discussion of results from Celia Jimenez-Cervantes below) however suggests that such receptors may undergo dimerisation and that in some cases point mutations in the receptors may lead not only to reduced signalling but also to agonist-independent activation of a range of intracellular signalling pathways. Moreover, the attenuation of signalling that occurs following long term agonist mediated activation of the MC1R receptor arises partly through phosphorylation-dependent de-sensitisation of the receptor and partly through receptor internalisation. The kinases likely to be responsible for this desensitisation are called GRKs (G protein-coupled receptor kinases), and they recognize the agonist-bound conformation of the receptor with much higher affinity than the free, resting state. Although these kinases exist as a family, little is known as to their target specificity, which is thought to be rather broad. The MC1R exhibits agonist-induced desensitisation in both mouse and human melanoma cells, and when expressed in heterologous systems such as HEK 293 cells. Human and mouse melanoma cells and melanocytes express at least two members of the family, GRK2 and GRK6. Co-expression of MC1R and GRK6 results in agonist-dependent receptor phosphorylation, enhanced MC1R desensitisation and an increased rate of receptor internalisation following agonist binding. Mutation of multiple potential GRK phosphorylation sites in the internal loops of the MC1R revealed that mutation of threonine 308 located in the C-terminal cytosolic extension interfered with desensitization and with agonist-mediated internalization. A T308D mutant mimicking the phosphorylated state of the residue showed a decreased responsiveness to agonist and an increased rate of internalisation. However, the T308A and T308D mutants are still able to undergo further desensitization when co-expressed with GRK6, suggesting that other residues in addition to T308 are targets for the kinase. Thus, the functional status of the MC1R seems controlled by GRK dependent phosphorylation of T308 and at least another Ser/Thr residue.

Celia Jimnenez Cervantes presented evidence for the oligomerisation of the melanocrtin 1 receptor MC1R. Results based on expression of epitope-tagged proteins in 293 cells indicated that the MC1R could form both dimers and higher order oligomers, though dimersiation did not promote any cooperativity in agonist binding. On the other hand, several loss-of-function variants of the MC1R behaved as dominant negative mutants, in that they decreased the availability of specific binding sites when coexpressed with the wild type receptor. Oligomerisation did not require di-sulphide bonds and did not appear to involve the C-terminal cytoplasmic domain but may be explained by a domain-swapping mechanism, based on functional trans-complementation experiments performed with various

loss-of-function mutants. The functional relevance of MC1R dimerisation is currently unclear but preliminary evidence suggests it may be related to early events in MC1R processing.

The effects of UV on the skin were discussed by Christine Duval. One of the key events occurring after UV irradiation of melanocyte/keratinocyte co-cultures is the activation of an inflammatory response and the release from keratinocytes of inflammation-associated molecules, particularly prostaglandins E2, F2a and I2. The UV-triggered release of the prostaglandins can be blocked with anti-inflammatory drugs and significantly such drugs also blocked by 50% the accumulation of pigment in response to UV irradiation. This effect could be also observed in reconstructed human pigmented epidermis. It was proposed that the effects of the keratinocyte-derived prostoglandins, that are known to signal via multiple pathways, act together with better characterised factors such as MSH and ET-1 to bring about the UV-mediated induction of pigmentation.

On a different note, Hiroaki Yamamoto presented results concering the role of thehomeodomain transcription factor OTX2 in eye development. *Otx2* is initially expressed in the optic vesicle (OV). Since Otx2-/- mice are inviable this study used a dominant-negative Otx2 (dnOtx2) expression vector that was targetted to the eye. In this case expression of dnOtx2 led to loss of expression of Mitf, a transcription factor necessary for development of the RPE. The expression of dnOtx2 in the eye also led to the ectopic expression of the ventral-specific gene Pax2 in the dorsal region of the optic cup (OC) as well as expression in the RPE of FGF8, a gene normally expressed in the NR and in the dorsal brain vesicle. Unexpectedly, at later-developmental stages, expression of dnOtx2 led to the presumptive RPE region exhibiting features characteristic of the telencephalon, expressing marker genes such as *Emx1* or *Nkx2.1*. The results presented supported the idea that Otx2 expression is required for the correct formation of the RPE, though the precise mode of action was not examined in detail. One attatractive possibility though is that Otx2 is a direct regulator of Mitf expression.

Plenary symposium VIII : Hair and Pigmentation
Chairs: Dr J Lambert, Dr J-J Panthier
Contributed by Dr J Lambert

D Tobin from Bradford opened this afternoon session on “Differential regulation of melanocyte biology in human epidermal and hair follicle pigmentary units”. He focused on the differences between melanocytes in epidermis versus hair follicle. Differences in size, dendrite formation, melanosomes, and microenvironmental issues like melanogenesis, melanocyte/keratinocyte unit interaction, melanosome transfer and melanin degradation were illustrated. In particular, it was shown that there is little expression of □-MSH, ACTH, and □-endorphin in epidermal melanocytes versus hair bulb melanocytes. Alopecia areata was brought forward as a possible model to study differences : are hair follicle melanocytes and epidermal melanocytes attacked to the same extent, why are hair bulb melanocytes preferentially targeted ? Can we differentially manipulate the melanogenic enzyme expression in hair follicle melanocytes and epidermal melanocytes ? *S Commo* from Clichy illustrated in a second talk “Unexpected characteristic of the human hair pigmentary system” that in hair follicle melanocytes TRP-2 expression is not detected in contrast to epidermal melanocytes. This was studied by confocal microscopy, western blot and RT-PCR. It is suggested that the lack of TRP-2 expression could be linked to transcriptional regulation under the influence of poor SOX10 expression. So, for eumelanogenesis in human hair TRP-2 is not required. *K Wakamatsu* from Toyoake, Japan presented data on “Relationship between chemical phenotype and visual phenotype in human hair pigmentation” and found a good correlation between red hair visual phenotype, MC1R mutation genotype and high pheomelanin chemical phenotype. 235 consenting volunteers were included in this study performed in collaboration with the University of Arizona. In a last talk *M Zoccola* of Biella introduced “A new approach in the determination of eumelanin in human hair”, the near infrared spectroscopy (NIR). Eumelanin detection by HPLC versus NIR showed a good correlation.

Plenary Symposium IX : UV Light and Skin, Update on Research
Chairs: Dr V Hearing, Dr R Schmidt
Contributed by Dr V Hearing

The purpose of this Symposium (which was sponsored by L'Oreal Recherche) was to update our understanding about the effects of UV on the skin. To that end, the organizers invited 2 speakers from within L'Oreal and 2 speakers from outside Institutions who are actively working in this area. In his opening comments, Dr. Rainer Schmidt noted that a moderate sun exposure is essential, not only for vitamin D production, but also for general health and survival. However, lengthy and repeated exposure to UV (e.g. to tan) involve short-term and a long-term health risks. UV stimulates skin pigmentation, a natural defense against UV exposure, but it also damages cells, in particular their DNA. Excessive sun exposure not only induces premature skin aging, but has become a major public health problem due to the dramatic increase in all types of skin cancers, including melanoma. Dr. Schmidt noted that this Symposium is dedicated to improving our knowledge of risks related to UV radiation, which will hopefully contribute to reducing abusive sun exposure and encouraging the use of efficient, protective sunscreens.

Dr. Vincent Hearing (National Cancer Institute, Bethesda, USA) then spoke about his group's work on the "Effects of UV on Melanocytes in Human Skin of Various Pigment Phenotypes". He began by introducing UV as a physiological agent which stimulates pigmentation in various types of human skin, but noted that the mechanism(s) whereby this increase in melanin production (tanning) occurs is not well understood. Although tanning is readily visible to the naked eye, few studies have examined the molecular consequences of UV on human skin in situ of various phototypes and racial backgrounds. His group has begun a systematic investigation of the effects of UV on human skin of different racial/ethnic groups and phototypes, examining the DNA damage that results, as well as rates of melanin formation and the expression of various melanocyte specific genes, before and at 7 min, 24 hr and 7 d after a single 1 MED UV exposure. Their data reveal that individual racial/ethnic origin and skin photosensitivity affects responses to UV, correlates inversely with DNA damage resulting from UV and is therefore a useful predictor for the risk of human skin cancer. They have characterized the similar densities of melanocytes at the epidermal:dermal border in different types of human skin and have measured the expression of various melanocyte specific proteins, including TYR, TYRP1, DCT, MART1, GP100 and MITF. They found that within 1 week, levels of melanin and the melanocyte markers have just begun to increase, and that the visible tan within that time essentially results from the increased distribution of melanin to upper layers of the skin.

Dr. Laurent Marrot (L'Oreal Advanced Research, Aulnay, France) then spoke about his group's study on "Epidermal Human Melanocytes in Culture: An Endpoint for Assessment of UVA Genotoxic Impact, Photoprotection and Phototoxicity". In human epidermis, melanocytes are particularly impacted by sunlight since they produce pigmentation to protect the skin, but this process can become deleterious when photoreactive pheomelanins are produced. UV is thought to be involved in the transformation of melanocytes, which leads to melanoma. His group has exposed cultured normal human melanocytes from Caucasian donors to UVA from a solar simulator (320-400 nm) at sub-lethal doses (similar to levels in natural sunlight). DNA breaks were then analyzed by the comet assay. They found that the antioxidant gene heme-oxygenase-1 was induced with 4 hr of exposure. Stimulation of melanogenesis (by adding tyrosine) significantly increased this photo-oxidative stress, suggesting that melanin and/or its chemical precursors behave as endogenous photo-sensitizers. This implies that melanocytes undergoing melanogenesis (and possibly skin undergoing tanning) need to be particularly well protected against UV radiation. Dr. Marrot's group used the comet assay to assess DNA damage in melanocytes protected by 2 different types of sunscreens, one protecting against UVB only and the other protecting against UVA and UVB. Melanocytes treated with the photoreactive drug lomefloxacin appeared to be more resistant than unpigmented skin cells to photocytotoxicity and photogenotoxicity following UVA exposure, although such treatment triggered melanogenesis as measured by an increase in tyrosinase activity. The sum of their results show that cultured melanocytes

are useful models to better understand oxidative effects of UV, to ensure adequate and safe photoprotection, and to study phototoxicity.

Dr. Françoise Bernerd (L'Oréal Recherche, Clichy, France) then spoke about her group's studies on the "Role of UVA Radiation in Biological Consequences of Sun Exposure". She noted that acute or chronic sun exposure of human skin elicits short term responses (such as sunburn) and long term effects (such as UV-induced skin cancer and photoaging). It was originally thought that only UVB (290-320 nm) radiation was responsible for those deleterious effects and that UVA (320-400 nm) was not biologically efficient. However, we now know that photodamage is linked to the biological effects of both UVA and UVB through different molecular mechanisms. Dr. Bernerd noted that UVB acts via direct induction of specific DNA lesions while UVA effects are mediated by the generation of reactive oxygen species. In addition, the high penetration of UVA allows it to reach deep layers of the skin and to elicit drastic effects in the dermis. Determination of early biological events in UV damage is especially important to understanding delayed effects as well as to designing more effective approaches to photoprotection (i.e. sunscreens). In vivo studies on human volunteers as well in vitro systems have proven the impact of UVA radiation on several distinct cellular parameters. The use of three dimensional skin models in vitro is a valuable tool for such studies, since they allow one to reproduce the main typical markers of sunburn reactions and to identify specific effects of UVA. Direct as well as indirect effects can be characterized with such models. Finally, it is possible to generate UV-hyperphotosensitive skin in vitro with xeroderma pigmentosum cells, thus representing a promising model to look at the effects of dysfunction of DNA repair.

Prof. Jean Krutmann (Heinrich-Heine-University, Düsseldorf, Germany) then addressed the topic of "Chronic Effects of UVA Radiation: Relevance for Photocarcinogenesis and Premature Skin Aging". He noted that there is increasing evidence that UVA plays a pivotal role in the pathogenesis of skin cancers and as well as in the photoaging of human skin. His group (and others) have analyzed the photobiological and molecular mechanisms by which UVA causes these detrimental effects. His group's studies initially focused on irradiation protocols, utilizing single exposures of human skin or skin cells to UVA. However, many detrimental effects induced by UVA result from chronic exposure, and thus they have developed a combined in vitro/in vivo model in which human skin or cultured primary human skin cells are exposed 3 times per day to relatively low doses of UVA over a 3 week period. Using that chronic exposure model, they found that UVA-induced damage is due, at least in part, to mutations in mitochondrial DNA. They report that significantly higher numbers of mutations in mitochondrial DNA are found in chronically sun-exposed skin compared to sun-protected skin. They demonstrated that mutations in mitochondrial DNA are induced in human skin cells by UVA in vitro as well as in vivo in human skin. They demonstrated that such mutations profoundly disturb mitochondrial functions and up-regulate expression of matrix metalloproteinase genes. This suggests an important role of UVA-induced mutations in mitochondrial DNA in premature skin photoaging and possibly in photocarcinogenesis. Protection of mitochondria against UVA-induced mutations and/or restoration of their functions in chronically photodamaged skin is a novel and promising approach to prevent and treat UVA-induced skin diseases.

Plenary Symposium X: Clinical aspects of Melanoma, i.e., Epidemiology, Diagnosis, Treatment
Chairs: Dr M-F Avril, Dr G Ghanem
Contributed by G Ghanem

Dr T. Dorval (Institut Curie, Paris) was the first contributor of the session. He highlighted the differences between uveal and cutaneous melanocytes in terms of antigen and gene expressions, specifically the melanoma associated gene families. He also pointed out the high resistance to chemotherapy as well as a new and promising treatment of the most frequent ocular melanoma metastases (50% of the patients) to the liver with IA Fotemustine on the top of different surgical approaches.

Dr S. Briganti (San Gallicano, Dermatological Inst., Rome) highlighted the role of antioxidants in the skin particularly catalase. The latter's activity is low in fair skins and importantly, is affected by UVA

particularly in the presence of pheomelanin. She pointed out the higher acidic isoform fraction of the enzyme in fair skin as well and, interestingly, in the large majority of melanoma patients. The authors proposed this approach to evaluate skin photodamage.
Dr R. Marais (Inst. Cancer Res, London) focused on the role of B-RAF in human melanoma since some mutations of this gene are able to immortalize melanocytes to acquire melanoma cells behaviour both in culture and in nude mice. The authors suggest a conformational model to explain B-RAF activation. They also pointed out the ability of some mutants to signal through MEK because they retain the possibility to activate WT C-RAF.
Dr S. Pavel (Leiden, University Medical Centre, Leiden) summarized the relationships between skin pigmentation, the number of pigmented nevi and the risk for developing melanoma. He presented a comparison between dysplastic naevi and normal melanocytes in terms of pigment synthesis, iron and calcium melanosome content. FACS as well as microarray analyses of these cells confirmed that they suffer from a higher and continuous oxidative stress that may play a role in malignant transformation.
E. Kinnaert (Institut J. Bordet, Brussels) used COMET assay to evaluate immediate DNA damage caused by ionizing radiation. The authors followed SSB and DSB separately when pigmentation was increased either by tyrosine addition or cystein depletion and observed the same protective effect on DNA; SSB protection being immediate and DSB delayed at 3 h after the irradiation. Cell survival fraction correlated with the number of cells expressing DSB and did not change over time suggesting - as expected- an efficient DNA repair mechanism. The latter may rescue the cells when not saturated by SSB repair; so pigmentation would facilitate these mechanisms by lowering or even preventing SSB.
Dr F.L. Meyskens (UCI Medical Center, Orange, CA) discussed data suggesting that changing the redox status of melanoma cells may lead to substantial phenotypic changes accompanied with reduced cell growth, increased dendricity, MHC class 1 antigen and Fas expression. The authors pointed to AP-1 as one of the key effectors behind their observations.
Dr M. Böhm (Ludwig Boltzmann Institute, Münster) highlighted the photoprotective role of MSH by preventing UVB-induced apoptosis by reducing the generation of cyclobutane pyrimidine dimers and possibly by enhancing nucleotide excision repair.

18 posters were linked to this session:

- **Oxidative stress:**
  - Benathan M. *et al.* Melanoma cell sensitivity resveratrol is modulated by glutathione levels and tyrosinase activity.
  - Borovansky J. *et al.* Free radical situation in melanoma-bearing animals.
  - Kim E. *et al.* Production of selenium peptide as an antioxidant.
- **Photobiology:**
  - Bivik C. *et al.* Cathepsin involvement in UVA/B induced apoptosis in human melanocytes.
  - Del Bino S. *et al.* Photoprotection afforded by constitutive pigmentation: relationship between skin typology and UV sensitivity.
  - Duval C. *et al.* Prevention of UV-induced skin pigmentation : in vitro and in vivo models.
  - Larsson P. *et al.* UVA/B induced redox alterations and activation of Redox regulated NF-kB in human melanocytes – protective effects of α-Tocopherol.
  - Fourtanier A-M. *et al.* UVA filters in sunscreens enhance the protection against photoimmuno-suppression.
- **Gene alteration DNA arrays:**
  - Malek O. *et al.* Construction of subtractive CDNA libraries to analyze carcinogenesis and induction of regression in the melim model of cutaneous melanoma.
  - Rodolfo M. *et al.* Braf alterations are associated with complex mutational profiles in malignant melanoma.
- **Melanoma models, pathology, therapy:**
  - Arnaudeau-Begard C. *et al.* The MeLiM swine model for familial melanoma: in vitro characterization and applications in studies of environmental risk factors to cancer.
  - Crechet F. *et al.* Is MC1R involved in swine melanoma: analysis of MC1R alleles in MeLiM families.

- Dierickx K. *et al.* Neutral metalloproteases and other proteases are involved in L-Prolyl-M-L-Sarcolysyl-L-P-Fluorophenylalanine-Ethylesther (PSF) processing.
- Ragnarsson-Olding B. *et al.* Ano-rectal melanomas; a national series in Sweden during 40 yrs.
- Li Z. *et al.* High-density oligonucleotide array analysis of Interleukin-6 sensitive and resistant human melanoma cells.

➢ **Varia:**

- Keighren M. *et al.* Transgenic reporter mice to map the expression pattern of MC1R.
- Roy R. *et al.* Molecular and histological analysis of the albinism of "snowflake".
- Cardinali G. *et al.* Keratinocyte growth factor promotes melanosome transfer to keratinocytes.

Plenary Symposium XI: New techniques in Pigment Cell Research
Chairs: Dr V Delmas, Dr J-C García-Borrón
Contributed by Dr J-C García-Borrón

Plenary Symposium XI on "New Techniques in Pigment Cell Research" started with a lecture presented by B. Bastian, entitled "**Distinct genetic pathways to melanoma depending on anatomic site and sun exposure patterns**". There are several types of melanoma distinguishable on the basis of histological features. Moreover, although UV radiation is a major determinant of melanoma, it is a common observation that melanomas also originate in relatively or totally protected areas. Melanomas were classified in four types: from sites with or without chronic sun damage (CSD), from non-haired acral skin, and from mucosa. Then the genetic changes in 126 primary melanomas from these groups were compared, with emphasis on genetic instability (as assessed from the proportion of genome altered, the number of changes in chromosome copy number, and the total number of amplifications) and on the status of two oncogenes, BRAF and RAS. Significant changes in the type and frequency of genetic changes were reported. Genetic instability was higher in melanomas on protected mucosa and glabrous skin, with amplifications of 11q13, gains of 6p and loss of 10p at the highest frequency in the latter, but gains in 1q and losses of 4q, 8p, 11p and 21q in the mucosal melanomas. Comparison of melanomas from skin with or without CSD also revealed differences. Losses of 10q and gains of 20q were found at a higher frequency in skin with no CSD, and changes in 4q, 11p more frequent in skin with CSD. Concerning mutations in BRAF and RAS, 57 % of melanomas did not show mutations in these genes. BRAF mutations were much more frequent in sites with no CSD than in the other type of melanoma, and were also frequently associated with losses of the PTEN region (10q). Mutations in the RAS genes were found only in samples without BRAF mutations. Loss of the CDKN2A region was frequent in cases with mutations in BRAF or RAS, but was not found in tumours without RAS of BRAF mutations. The distinct patterns of genetic changes in tumours of different anatomic origin and history of sun exposure suggest that there is more than one pathway leading to melanoma.

The second lecture, by F. Beermann, was entitled "**Transgenic mice to study pigmentation genes and melanoma**". This lecture dealt with two different topics. The first one was the generation of a knockout mouse model null for dopachrome tautomerase (Dct). This model is interesting because the natural *slaty* mouse retains measurable Dct activity (10-30% of wild type), which complicates studies on the physiological role of Dct. Dct null mice are viable, excluding a major role of the early expression of Dct in the brain, and no apparent abnormalities in sites of Dct expression (skin, retinal pigment epithelium or brain) were detected. Dct-null melanocyte cultures could be established and did not show significant impairment of growth. The location of Typr1 and Tyr was also investigated and, again, Dct-null melanocytes behaved essentially as wild type. The only apparent phenotype in the Dct knockouts was therefore the coat color, similar to *slaty*, but interestingly, melanocytes from the knockouts contained more melanin than the natural *slaty* mutants. The second part of the lecture was devoted to a novel mouse model for melanoma that would mimic more closely the behaviour of human melanomas than other existing models, in that it would display an aggressive and metastatic phenotype. This model relies on the melanocyte-specific expression of human N-Ras^{Q61K} oncogene placed under the control of the tyrosinase promoter/enhancer. Transgenic mice display constitutive activation of the MAPK pathway, and show hyperpigmentation of the skin, with melanin deposits in

papillary dermis and subcutaneous fat layer. Albino transgenic animals also present histological abnormalities in the skin, but this is not the case for animals lacking melanocytes, suggesting that the histological changes are melanocyte-dependent. The transgenic mice develop melanoma with frequent metastases. Deletion of the INK4A-ARF locus by appropriate crosses increased the incidence of melanomas and reduced the latency of tumour appearance. Melanomas were melanotic, expressed tyrosinase, and metastasised to lymph nodes, lung and liver. Cells from these melanomas were able to grow in soft agar and were metastatic when injected into mice. In lung metastases, the cell population appeared heterogeneous in that some cells were positive for a stem cell marker, nestin, whereas others were negative. The impact of other melanoma candidate genes such as the β-catenin gene is currently being investigated.

L. Montoliu then presented the third lecture entitled "**New animal models to study visual abnormalities found in albinism**". Type I oculocutaneous albinism (OCAI) is associated with severe visual abnormalities, including an under-development of central retinal regions with lack of fovea, defects in the embryonic development of the neuroretina, abnormal chiasmatic projections with lack of stereoscopic vision, and reduction in rod photoreceptor numbers with partial night vision loss. Introduction of functional copies of the tyrosinase gene in albino mice corrects all these defects, in particular abnormal axonal chiasmatic projections of ganglion cells, as demonstrated by anterograde staining of the optic chiasm with fluorescent labels. In one transgenic model with inducible Tyrosinase gene expression a regulated rescue of abnormal chiasmatic projections found in albinism was also demonstrated. This shows that adequate tyrosinase activity is sufficient to restore normal retinal development, but does not allow distinguishing whether this phenotypic rescue is due to the presence of pigments, or to intermediates of the melanogenic pathway. To overcome this problem, a new transgenic model was obtained, with neural tyrosine hydroxylase (an enzyme able to transform tyrosine into dopa, but lacking dopa oxidase and melanin formation activity) under the control of the tyrosinase locus control region. The construct employed for the generation of the transgenic mice was bicistronic, containing an IRES and the gene encoding for GTP-cyclohydrolase I, an enzyme necessary for the biosynthesis of the tetrahydrobiopterin cofactor of tyrosine hydroxylase. When expressed in tyrosinase-negative melan-c cells, this construct yielded measurable levels of tyrosine hydroxylase activity, but no melanin production. Transgenic mice with an albino background lacked melanin pigments, but the presence of tyrosine hydroxylase and its enzymatic activity could be demonstrated, and dopa was detected in eye extracts by HPLC. Expression of tyrosine hydroxylase restored normal cell proliferation in the retina, photoreceptors number, and corrected the pattern of axonal chiasmatic projections. Therefore, the visual abnormalities in albino mice seem due to the absence of a melanogenic intermediate, rather than to the absence of pigment. To gain further insight on the molecular events associated with the visual abnormalities in OCAI, genes whose expression in the eye is changed in the transgenic mice were analyzed by microarray technology, using an array of approximately 16,000 cDNAs including most known genes involved in development or regulation of the cell cycle. Preliminary data indicate changes in the levels of expression of several genes related with the stress response to hypoxia, maybe as a result of increased cell proliferation.

The last lecture, by A. Alonso from the BD Biosciences Scientific Support Team, was entitled "**BD living colors fluorescent proteins: revolutionary reporters for investigating biological events in living cells**". New fluorescent proteins with quantum yields approaching unity were presented. These were the oligomeric Reef Coral Fluorescent Proteins (RCFPs) and a monomeric green protein from Aequorea coerulescens, the Novel Fluorescent Protein AcGFP. These proteins are more stable than previously used fluorescent proteins, and have a wide range of applications including flow citometry, and monitoring by fluorescence microscopy imaging cell migration, protein localization and protein translocation. Vectors encoding fusions of the fluorescent proteins to localization signals or subcellular structural proteins are available and allow visualizing specific organelles. These expression vectors are suitable for localization studies. The new fluorescent proteins seem promising tools for the study of several aspects of pigment cell biology, such as the contribution of microtubules to melanocyte motility, the cellular localization of melanogenic proteins, and the intracellular movement of melanosomes.

Plenary Symposium XII: Immunotherapy
Chair: Dr T Boon
Contributed by Dr S De Schepper

Dr Thierry Boon presented his work on "T cell responses of melanoma patients vaccinated with defined tumor antigens".
The group of Dr Boon has been investigating the use of vaccinations in melanoma treatment for over 10 years. The first vaccinations were directed against MAGE-3 antigens (MAGE3A1 peptide, MAGE3 protein +QS21+MPL, ALVAC MAGE1/3 Minigine) but all of these resulted in only a few regressions and the majority of vaccinations failed. In the cases with tumor regression only a low frequency of anti-vaccine cytotoxic T-lymphocytes (CTL) but high frequencies of anti-tumor CTL directed against other tumor antigens could be detected. In one regressor patient it was seen that in metastases after vaccination the frequency of anti-vaccine CTL was low but some new anti-tumor T-cell clonotypes emerged. This could mean that the low levels of anti-vaccine CTL attack the tumor but only cause limited damage to the tumor cells. They could however in this way create conditions for a new wave of anti-tumor CTL, reactivating the anti-tumor T cell respons.
In conclusion, it was stated that not the number of T cells is important in vaccination-induced respons in melanoma, but their functional properties. Therefore a combination of different peptides or different clones for vaccination might be an option. In addition, a combination of vaccination with immunosuppressive therapies (depletion of T cells just before vaccination) or anti-TGF-β therapy should be investigated for future melanoma treatment.

Dr Das spoke about "Vitiligo and melanoma: in roads to immunotherapy"
Anti-melanocytic T cell responses in vitiligo as well as melanoma occur in an MHC class I and II restricted manner and activation of dendritic cells plays a crucial role in both diseases.
In vitiligo self-reactive T cells destroy melanocytes and, moreover, pigmentation associated melanocytic antigens, generally sequestered in melanosomes, may render these melanocytes highly immunogenic during inflammation. In addition MART-1 reactive cells infiltrate vitiligo marginal skin. The reduced viability of vitiligo melanocytes appears to be a consequence of increased sensitivity to oxidative stress.
Dr Das concludes that immunotherapy in vitiligo and melanoma has potential and will always have potential.

Plenary Session XIII: Trafficking and Transfer of melanosomes
Chairs: Dr G Raposa, Dr J-M Naeyaert
Contributed by Dr S De Schepper

M. Marks illustrated the critical role of Pmel17 in fibrillogenesis of stage II melanosomes. Pmel17 is synthesized as an integral membrane protein that has to pass through multivesicular stage I melanosomes. Pmel17 is cleaved in a post-Golgi compartment into two disulfide-linked subunits: a large luminal subunit, M alpha, and an integral membrane subunit M beta. After cleavage the luminal fragment is incorporated into the fibrils which initiates fibrillogenesis.
The group of Dr Marks has been working on protein sorting and localization to premelanosomes, melanosomes and lysosomes for many years, resulting in numerous high impact publications (J Cell Biol 2001; 152(4):809-24; Nat Rev Mol Cell Biol 2001; 2(10):738-48; Mol Biol Cell 2001; 12(11): 3451-64; Traffic 2002; 3(4): 237-48; J Cell Biol 2003; 161(3): 521-33; J Invest Dermatol 2003; 121(4): 821-30). They recently decribed a novel splice variant of Pmel17 lacking some of the internal repeats and suggest that this either alters fibrilligenic activity or the interaction of Pmel17 with melanin intermediates.

M. Seabra discussed the tripartite complex whereby melanosomes interact with the subcortical actin cytoskeleton: Rab 27a, melanophilin and Myosin Va. Loss of function mutations in these genes cause Griscelli syndrome, resp type II, III and I.
A similar tripartite complex is present in the retinal pigment epithelium and is formed by Rab 27a, a melanophilin homolog MyRIP and myosin VIIa.
Rab 27a seems to have a broad distribution in different tissues, e.g. sebaceous glands. The terminal coiled coil of rab 27a seems to be important for activation of Myosin Va. Rab 27a and melanophilin are present on melanosomes trafficking over microtubules, i.e. without attached myosin Va.
Rab 27a is an excellent marker for mature stage IV melanosomes.

E. Coudrier described the roles of Rab 8 and Myosin 1b in melanosome trafficking/biogenesis. Rab 8 could have a role in undocking melanosomes from actin fibers through a yet unknown effector.
Myo 1b over- or downexpression affects the cellular distribution and the morphology of the sorting multivesicular endosomes involved in the biogenesis of melanosomes. Also, over-expression of Myo1b reduced the proteolytic cleavage of Pmel 17 with resulting deficit in fibrillogenesis in stage II melanosomes. Myo 1b co-immunoprecipitates with Pmel 17 and is therefore important in the biogenesis of melanosomes.

V. Gelfand discussed signaling pathways of importance in movement of frog melanophores (dispersion/aggregation). Several motor proteins contribute to their transport: kinesin II and cytoplasmic dynein for transport along microtubules and myosin Va that counteracts dynein-driven transport towards the cell center and is important for the homogeneous distribution of organelles in the cytoplasm. In addition to cAMP-dependent regulation, aggregation is blocked by inhibition of the MAPkinase pathway. Melatonin inhibits pKA with downstream activation of MEK/MAPK.

X.S. Wu introduced a novel 22kDA protein called dilute suppressor (dsup). Loss of expression of dsup rescues the coat color phenotype of dilute (Myosin Va^{-}), ashen ($Rab27a^{-}$), leaden ($melanophilin^{-}$) mice, or, in other words, dilute suppressor is an extragenic suppressor of dilute, ashen and leaden.

	Myosin Va	Dsup	Coat color
Wild type	+	+	black
Dilute	-	+	grey
Dilute suppressor	-	-	black

Dilute melanocytes that are also homozygous for dilute suppressor show spreading of melanosomes throughout the cytoplasm but never have accumulation in the dendrite tips, a strictly myosin Va-dependent process.
Their group suggests that dsup potentiates the dynein-dependent minus end directed movement of melanosomes and that absence of dsup shifts the balance between plus and minus end directed movement towards the plus end causing spreading of pigmentation.
They are now further characterizing dsup and suggest that it may be a specialized melanosomal membrane microdomain.

Graça Raposo spoke about the role of AP3 and AP1 in melanosome biogenesis.
The group of Dr Raposo has been working on premelanosome biogenesis for many years. As mentioned earlier cleavage and sorting of Pmel 17 forms intraluminal striations and is essential for premelanosome formation. Maturation of melanosomes (stages III and IV) depend on melanogenic enzymes such as tyrosinase and tyrosinase-related protein 1. Mutations in the adaptor AP3 have been found in Hermansky-Pudlak syndrome and in melanocytes from Hermansky-Pudlak patients sorting of tyrosinase is affected.
The data of Dr Raposo suggest that there is an AP3/AP1 dependent sorting of tyrosinase.

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Dr. A. Napolitano)

A series of papers from different groups (Chicharro *et al*; Gonzales *et al*. ; Tatsuma *et al*) report on the use melanin-based electrodes as amperometric detectors for quantitation of catecholamines. The use of a tyrosinase-melanin electrode responding amperometrically to oxygen is also described.
Another paper by the group at the University of Saint Paul in Brasil (Da Silva *et al.*) has appeared which describes a scanning probe microscopy investigation of the structural and electronic organization of hydrated melanin films synthetized from DOPA. It is shown that the hydration process produces a restructuring of melanin observed not only through topogical variations, but also through the creation of areas with different electronic properties.
The binding properties of melanin remain an issue of continuous interest. The affinity for zinc of the pigments in brown irides is compared to that of blue irides in search for a possible explanation of the different prevalence of age-related macular degeneration in Caucasian with respect to Africans. (Kokkinou *et al*). Raman resonance spectroscopy was employed by Simon and coworkers to identify the iron III binding sites of sepiomelanin as catechol like structural units.
An overview of the stuctural and chemical properties of melanosomes (Liu *et al*) from black and red hair is offered by combined use of different techniques. In addition to the usual chemical degradation procedures and spectrophotometric analysis of the pigment following Soluene solubilization, new insights into the structural features of melanosomes are gained by solid state NMR and IR spectroscopy.
An interesting topic which is emerging in the literature revolves on the photosensitizing properties of melanins. In this connection the in vivo study by Takeuchi *et al* showed that TUNEL-positive cells in black and yellow mice were about 3-times more frequent than in albino mice after UVB or UVA irradiation, whereas DNA lesions and apoptosis were similar for the three strains. The conclusion is that UV-irradiated melanin, particularly pheomelanin, photosensitizes adjacent cells to caspase-3 independent apoptosis, and this occurs at a frequency greater than the apoptosis induced by direct DNA absorption of UV. The role of melanins in solar UVA irradiation is also addressed in another paper by Haywood and Linge using a soluble eumelanin from Dopa. The controversial role of melanocyte melanins with respect to UVA or UVB radiation is raised by Kwam and Dahle.
Various reports deal with the activity of various melanogenic inhibitors such as the fungal metabolite terrein (Park *et al*), hydroxystilbenes from Veratrum Patulum (Dong Hyun *et al*.), and methly *p*-coumarate (Kubo *et al.*).

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OTHER PIGMENTS

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Characterization of the melanin pigment of a cosmopolitan fungal endophyte. Mycol Res. 108(Pt 8):974-8, 2004.

2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

The initial step in the evaluation of the melanocyte biology is the isolation and the culture in vitro of the melanocyte itself. However, few paper are directly dedicated to the culture methods. So, it is relevant the study of **Da-Guang Wang** group which focused on the protocol of isolation and culture of the amelanotic melanocytes obtained from human hair follicles. The crucial role exerted by the different supplements of the medium is presented by **Oka**. He focused the attention on the anti-apototic activity of insulin, TPA, bFGF, and IBMX providing well-dressed functional and molecular evidence for a mitochondrial dependent and independent control of the apoptotic process in normal melanocytes. A further contribute to the understanding of the melanogenetic pathway is provided by Schallreuter group which investigated the link between catecholamin pathway and melanine synthesis. Indeed, based on a functional (immunofluoroscence and immunohistochemistry) receptor saturation assay) and molecular (RT-PCR) approaches, **Gillbro** provides further evidence for a link between catecholamine and melanine pathways through a receptorial mechanism. The author suggests that the epinephrine released by keratinocytes induces cAMP activation and then melanine synthesis via □2-AR. An other factor involved in the melanogenesis is nitric oxide. The relationship between nitric oxide, metallothionein and melanogenesis was investigated by **Sasaki**.

At the extreme of the pigmentary disorders study there is the in vivo instrumental non-invasive methods for the evaluation of the skin pigmentation, as puntually reported by **Stamatas**. The study proposes an overview of the the main non-invasive approaches to provide a quantitative or semi-quantitative measure of the skin colour, taking in account the possibile source of bias, the suitability for the skin study, the tecnichal basis, and the cost.

The role of the MC1R pathway in pigmentary disorders and in inflammatory diseases is under continuous study.

Foster reports an increased risk for vulvar vestibulitis syndrome associated with variants of IL1RB and MC1R, and combined genetic effects are associated with additive risk. This study supports a genetic contribution to VVS, suggests an increased risk of VVS in women with fair skin, and indicates potential new treatment and primary prevention options.

A role for adrenocorticotropic hormone and α-melanocyte hormone in the regulation of the human follicular pigmentary unit has not completely determined. **Kauser** designed a study to examine the involvement of the adrenocorticotropic hormone and α-melanocyte stimulating hormone/melanocortin-1 receptor system in human follicular melanocytes biology. To address this question they employed RT-PCR and immunohisto/cytochemistry. The functional role for these POMC peptides was assessed in follicular melanocyte cultures. They suggest a possible role for POMC peptides in regulating human hair follicle melanocyte differentiation.

Glutathione and its precursor cysteine play an important role in cellular defence mechanisms against oxidative stress. In melanocytes these compounds are also important precursor of the pheomelanin. **Kinnaert** aimed to assess the role of the pigment in the cellular radioprotective mechanism using a human melanoma cell model under cysteine or GSH depletion. Intracellular cysteine depletion causes melanogensis switch to eumelanin synthesis. In the same condition the cells resulted less suscettible to X-rays exposure. On the other hand, treatment with buthionine-S-sulfoximine decreased GSH level without changes in pigmentation whereas the cells appeared more sensitive to radiation.

Regarding the pigmentary disorders a wide production of papers can be detected in PubMed. However, I want to remember here some studies focused on melanoma and vitiligo. A common single nucleotide polymorphism (SNP) in the untranslated region (5'UTR) of the epidermal growth factor (EGF) gene modulates the level of transcription of this gene. This variant may be associated with melanoma risk, but conflicting findings have been reported. An Australian melanoma case-control sample was typed for EGF + 61A>G transversion (rs4444903) by the **James** group. The EGF + 61 SNP was not found to be significantly associated with melanoma or with development of nevi or freckles. Among melanoma cases, however, G homozygotes had thicker tumours, in keeping with other studies. The authors conclude that EGF polymorphism does not appear to predispose to melanoma or nevus development, but its significant association with tumour thickness implies that it may be a useful marker of prognosis. **Li** suggests, by means of an immunohistochemical study, a new possible candidate for the early diagnosis of melanoma. The authors indicated that two crucial proteins involved in the cell cycle progression are differently expressed in melanoma. Indeed, in melanoma p27 (promoting the G1 arrest) and skp2 (controlling the p27 degradation) are down-regualted and up-regulated, respectively. Moreover, the different expression of the two proteins correlates with the clinicopathology features. Based on western blot and northern blot analysis, **Mangahas** showed that endothelin-1, produced by keratinocytes, promotes the over-expressiion of MCAM on melanocytes favouring cell invasion. Moreover, even the effect of endothelin-1 on MCAM appears to be mediated by the specific receptor (EtbR), since the blocking antibody inhibits the MCAM up-regulation. An intersting new approach was proposed by **Blum** for the diagnosis of melanoma within the melanocytic lesions. He provides a matemathical method based on an algorithm demonstrating that computer analysis can be caracterized by an accurancy comparable to those of dermoscopic analysis. In any case the interpretation of the diagnostic algorithm requires a qualified medical expert.

A further evidence for the occurrence, at epidermal level, of an oxidative stress (increased production of MDA and decresed antioxidant enzymatic activities) during vitiligo was provided by **Yildirim**. The relevance of the epidermal unit was indicated by **Lee** which shows, through immunohistochemistry and western blot methods, an increased susceptibility of keratinocytes to physical trauma in vitiligo areas. The author suggests that an altered ratio between pro-apoptotic (p53, caspases) and anti-apototic (Bcl-2) factors can cause the apoptotic death of the keratinocytes with the subsequent reduced production of the melanocyte growth factors. A clinical trial presented by **Kullavanijaya** shows the effectiveness of calcipotriol as support therapy for vitiligo patients treated with UVB phototherapy. Beside the good clinical results obtained through UVB phototherapy, the mechanism of the repigmentation is still uncertain. **Wu**, in a in vitro model,

demonstrates that narrow-band UVB induces melanocytes migration due to the release of bFGF and ET-1 by sorrounding keratinocytes and to modulation of the focal adhesion kinase ($p125^{FAK}$) in melanocytes. The final effect is an increased cell migration and a modified adhesion resulting in the colonization of the epidermis by outer root sheat melanocytes.
The relation between melanin content and type and UV-sensitivity was investigated by Rijken and Takeuchi. A higher melanin content and a different melanosomal dispersion pattern in the epidermis of high phototype subjects are thought to be responsible for the high resistance to UV damage. To further investigate the photoprotective properties of the black skin, the purpose of the work of **Rijken** was to compare responses to solar-simulating radiation in black (phototype VI) and white (phototype II) skin. In skin biopsies from the two different groups differences in DNA photodamage, infiltrating neutrophils, proteolitic enzymes induction, keratinocyte activation and IL-10 expression were evaluated. The modification of these parameters was significantly more pronounced in low with respect high phototype group.
The in vivo photosensitising acitivity of the melanin has been analysed by **Takeuchi** in a mice (albino, yellow, and black coat) model. He demonstrated that melanin acts as a potent UVB photosensitiser causing cell death and DNA lesions.

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3. MSH, MCH, other hormones, differentiation

(Dr. R. Morandini)

There are two main fields that retain the attention of the authors and remain an issue of continuous interest, the relationship between melanocyte stimulating hormone and

1. Obesity

Donaldson *et al.* reported a correlation between plasma MSH concentration and body mass index in horses that are older than ten 10 years but not with younger ones. Another paper from the same group (McFarlane *et al.*) highlighted the seasonal varaition of alpha-melanocyte stimulating hormone concentrations in horses and ponies : a significantly higher plasma alpha-MSH concentration was found in September in ponies (11-fold) and horses (2-fold), compared with values measured in Spring.
Another interesting paper (Savontaus *et al.*) in this field focuses and supports the hypothesis that long-term melanocortinergic activation could serve as a potential strategy for anti-obesity and/or antidiabetic therapy. In the same species, Tian *et al.* makes a link between the failure of hypothalamic anorexigenic peptides CART (cocaine and amphetamine-regulated transcript) and alpha-MSH to increase their content in response to high-fat diet. This may play a key role in excessive high energy consumption resulting in obesity.
In the treatment of obesity, the synthesis of MCH-R antagonists gained a great deal of interest from pharmaceutical companies. A related review is made by Kowalski *et al*.

2. Anti-inflammatory therapy

Cragnolini *et al.* shows that the anti-inflammatory effect of alpha-MSH acts through MC3 and MC4 receptors. Another study by Lam *et al.* focuses on the MC3 receptor and suggests that specific small molecule agonists directed at MC3-R could be potential novel therapeutics for inflammatory conditions.
Zou *et al.* observes that in scrape-wounded cells, H_2O_2 inhibited wound restitution, and this was partially restored by cotreatment with alpha-MSH. This interesting paper is in accordance with the data found by Haycock *et al.* (J Biol Chem. 2000 May 26;275(21) using melanoma cells.

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4. Photobiology

(Dr. N. Smit)

The role of UV radiation is of limited importance in the development of melanoma. Genetic factors leading to naevogenesis and large numbers of naevi are the highest risk factors and development of naevi is again strongly dependant on skin pigmentation. This is described in our paper (in Dutch. Pavel and Smit). Nevertheless, inducing damage by UV irradiation in model systems from pure monocultures to transgenic mice models is a very helpful tool for us to understand the molecular events leading to the malignant transformation of melanocytes. Kvam And Dahle summarize in a short paper how melanin production in melanocytes may contribute to oxidative damage by UVA and offers protection against direct DNA damage by UVB. Hayword and Linge describe a model system by which they can measure the photosensitizing effects of soluble eumelanin exposed to UVA at various concentrations. They would consider their results of biological relevance in the case that soluble melanin were shown to exist in vivo. In this respect it is interesting that Ou-Yang et al reported earlier this year (JID 2004, 122: 492-6) about the importance of soluble melanin in UVA induced pigment in skin. Thus the photosensitizing properties of melanin and/or melanin precursors could play an important part in damage generated in melanocytes and in naevi. Treatment with antioxidants should theoreticaly lead to reduction of oxidative damage in melanin producing cells. Indeed we have found that DNA damage in melanocytes can be significantly reduced by preincubation of the cells with vitamin E and C in combination with carotenoid containing extracts (Smit et al). At the ESPCR meeting in Paris, Meyskens also presented work on the positive effects of the antioxidant resveratrol (present in red wine and grape seed) on cell signalling in melanoma cells. A paper by Aziz et al now (on line) also describes protective effects of resveratrol in mouse skin against UVB induced damage. According to de Fabo et al it is UVB irradiation that is responsible for initiation of melanoma. Once again we have to realize that this work was done in the hgf/scatter factor mouse model, mice that are already prone to generate melanomas. De Gruijl et al describe an overview of all the mouse models that have been used to study melanoma induction by chemical treatments and UV. The pathways involved are discussed with a special emphasis on the RTK/RAS pathway. These authors conclude that the impact of UV irradiation needs to be further ascertained by varying wavelengths - UVB versus UVA – and exposure schedules. Finally, Sarkar-Agarwal et al, who describe melanoma as a UV-induced tumor, used a mouse fibroblast model system where the cells lacked either $p16^{INK4A}$ or $p19^{ARF}$. A host cell reactivation assay was used to study DNA repair capacities of the cells which was reduced for both mutants. Thus, INK4A/ARF mutations may influence DNA repair and play a role in hypermutability often observed in (atypical) naevi. The question still arises when the repair deficiency is present in the fibroblast and all other cells lacking normal p16 pr p19, why does the problem occur especially in the melanocytes. Does the specific property of this cell, the synthesis of melanin, play an important part in the increased risks for the cell type or are melanocytes simply highly sensitive to changes in a specific signaling pathway ?

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5. Neuromelanins

(Prof. M. d'Ischia)

Interest in the metal-neuromelanin interaction continues unabated. In a study of the role of iron- and copper-containing molecules in the vulnerability of locus coeruleus and substantia nigra neurones during aging, Zecca et al. (2004) measured the levels of these metals and their major molecular forms like ferritins, ceruloplasmin, neuromelanin, manganese-superoxide dismutase (SOD), and copper/zinc-SOD in normal subjects at different ages. The results indicated a lower iron content in the locus coeruleus compared to the substantia nigra, and a higher heavy-chain ferritin/iron, suggesting that in locus coeruleus neurones the iron mobilization and toxicity is lower than that in the substantia nigra and is efficiently buffered by neuromelanin. On the basis of this and other data, the authors suggested that the difference in iron mobilization may be one of the reasons underlying the lower vulnerability of the locus coeruleus compared to the substantia nigra in Parkinsonian syndromes. The many possible roles of metal ions in neurodegenerative disorders and ageing are summarized and critically discussed in a review by Doraiswamy and Finefrock (2004).
In another paper, the effects of neuromelanin from the human substantia nigra on the proteasome activity in human dopaminergic SH-SY5Y cells was investigated in relation to the recent implication of an impairment of the ubiquitin-proteasome system in neuronal death in ageing and Parkinson's disease (Shamoto-Nagai et al., 2004). The results showed an effect of neuromelanin on the 26S proteasome, but not on the 20S proteasome activity in vitro, which may be responsible at least in part for the selective vulnerability of dopamine neurones in ageing and related disorders.

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Abstract: In this study, a comparative anal. of metal-related neuronal vulnerability was performed in two brainstem nuclei, the locus coeruleus (LC) and substantia nigra (SN), known targets of the etiol. noxae in Parkinson's disease and related disorders. LC and SN pars compacta neurons both degenerate in Parkinson's disease and other Parkinsonisms; however, LC neurons are comparatively less affected and with a variable degree of involvement. In this study, iron, copper, and their major mol. forms like ferritins, ceruloplasmin, neuromelanin (NM), manganese-superoxide dismutase (SOD), and copper/zinc-SOD were measured in LC and SN of normal subjects at different ages. Iron content in LC was much lower than that in SN, and the ratio heavy-chain ferritin/iron in LC was higher than in the SN. The NM concn. was similar in LC and SN, but the iron content in NM of LC was much lower than SN. In both regions, heavy- and light-chain ferritins were present only in glia and were not detectable in neurons. These data suggest that in LC neurons, the iron mobilization and toxicity is lower than that in SN and is efficiently buffered by NM. The bigger damage occurring in SN could be related to the higher content of iron. Ferritins accomplish the same function of buffering iron in glial cells. Ceruloplasmin levels were similar in LC and SN, but copper was higher in LC. However, the copper content in NM of LC was higher than that of SN, indicating a higher copper mobilization in LC neurons. Manganese-SOD and copper/zinc-SOD had similar age trend in LC and SN. These results may explain at least one of the reasons underlying lower vulnerability of LC compared to SN in Parkinsonian syndromes.

6. Genetics, molecular and developmental biology

(Dr. F. Beermann)

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on the absence of melanin pigment in sdy. Electroretinograms showed that the dark-adapted retinal network recovers sensitivity to a pulse of light more slowly in sdy mutants than in wild types. This failure is localized in the retinal neural network, postsynaptic to photoreceptors. We propose that retinal pigment epithelium (which normally expresses tyrosinase) secretes a modulatory factor, possibly L-DOPA, which regulates light adaptation in the retinal circuitry.

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Effects of G-protein mutations on skin color. Nat Genet 36(9):961-968, 2004.
Abstract: A new class of dominant dark skin (Dsk) mutations discovered in a screen of approximately 30,000 mice is caused by increased dermal melanin. We identified three of four such mutations as hypermorphic alleles of Gnaq and Gna11, which encode widely expressed Galphaq subunits, act in an additive and quantitative manner, and require Ednrb. Interactions between Gq and Kit receptor tyrosine kinase signaling can mediate coordinate or independent control of skin and hair color. Our results provide a mechanism that can explain several aspects of human pigmentary variation and show how polymorphism of essential proteins and signaling pathways can affect a single physiologic system.

- Vance KW, Goding CR.
The transcription network regulating melanocyte development and melanoma. Pigment Cell Res 17(4):318-325, 2004.

- Wilson YM, Richards KL, Ford-Perriss ML, Panthier JJ, Murphy M.
Neural crest cell lineage segregation in the mouse neural tube. Development Nov 17, Epub 2004.
Summary: The experiments provide evidence for existence of neural crest subpopulations and thus for neural crest lineage segregation already in the neural tube. For example cells expressing Kit in the neural tube will exclusively migrate into the developing dermis, where they express melanocyte markers, and are destined as melanocyte precursors.

- Yang CT, Sengelmann RD, Johnson SL.
Larval melanocyte regeneration following laser ablation in zebrafish. J Invest Dermatol 123(5):924-929, 2004.

7. Tyrosinase, TRPs, other enzymes

(Prof. J.C. Garcia-Borron)

- Beermann F, Orlow SJ, Lamoreux ML.
  The Tyr (albino) locus of the laboratory mouse. Mamm Genome. 15(10):749-58, 2004.
  The albino mouse was already known in ancient times and was apparently selectively bred in Egypt, China, and Japan. Thus, it is not surprising that the c or albino locus (now the Tyr locus) was among the first used to demonstrate Mendelian inheritance in mammals at the dawn of the past century. This locus is now known to encode tyrosinase, the rate-limiting enzyme in the production of melanin pigment, and the molecular basis of the albino (Tyr(c)) mutation is known. Here we describe the congenic series of Tyr-locus alleles, from wild type to null (albino). We compare eye and skin pigmentation phenotypes and the genetic lesions that cause each. We suggest that this panel of congenic mutants contains rich, untapped resources for the study of many questions of basic cell biological interest.

- Casella L, Granata A, Monzani E, Pievo R, Pattarello L, Bubacco L.
  New aspects of the reactivity of tyrosinase. Micron. 35(1-2):141-2, 2004.
  Tyrosinase was found to be active in the sulfoxidation of thioanisol, producing the (R)-sulfoxide with high enantiomeric excess. The activity of the enzyme with phenolic and diphenolic substrates in a mixed aqueous Hepes buffer pH 6.8-methanol-glycerol solvent was also investigated over a range of temperatures. These experiments enabled us to deduce the thermodynamic parameters associated with substrate binding to the enzyme and the activation parameters associated with the rate determining step of the enzymatic reaction.

- Cerenius L, Soderhall K.
  The prophenoloxidase-activating system in invertebrates. Immunol Rev. 198:116-26, 2004.
  A major innate defense system in invertebrates is the melanization of pathogens and damaged tissues. This important process is controlled by the enzyme phenoloxidase (PO) that in turn is regulated in a highly elaborate manner for avoiding unnecessary production of highly toxic and reactive compounds. Recent progress, especially in arthropods, in the elucidation of mechanisms controlling the activation of zymogenic proPO into active PO by a cascade of serine proteinases and other factors is reviewed. The proPO-activating system (proPO system) is triggered by the presence of minute amounts of compounds of microbial origins, such as beta-1,3-glucans, lipopolysaccharides, and peptidoglycans, which ensures that the system will become active in the presence of potential pathogens. The presence of specific proteinase inhibitors prevents superfluous activation. Concomitant with proPO activation, many other immune reactions will be produced, such as the generation of factors with anti-microbial, cytotoxic, opsonic, or encapsulation-promoting activities.

- Chen P, Solomon EI.
  O2 activation by binuclear Cu sites: noncoupled versus exchange coupled reaction mechanisms. Proc Natl Acad Sci U S A. 101(36):13105-10, 2004. Epub 2004 Aug 30.
  Binuclear Cu proteins play vital roles in O(2) binding and activation in biology and can be classified into coupled and noncoupled binuclear sites based on the magnetic interaction between the two Cu centers. Coupled binuclear Cu proteins include hemocyanin, tyrosinase, and catechol oxidase. These proteins have two Cu centers strongly magnetically coupled through direct bridging ligands that provide a mechanism for the 2-electron reduction of O(2) to a mu-eta(2):eta(2) side-on peroxide bridged Cu(II)(2)(O(2)(2-)) species. This side-on bridged peroxo-Cu(II)(2) species is activated for electrophilic attack on the phenolic ring of substrates. Noncoupled binuclear Cu proteins include peptidylglycine alpha-hydroxylating monooxygenase and dopamine beta-monooxygenase. These proteins have binuclear Cu active sites that are distant, that exhibit no exchange interaction, and that activate O(2) at a single Cu center to generate a reactive Cu(II)/O(2) species for H-atom abstraction from the C-H bond of substrates. O(2) intermediates in the coupled binuclear Cu enzymes can be trapped and studied spectroscopically. Possible intermediates in noncoupled binuclear Cu proteins can be defined through correlation to mononuclear Cu(II)/O(2) model complexes. The different intermediates in these two classes of binuclear Cu proteins exhibit different reactivities that correlate with their different electronic structures and exchange coupling interactions between the binuclear Cu centers. These studies provide insight into the role of exchange coupling between the Cu centers in their reaction mechanisms.

- Commo S, Gaillard O, Bernard BA.
  Human hair greying is linked to a specific depletion of hair follicle melanocytes affecting both the bulb and the outer root sheath. Br J Dermatol. 150(3):435-43, 2004.
  BACKGROUND: Although hair greying is a very common phenomenon characterized by loss of pigment in the hair shaft, the events that cause and control natural hair whitening with age in humans are still unclear. OBJECTIVES: To decipher the origin of natural hair whitening. METHODS: Human hair melanocytes were immunohistochemically characterized at different stages of whitening. RESULTS: Loss of hair shaft melanin was found to be associated with a decrease in both bulb melanin content and bulb melanocyte population. Although few melanocytes were present in the bulbs of grey hair, they still expressed tyrosinase and tyrosinase-related protein-1, synthesized and transferred

melanins to cortical keratinocytes as seen by the presence of melanin granules. In white hair bulbs, no melanocytes could be detected either with pMel-17 or vimentin labelling. Pigmented hair follicles are known to contain inactive melanocytes in the outer root sheath (ORS), and grey and white hairs were also found to contain some of these quiescent melanocytes. However, their population was decreased compared with pigmented hair follicles, ranging from small to nil. This depletion of melanocytes in the different areas of white hairs was detected throughout the hair cycle, namely at telogen and early anagen stages. In contrast, the infundibulum and sebaceous gland of both pigmented and white hairs showed a similar distribution of melanocytes. Furthermore, other distinct cell populations located in the ORS, namely putative stem cells, Merkel cells and Langerhans cells were equivalently identified in pigmented and white hairs. CONCLUSIONS: Thus, hair greying appears to be a consequence of an overall and specific depletion of bulb and ORS melanocytes of human hair.

- Commo S, Gaillard O, Thibaut S, Bernard BA.
Absence of TRP-2 in melanogenic melanocytes of human hair. Pigment Cell Res. 17(5):488-97, 2004.
Skin and hair colour mostly depend on the activity of melanogenic melanocytes. Numerous proteins involved in melanocyte function have been identified including pMel-17, Mitf-M, Sox10, tyrosinase, tyrosinase related proteins-1 (TRP-1) and -2 (TRP-2). In the hair, melanogenic activity occurs only during the anagen phase of the hair cycle. In order to evaluate the implications of some known melanogenic proteins in human hair pigmentation, we performed immunohistochemical studies to reveal the expression of pMel-17, Mitf-M, tyrosinase, TRP-1 and TRP-2 in active bulb melanocytes of eumelanic brown and black anagen hairs of different ethnic origins, e.g. brown Caucasian, black Asian and African hairs. The labelling was compared with that observed in Caucasian and African scalp epidermis (interfollicular epidermis) melanocytes. We found that while pMel-17, TRP-1 and TRP-2 were expressed in epidermal melanocytes irrespective of ethnic origin and melanin content of the scalp epidermis, Mitf-M and tyrosinase expression were clearly evidenced only in pigmented epidermis, e.g. African scalps. Regarding human hair, pMel-17, Mitf-M, tyrosinase and TRP-1 were detected in a similar manner in active bulb melanocytes of brown and black hairs. In contrast and unexpectedly, TRP-2 could not be detected in hair bulb melanocytes, whatever the hair colour and ethnic origin. The lack of TRP-2 was further confirmed by western blot analyses. Reverse transcriptase-polymerase chain reaction (RT-PCR) performed on hair bulb mRNA demonstrated that Mitf-M, tyrosinase and TRP-1 amplimer signals were easily detected, whereas the TRP-2 amplimer signal was barely detectable. Furthermore Sox10 was not detected in hair bulb. Altogether our results suggest that the absence of detectable level of TRP-2 is due to transcriptional control in active melanocytes of human eumelanic hair bulbs. According to the absence of TRP-2 in melanin-producing melanocytes of brown and black hair bulbs, one must consider that eumelanogenesis as well as brown and black colour do not require TRP-2 expression in human hair.

- Fenoll L, Penalver MJ, Rodriguez-Lopez JN, Garcia-Ruiz PA, Garcia-Canovas F, Tudela J.
Deuterium isotope effect on the oxidation of monophenols and o-diphenols by tyrosinase. Biochem J. 380(Pt 3):643-50, 2004.
A solvent deuterium isotope effect on the catalytic affinity (km) and catalytic constant (kcat) of tyrosinase in its action on different monophenols and o-diphenols was observed. The catalytic constant decreased in all substrates as the molar fraction of deuterated water in the medium increased, while the catalytic affinity only decreased for the o-diphenols with an R group in C-1 [-H, -CH3 and -CH(CH3)2]. In a proton inventory study of the oxidation of o-diphenols, the representation of kcat fn/kcat f0 against n (atom fractions of deuterium), where kcat fn is the catalytic constant for a molar fraction of deuterium (n) and kcat f0 is the corresponding kinetic parameter in a water solution, was linear for all substrates, indicating that only one of the four protons transferred from the hydroxy groups of the two molecules of substrate, which are oxidized in one turnover, is responsible for the isotope effects, the proton transferred from the hydroxy group of C-4 to the peroxide of the oxytyrosinase form (Eox). However, in the representation of Km fn/Km f0 against n, where Km fn represents the catalytic affinity for a molar fraction of deuterium (n) and Km f0 is the corresponding kinetic parameter in a water solution, a linear decrease was observed as n increased in the case of o-diphenols with the R group [-H, -CH3 and -CH(CH3)2], and a parabolic increase with other R groups, indicating that more than one proton is responsible for the isotope effects on substrate binding. In the case of monophenols with six protons transferred in the catalytic cycle, the isotope effect occurs in the same way as for o-diphenols. In the present paper, the fractionation factors of different monophenols and o-diphenols are described and possible mechanistic implications are discussed.

- Gasowska B, Kafarski P, Wojtasek H.
Interaction of mushroom tyrosinase with aromatic amines, o-diamines and o-aminophenols. Biochim Biophys Acta. 1673(3):170-7, 2004.
3-Amino-L-tyrosine was found to be a substrate of mushroom tyrosinase, contrary to what had previously been reported in the literature. A series of amino derivatives of benzoic acid were tested as substrates and inhibitors of the enzyme. 3-Amino-4-hydroxybenzoic acid, 4-amino-3-hydroxybenzoic acid and 3,4-diaminobenzoic acid were oxidized by this enzyme, as previously reported for Neurospora crassa tyrosinase, but 4-aminobenzoic acid and 3-aminobenzoic acid were not. Interestingly, 3-amino-4-hydroxybenzoic acid was oxidized five times faster than 4-amino-3-hydroxybenzoic acid, confirming the importance of proton transfer from the hydroxyl group at C-4 position. All compounds inhibited the monophenolase activity but their effect on the diphenolase activity was small or negligible. 3-Amino-4-hydroxybenzoic acid was a stronger inhibitor than 4-amino-3-hydroxybenzoic acid, indicating

their different binding affinity to the oxy form of the enzyme. Both, however, were weaker inhibitors than 3-amino-L-tyrosine, 4-methoxy-o-phenylenediamine and 3,4-diaminobenzoic acid, which was the strongest inhibitor from among the compounds tested. These results show that the relative positioning of the amino group and the hydroxy group in o-aminophenols with respect to the side chain is important both for binding to the dicopper center and for catalysis.

- Girelli AM, Mattei E, Messina A, Tarola AM.
Inhibition of polyphenol oxidases activity by various dipeptides. J Agric Food Chem. 52(10):2741-5, 2004.
In an effort to develop natural and nontoxic inhibitors on the activity of mushroom polyphenol oxidase (PPO) the effect of various glycyl-dipeptides (GlyAsp, GlyGly, GlyHis, GlyLeu, GlyLys, GlyPhe, GlyPro, GlyTyr) was investigated. The inhibition study with dihydroxyphenylalanine (DOPA) as substrate is based on separation of the enzymatic reaction components by reversed phase HPLC and the UV detection of the dopachrome formed. The results have evidenced that several of tested dipeptides inhibited PPO activity in the range of 20-40% while GlyPro and GlyLeu had no effect. The study has also permitted the characterization of the following kinetic pattern: a linear-mixed-type mechanism for GlyAsp, GlyGly, GlyLys, and GlyPhe and a hyperbolic-mixed-type for GlyTyr. It was not possible to identify the inhibition mechanism for GlyHis, although it affects PPO activity. In addition the effects of GlyAsp, GlyLys and GlyHis were evaluated for lessening the browning of fresh Golden Delicious apple and Irish White Skinned potato. The effectiveness of such inhibitors was determined by the difference between the colors observed in the dipeptide-treated sample and the controls using the color space CIE-Lab system. The % browning inhibition on potato (20-50%) was greater than of apple (20-30%) by the all tested dipeptides. Only GlyLys presented the significant value of 50%.

- Haghbeen K, Saboury AA, Karbassi F.
Substrate share in the suicide inactivation of mushroom tyrosinase. Biochim Biophys Acta. 1675(1-3):139-46, 2004.
To address the real cause of the suicide inactivation of mushroom tyrosinase (MT), under in vitro conditions, cresolase and catecholase reactions of this enzyme were investigated in the presence of three different pairs of substrates, which had been selected for their structural specifications. It was showed that the cresolase activity is more vulnerable to the inactivation. Acetylation of the free tyrosyl residues of MT did not cure susceptibility of the cresolase activity, but clearly decreased the inactivation rate of MT in the presence of 4-[(4-methylbenzo)azo]-1,2-benzenediol (MeBACat) as a catecholase substrate. Considering the results of the previous works and this research, some different possible reasons for the suicide inactivation of MT have been discussed. Accordingly, it was proposed that the interruption in the conformational changes in the tertiary and quaternary structures of MT, triggered by the substrate then mediated by the solvent molecules, might be the real reason for the suicide inactivation of the enzyme. However, minor causes like the toxic effect of the ortho-quinones on the protein body of the enzyme or the oxidation of some free tyrosyl residues on the surface of the enzyme by itself, which could boost the inactivation rate, should not be ignored.

- Ji C, Wang Y, Guo X, Hartson S, Jiang H.
A pattern recognition serine proteinase triggers the prophenoloxidase activation cascade in the tobacco hornworm, Manduca sexta. J Biol Chem. 279(33):34101-6, 2004. Epub 2004 Jun 09.
A serine proteinase cascade in insect hemolymph mediates prophenoloxidase activation, a defense mechanism against pathogen or parasite infection. Little is known regarding its initiating proteinase or how this enzyme is activated in response to invading microorganisms. We have isolated from the tobacco hornworm, Manduca sexta, a cDNA encoding a modular protein designated hemolymph proteinase 14 (HP14). It contains five low density lipoprotein receptor class A repeats, a Sushi domain, a unique Cys-rich region, and a proteinase-catalytic domain. The HP14 mRNA exists in fat body and hemocytes of the naive larvae, and its level increases significantly at 24 h after a bacterial challenge. We expressed proHP14 with a carboxyl-terminal hexahistidine tag in a baculovirus/insect cell system and detected the recombinant protein in two forms. The 87-kDa protein was primarily intracellular, whereas the 75-kDa form was present in the medium. Interaction with peptidoglycan resulted in proteolytic processing of the purified zymogen and generation of an amidase activity. Supplementation of hemolymph with proHP14 greatly enhanced prophenoloxidase activation in response to Micrococcus luteus. These data suggest that proHP14 is a pattern recognition protein that binds to bacteria and autoactivates and triggers the prophenoloxidase activation system in the hemolymph of M. sexta.

- Jimenez-Atienzar M, Cabanes J, Gandia-Herrero F, Garcia-Carmona F.
Kinetic analysis of catechin oxidation by polyphenol oxidase at neutral pH. Biochem Biophys Res Commun. 319(3):902-10, 2004.
Catechin oxidation by peach polyphenol oxidase was performed in a pH range of 3.5-8.0. At acidic pH, maximal spectral changes were observed at 390nm and at pH 7.5, at 430nm. Catechin oxidation was studied at pH 7.5 to avoid the formation of free radicals. The results obtained allowed us to propose a pathway for the enzymatic oxidation of catechin, according to which enzymatic oxidation produces the corresponding catechin-o-quinone, which suffers the nucleophilic attack of another catechin unit, leading to the formation of a dimer. This dimer is then oxidized by the enzymatically generated o-quinone. The progress curves obtained for catechin oxidation by PPO showed a lag period, whose length changed with enzyme and substrate concentrations, and which must have been caused by the chemical

reactions taking place after the enzymatic reaction. The results obtained by simulation of the model produced the same qualitative dependences as obtained experimentally.

- Kanost MR, Jiang H, Yu XQ.
  Innate immune responses of a lepidopteran insect, Manduca sexta. Immunol Rev. 198:97-105, 2004.
  Many innate immune mechanisms are conserved throughout the animal kingdom. Manduca sexta, a widely used model for insect biochemical research, employs these mechanisms to defend against invading pathogens and parasites. We have isolated from M. sexta hemolymph a group of proteins (hemolin, peptidoglycan recognition proteins, beta-1,3-glucan recognition proteins, and C-type lectins), which serve as a surveillance mechanism by binding to microbial surface molecules (e.g. peptidoglycan, lipopolysaccharide, lipoteichoic acid, and beta-1,3-glucan). The binding triggers diverse responses such as phagocytosis, nodule formation, encapsulation, melanization, and synthesis of anti-microbial peptides/proteins. Some of these responses are mediated and coordinated by serine proteinase cascades, analogous to the complement system in mammals. Our current research is focused on the proteolytic activation of prophenoloxidase (proPO)--a reaction implicated in melanotic encapsulation, wound healing, and protein cross-linking. We have isolated three proPO-activating proteinases, each of which requires serine proteinase homologs as a cofactor for generating active phenoloxidase. The proteinases and proteinase-like molecules, containing one to two clip domains at their amino-terminus, are acute-phase proteins induced upon an immune challenge. Inhibitory regulation of the proteinases by serpins and association of the proteinase homologs with a bacteria-binding lectin are important for ensuring a localized defense response. Additional serine proteinases expressed in M. sexta hemocytes and fat body have been discovered. Future research efforts will be aimed at elucidating the proteinase cascade for proPO activation and investigating the roles of proteinases in other immuneresponses such as processing of plasmatocyte-spreading peptide.

- Kaplan J, DeGrado WF.
  De novo design of catalytic proteins. Proc Natl Acad Sci U S A. 101(32):11566-70, 2004. Epub 2004 Aug 03.
  The de novo design of catalytic proteins provides a stringent test of our understanding of enzyme function, while simultaneously laying the groundwork for the design of novel catalysts. Here we describe the design of an O(2)-dependent phenol oxidase whose structure, sequence, and activity are designed from first principles. The protein catalyzes the two-electron oxidation of 4-aminophenol (k(cat)/K(M) = 1,500 M(-1).min(-1)) to the corresponding quinone monoimine by using a diiron cofactor. The catalytic efficiency is sensitive to changes of the size of a methyl group in the protein, illustrating the specificity of the design.

- Lee SY, Lee BL, Soderhall K.
  Processing of crayfish hemocyanin subunits into phenoloxidase. Biochem Biophys Res Commun. 322(2):490-6, 2004.
  Hemocyanin and phenoloxidase are both copper-binding proteins involved in the immune system for a wide range of animal species. In crayfish, these proteins were purified and characterized from plasma and hemocytes, respectively. Recently, we have reported that the processing of one of the hemocyanin subunits occurs by a proteolytic cleavage under acidic conditions which results in the release of an antibacterial peptide designated as astacidin 1 from the C-terminus [J. Biol. Chem. 278 (2003) 7927]. In the present paper, we show that cleavage of crayfish hemocyanin subunit 2 at the N-terminal part results in that the processed hemocyanin exhibits phenoloxidase activity. The calculated mass of the cloned hemocyanin 2 is 78,372Da, which corresponds to the size obtained after SDS-PAGE under reducing conditions of the purified hemocyanin and pI is estimated to be 5.70. The complete hemocyanin 2 sequence shows 74% and 44% similarity with hemocyanin 1 and prophenoloxidase of crayfish, respectively. Crayfish hemocyanin exhibited phenoloxidase activity in presence of trypsin, but no activity could be detected if treated with sodium dodecyl sulfate. These results show that hemocyanin of crayfish is involved in several immune responses such as an oxygen carrier protein, as a precursor for an antibacterial peptide, and a molecule with phenoloxidase function.

- Lopez-Serrano D, Solano F, Sanchez-Amat A.
  Identification of an operon involved in tyrosinase activity and melanin synthesis in Marinomonas mediterranea. Gene. 342(1):179-87, 2004.
  The genomic region of Marinomonas mediterranea containing the genes required for tyrosinase activity and melanin synthesis has been cloned by marker rescue using the transposon-generated, amelanogenic strain T105. Five ORFs, two incomplete and three complete, have been sequenced in the genomic region where the transposon was inserted. RT-PCR analysis indicates that ORF 3, coding for tyrosinase, and ORF4, coding for a protein of 250 amino acids, are in the same transcriptional unit, constituting an operon whose promoter region has been determined by 5'-RACE. This operon has been sequenced in the wild-type and several mutant strains, indicating that both ORFs are required for expression of tyrosinase activity and melanin synthesis. The nitrosoguanidine generated, amelanogenic mutant ng56, shows a nonsense mutation in ORF3 coding for the tyrosinase. On the other hand, in the strain T105 the transposon is inserted in ORF4. The product of this gene is related to copper metabolism, since the addition of this metal ion to cell extracts or culture media partially restores melanin synthesis and tyrosinase activity in the strain T105. However, it does not show significant sequence similarity to previously characterized metallochaperones and hence may be an example of a new kind of those proteins. The operon has been denoted as ppoB, taking into consideration that ppoA denotes the M. mediterranea gene coding for the previously cloned polyphenol oxidase with laccase activity. This is the first demonstration of the tyrosinase gene forming part of an operon in a Gram-negative bacterium.

- Page-McCaw PS, Chung S, Muto A, Roeser T, Staub W, Finger-Baier K, Korenbrot JI, Baier H.
Retinal network adaptation to bright light requires tyrosinase. Nat Neurosci. 2004 [Epub ahead of print].
The visual system adjusts its sensitivity to a wide range of light intensities. We report here that mutation of the zebrafish sdy gene, which encodes tyrosinase, slows down the onset of adaptation to bright light. When fish larvae were challenged with periods of darkness during the day, the sdy mutants required nearly an hour to recover optokinetic behavior after return to bright light, whereas wild types recovered within minutes. This behavioral deficit was phenocopied in fully pigmented fish by inhibiting tyrosinase and thus does not depend on the absence of melanin pigment in sdy. Electroretinograms showed that the dark-adapted retinal network recovers sensitivity to a pulse of light more slowly in sdy mutants than in wild types. This failure is localized in the retinal neural network, postsynaptic to photoreceptors. We propose that retinal pigment epithelium (which normally expresses tyrosinase) secretes a modulatory factor, possibly L-DOPA, which regulates light adaptation in the retinal circuitry.

- Serafino A, Sinibaldi Vallebona P, Lazzarino G, Tavazzi B, Rasi G, Pierimarchi P, Andreola F, Moroni G, Galvano G, Galvano F, Garaci E.
Differentiation of human melanoma cells induced by cyanidin-3-O-beta-glucopyranoside. FASEB J. 2004 [Epub ahead of print].
Great attention has been recently given to a flavonoid of the anthocyanin class, cyanidin-3-O-beta-glucopyranoside (C-3-G), which is widely spread throughout the plant kingdom, and is present in both fruits and vegetables of human diets. In this study, we investigated the effect of C-3-G on proliferation and differentiation of human melanoma cells. Both morphological and functional parameters were evaluated, using electron and confocal microscopy, cytofluorometric analysis, HPLC assay, Western blot analysis, and enzymatic assay, as appropriate. A treatment with a single dose of C-3-G decreased cell proliferation without affecting cell viability and without inducing apoptosis or necrosis. The mitotic index and cell percentage in S phase were significantly lower in C-3-G treated cells compared with untreated control. C-3-G treatment induced, in a dose- and time-dependent manner, melanoma cell differentiation characterized by a strong increase in dendrite outgrowth accompanied with a remodeling of the microtubular network, a dramatic increase of focal adhesion and an increased expression of "brain specific" cytoskeletal components such as NF-160 and NF-200 neurofilament proteins. C-3-G treatment also induced increase of cAMP levels and up-regulation of tyrosinase expression and activity resulting in an enhanced melanin synthesis and melanosome maturation. Up-regulation of the melanoma differentiation antigen Melan-A/MART-1 in treated cells respect to the untreated control was also recorded. Data obtained provide evidence that a single treatment with C-3-G is able to revert the human melanoma cells from the proliferating to the differentiated state. We conclude that C-3-G is a very promising molecule to include in the strategies for treatment of melanoma; also because of its nutritional relevance.

- Smith-Thomas L, Moustafa M, Spada CS, Shi L, Dawson RA, Wagner M, Balafa C, Kedzie KM, Reagan JW, Krauss AH, Woodward DF, MacNeil S.
Latanoprost-induced pigmentation in human iridial melanocytes is fibroblast dependent. Exp Eye Res. 78(5):973-85, 2004.
The prostaglandin F2alpha derivative, latanoprost (LT), used in glaucoma treatment, can induce pigmentation in irises of patients with hazel or heterochromatic eye colour. The mechanism by which LT induces pigmentation in the iris is not yet established, although it does not appear to induce proliferation of iridial melanocytes. The purpose of this study was to develop an in vitro model in which to investigate this mechanism. The pigmentary responses to LT and prostaglandin F(2alpha) (PGF(2alpha)) were examined in human iridial melanocytes alone or in co-culture with epithelial cells (non-ocular human epidermal keratinocytes and iris pigment epithelial cells) or mesenchymal cells (non-ocular dermal fibroblasts or iridial fibroblasts). Melanogenesis was assessed after 4 days culture with prostanoids, using dopa oxidase activity. Prostaglandin FP expression on human iridial fibroblasts and melanocytes was investigated using an immunofluorescent technique employing antibody to PGF(2alpha) receptor and RT-PCR. Iridial melanocytes did not show a convincing increase in dopa oxidase when cultured alone but in the presence of fibroblasts (ocular or non-ocular) there was a significant increase (25-30%) in dopa oxidase activity in response to 10(-7)-10(-5)m LT and PGF(2alpha). Co-culture of melanocytes with epithelial cells, while leading to increased dopa oxidase activity, did not lead to any melanogenic response to LT or PGF(2alpha). FP receptor expression was detected on fibroblasts but not iridial melanocytes by immunocytochemistry and RT-PCR. The melanocyte/fibroblast co-culture model developed in this study also showed that LT and PGF(2alpha) increased dopa oxidase activity in melanocytes from donors with brown but not blue eyes. These results suggest that LT may be inducing pigmentation in the human iris indirectly through the FP receptor on adjacent fibroblasts.

- Takeyama R, Takekoshi S, Nagata H, Osamura RY, Kawana S.
Quercetin-induced melanogenesis in a reconstituted three-dimensional human epidermal model. J Mol Histol. 35(2):157-65, 2004.
Quercetin (3,5,7,3',4'-pentahydroxyflavone) is one of the most abundant natural flavonoids. It is present in various common vegetables and fruits. In this report, we examined the effect of quercetin on melanogenesis using a three-dimensional reconstituted human epidermal culture model, MelanoDerm, which is a new commercially-available cultured human epidermis containing functional melanocytes. Treatment with 10 microM quercetin induced an increase of tyrosinase activity in cultured epidermis after 3-5 days in time-dependent manner. In the quercetin-treated

epidermis, furthermore, melanin content and tyrosinase expression were markedly increased, as shown by immunohistochemistry after a 7-day culture period. Ultrastructural studies clearly indicated an accumulation of mature melanosomes (stages III and IV) inside the basal layer of the cultured epidermis after the quercetin treatment. In addition, the dendrites of melanocytes extended further towards the adjacent keratinocytes after quercetin treatment. These results suggest that quercetin has an effect on maturation of melanosomes and that quercetin has the potential to induced melanogenesis in human epidermis.

- Van Neste D, Tobin DJ.
Hair cycle and hair pigmentation: dynamic interactions and changes associated with aging. Micron. 35(3):193-200, 2004.
The tight coupling of hair follicle melanogenesis to the hair growth cycle dramatically distinguishes follicular melanogenesis from the continuous melanogenesis of the epidermis. Cyclic re-construction of an intact hair follicle pigmentary unit occurs optimally in all scalp hair follicles during only the first 10 hair cycles, i.e. by approximately 40 years of age. Thereafter there appears to be a genetically regulated exhaustion of the pigmentary potential of each individual hair follicle leading to the formation of true gray and white hair. Pigment dilution results primarily from a reduction in tyrosinase activity within hair bulbar melanocytes. Thereafter, sub-optimal melanocyte-cortical keratinocyte interactions, and defective migration of melanocytes from a reservoir in the upper outer root sheath to the pigment-permitting microenvironment close to the follicular papilla of the hair bulb, will all disrupt normal function of the pigmentary unit. Evidence from studies on epidermal melanocyte aging suggest that reactive oxygen species-mediated damage to nuclear and mitochondrial DNA may lead to mutation accumulation in bulbar melanocytes. Parallel dysregulation of anti-oxidant mechanisms or pro/anti-apoptotic factors is also likely to occur within the cells. Pigment loss in canities may also affect keratinocyte proliferation and differentiation, providing the tantalizing suggestion that melanocytes in the hair follicle contribute far more that packages of pigment alone. Here, we review the current state of knowledge of the development, regulation and control of the aging human hair follicle pigmentary system in relation with hair cycling. The exploitation of recently available methodologies to manipulate hair follicle melanocytes in vitro, and the observations that melanocytes remain in senile white hair follicles that can be induced to pigment in culture, raises the possibility of someday reversing canities. The perspective of rejuvenation of the whole hair follicle apparatus are still part of the dream but optimising its functional properties is clinically relevant and is close to reality. Finally as hair color influences its visibility when optical methods such as scalp photography are used to count hair fibers, the attention is drawn to possible interpretations of statistically significant changes in visible hair. Such changes may not exclusively be related to improved hair growth itself but also to changes in natural hair color that makes the hair more visible with the method used to count hairs.

- Wang Q, Shi Y, Song KK, Guo HY, Qiu L, Chen QX.
Inhibitory effects of 4-halobenzoic acids on the diphenolase and monophenolase activity of mushroom tyrosinase. Protein J. 23(5):303-8, 2004.
The effects of 4-halobenzoic acids (4-fluorobenzoic acid, 4-chlorobenzoic acid, and 4-bromobenzoic acid) on the activity of mushroom tyrosinase have been studied. The results show that 4-halobenzoic acids can strongly inhibit both monophenolase activity and diphenolase activity of the enzyme, and the inhibition displays a reversible course. The IC50 values were estimated as 0.26, 0.20, and 0.18 mM for diphenolase activity and as 1.03, 0.75, and 0.60 mM for monophenolase activity, respectively. Kinetic analyses show that the inhibition mechanism of all three 4-halobenzoic acids is noncompetitive inhibition to the diphenolase activity, and the inhibition constants (K1) were determined to be 0.25, 0.20, and 0.17 mM, respectively. The lag time of the monophenolase was obviously lengthened by these three 4-halobenzoic acids. When the concentration of inhibitors reached 1.4 mM, the lag time was lengthened from 30 s to 120, 125, and 150 s, respectively.

- Yamazaki S, Morioka C, Itoh S.
Kinetic evaluation of catalase and peroxygenase activities of tyrosinase. Biochemistry. 43(36):11546-53, 2004.
Tyrosinase is a copper monooxygenase containing a coupled dinuclear copper active site (type-3 copper), which catalyzes oxygenation of phenols (phenolase activity) as well as dehydrogenation of catechols (catecholase activity) using O(2) as the oxidant. In this study, catalase activity (conversion of H(2)O(2) to (1/2)O(2) and H(2)O) and peroxygenase activity (H(2)O(2)-dependent oxygenation of substrates) of mushroom tyrosinase have been examined kinetically by using amperometric O(2) and H(2)O(2) sensors. The catalase activity has been examined by monitoring the initial rate of O(2) production from H(2)O(2) in the presence of a catalytic amount of tyrosinase in 0.1 M phosphate buffer (pH 7.0) at 25 degrees C under initially anaerobic conditions. It has been found that the catalase activity of mushroom tyrosinase is three-order of magnitude greater than that of mollusk hemocyanin. The higher catalase activity of tyrosinase could be attributed to easier accessibility of H(2)O(2) to the dinuclear copper site of tyrosinase. Mushroom tyrosinase has also been demonstrated for the first time to catalyze oxygenation reaction of phenols with H(2)O(2) (peroxygenase activity). The reaction has been investigated kinetically by monitoring the H(2)O(2) consumption rate in 0.5 M borate buffer (pH 7.0) under aerobic conditions. Similarity of the substituent effects of a series of p-substituted phenols in the peroxygenase reaction with H(2)O(2) to those in the phenolase reaction with O(2) as well as the absence of kinetic deuterium isotope effect with a perdeuterated substrate (p-Cl-C(6)D(4)OH vs p-Cl-C(6)H(4)OH) clearly demonstrated that the oxygenation mechanisms of phenols in both systems

are the same, that is, the electrophilic aromatic substitution reaction by a micro-eta(2):eta(2)-peroxo)dicopper(II) intermediate of oxy-tyrosinase.

- Yoneta A, Yamashita T, Jin HY, Kondo S, Jimbow K.
  Ectopic expression of tyrosinase increases melanin synthesis and cell death following UVB irradiation in fibroblasts from familial atypical multiple mole and melanoma (FAMMM) patients. Melanoma Res. 14(5):387-94, 2004.
  Patients with familial atypical multiple mole and melanoma (FAMMM) [so-called familial dysplastic naevus syndrome (FDNS)] have a high risk for the development of malignant melanoma. The underlying gene defect has an autosomal dominant inheritance with variable expression and incomplete penetrance. Fibroblasts derived from FAMMM patients have high sensitivity to UVC and mutagens, e.g. 4-nitroquinoline-1-oxide. We were interested in identifying how the combination of inherent sensitivity to UV light and abnormal melanin synthesis interacts in the development of melanoma in FAMMM patients. Intermediates of melanin synthesis produce free radicals that are toxic to cells. Atypical moles (dysplastic naevi) are engaged in the biosynthesis of abnormal melanin pigments. This study examined whether there was any abnormal melanin pigmentation or cell damage after the ectopic expression of tyrosinase in fibroblasts from FAMMM patients when compared with fibroblasts from normal subjects. Fibroblasts from FAMMM patients (3012T and 3072T) were associated with a higher sensitivity than normal human fibroblasts to the toxicity of UVB. When cells were infected with tyrosinase-expressing adenovirus (Ad-HT) and irradiated with UVB, FAMMM fibroblasts showed higher tyrosinase activity, produced more melanin pigments and were degraded more significantly than normal human fibroblasts. Western blot analysis revealed that Ad-HT-infected 3072T produced a larger amount of tyrosinase protein than did Ad-HT-infected normal fibroblasts after UVB irradiation. Our findings suggest: (1) that FAMMM fibroblasts have an unknown machinery which enhances tyrosinase expression by UVB irradiation; and (2) that the resulting increase in melanin synthesis affects the cytotoxicity of UVB to FAMMM fibroblasts. All of these processes may be involved in the genomic instability and development of melanoma in FAMMM patients.

8. Melanosomes

(Dr. J. Borovansky)

Brief (*Tomita&Suzuki*) and detailed (*Slominski et al*) reviews have recently appeared. Aberrant melanosomes have been again observed in naevus cells (*Ide et al*), in melanoma cells (*Kazakov et al*) and in astrocytoma (*Krossnes et al*). Processing and sorting of gp100 protein and of tyrosinase in amelanotic cells were studied by *Yasumoto et al* and by *Watabe et al*, respectively. HMB-45 antibody has been considered to be melanosome specific (*Schaumburg-Lever et al / J Cutaneous Pathol 18(6): 432, 1991; Taatjes et al / Arch Pathol Lab Med 117(3): 264, 1993*) and has recently been shown to recognize the cleaved form of the gp100 protein in stage II melanosomes (*Yasumoto et al – see below*). Three papers showing that angiomyolipoma, tumour consisting of smooth muscle cells, adipose tissue and blood vessels, has been constantly HMB-45 positive as for its smooth muscle component (*Lin et al, Yaldiz et al, Zhang et al*) which suggests that HMB-45 is not a melanoma-restricted marker. Melanogenesis can be influenced by metallothionein (*Sasaki et al*). Role of both ß-endorphine and µ-opiate receptor in intramelanosomal regulation was suggested (*Kauser et al*). An attempt to model UVA-reactions in melanosomes was done (*Haywood&Linge*). Prof. Simon´s group has continued in introducing new techniques into melanosome research – this time photoelectron emission microscopy (*Samokhvalov et al).* Two papers studied degradation of melanosomes (*Mammone et al, Takahashi&Nakamura*).

- Haywood RM, Linge C.
  An experimental and theoretical model for solar UVA-irradiation of soluble eumelanin: towards modelling UVA-photoreactions in the melanosome? J Photochem Photobiol B76 (1-3): 19-32, 2004.
  Comments : A model was developed for the UVA-irradiation of soluble synthetic eumelanin exposed to the levels of irradiation comparable to sunlight. Radical production was determined by means of ESR and indirectly using a spin trap. The model would be biologically relevant if soluble forms of eumelanin were shown *in vivo*. The authors hope that eumelanin within the melanosome might be in a colloidal form due to its binding to protein(s).

- Ide F, Obara K, Enatsu K, Mishima K, Saito I.
  Balloon cell nevus of the soft palate: An immunohistochemical and ultrastructural study. Pathology Int 54(11): 872-876, 2004.
  Comments: Case report. The lesion was composed of conventional naevus cells, balloon cells and transitional forms between them. Progressive vacuolization of melanosomes in naevomelanocytes may be responsible for the formation of peculiar ballooning appearance. Ballooning phenomenon was not evident in melanophages containing melanosome complexes.

- Kauser S, Thody AJ, Schallreuter KU, Gummer CL, Tobin DJ.
  ß-Endorphin as a regulator of human hair follicle melanocyte biology. J Invest Dermatol 123(1):184-195, 2004.
  Comments: Immunoelectron microscopic studies revealed a close association of both ß-endorphin expression and µ-opiate receptor labelling with stage II and stage III melanosomes in cultured follicular melanocytes. Functional studies demonstrated that ß-endorphin is a modifier of hair follicle melanocytes via its ability to upregulate melanogenesis, dendricity and proliferation. Possibility of internalization of the receptor/ligand complex was suggested which might facilitate intramelanosomal regulation and subsequent melanogenesis.

- Kazakov DV, Rűtten A, Kampf W, Michal M.
  Melanoma with prominent pigment synthesis (animal-type melanoma). A case report with ultrastructural studies. Am J Dermatopathol 26(4): 290-297, 2004.
  Comments: First ultrastructural characterization of a rare melanoma type – animal-type melanoma /ATM/ (melanoma with prominent pigment synthesis) in man demonstrating that ATM belongs to neoplasms of melanosome-producing cells (other tumours of this category are summarized in the Discussion). Melanosomes were mainly ellipsoidal and spherical in shape with a marked predominance of stage II/III organelles. In addition to normal melanosomes there was a high number of aberrant melanosomes with deranged internal structures, eccentric melanin deposition, concentric lamellar matrix and vesicles in their interior.

- Krossnes BK, Mella O, Wester K, Mork SJ.
  Pigmented astrocytoma with suprasellar location: case report and literature review. Acta Neuropathol 108(5): 461-466, 2004.
  Comments: Fifth published case of pigmented astrocytoma. Glial tumour cells contained large numbers of mature, often aberrant, melanosomes and also some stage II and III melanosomes. Neuromelanin or lipofuscin granules were not found.

- Lin KJ, Eng HL, Lu SN, Chiu KW, Kuo FY.
  Hepatic angiomyolipoma: report of two cases with emphasis on smear cytomorphology and the use of cell block with immunohistochemical stains. Diagn Cytopathol. 31(4): 263-266, 2004.

- Mammone T, Marenus K, Muizzuddin N, Maes D.

Evidence and utility of melanin degrading enzymes. J Cosmetic Sci 55(1): 116-117, 2004.
Comments: Mechanism(s) of degradation the of melanin moiety of melanosomes has remained unsolved although some evidence suggests that it is rather an oxidative than a hydrolytic process /*see Borovanský&Elleder/ Pigment Cell Res 16:280,2003* /. In this study keratinocytes grown *in vitro* were pulse-labelled with melanin synthesized from ^{14}C-DOPA and in 2-18hr period the counts in both the cells and media were decreasing. The process of degradation was inhibited by chloroquine and by cycloheximide. The extracts from Aspergillus fumigatus and from Saccharomyces cerevisiae, when applied to human skin, caused a significant reduction in UVB-induced pigmentation. Since no technical details are mentioned, it is impossible to assess the importance of the reported data.

- Samokhvalov A, Hong L, Liu Y, Garguilo J, Nemanich RJ, Edwards GS, Simon JD.
Oxidation potentials of human eumelanosomes and pheomelanosomes. Photochem Photobiol 2004 – Epub ahead of print.
Comments: The technique of free electron laser/photoelectron emission microscopy was employed to ascertain ionisation thresholds of isolated hair melanosomes (= 4.6eV /eumelanosomes/ and 3.9 eV /as for phaeomelanosomes/); subsequently the corresponding values of oxidation potentials (-0.2V and 0.5V, respectively) were calculated. Experiments examining the reduction of Fe(III)-cytochrome confirmed that phaeomelanosomes are more pro-oxidant than eumelanosomes.

- Sasaki M, Kizawa K, Igarashi S, Horikoshi T, Uchiwa H, Miyaki Y.
Suppression of melanogenesis by induction of endogenous intracellular metallothionein in human melanocytes. Exp Dermatol 13(8): 465-471, 2004.
Comments: Melanosome-enriched fractions were prepared from melanocytes in which melanogenesis was suppressed by the induction of metallothionein. Tyrosinase activity was found to be decreased in these fractions and could be restored by the addition of antibody against metallothionein.

- Slominski A, Tobin DJ, Shibahara S, Wortsman J.
Melanin pigmentation in mammalian skin and its hormonal regulation.
Physiol Rev 84(4): 1155-1228, 2004.
Comments: A modern and the most extensive review of 2004 summarizing contemporary situation in pigment cell research with an adequate attention to melanosomes.

- Takahashi T, Nakamura K.
A study of the photolightening mechanism of blond hair with visible and ultraviolet light. J Cosmet Sci 55: 291-305, 2004.
Comments: Visible and ultraviolet light lightened to a similar degree melanin granules isolated from blond hair and decomposed melanin granules that were exposed on a cross section of blond hair. Visible light destroyed the structure of isolated melanin granules, UV light did not act in a similar manner, but the lightening rates induced by both lights were similar.

- Tomita Y, Suzuki T.
Genetics of pigmentary disorders. Am J Med Genetics 131C(1): 75-81, 2004.
Comments: Brief review accompanied by beautiful colour figures and clearly arranged tables: Disorders of melanoblast migration, disorders of melanosome formation, disorders of melanin synthesis in the melanosome and disorders of mature melanosome transport are covered.

- Watabe H, Valencia JC, Yasumoto K, Kushimoto T, Ando H, Muller J, Vieira WD, Mizoguchi M, Appella E, Hearing VJ.
Regulation of tyrosinase processing and trafficking by organellar pH and by proteasome activity. J Biol Chem 279(9): 7971-7981, 2004.
Comments: Processing of tyrosinase can be altered in amelanotic melanoma cells. The authors demonstrated that retention of tyrosinase in the ER can be corrected in the presence of protonophore or proton pump inhibitors, which increase the pH of intracellular organelles. Tyrosinase is then transported correctly from the ER to the Golgi, glycosylated and finally sorted to melanosomes. The expression of TYRP1 (facilitating tyrosinase processing in the ER) was downregulated in amelanotic cells.

- Yaldiz M, Kilinc N, Ozdemir E.
Strong association of HMB-45 expression with renal angiomyolipoma.
Saudi Med J 25(8): 1020-1023, 2004.

- Yasumoto K, Watabe H, Valencia JC, Kushimoto T, Kobayashi T, Appella E, Hearing VJ.
Epitope mapping of the melanosomal matrix protein gp100 (PMEL17). J Biol Chem 279(27): 28330-28338, 2004.
Comments: Protein gp100 was found to be processed and sorted in a manner distinct from other melanosomal proteins: it is delivered directly to stage I melanosomes following its processing in the ER and cis-Golgi. After its delivery

gp100 is cleaved at both termini in a series of specific steps that result in the transition of stage I to stage II melanosomes, with subsequent delivery of melanosomal enzymes to the organelles.

- Zhang SH, Cong WM, Xian ZH, Wong WQ, Dong H, Wu MC
  Morphologic variants and immunohistochemical features of hepatic angiomyolipoma. /*In Chinese*/
  Zhongua Bing Li Xue Za Zhi 33(5): 437-440, 2004.

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2005 8th International Conference on Solar Energy and Applied Photochemistry

February 20-26, Photoenergy Center, Upper Egypt [Luxor/Aswan]
Contact: Prof. Sabry Abdel-Mottaleb
Fac. of Science, Ain Shams University,
Abbassia, 11566 Cairo, Egypt
Cellular: + 2012 216 9584
Fax: + 202 484 5941 OR + 202 634 7683
E-mail: solar05@photoenergy.org
Web: www.photoenergy.org

2005 10th World Congress on Cancers of the Skin

May 13-17, Vienna, Austria
Contact: Elfriede Pomp
Dept of Dermatology, Vienna General Hospital
University of Vienna
Währinger Gürtel 18-20
A - 1090 Vienna
Tel: +00431 40400 7707 Fax: +00431 40400 7699
E-mail: info@wccs.at
Web: www.wccs.at

2005 4th International EUROSKIN Conference

May 18-20
Contact:
E-mail l: euroskin@t-online.de
Web: www.euroskin.info

2005 IVth IACD World Congress - International Academy of Cosmetic Dermatology

Juli 03-05 Palais des Congrès de Paris 75017 Paris (France)
Contact: MCI France / IACD 2005
11, rue de Solférino
75007 Paris
France
Tel.: 33 (0)1 53 85 82 51 - Fax.: 33 (0)1 53 85 82 83
E.mail : iacd2005@mci-group.com

Web: www.iacd-paris2005.com

2005 XIVth International Pigment Cell Conference (IPCC)

September 18-23, Reston, Virginia, USA
Contact: Dr. V. HEARING
E-mail: hearingv@nih.gov
Web: www.ipcc.info

2005 35th Annual ESDR Meeting

September 22-24, Tübingen, Germany
Contact:
E-mail: office@esdr.org
Web: www.esdr.ch

2005 14th Congress of the European Academy of Dermatology and Venereology

October 12-16, London, United Kingdom
Contact: CTS
Data House
Curriers Close
Tile Hill
Coventry CV4 8AW
UK
Tel: +44 (0)870 429 4612
Fax: +44 (0)870 429 4613
Email: eadv@ctsnet.co.uk
web: www.eadv2005.com

2006 36th Annual ESDR Meeting

September 7-9, Paris, France
Contact:
E-mail: office@esdr.org
Web: www.esdr.ch

2006 XIIIth Meeting of the ESPCR

Barcelona, Spain
Contact: Dr. L. Montoliu
E-mail: montoliu@cnb.uam.es
Web : www.cnb.uam.es/~espcr06/

2007 37th Annual ESDR Meeting

September 6-8, Zurich, Switzerland
Contact:
E-mail: office@esdr.org
Web: www.esdr.ch

2007 21ST World Congress of Dermatology

October 1-5
Contact:
E-mail: info@dermato2007.org
Web: www.dermato2007.org

2008 International Investigative Dermatology (Joint Meeting of the ESDR, SID and JSID)

May 14-17 , Kyoto, Japan

Contact:

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

To the members of the Pigment Cell Societies
(ESPCR, JSPCR, PASPCR, ASPCR)

Special Interest group on Vitiligo Satellite Meeting,
IPCC Hyatt Regency Reston, Reston, Virginia, USA,
23 sept 2005

Dear Colleagues,

Vince Hearing, who is organizing the next IPCC meeting , has offered the possibility for the Vitiligo research community to meet after the next international pigment cell conference. We think that this is a very welcome occasion to share our views and projects on an international basis around this puzzling and disfiguring disease. As co-chairs of the SIG on Vitiligo, we will be in charge of organising a one day satellite meeting, on Friday 23 Sept 2005.

Vitiligo is one of the new frontiers in pigment cell research, due to the large amount of information gathered in melanocyte biology over the last two decades. Pigment cell biologists have manifested a keen interest in this disease at the last ESPCR meeting, held in Paris last September, and are willing to confront their views with those of clinical investigators at this next meeting, which promises to be exciting. Other topics to be covered will include methods of assessment and outcome measures, medical and surgical therapies, and an exchange forum on the international burden of the disease including representatives from patient's support groups .
We plan to post a page on the IPCC website http://www.palladianpartners.com/IPCC05 in December with a preliminary program.

We welcome short contributions to be adapted to a workshop format which will fit the delineated program so please feel free to contact us We would also be happy to welcome at this Satellite symposium other colleagues interested in vitiligo research who do not plan to go to the IPCC. Please send us their electronic addresses to inform them.

We are looking forward to your input and any further suggestions you may have in order to make this symposium lead us to better approaches for our patients,

Best regards,

Alain Taïeb alain.taieb@chu-bordeaux.fr
Mauro Picardo dirsci.isg1@ifo.it

ANNOUNCEMENT
ESPCR COUNCIL ELECTIONS

Dear ESPCR members,

The election process to cover two vacancies in the ESPCR Council was completed at the end of October. The re-elected candidates are:

- Dr. Colin Goding, from the MCRI, Oxted.
- Dr. Lionel Larue, from the Institut Marie Curie, Paris.

The new composition of the Council will be presented for approval to the next General Assembly, to be held in Reston, Virginia during the XIXth IPCC.

This new composition will be:

OFFICERS

President: J. C. García-Borrón (Murcia)
Secretary: J.M. Naeyaert (Ghent)
Treasurer: J. Lambert (Ghent)

COUNCIL

F. Beermann (Lausanne)
Dorothy Bennett (London)
G. Ghanem (Brussels)
C.R. Goding (Oxted)
Lionel Larue (Paris)
M. Picardo (Rome)
N. Smit (Leiden)
A. Taïeb (Bordeaux)

THE VITILIGO SOCIETY

CALL FOR RESEARCH PROJECTS

The Vitiligo Society of the UK has been reorganised such that a greater emphasis will in future be placed on research into the causes of vitiligo, its treatment and hopefully a cure. To these ends the Society is calling for potential research projects concerned with the basic mechanisms of pigment formation and destruction or possible new lines of treatment of vitiligo that require funding in order to proceed. Research projects at pre- and post-doctoral levels are eligible and will be considered by the Society's Medical and Scientific Committee. It is unlikely that funding will be available before the middle of 2005.

A Research Application form can be downloaded from the Society's web site at www.vitiligosociety.org.uk or by post from the Secretary of the Society at 125 Kennington Rd., London, SE11 6SF.

IPCC Update - December, 2004

The International and Local IPCC Program Committees have finished selecting all Invited Speakers for the 19th IPCC; the full list can be seen at [www.ipcc.info]. We have 9 Keynote Speakers and 22 Plenary Symposium speakers. The balance of lectures in the Plenary Symposia and Concurrent Sessions will be selected from the abstracts submitted, and up to 160 oral presentations will be selected in that manner. Abstracts will be rated blindly by the 3 co-chairs of each session so to maximize your chance of being selected for an oral presentation, plan and write your abstract well, paying particular attention to the topic of the intended Plenary Symposium or Concurrent Session. When you submit your abstract online, you will have the option to have your abstract considered first for one Plenary Symposia, then for one Concurrent Session; abstracts not selected for oral presentation will be scheduled for a poster presentation. The top 5 abstracts submitted will be presented in the Hot Topics Symposium. You may be listed as a coauthor on multiple abstracts but you may be the presenting author on only 1 abstract at this meeting.

Scientific Program - The expanded scientific program can now be accessed from the IPCC web site [www.ipcc.info]. It will not be further updated until after the abstract deadline and oral presentations have been added (approximately in mid-June). The names of all invited speakers and co-chairs of sessions along with tentative scheduled times for all scientific, political and social activities are listed.

Social Program - The social program is currently being designed but will include an Opening Reception on Sunday, Sept 18th and the Conference Tour/Awards Banquet at the J.F. Kennedy Center for the Performing Arts on Wednesday, Sept 21st. We are able to accommodate up to 500 people at the Conference Banquet; this should be sufficient (we hope) but if registration exceeds that number, only the first 500 registrants will be provided a ticket to the Banquet.

Registration - will cover: attendance at all scientific sessions, a copy of the Program/Abstracts (to be published as a Supplement in Pigment Cell Research), continental breakfasts each day, attendance at social events as noted above (and as listed on the web site) and Satellite Symposia scheduled for Friday, Sept 23rd (currently Genetics & Developmental Biology, Melanoma, Photobiology, and Vitiligo sessions are scheduled). Please note that registration fees will be discounted from January 1st until May 1st after which they will be set at the standard rate, as noted on the web site. After August 24th, discounted hotel reservations at the Conference venue will be discontinued and a late registration penalty will be added. Students, junior faculty and retired scientists will be eligible for reduced registration fees. Please also note that active members of the 3 regional pigment cell societies are eligible for reduced registration fees; members should ensure that their dues for 2005 have been paid and that they are in good standing in their society before registering.

Hotel Reservations - special rates at the Conference hotel (the Hyatt Regency Reston) can be accessed online via the IPCC web site. Be sure to use the conference code to get the discounted rate ($175), which is a 30-40% discount over their standard rate. The hotel will be fully booked by the time of the meeting so attendees are encouraged to reserve their room as soon as possible. The hotel is offering the discount rate to IPCC attendees for up to 3 days before or after the meeting for those who would like to arrive early or stay late for sightseeing, etc. The cost of the room is the same for 1 or 2 people, so sharing a room is an excellent option to save your budget. You can browse the hotel facility and its amenities at [reston.hyatt.com] and the surrounding pedestrian mall with many shops and restaurants at [www.restontowncenter.com].

Online Web Site Functions - Abstract submission, early Registration, Travel Stipend and Hotel reservations will open online as of January 1, 2005. The deadline for Abstract submission and Travel Stipend applications is May 1, 2005. Instructions for size and formatting of your abstract can be found on the web site, as can the requirements and procedures for students/junior faculty to apply for Travel

Stipends. You will be notified of the disposition and scheduling of your abstract by the end of June and those awarded Travel Stipends will be notified at that time as well.

Important Dates:

- Jan 1, 2005 - Web site [www.ipcc.info] becomes fully functional for Abstract submission, early Registration, Travel Stipend application and Hotel reservations.
- May 1, 2005 - deadline for Abstract submission, Travel Stipend applications and early Registration.
- June 30, 2005 - notification of Abstract scheduling and decisions on Travel Stipends.
- Aug 24, 2005 - deadline for normal Registration and Hotel reservations.
- Sept 18-22, 2005 - the 19th International Pigment Cell Conference
- Sept 23, 2005 - Satellite Symposia

We look forward to welcoming you at this meeting, and please contact me if you have any questions about this Conference,

Vince Hearing

From the 19th International Pigment Cell Conference

*** * * WWW.IPCC.INFO * * ***

The 19th IPCC is just about 1 year away and active planning of the scientific and social program by the Local Organizing and International Scientific Program Committees continues at a fast pace. We have selected 6 Outstanding Keynote Speakers who will present lectures at various times during the course of the IPCC (Prof. Elizabeth Blackburn, Dr. Francis Collins, Dr. Jennifer Lippincott-Schwartz, Prof. Shin-ichi Nishikawa, Prof. Jonathan Rees and Prof. Robert Weinberg). Add to that stellar list the names of Prof. Dorothy Bennett (who will present the IFPCS Presidential lecture) and Prof. Greg S. Barsh (who will present the Aaron B. Lerner lecture) as well as the Seiji lecturer (yet to be determined). Those speakers alone could constitute an outstanding Conference but we aren't finished yet. Each of the 13 Plenary Symposia will also have 2 outstanding Plenary Lecturers who are currently being selected, and whose names will probably be posted in the Scientific Program on the web site (www.ipcc.info) by the time you read this.

Each of those Plenary Symposia will also feature 3 or 4 oral presentations selected by the co-chairs of the various sessions from the abstracts submitted. In addition, there will be 14 Concurrent Sessions, each also featuring 8 oral presentations selected by the co-chairs from the abstracts submitted. A planned Poster Session combined with a wine & cheese session should prove lively as will Sunrise Sessions by experts in the field who will provide background each day for those not expert in those topics.

The IPCC Conference site, the Hyatt Regency Hotel in Reston, Virginia, is a magnificent conference facility with quick (10 min) and reasonable (free) access to Dulles International Airport. It is located on a pedestrian mall with plenty of shops and restaurants for diversion and culinary choices.

We have not ignored the Social aspects of the meeting and planning is underway for an Opening Cocktail reception (Sunday, Sept 18th), a Wine & Cheese poster session (Tuesday, Sept 20th), a Tour and Conference banquet (Wednesday, Sept 21st) and a farewell drink (Thursday, Sept 22nd). Friday (Sept 23rd) will feature a number of Satellite Sessions to be arranged by various groups and anyone wanting to host one of those should contact the Organizer as soon as possible. Preference will be given to IFPCS special interest groups and already we are scheduling Satellite sessions on Melanoma, Vitiligo, Photobiology and a Genetics Workshop. Plan to bring along an accompanying person; we are arranging an activity each day for them, and their registration fees will cover those activities along with the other social functions mentioned above.

We are soliciting funds from societies, government institutions and corporate sponsors to make this meeting as affordable as possible. Travel stipends will be available to students and junior faculty that are members of one of the regional pigment cell societies. However, pay attention to the deadlines below as fees and hotel expenses will increase significantly as the meeting approaches.

IMPORTANT DATES:

- January 1, 2005 - web site (www.ipcc.info) becomes active for abstract submission, early registration and hotel reservations
- April 1, 2005 - deadline for abstract submission
- July 1, 2005 - deadline for early registration (prices then increase by $100)
- Aug 1, 2005 - deadline for hotel reservation discount (prices ~double)
- Sept 1, 2005 - deadline for normal registration (prices then increase again by $100)

IFPCS AWARDS

It is a very great pleasure to announce to you the winners of the two IFPCS awards to be presented at the 19th IPCC next year, as determined by voting by the IFPCS Awards Committee, which concluded at the end of October. Both seem very well deserved.

One of you already knows at least one of these results, since the Seiji Lectureship is awarded to Prof Shigeki Shibahara.

Secondly, the Myron Gordon Award winner is Prof Masako Mizoguchi, so general congratulations to JSPCR for taking both awards this year.

Prof. D.C. Bennett
President, IFPCS