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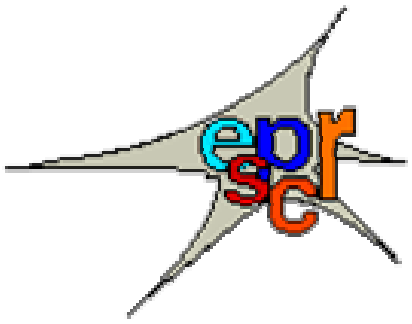

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH *BULLETIN*

N° 41 - December 2001

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*** HAPPY NEW YEAR 2002 ***

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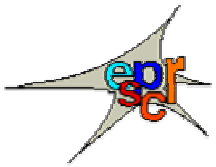

LETTER TO THE EDITOR
DISCUSSION, REVIEW,
SHORT COMMUNICATION, ...

MEETING REPORT

10th MEETING of the ESPCR

26-29 Sept 2001, Rome, Italy

INTRODUCTION

Contributed by Mauro Picardo

With great pleasure, this year, we organized and hosted the 10th Meeting of the European Society for Pigment Cell Research that, after thirteen years, has been held again in Italy. The inaugural Meeting indeed took place in Sorrento under the organization of one of the founders of the Society. We have tried to renew the same warm and friendly atmosphere and, must be said, Rome contributed to this wish with a extraordinary sunny September. Unfortunately, on 11th September, a date that everybody will never forget, the world was shocked by terrorist attacks in U.S.A., whose cruelty was of an exceptional extent. In spite of the discouraging circumstances, the overall attendance to the congress was only slightly affected and we did appreciate the participation of those who traveled to Rome, especially from overseas countries. We also understood fully some cancellations. For this reason, and for many others, on behalf of the organizing and scientific committees, I would love to thank the 210 scientists who participated in the 10th ESPCR Meeting, of whom 40 were non-ESPCR members.

The satellite symposium on hyper- and hypo- pigmentary disorders that preceded the opening of the meeting gave me the opportunity to introduce in a new dress the Dermatological Institute S. Gallicano that participated to the organization of the event. The goal of the clinically-oriented satellite symposium was to gather expertise of excellent clinicians-scientists in the treatment of benign pigmentary disorders to be transmitted to dermatologists. The satellite symposium, for its great deal of valuable contributions, represented a good premise to the following ESPCR meeting that was opened the day after in the Aula Minor at Angelicum University. In keeping with the interdisciplinary tradition of the Society, the scientific program foresaw numerous - 42 - invited contributions from excellent European and non-European researchers, in an attempt to offer overviews on very different areas of pigmentation to participants. The program was divided in eight sessions and two round tables that were all well attended, and discussions stemmed at the end of each presentation, always lively and rich of interesting cues. Organizers and scientific committee agreed in the introduction of innovative issues related to the regulation of melanogenesis, such as oxidative stress. In line with the latter, we judged of great interest an overview on the involvement of mitochondrial aberrant redox mechanisms in the pathogenesis of several diseases, held by Giuseppe Rotilio. Celebrative and of high scientific significance was the Fritz Anders Memorial Lecture on the genetic link between skin colour and susceptibility to skin cancer delivered by Richard Sturm. Very interesting submitted papers were also numerous and were selected for 46 oral presentations and 54 (plus 3 late) poster presentations. We tried to mirror the wider view on advances in the pigmentary cell research given during the meeting with an “overview” offered by the terrace on Via dei Fori Imperiali on the historical traces left by the ancients that, hopefully, was enjoyed by attendants.

We hope that the displayed continuous progress and improvement of our Society will further contribute to the success of the forthcoming meetings.

Mauro Picardo and the Organizing Committee

Session I. EUMELANINS, PHEOMELANINS AND SUSCEPTIBILITY TO UVR
Chaired by Marco d'Ischia, Stan Pavel and Tadeusz Sarna.
Contributed by Marco d'Ischia

The opening session of the 10th ESPCR meeting in Rome addressed at a multidisciplinary level the role of the different types of melanins in the individual susceptibility to UV radiation. The theme was brought to focus by three inspiring invited lectures and was further discussed in three proffered platform presentations. The opening lecture by G. Prota (Naples) was basically a keynote address in which the emerging complexities in the structures of melanin pigments from human hair were briefly overviewed. By means of improved microanalytical methodologies, based on hydrogen peroxide degradation, it was shown that the variety of human hair colour is due to at least four types of melanins. Such a diversity depends both on the well known branching point in the biosynthetic pathway, due to the intervention of cysteine leading to pheomelanins, and the occurrence of post-biosynthetic modifications due chiefly to hydrogen peroxide. This can cause even dramatic alterations of the basic polydihydroxyindole and polybenzothiazine structures of eumelanins and pheomelanins, whereby pyrrolic and (benzo)thiazolic moieties become prevailing.

The discussion that followed touched on several aspects of the presentation from the sensitivity of analytical methods to the possible consequences of the structural variety in relation to UV susceptibility. The emerging view was that melanins are not directly responsible for skin susceptibility but are rather markers of the genetic and biochemical features of the melanocytes from which they are produced.

The subsequent presentation by S. Rosso (Turin) provided an overview of epidemiological ongoing approaches to determine relationships between skin type, melanins and melanoma risk. The author stressed that the relationship between genes polymorphism, molecular markers and risk of skin neoplasm in human population must be investigated with an appropriate epidemiological study design. To this aim, the GEM and HELIOS2 studies in molecular epidemiology maximise their efficiency in studying low prevalence markers, comparing sporadic versus multiple melanoma (GEM), and melanoma, basal-cell, squamous-cell carcinoma versus health controls (HELIOS2). As the author pointed out, molecular epidemiology of skin cancer is necessarily a work in progress, as from laboratory research new suggestions can be obtained on genes and molecular markers involved in carcinogenesis, and their biological mechanism. The presentation was well balanced and offered interesting opportunities for discussion. The third invited lecture was delivered by E. Kvam (Oslo), who studied the role of melanin or melanin precursors in UVA-induction of oxidative damage in human and mouse melanocytes and melanoma cells. He reported that some types of cells, in which melanin synthesis was induced prior to UVA-irradiation, were sensitized to UVA-induction of the premutagenic oxidative DNA base damage, 8-oxo-guanine. Since only cells producing pheomelanin were sensitized, pheomelanin synthesis is apparently associated with increased susceptibility to UVA-induction of premutagenic DNA damage.

In the first proffered paper, J.-F. Doré (Lyon) presented results from a collaborative study involving four groups in Brussels, Lyon, Tokyo and Milan, showing that individual response to UV light, as measured by apoptosis induced in peripheral blood lymphocytes by low dose UV radiation, constitutes a novel risk factor for melanoma at an early age, independently of other known risk factors. The discussion raised the possible relation between the functional assay and a polymorphism in DNA repair pathways. Considerable interest was also raised by the last two papers. In a joint contribution from Bradford University and Procter-Gamble, the effect of pro-opiomelanocortin (POMC) peptides was investigated for the first time in cultured human scalp hair follicle melanocytes. The results were reported by S. Kauser (Bradford) and indicated potent mitogenic, melanogenic and dendrogenic effects suggesting involvement in the regulation of differentiation and melanogenesis. An attractive, though still speculative possibility, is that a chronic alteration of POMC peptide homeostasis may be involved in hair greying, thus providing a new focus for dermocosmetic research in this area. Finally F. Rouzaud from Dr. Hearing's lab at NIH (Bethesda) presented recent data on the effects of alpha-MSH on the extension gene transcripts. The remarkable finding was that alpha-MSH induces two types of transcripts, the second of which, designated T2, is shorter that the first (T1). This may help define the

role of specific nucleotide sequences in each transcript and the significance of post transcriptional processes.
The central relevance of the theme to the whole field of pigment cell research, the multidisciplinary approaches surveyed, and the high quality of the presentations were undoubtedly strong incentives for the lively discussions during the session. Dissecting the complex interplay of etiological and contributory factors that emerged from the various presentations is certainly a primary goal in studies of UV susceptibility and is likely to open new perspectives for future research in this area.

Session II. REGULATORY MECHANISMS OF MELANOGENESIS
Chaired by José Carlos García-Borrón, Sheila Mac Neil and Anthony Thody
Contributed by José Carlos García-Borrón

The Session started with three consecutive invited lectures. The first one was presented by Robert Ballotti (Nice), who reviewed cAMP signalling in melanocytes. This signalling is complex and involves an interplay of several intracellular cascades. The best known is the one leading to protein kinase A-mediated activation of CREB and transcriptional up-regulation of Microphthalmia (Mitf). Mitf then binds to and activates the promoters of several melanogenic genes, notably TYR. But cAMP does much more than this. It activates the MAP kinase pathway, whose sustained activation inhibits melanogenesis by inducing a phosphorylation-dependent Mitf degradation. Therefore, the same signal mediates both stimulatory and inhibitory responses, that may allow for a limitation of the melanogenic response to αMSH. In addition ot this effect of cAMP on MAP kinases, cAMP also interacts with the phosphatidyl inositol 3-kinase pathway and with Rho protein, which accounts for its influence on melanocyte dendricity. The overall picture that emerges is that of a complex network of interactions and cross-talk between signalling cascades governing virtually all aspects of melanocyte biology.
The next invited lecture was delivered by Colin Goding (Oxted) who presented data on the differential regulation of Mitf in melanocytes and melanoma cells. Since Mitf is a key regulator of melanocyte development and differentiation, the control of its expression is a major aspect of melanocyte biology. The transcription factors positively controlling the Mitf promoter are relatively well characterized. However, much less is known about repressors of the Mitf promoter activity. Melanoma cells display aberrant patterns of tyrosine kinase activity and a constitutive activation of the Wnt signalling pathway. Dr. Goding presented strong evidence showing that Brn-2, a member of the POU domain family of transcription factors binds to and represses Mitf promoter activity, both in vitro and in vivo, as shown by chromatin immunoprecipitation experiments. Interestingly, the levels of expression of Brn-2 are very low in normal melanocytes, but high in melanoma cells. This can be related with the positive control of Brn-2 expression by Ras, Rho and β-catenin signalling, three pathways deregulated in melanoma.
The last invited lecture of the Session was presented by Jose Carlos García-Borrón (Murcia) and dealt with structure-function relationships in the tyrosinase family proteins. The main question that was addressed was the identification of structural elements accounting for the very different enzymatic activities of tyrosinase, Tyrp2/Dct and Tyrp1. Concerning Tyrp2, its dopachrome tautomerase activity is explained by the binding of zinc, rather than copper, as the metal cofactor. However, the structure of the metal ion binding sites of tyrosinase and Tyrp2 is very similar, and mutation of selected residues of Tyr copper binding sites to mimic the Tyrp2 sites fails to switch the metal cofactor specificity. Therefore, the determinants of a differential metal ion specificity may lay outside the active site and be related to the interaction with different chaperones. Evidence was also obtained for a differential docking of monophenolic and diphenolic substrates to the active site or Tyr, since the effects on the hydroxylase and oxidase activities of some site-directed mutants were markedly different. Finally, Tyrp1 does not display detectable enzymatic activities when expressed in heterologous cells, but the protein is not adequately processed to the final glycosylation isoform. This is in contrast to Tyr, whose glycosylation pattern is normal after transient expression in the same host cells. Therefore, the requirements for efficient processing of Tyr and Tyrp1 are different. Overall, the data presented suggest that important determinants of full enzymatic activity are located outside the highly conserved

metal ion binding sites, but do have a strong effect on the glycosylation status of these sites and on their metal binding specificity.
The next presentation, by J. Vachtenheim (Prague) highlighted the complexity of the regulation of the chromatin-integrated tyrosinase gene. In human melanoma cells, Tyr mRNA levels correlate very poorly with MITF protein levels. Moreover, evidence for still uncharacterised mechanisms of transcriptional activation of the Tyr gene was obtained from studies of E1a mutants.
The next two papers came from A. Thody's laboratory (Bradford). In the first one, J. Ancans discussed the possible implications of the genetic background in P-locus product abundance. The P protein appears to activate melanogenesis by controlling the melanosomal pH. Melanocytes from skin types I to IV show little or no difference in the levels of P mRNA, and the same is true for melanocytes from Caucasian or Asian donors. Conversely, P mRNA abundance increases upon treatment with αMSH or sun exposure. The data suggest that the presence of P gene polymorphisms, rather than differences in the levels of expression, might act as genetic determinants of skin colour. The second paper, presented by M. Hoogduijn discussed the regulation of calcium fluxes in human melanocytes. The striking feature in this presentation was the demonstration of spontaneous fluctuations in intracellular calcium concentrations within individual melanocytes, whose significance remains to be elucidated.
The last talk of the Session was delivered by Messod Benathan (Lausanne), and dealt with the effects of glutamine on thiol availability and tyrosinase activity in human melanocytes and melanoma cells. Cells grown in media containing a low glutamine concentration contained high levels of free cysteine and 5-S-cysteinyldopa (5-S-CD), but low tyrosinase activity. In the presence of higher concentrations of glutamine, cysteine and 5-S-CD decreased in a concentration-dependent manner, whereas tyrosinase activity increased. These effects of glutamine might be dependent on the conversion into glutamate.

Session III. GENETICS
Chaired by Miguel Seabra and Eugene Healy
Contributed by Eugene Healy

The first talk of this session was by Eugene Healy (Southampton) who discussed the causal association between melanocortin 1 receptor (*MC1R*) gene variants and red hair and fair skin type. Following on from the original association studies, more recent work involving transfected cell lines demonstrated that *MC1R* variants compromise signalling via cAMP in melanoma cells. The generation of recessive yellow mice transgenic for human *MC1R* confirmed that *MC1R* variants alter the pigmentation phenotype *in vivo*, and preferentially result in the synthesis of phaeomelanin.
Valeria Marigo (Naples) reported on Oa1-deficient mice generated by gene targeting as a model for ocular albinism type 1, an X-linked form of albinism affecting the eye. Although the Oa1-deficient mice are phenotypically indistinguishable from wild type mice, abnormally large melanosomes are detected by microscopic examination of the retinal pigment epithelium of the affected mouse. Similar to that seen in human OA1 patients, a reduction in ipsilateral optic nerve fibres is seen in the Oa1 mouse, and the model may therefore help in the elucidation of the mechanism whereby this abnormality arises.
Celia Jiménez-Cervantes (Murcia) discussed two new *MC1R* variants which had recently been identified in the Spanish population. Cell transfection experiments suggested that the Ile40Thr and Val122Met, which were seen more frequently in subjects with skin types I-II, are likely to affect the ability of alpha-MSH to signal via MC1R. Both variant receptors had slightly lower binding affinities than the wild type receptor, and resulted in attenuated cAMP responses, consistent with these variants being partial loss of function mutations.
Jochen Utikal (Ulm) showed evidence, using fluorescence in situ hybridization (FISH), for additional copies of Cyclin D1 being present in short term cultures of primary and metastatic melanomas. In addition, high quality pictures of interphase FISH on paraffin-embedded sections were presented, which demonstrated that primary uncultured melanomas contained extra copies of Cyclin D1 (as compared with a centromere 11 probe). The results suggested that aberrations of Cyclin D1 may be important in the pathogenesis of cutaneous melanoma.

The next presentation by Tomonori Motokawa (Nagoya) reported on the expression of several pigment-related genes in lentigo senilis. Increased expression of Tyr, TRP-1, TRP-2, Pmel-17, P, and MITF was detected in cells matching the distribution of melanocytes, whereas POMC was over-expressed in epidermal keratinocytes. The possibility of increased POMC expression resulting in the transcription of the other pigment-related genes in the melanocytes was discussed. However, it was noted that the frequent observation of lentigo senilis in red haired subjects, many of whom have two variant *MC1R* alleles, might suggest that the higher POMC expression was not a causal event.
Paola Grammatico (Rome) spoke about CDNK2 mutations in patients with melanoma, and reported that three novel mutations had been identified, as well as five previously described mutations, in Italian subjects with this condition. It was noted that subjects with CDNK2 mutations more frequently had multiple melanoma, and that CDNK2 mutations were sometimes present in families where only one or two members had developed melanoma.
In the final talk, Annerose Anders (Giessen) presented interesting data on melanomagenesis in a Xiphophorus fish line. In this model, more melanomas developed in successive generations of fish who were descendants of those exposed to UVB and X-ray irradiation (although the offspring themselves had not been exposed to these noxious stimuli) than the numbers of melanomas arising in descendents of fish not exposed to radiation. Paragenetic elements, possibly involving a retrotransposon, are likely to be responsible, and result in anticipation with earlier onset of melanoma in later generations of fish.

Session IV. PIGMENT CELL DEVELOPMENT
Chaired by Elisabeth Dupin, Colin Goding and Lionel Larue
Contributed by Lionel Larue

This session regrouped two aspects of development. The first one was associated to the embryological development and the second one to the technological development. The first presentation of this session associated both aspects. Unfortunately, William Pavan (IL23) cancelled his trip to Rome after the events of September 11th, 2001. Bill was supposed to present his work on a functional genomic approach to discover genes involved in the development and the transformation of melanocytes. They produced cDNA micro array containing thousands of melanocyte expressed EST and known genes associated with melanocytes. On these micro arrays we can find Endothelin-3 (ET-3) and Steel (Sl) ligands as well as their corresponding receptors (Ednr-B and Kit). After hybridization, they could define different "blocks" of cDNA. Importantly, they developed an efficient method to study the function of these EST-cDNAs in melanoblast-derived cells. Two papers were given on the role of one of the major ligands/receptors of the melanocyte development. These papers may lead to the idea that during evolution, the signaling and the role associated to ET-3 and Steel evolved. In birds, using the well established neural crest cell in vitro culture, Dupin et al. (IL24) showed the importance of ET-3 during proliferation and survival of the glia-melanocyte bipotent cells and during the melanocyte differentiation. A precise study on this aspect of the development of melanocytes was presented. In mammals, only one EdnrB was cloned, the chance that a second receptor exists is very low. In birds, these authors isolated a second receptor (Ednr-B2); the effect of ET-3 is mediated by Ednr-B and Ednr–B2, which are temporally and spatially regulated differently. Pla and Larue (SP20) showed the importance of Ednr-Bs during the migration of neural crest cells. They used a novel migration assay in which they graft various ES cell mutants in the chicken embryo. They could show that Ednr-B2 can direct the migration towards the dorso-lateral pathway. Another aspect of the migration was presented by Wehrle-Haller et al. (SP36). The role of the cell adhesion molecule, αvβ3 integrin, was studied using a time-lapse approach. This approach allowed the authors to show the importance of the local density of this protein. The take home message is that a slow turnover of αvβ3 is associated to low-density contacts and to a moderate migration, and a high turnover of αvβ3 is associated to a high-density contact and to a high migration. Originally, beta-catenin was found to be associated to the cell adhesion molecule and tumor-suppressor, E-cadherin. An oncogenic form of β-catenin was detected in carcinomas. Martinozzi et al. (SP18) expressed oncogenic forms of β-catenin in melanocytes in vivo.

At the time of this presentation, no melanoma was observed. An overall down-pigmentation and white-belly spots were observed. The molecular mechanisms remain unclear. Szabad et al. (SP21) isolated and characterized an immortalized human epidermal melanocyte cell line. This cell line possesses effectively a large number of melanocyte characteristics. However and interestingly, it has to be noticed that Cyclin D1 is overexpressed, tyrosinase is not well expressed and that a defect at the level of chromosome 15 was discovered. This cell line will certainly be useful for many laboratories in the future. Finally, Beck et al. (SP19) focused their interest in the proper coculture (melanocyte/keratinocyte) cell growth conditions. It is known for a long time that the proper heterophilic cell-cell interactions are crucial to study the normal and pathological behavior of melanocytes. They discover that Green's medium with collagen I or Defined keratinocyte medium with the plasma polymer facilitate effective co-culture of keratinocytes and melanocytes.

Session V. OXIDATIVE STRESS AND MELANOGENESIS
Chaired by Robert Ballotti, Mauro Picardo, Nico Smit
Contributed by Nico Smit

In this session several factors involved in the induction of oxidative stress in melanocytes were discussed.

Mac Neil and Haycock (Sheffield) presented their work on the different physiological responses of α-MSH especially with regard to its function mediating the defence mechanism of melanocytes against inflammation and oxidative stress. The effect of α-MSH on cAMP levels was measured in melanocyte cultures and melanoma cell lines. When the cAMP pathway is inhibited using an adenosine agonist a calcium response can be found when the cells are treated with α-MSH. This is in accordance with results presented by Hoogduijn in another session (II) who also showed influences on calcium levels in individual cells by α-MSH and ACTH. Other responses of α-MSH may depend on the ratio of the cAMP and calcium signalling. One of these responses next to effects on pigmentation (and tyrosinase activation) is the inhibition of cytokine (TNF-α) induced NFκB. This induction of NFκB was nicely demonstrated by Dr Haycock using digital imaging of the NFκB/p65 subunit by immunofluorescence microscopy. α-MSH and MSH 11-13 tripeptide were shown to inhibit the induction of NFκB. In HBL melanoma cells this inhibition was strong whereas in C8161 melanoma cells no inhibition was found. The HBL cells show a good cAMP response and a limited calcium response. For the C8161 cells the opposite was found suggesting that the signalling via cAMP or calcium may influence the further biological responses to MSH such as the defence against inflammation and oxidative stress.

A relevance for induction of NFκB in melanocytes was obvious from the paper presented by McNulty (Irvine). In this case the observed increased expression of NFκB in nevi and melanoma could have also been induced by cytokines or directly, as a response to (oxidative) stress. Melanoma cells exhibit constitutive NFκB binding activity by RelA. Nevi showed activated RelA staining in the cytoplasm while metastatic melanomas showed both nuclear and cytoplasmic staining. In comparison to nevi the melanomas also showed reduced staining of NFκB inhibitory proteins IκB-alpha and –epsilon. This loss of the inhibitory proteins may be responsible for accumulation of RelA in the nucleus.

Next to the effects of oxidative stress at the transcriptional level that influence cell cycle regulation, mutations in certain genes may be responsible for the development of melanoma from the normal melanocyte in skin and atypical nevi. Events that could lead to hypermutability in atypical nevus cells may be related to increased oxidative stress in these cells. This was the topic of the two papers from our laboratory by Smit and Van Nieuwpoort (Leiden). Different fluorescent probes were used for demonstration of hydroperoxides in the cell. The non fluorescent dihydrodichlofluorescein diacetate and dihydrorhodamine 123 can react with intracellular hydrogen peroxide (http://www.probes.com) to form the fluorescent dichlorofluorescein or rhodamine 123. Results indicate that basic levels of ROS detected with these probes are high in cultured melanocytes of skin type VI as compared to skin type I and II melanocytes. Furthermore the melanocytes from an atypical nevus showed higher fluorescence intensity than the corresponding normal melanocytes from the same individual. Hydrogen peroxide

may cause high levels of oxidative DNA damage (8-hydroxy-2'-deoxyguanosine) especially when iron is present at the site of the nucleus. Using methylene blue as a photosensitizer very strong induction of 8OHdG was found in lightly pheomelanic M14 melanoma cells after UVA irradiation and much lower levels in the eumelanic An melanoma cells. This could be a result of the photosensitising properties of pheomelanin. The paper by Maresca (Rome) indicates such properties of pheomelanin, since this caused changes in the electrophoretic behaviour of catalase as shown by zymography, whereas eumelanin did not induce any modification.

Session VI. EXTRACUTANEOUS MELANINS
Chaired by Dan-Ning Hu, Giuseppe Prota and Francisco Solano
Contributed by Francisco Solano

The session was opened by Prof. Prota, who emphasized the importance of the advances about the structure and functions of extracutaneous melanins in the context of the new markers identified from oxidative degradation studies (further details in his presentation IL15, Session 1). He then introduced the first speaker, Dr. Sarna, who presented new data concerning the properties and function of ocular melanin. Dr. Sarna (Krakow) and collaborators have characterized melanosomes from RPE donors of very different age by atomic force microscopy. The presentation clearly showed that the surface of melanosomes is coated with lipid material, mainly lipofuscin. This coating material increases a lot with aging, decreasing the accessibility of melanin to putative cytotoxic intracellular compounds. Thus, RPE melanosomes of aged donors loose part of their antioxidant and photoprotective efficiency. Next, Dr. Hu, from the New York Medical College, presented some data also concerning ocular melanins, although the talk was initiated with an emotive mention to the Twin Towers catastrophic event of past September 11th, less than 3 miles away from the Eye and Ear Infirmary Hospital where Dr. Hu is working. He established differences between the two different pigment cells in the eye concerning melanin content, variability with the iris color and expression of the melanogenic enzymes. Using cultures of uveal melanocytes, Dr. Hu has studied the effect of a plethora of agents including growth factors, cytokines, hormones, neurotransmitters and prostaglandins. Most of them affect pigmentation, so that the pathogenesis of changes in the iris color can be explained by alteration of these factors. The last invited lecture of the Session was delivered by Dr. Rosei from Univ. Roma, "La Sapienza", who presented an interesting study of synthetic melanins studied by Micro-Raman spectroscopy in comparison with other models, such as amorphous carbon films. Surprisingly, all synthetic polymers presented two main characteristic bands independently of the preparation method or the presence of peptidic bonds. However, Raman spectra were dependent on the excitation wavelength. In general, the phonon modes of melanins closely resemble those observed in carbon films. Thus, this technique may be used to estimate the size of carbon clusters in this untreatable material.

After the 3 invited lectures, the session accumulated a considerable delay, and the invited presentations were initiated by Dr. Miranda (Rome) presenting the purification and kinetic characterization of a tyrosinase from truffles (*Tuber melanosporum*). This tyrosinase is one of the scarce plant catechol oxidases presenting tyrosine hydroxylase and dopa oxidase activities. The enzyme is reversibly inhibited by dimethyl-sulfide and methyldithiometane. Both chemicals are present in the endogenous flavour of truffles. So, Dr. Miranda and his Italian team demonstrated that the tyrosinase activity decreases as the fruit matures and becomes flavoured, and the correlation between tyrosinase activity and the color of the truffle, from black to whitish. These studies might have a great economic interest for the truffle's market.

The excursion to plant melanin was terminated to come back to animal melanins with the new presentation, performed by Dr. S. Svensson (Linköping), who presented a method for screening the melanin binding ability of a number of antineoplastic agents. In fact, they used commercial melanin from *Sepia officinalis* to test the capacity of this polymer to bind the chemotherapeutic agent cisplatin and the antibiotics doxorubicin and daunorubicin. According to the presented results, the first agent did not bind to melanin, but the latter antibiotics do.

Due to the delay accumulated, the session was interrupted for lunchtime. The other two scheduled presentations, by Dr I. Canton (collaboration between Univ. Sheffield, UK and Univ. Basque Country, Spain) and Dr. D.J. Tobin (Bradford Univ, UK) were postponed to the afternoon session. Those presentations dealt with the inhibitory effect of a-MSH on the invasion of ocular melanoma cells and the consideration of the human hair follicle as a suitable target for pro-POMC peptides.

Session VII. MELANOSOMES AND MULTIORGANELLAR DISEASE
Chaired by Ghanem Ghanem and Shosuke Ito
Contributed by Ghanem Ghanem

- First, some most interesting work on melanosome biology and transport has been presented by M. Seabra. The importance of *Rab27a* (ashen) and *Myo5a* (dilute) genes in melanosome transport and clustering has been studied in mouse mutant models of Griscelli syndrome. It has been found that Rab27a is associated with melanosomes as well as myosin V as demonstrated by fluorescent antibody studies. However, myosin V (product of *Myo5a*) did not colocalize with melanosomes in ashen or leaden models. Rab27a is mutated in *ashen* mice. It has been also found that melanophilin is the product of the *leaden* gene. On the other hand, Rab27a seems necessary for the secretion of CTL lytic granules leading to a lack in CTL killing activity in Griscelli and ashen models. Likewise itself, myosin Va and Rab27 appear to play a role in melanosome capture at the cell periphery. Rab27a is suggested as a marker for lysosome-related organelles like melanosomes.
- The second contribution, presented by S. Höning, discussed the function of AP3 adaptor complex in melanosome biogenesis. Lysosomal and melanosomal membrane proteins like tyrosinase and TYRPs may interact with cytosolic adaptor complexes AP1→4. After the binding, coated vesicles may be formed and and transport the proteins from one cellular compartment to another, possibly via endosomes. In melanosomes, AP complexes bind to tyrosinase tail and AP1 and AP2 bind to TYRP-1. AP3 complexes colocalize with tyrosinase and show more than 80% labelling in melanosomes. The authors propose that sorting of tyrosinase to endosomes and melanosomes is a variation of the intracellular membrane proteins sorting to lysosomes.
- K. Lefort presented data supporting a GADD45 activation by UVB specific to melanocytes as compared to skin fibroblasts and keratinocytes. The authors focused on GADD45 functions by also on the analysis of the promoter activity. They were able to localize a minimal promoter region of 50 bp responsible for GADD45 activation by UVB, and that binds to the POU family gene products oct-1 and N-oct3. The latter is poorly expressed in melanocytes leading the authors to conclude to an original alternative mechanism for UVB response in these particular cells.
- A. Calcabrini investigated the resistance mechanisms to melphalan in a series of M14 melanoma cell lines resistant to doxorubicin known to be due to P-gp expression. The authors found by clonogenic assays that the same is true with melphalan and that this resistance can be reversed by the use of cyclosporin A. They suggest that melphalan is actively transported out of the cell by P-gp thus explaining the observed resistance to the drug.

Two additional contributions have been moved from the previous session due to a lack of time and were presented:

- I. Canton showed data suggesting that MSH is able to significantly inhibit the invasion of ocular melanoma cells at concentrations as low as 10^{-11} M. The authors stressed that MSH is present in the aqueous humour and that its immunosuppressive properties may explain why primary melanoma of the iris is less aggressive than the one arising in the posterior compartment of the eye.
- D.J. Tobin focused on the human hair follicle pigmentary unit and studied the presence to different POMC derived peptides by immunohistochemistry. ACTH, α-MSH and β-endorphin expression was evaluated in gp100 positive melanocytes. The authors concluded that all three immunoreactive peptides are differentially expressed in the fully functioning anagen hair follicle pigmentary unit. ACTH and α-MSH may function during the critical stages of hair follicle regeneration during the hair growth cycle. They finally highlighted the expression of β-endorphin in hair bulb melanocytes explaining the maintenance of high melanogenic activity during the anagen phase.

Session VIII. NEW TRENDS IN MELANOMA-BASIC ASPECTS
Chaired by Jean Marie Naeyaert and Pier Giorgio Natali
Contributed by Jean Marie Naeyaert

Dorothy Bennett (London) discussed cellular senescence in the melanocytic system. Understanding cellular senescence is of major importance since it constitutes a barrier to tumour development. It is controlled by telomere shortening and by the tumour suppressor gene CDKN2A that encodes two distinct cell cycle inhibitory proteins, p16 (INK4a) and p14 (ARF). P16 activates the RB1 pathway while ARF is an activator of the p53 pathway. In mouse melanocytes, p16 can induce senescence without ARF, while immortalized cells expressed ARF but not p16. Two independent strains of p16-null, ARF-positive human melanocytes showed severe abnormalities of senescence. Immortalization was independent of the p53 pathway. This could help explain the rarity of p53 mutations in sporadic melanoma. These studies are in favour of a predominance of the p16/RB1 pathway in effecting melanocyte senescence.
Marco Paggi (Rome) presented work on the in vitro and in vivo tumour growth inhibition by a p16-mimicking peptide in p16-defective, pRB-positive human melanoma cells (A375M). A2058 cells (p16-positive, pRB-defective) were refractory to the action of the p16-mimicking peptide. The authors conclude that p-16 mimicking peptide may be a promising tool for targeted cancer therapy in selected melanoma phenotypes.
Böhm and coworkers (Münster) presented data on the activation status of mitogen-activated protein kinases (MAPK) in melanoma cells in vitro and in situ. Using Western blotting with phospho-specific MAPK antibodies and kinase assays, they demonstrated constitutive activation of MAPK-1 and MAPK-2 in melanoma cells as compared to normal human melanocytes. This was not due to increased protein expression or to suppression of the MKP-1 phosphatase. In situ MAPK-1/2 tyrosine phosphorylation correlated with invasiveness of primary cutaneous melanoma. Melanoma metastases showed a heterogeneous staining pattern.
Malorni et al (Rome) discussed subcellular mechanisms responsible for interferon-induced sensitisation to cisplatin-induced apoptosis in melanoma cells. By CIFN gene transfer into melanoma cells they could demonstrate a direct effect on mitochondria, i.e. a transient hyperpolarisation, that sensitises these cells towards type II apoptotic stimuli (cisplatin, staurosporin, radiation), but not to type I triggers (FAS, TRAIL).
Dr McNulty (Irvine) presented data on the reactive oxygen species (ROS) regulation of NFkB and AP-1 in melanoma cells. It looks like the binding activity of both transcription factors is still responsive to ROS (NFkB to intracellular hydrogen peroxide and AP-1 to superoxide anion). However, there is no concomitant control of apoptosis, maybe providing an explanation for the fact that melanoma cells can escape the noxious injury of chemotherapeutic agents.
The final paper of the Session was presented by Baldi et al (Rome). They performed cDNA array analysis on two cell lines from one patient, one from a primary cutaneous melanoma and from a metastatic lymph node. 31 differentially expressed genes were identified, 27 down-regulated and 4 up-regulated in the metastatic cell phenotype. The authors chose a down-regulated gene, PRSS11, for further study. Its gene product is a human serine protease homologue of the E coli protease htrA. Metastatic clones stably transfected with PRSS11 cDNA showed a significant decrease in proliferation and chemotaxis. The studies suggest that decrease of PRSS11 expression could be an indicator of melanoma aggressiveness.

Session IX. DIAGNOSTIC, PROGNOSIS AND TREATMENT OF MELANOMA
Chaired by Jean François Doré, Silvia Moretti and Alain Taieb
Contributed by Alain Taieb

K. Teuchner et al (Berlin) presented a promising new diagnostic technique based on femtosecond pulse stepwise two photon excitation of the fluorescence of pigmented tissues. This mode of excitation

yields a selective fluorescence for melanin in tissues in vivo, which has been applied to clinical samples including excised primary melanomas and melanocytic nevi. There is a modification of the fluorescence spectrum, with a pronounced shift in the red for primary melanomas. The distinction between nevus and melanoma remains to be studied on a larger scale.

The strategies used in chemotherapy to obtain a better efficacy/tolerance ratio could be influenced by the studies presented by Leonetti et al (Rome), which suggest that the concomitant administration of vitamin E (4,3 mg/kg) and cisplatin in mice injected with a human melanoma cell line reduces the overall toxicity of cisplatin and more specifically its peripheral nerve toxicity, without affecting its efficacy. If these findings can be generalized, they offer a simple and readily available therapeutic improvement in the management of metastatic melanoma.

A systematic comparison of cutaneous and extracutaneous melanoma is currently carried out at the Karolinska Institute of Stockholm and was presented by B. Ragnarsson-Olding et al. New data were presented at this meeting concerning the rare vulvar melanomas as compared to common skin melanomas for p53 gene mutations and protein expression studied by immunohistochemistry. The data were similar, with immunopositivity in 42% of cases and controls as wells as no differences for mutations. This type of approach, although here negative, may provide clues for the understanding of the molecular mechanisms involved in melanoma, especially to detect environmental influences such as UV irradiation.

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NOT AVAILABLE

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(Dr. M. Picardo)

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3. MSH, MCH, other hormones, differentiation

(Dr. B. Loir)

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(Dr. E. Wenczl)

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5. Neuromelanins

(Prof. M. d'Ischia)

Virtually all of the papers dealing with neuromelanin and Parkinson's disease that appeared during the last few months centred on the mechanisms of the iron-neuromelanin interaction and the possible relevance to neurodegeneration and Parkinson's disease. Several techniques were used to determine iron levels and state in substantia nigra, neuromelanin granules and single nerve cells. These included instrumental neutron activation analysis and X-ray fluorescence (XRF) using a synchrotron radiation (SR) microbeam. The results confirmed the accumulation of iron in neuromelanin granules, and indicated in one case a considerably higher Fe3+/Fe2+ ratio in a glial cell compared to that of neuromelanin granules. Iron and oxidative stress are thus increasingly implicated as contributory factors in neuronal degeneration.

- Ektessabi Ali, Shikine Shunsuke, Yoshida Sohei.
  Quantitative analysis of biomedical samples using synchrotron radiation microbeams. AIP Conf. Proc. 576 (Applications of Accelerators in Research and Industry), 720-723, 2001.
  Comments: X-ray fluorescence (XRF) using a synchrotron radiation (SR) microbeam was applied to investigate distributions and concns. of elements in single neurons of patients with neurodegenerative diseases. In this paper we introduce a computer code that has been developed to quantify the trace elements and matrix elements at the single cell level. This computer code has been used in studies of several important neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and parkinsonism-dementia complex (PDC), as well as in basic biol. expts. to det. the elemental changes in cells due to incorporation of foreign metal elements. The substantia nigra (SN) tissue obtained from the autopsy specimens of patients with Guamanian parkinsonism-dementia complex (PDC) and control cases were examd. Quant. XRF anal. showed that neuromelanin granules of Parkinsonian SN contained higher levels of Fe than those of the control. The concns. were in the ranges of 2300-3100 ppm and 2000-2400 ppm resp. On the contrary, Zn and Ni in neuromelanin granules of SN tissue from the PDC case were lower than those of the control. Esp. Zn was less than 40 ppm in SN tissue from the PDC case while it was 560-810 ppm in the control. These changes are considered to be closely related to the neuro-degeneration and cell death.

- Fujisawa S., Ektessabi A.M., Yoshida S.
Chemical state analysis of iron in nerve cells. AIP Conf. Proc. 576 (Applications of Accelerators in Research and Industry), 707-710, 2001.
Comments: The chem. states of Fe contained in tissues obtained from a patient with parkinsonism-dementia complex (PDC) were studied using micro beams from a synchrotron radiation source. XRF analyses were performed at energies 7.160 keV, highly above the Fe absorption edge, and 7.120 keV, slightly above the Fe2+ absorption edge to suppress the excitation of Fe3+. Iron was detected in neuromelanin granules and one of glial cells in the PDC tissue. The results show differences in the chem. state of the Fe in the neuromelanin granules and the glial cells. The Fe3+/Fe2+ ratio of Fe contained in the glial cell is considerably higher than that of the neuromelanin granules.

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Iron and oxidative stress in nigral neurons of guamanian ALS/PDC: Chemical state imaging using synchrotron radiation. Int. Congr. Ser. 1221(Molecular Mechanism and Therapeutics of Amyotrophic Lateral Sclerosis), 227-233, 2001.
Comments: We report the distribution and chem. imaging of iron (Fe) in and around melanized neurons within the substantia nigra of one control and one patient with parkinsonism-dementia (PDC) from Guam, using ordinary iron stainings and synchrotron radiation X-ray fluorescence spectrometry (SRXRF). Iron stainings revealed that Ferric (Fe3+) iron was more heavily deposited in neuromelanin granules in and around the nigral neurons of the PDC case than those of the control. SRXRF results revealed that Fe content of PDC was 2-3 times higher than that of the control, although Fe chem. imaging within melanized neurons of both PDC and control cases showed an intermedaite form between the ferrous (Fe2+) and ferric (Fe3+) form. Free-neuromelanin granules showed this difference: in the PDC case, excessive Fe accumulation can be seen in the glial cells surrounding neuromelanin aggregates. In this area, the Fe chem. state has shifted from a ferrous (Fe2+) to ferric (Fe3+) form. These results suggest that oxidative stress might play an important role in the underlying process of nigral degeneration in the Guamanian PDC and related neurodegenerative diseases.

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Combined biochemical separation and INAA for the determination of iron and other metals in Neuromelanin of human brain Substantia Nigra. J. Radioanal. Nucl. Chem. 249(2),449-454, 2001.
Comments: In Parkinson disease Fe and other metals increase in Substantia Nigra (SN) and other basal nuclei. Since Fe can generate cytotoxic free radicals. Neuromelanin (NM) could play an important protective role in neurons. In this work an original procedure for sepn. of NM, prepn. of samples and anal. is presented. The detn. of SN and its NM elemental content was carried out by instrumental neutron activation anal. Several actions were taken to reduce the metal contaminations: use of high purity reagents, dissection of tissues with titanium coated tools and adequate processing of samples.

6. Genetics, molecular and developmental biology

(Dr. F. Beermann)

Underwhite / AIM1 / MATP: underwhite is a recessive pigmentation mutant in the mouse, which was first described in 1964 (Dickie MM, Mouse News Letters 30:30, 1964), and which manifests as altered pigmentation of both eye and fur. The recent months saw the identification of the gene encoded at the locus, which is the homolog of a gene recently isolated and identified from a pigmentation mutant in the Medaka fish (Fukamachi et al., 2001). The gene (called AIM1) encodes a transmembrane transport protein which is implicated in melanin synthesis. Subsequent analyses have shown that AIM1 is located at the underwhite locus in the mouse (Du and Fisher, 2001). Independent experiments have identified the same gene product in human (called then MATP) where it might cause a new form of oculocutaneous albinism, OCA4 (Newton et al., 2001).

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7. Tyrosinase, TRPs, other enzymes

(Prof. J.C. Garcia-Borron)

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Inverse correlation between pink-eyed dilution protein expression and induction of melanogenesis by bafilomycin A1. Pigment Cell Res. 14(5):362-7, 2001.

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Transforming growth factor beta1 mediates hypopigmentation of B16 mouse melanoma cells by inhibition of melanin formation and melanosome maturation. Int J Biochem Cell Biol. 33(10):971-83, 2001.

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Abnormal translocation of tyrosinase and tyrosinase-related protein 1 in cutaneous melanocytes of Hermansky-Pudlak Syndrome and in melanoma cells transfected with anti-sense HPS1 cDNA. J Invest Dermatol. 117(3):641-6, 2001.

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Lactoferrin-melanin interaction and its possible implications in melanin polymerization: Crystal structure of the complex formed between mare lactoferrin and melanin monomers at 2.7-A resolution. Proteins. 45(3):229-36, 2001.

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The mechanism of epidermal hyperpigmentation in dermatofibroma is associated with stem cell factor and hepatocyte growth factor expression. J Invest Dermatol. 117(3):627-33, 2001.

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Glycosphingolipids are required for sorting melanosomal proteins in the Golgi complex. J Cell Biol. 155(3):369-80, 2001.

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Oculocutaneous albinism types 1 and 3 are ER retention diseases: mutation of tyrosinase or Tyrp1 can affect the processing of both mutant and wild-type proteins. FASEB J. 15(12):2149-61, 2001.

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Production of melanocyte-specific antibodies to human melanosomal proteins: expression patterns in normal human skin and in cutaneous pigmented lesions. Pigment Cell Res. 14(4):289-97, 2001.

- Zhao X, Murata T, Ohno S, Day N, Song J, Nomura N, Nakahara T, Yokoyama KK.
  Protein kinase Calpha plays a critical role in mannosylerythritol lipid-induced differentiation of melanoma B16 cells. J Biol Chem. 276(43):39903-10, 2001.

8. Melanosomes

(Dr. J. Borovansky)

The majority of recent papers dealt with melanosome biogenesis (*Huizing et al.*, *Kushimoto et al., Marks & Seabra, Raposo et al., Shinoda et al., Sprong et al.*) and with other aspects concerning melanosomes (*Gomez et al., Martinez-Esparza et al., Minwalla et al, Virador et al.*). Traditionally there were papers devoted to melanosome transport (reviews by *Deacon & Gelfand* and *Westbroek et al.*). The role of intramelanosomal pH as a possible control factor for melanogenesis (*Ancans et al.*) and possible participation of oxidative mechanisms in melanin and melanosome degradation (*Elleder & Borovansky*) were also investigated.

- Ancans J, Tobin DJ, Hoogduijn MJ, Smit NP, Wakamatsu K, Thody AJ.
  Melanosome pH controls rate of melanogenesis , eumelanin/phaeomelanin ratio and melanosome maturation in melanocytes and melanoma cells. Exp Cell Res 268: 26-35, 2001.
  Comment: The role of intramelanosomal pH as a possible control mechanism for melanogenesis was studied in 11 human melanocyte cultures and 9 melanoma cell lines. Near normal melanosomal pH was found to be optimal for human tyrosinase activity and melanogenesis, low pH suppressed melanogenesis. The ratio eu-/phaeomelanin production and maturation rate of melanosomes can be regulated by melanosomal pH. The role of P protein in neutralization of melanosomal pH was reemphasized.

- Deacon SW, Gelfand VI.
  Of yeast, mice, and men: Rab proteins and organelle transport. J Cell Biology 152(4): F21-F24.
  Comments: A brief review summarizing recent articles describing that Rab proteins on the surface of organelles can function as a part of the recognition for motor proteins. Special attention is paid 1) to Rab27a which is involved in targetting of myosin Va to melanosome membranes and 2) to genetic disorders regarding Rab protein and myosin in mice and men.

- Elleder M, Borovanský J.
  Autofluorescence of melanins induced by ultraviolet radiation and near ultraviolet light. A histochemical and biochemical study. Histochemical Journal 33: 273-281, 2001.
  Comments: The phenomenon of autofluorescence induced by UV (330-380nm) and near UV light (400-440nm) irradiation was observed in melanin polymers (eumelanin, phaeomelanin, neuromelanin, ochronotic pigment) both of natural and synthetic origin and in in situ and isolated melanosomes embedded in Immu-Mount. The induction of fluorescence was inhibited by anhydrous conditions, sodium azide and catalase. In parallel experiments rapid degradation of melanins and melanosomes with an intermediate fluorescence stage was achieved in UV-irradiated sections or samples mounted in media artificially enriched with hydrogen peroxide or directly in aqueous solutions of hydrogen peroxide, sodium peroxide or periodic acid. These results suggest that oxidative rather than hydrolytic reactions may be involved in melanin and melanosome degradation.

- Gomez PF, Luo D, Hirosaki K, Shinoda K, Yamashita T, Suzuki J, Otsu K, Ishikawa K, Jimbow K.
  Identification of rab7 as a melanosome-associated protein involved in the intracellular transport of tyrosinase-related protein 1. J Invest Dermatol 117: 81-90, 2001.
  Comments: Modern cell biology techniques enabled to identify several GTP-binding proteins (rab3, rab7, rab8) among melanosomal proteins that may be involved in the control of the melanogenesis process. It was suggested that rab7 is a melanosome-associated molecule, that TRP1 is present in late-endosome delineated granules and that rab7 is involved in the transport of TRP1 from these granules to the melanosome.

- Huizing M, Anikster Y, Gahl WA.
  Hermansky-Pudlak syndrome and Chediak-Higashi syndrome: Disorders of vesicle formation and trafficking. Thromb Haemost 86: 233-245, 2001.
  Comments: An extensive and detailed review summarizing clinical and laboratory findins, diagnosis, treatment, genetic and cell biology features of HP and CH syndromes. The authors propose that HPS and CHS represent extremes of disease entities that correspond to discrete steps in the genesis and movement of vesicles of lysosomal lineage. HPS appears to be a disorder of vesicle formation and CHS a defect in vesicle trafficking.

- Kushimoto T, Basrur V, Valencia J, Matsunaga J, Vieira WD, Ferrans VJ, Muller J, Appella E, Hearing VJ.
  A model for melanosome biogenesis based on the purification and analysis of early melanosomes.
  Proceedings of the Nat. Acad. Sci USA 98(19): 10698-10703, 2001.

Comments: Melanosome biogenesis and distribution of catalytic and specific proteins were investigated after purification of early stage melanosomes. A method for their separation (including free flow electrophoresis purification step) is reported. . Fraction rich in Stage I melanosomes contained only TYRP-1 and GP100, whereas tyrosinase, DCT and MART-1 were detected only after the formation of stage II melanosomes. which may depend on the proteolytic processing of GP100. An abstract is also available in J Invest Dermatol 117(2):509, 2001.

- Marks MS, Seabra MC.
The melanosome: Membrane dynamics in black and white. Nature Reviews Molecular Cell Biology 2(10): 738-748, 2001.
Comments: The biogenesis and intracellular movement of melanosomes and related organelles are disrupted in several genetic diseases in mice and humans. The characterization of genes defective in these diseases reinvigorated interest in the melanosome as a model system for understanding the molecular mechanisms that underlie intracellular membrane dynamics.

- Martinez-Esparza M, Ferrer C, Castells MT, Garcia-Borron JC, Zuasti A.
Transforming growth factor beta 1 mediates hypopigmentation of B16 melanoma cells by inhibition of melanin formation and melanosome maturation. Int Journal of Biochemistry & Cell Biology 33(10): 971-983, 2001.
Comments: The effects of TGF beta1 and alpha MSH on melanosome number, volume density and maturation density was studied by means of quantitative image analysis technique.TGF beta 1 increased the percentage of stage III melanosomes.

- Minwalla L, Zhao Y, LePoole IC, Wickett RR, Boissy RE.
Keratinocytes play a role in regulating distribution patterns of recipient melanosomes in vitro.
J Invest Dermatol 117(2): 341-347, 2001.
Comments: Size and distribution of melanosomes in keratinocytes of Black and Caucasian skin are typically different. Electron microscopic investigation of cocultures of melanocytes and keratinocytes derived from Black and Caucasian skin in various combinations suggested that the recipient melanosomes regardless of origin were predominantly distributed individually by keratinocytes from dark skin and in membrane-bound clusters by those from light skin. The regulatory factor(s) within the keratinocyte seem to determine recipient melanosome distribution pattern. Melanosome size was not related to their distribution in keratinocytes.

- Raposo G, Tenza D, Murphy DM, Berson JF, Marks MS.
Distinct protein sorting and localization to premelanosomes, melanosomes, and lysosomes in pigmented melanocytic cells. J Cell Biology 152(4): 809-823, 2001.
Comments: Premelanosomes and melanosomes represent a distinct lineage of organelles separable from conventional endosomes and lysosomes. Melanosome resident proteins Pmel17 and TRP1 localized to separate vesicular structures that were distinct from those enriched in lysosomal proteins LAMP1 and cathepsin D. Pmel17 was most enriched along the intralumenal striations of premelanosomes. Increased pigmentation was accompanied by a decrease of Pmel17 and by an increase in TRP1 in limiting membranes. Premelanosomal proteins segregated from endocytic markers within an unusual clathrin-coated endosomal compartment.

- Shinoda K, Wada I, Jin HY, Jimbow K.
A melanosome-associated monoclonal antibody J1 recognizes luminal membrane of prelysosomes common to biogenesis of melanosomes and lysosomes. Cell Structure and Function 26(3): 169-177, 2001.
Comments: Study aiming to identify how and to what extent the endosome-lysosome system is involved in melanosome biogenesis by utilizing a novel melanogenesis marker J1. The antigenic epitope of MoAb J1 was expressed primarily by granular structures localized in regions proximal to the Golgi complex; these structures contained also tyrosinase and TRP1 and a lysosomal marker LPG85. MoAb J1 did not react with vesicles with late/early (syntaxin 8/ EEA) endosomal markers. The authors suggest that tyrosinase and TRP1 are transported to melanosomes from TGN via the prelysosomal granules after being transiently transported to late endosomes.

- Sprong H, Degroote S, Claessens T, van Drunen J, Oorschot V, Westerink BHC, Hirabayashi Y, Klumperman J, van der Sluijs, van Meer G.
Glycosphingolipids are required for sorting melanosomal proteins in the Golgi complex. J Cell Biology 155(3): 369-379, 2001.
Comments: Glycosphingolipid-deficient GM95 mouse melanoma cell line was studied to explore the role of glycosphingolipids in membrane transport. GM95 cells did not produce melanin because tyrosinase was not targeted to melanosomes but accumulated in the Golgi complex. TRP1 still reached melanosomal compartment via the plasma membrane instead of direct pathway from the Golgi. Delivery of lysosomal enzyme s from the Golgi complex to endosomes was normal. Transfection of ceramide glucosyltransferase or addition of glucosylsphingosine restored tyrosinase transport and pigmentation.

- Virador V, Matsunaga N, Matsunaga J, Valencia J, Oldham RJ, Kameyama K, Peck GL, Ferrans VJ, Vieira WD, Abdel-Malek ZA, Hearing VJ.
  Production of melanocyte-specific antibodies to human melanosomal proteins: Expression patterns in normal human skin and in cutaneous pigmented lesions. Pigment Cell Res 14(4): 289-297, 2001.
  Comments: Having generated polyclonal antibodies against human tyrosinase, TRP1, dopachrome tautomerase and Pmel17 the authors determined the distribution and function of melanosomal proteins in normal human skin and in various pigmented cutaneous lesions.

- Westbroek W, Lambert J, Naeyaert JM.
  The *Dilute* locus and Griscelli syndrome: Gateways towards a better understanding of melanosome transport. Pigment Cell Res 14(5): 320-327, 2001.
  Comments: Based on their rich research experience the authors set up a review summarizing molecular events involved in and determining the process of melanosome transport, describing in detail the function of myosin Va, Rab 27A, kinesin, kinectin, dynein and other proteins.

9. Melanoma experimental, Cell culture

(Dr. N. Smit)

Melanocyte culture

Bandyopadhyay et al discuss in their mini review the differences in behaviour of melanocytes, keratinocytes and fibroblasts with regard to senescence. It is indicated that culture conditions and skin type play an important role in the time of onset of senescence or the increase in population doubling time. Introduction of telomerase (hTERT) has resulted in a marked life span extension of melanocytes (>45 PD compared to 13-15 PD , normally). Also in the paper by **Arbiser et al** telomerase has been used to generate the first cell line from a renal angiomyolipoma.
De Leeuw et al have compared the sensitivity of cultured human melanocytes from different skin phototypes. Using the clone forming ability assay it is shown that considerable differences in survival can be found for the cultures after UVB irradiation which depends on the pigmentation of the melanocytes. In another study (**Smit et al**) we have used melanocyte cultures and increased their pigmentation by using higher tyrosine concentrations in the culture medium. It was found in all cases that the increase in melanin content offers protection against induction of CPDs and 6-4PPs by UVB irradiation. Especially good correlation was found between eumelanin content of the cells and protection against DNA damage. In the paper by **Sahm et al** cultured effects of UV radiation (280 –380 nm) was studied in cultures of choroidal melanocytes and iris pigment epithelial cells. Results suggest that the more densely pigmented cells showed the best survival of the UV treatment and no induction of melanogenesis could be demonstrated.
Duval et al describe the effects of UVB on different culture systems containing melanocytes (monocultures, co-cultures with keratinocytes and reconstructed epidermis). Results indicate that UVB induced melanogenesis dose dependently (from 5 to 30 mJ/cm^2) whereas the response for the melanocyte monocultures showed a threshold between 50 and 60 mJ/cm^2. The response to UVB in the melanocyte – keratinocyte co-cultures was not found in the absence of 7 growth factors that were normally present in the coculture medium. **Lee et al** also produced a reconstructed epidermal system using HaCat cells as a source of (immortalized) keratinocytes. Unfortunately the melanocytes do not remain on the basal layer in this system although they do seem to transport melanosomes to the HaCat cells.
Next to **Ancans et al** also **Manga and Orlow** used bafilomycin A1. In p-null melanocytes this also resulted in an induction of melanin synthesis.

- Ancans J, Tobin DJ, Hoogduijn MJ, Smit NP, Wakamatsu K, Thody AJ.
  Melanosomal pH controls rate of melanogenesis, eumelanin/phaeomelanin ratio and melanosome maturation in melanocytes and melanoma cells. Exp. Cell Res. 268:26-35, 2001.
  Comments: All melanocyte cultures (9 of 9) from Caucasian skin as well as two melanoma cell lines with comparable melanogenic activity showed rapid (within 24 h) increases in melanogenesis in response to neutralization of melanosomal pH by treatment with the melanosomal proton pump inhibitors concanamycin A or bafilomycin A1.

- Arbiser JL, Yeung R, Weiss SW, Arbiser ZK, Amin MB, Cohen C, Frank D, Mahajan S, Herron GS, Yang J, Onda H, Zhang HB, Bai X, Uhlmann E, Loehr A, Northrup H, Au P, Davis I, Fisher DE, Gutmann DH.
  The generation and characterization of a cell line derived from a sporadic renal angiomyolipoma: use of telomerase to obtain stable populations of cells from benign neoplasms. Am. J. Pathol. 159:483-491, 2001.
  Comments: Angiomyolipomas are benign tumors of the kidney derived from putative epithelioid cells that may differentiate into different cell types. A human angiomyolipoma cell line was generated by introducing SV40 large T antigen and human telomerase. The cell line shows characteristics of different cell types including that of melanocytes (by microphthalmia expression).

- Bandyopadhyay D, Timchenko N, Suwa T, Hornsby PJ, Campisi J, Medrano EE.

The human melanocyte: a model system to study the complexity of cellular aging and transformation in non-fibroblastic cells. Exp. Gerontol. 36:1265-1275, 2001.

- Brown DA.
Skin pigmentation enhancers. J. Photochem. Photobiol.B 63:148-161, 2001.
Comments: In this review different agents are compared with regard to their efficacy of increasing skin pigmentation in melanoma cells, melanocytes and skin. Such pigment enhancers are under development in order to safely induce a photoprotective tan.

- Castel H, Vaudry H.
Nitric oxide directly activates GABA(A) receptor function through a cGMP/protein kinase-independent pathway in frog pituitary melanotrophs. J. Neuroendocrinol. 13:695-705, 2001.
Comments: In cultured frog melanotrophs the regulation of GABA(A) receptor function was investigated by studying the effects of nitric oxide donors and oxidizing agents such as H2O2 and dithiobisnitrobenzoic acid (DTNB).

- De Leeuw SM, Smit NP, Van Veldhoven M, Pennings EM, Pavel S, Simons JW, Schothorst AA.
Melanin content of cultured human melanocytes and UV-induced cytotoxicity. J. Photochem. Photobiol. B 61:106-113, 2001.

- Duval C, Regnier M, Schmidt R.
Distinct melanogenic response of human melanocytes in mono-culture, in co-culture with keratinocytes and in reconstructed epidermis, to UV exposure. Pigment Cell Res. 14:348-355, 2001.

- Fink D, Schlagbauer-Wadl H, Selzer E, Lucas T, Wolff K, Pehamberger H, Eichler HG, Jansen B.
Elevated procaspase levels in human melanoma. Melanoma Res. 11:385-393, 2001.

- Gilhooly EM, Morse-Gaudio M, Bianchi L, Reinhart L, Rose DP, Connolly JM, Reed JA, Albino AP.
Loss of expression of protein kinase C beta is a common phenomenon in human malignant melanoma: a result of transformation or differentiation? Melanoma Res. 11:355-369, 2001.

- Graeven U, Rodeck U, Karpinski S, Jost M, Philippou S, Schmiegel W. **Modulation of angiogenesis and tumorigenicity of human melanocytic cells by vascular endothelial growth factor and basic fibroblast growth factor**. Cancer Res. 61:7282-7290, 2001.
Comment: The study reports on the relative contribution of VEGF and bFGF to in vitro and in vivo growth of a tumorigenic melanoma cell line (WM164) and nontumorigenic, immortalized melanocytes (FM516SV).

- Hu DN, McCormick SA, Woodward DF.
A functional study on prostanoid receptors involved in cultured human iridal melanocyte stimulation. Exp. Eye Res. 73:93-100, 2001.

- Huizing M, Sarangarajan R, Strovel E, Zhao Y, Gahl WA, Boissy RE.
Ap-3 mediates tyrosinase but not trp-1 trafficking in human melanocytes. Mol. Biol. Cell. 12:2075-2085, 2001.

- Kawaguchi Y, Mori N, Nakayama A.
Kit(+) melanocytes seem to contribute to melanocyte proliferation after UV exposure as precursor cells. J. Invest Dermatol. 116:920-925, 2001.

- Lee DY, Park KC, Cho KH.
In a skin equivalent HaCaT cells have a preserved capacity to receive melanosomes but melanocytes do not remain in the basal location. Arch. Dermatol. Res. 293:268-272, 2001.

- Manga P, Boissy RE, Pifko-Hirst S, Zhou BK, Orlow SJ.
Mislocalization of melanosomal proteins in melanocytes from mice with oculocutaneous albinism type 2. Exp. Eye Res. 72:695-710, 2001.

- Manga P, Orlow SJ.
Inverse correlation between pink-eyed dilution protein expression and induction of melanogenesis by bafilomycin A1. Pigment Cell Res. 14:362-367, 2001.

- Minwalla L, Zhao Y, Cornelius J, Babcock GF, Wickett RR, Le Poole IC, Boissy RE.
Inhibition of melanosome transfer from melanocytes to keratinocytes by lectins and neoglycoproteins in an in vitro model system. Pigment Cell Res. 14:185-194, 2001.

- Mouriaux F, Chahud F, Maurage CA, Malecaze F, Labalette P.
Implication of stem cell factor in the proliferation of choroidal melanocytes. Exp. Eye Res. 73:151-157, 2001.

- Ooka S, Kawa Y, Ito M, Soma Y, Mizoguchi M.
Establishment and characterization of a mouse neural crest derived cell line (NCCmelan5). Pigment Cell Res. 14:268-274, 2001.

- Sahm M, Loeffler KU, Seifert P, Spitznas M.
Effect of ultraviolet radiation on melanogenesis in four different types of cultured bovine ocular pigmented cells. Graefes Arch. Clin. Exp. Ophthalmol. 239:302-309, 2001.

- Smit NP, Vink AA, Kolb RM, Steenwinkel MJ, van den Berg PT, van Nieuwpoort F, Roza L, Pavel S.
Melanin offers protection against induction of cyclobutane pyrimidine dimers and 6-4 photoproducts by UVB in cultured human melanocytes. Photochem. Photobiol. 74:424-430, 2001.

- Smith-Thomas LC, Moustafa M, Dawson RA, Wagner M, Balafa C, Haycock JW, Krauss AH, Woodward DF, MacNeil S.
Cellular and hormonal regulation of pigmentation in human ocular melanocytes. Pigment Cell Res. 14:298-309, 2001.

- Sviderskaya EV, Hill SP, Balachandar D, Barsh GS, Bennett DC.
Agouti signaling protein and other factors modulating differentiation and proliferation of immortal melanoblasts. Dev. Dyn. 221:373-379, 2001.

- Tronnier M, Alexander M, Lontz W, Brinckmann J.
UV light does not induce p53 mutation in melanocytes in vitro. Dermatology. 202:339-340, 2001.

- Xu C, Green A, Parisi A, Parsons PG.
Photosensitization of the sunscreen octyl p-dimethylaminobenzoate by UVA in human melanocytes but not in keratinocytes. Photochem. Photobiol. 73:600-604, 2001.

- Yoshimura K, Tsukamoto K, Okazaki M, Virador VM, Lei TC, Suzuki Y, Uchida G, Kitano Y, Harii K.
Effects of all-trans retinoic acid on melanogenesis in pigmented skin equivalents and monolayer culture of melanocytes. J. Dermatol. Sci. 27 Suppl 1:S68-75.:S68-S75, 2001.

Melanoma Experimental Treatment

- Agarwala SS.
Chemotherapy Foundation Symposium XVIII: Session on malignant melanoma. Expert. Opin. Investig. Drugs 10:381-385, 2001.

- Brown CK, Kirkwood JM.
Targeted therapy for malignant melanoma. Curr. Oncol. Rep. 3:344-352, 2001.
Comments: This review focuses on three different approaches of targeted melanoma therapy. 1; Immunotherapy aimed at generating specific antimelanoma immunity. 2; Targeting making use of the specific property of melanoma cells synthesizing melanin and 3: making use of genes identified that may be responsible for the malignant transformation of the melanocyte.

- Certa U, Seiler M, Padovan E, Spagnoli GC.
High density oligonucleotide array analysis of interferon- alpha2a sensitivity and transcriptional response in melanoma cells. Br. J. Cancer 85:107-114, 2001.

- Chen Y, Kramer DL, Diegelman P, Vujcic S, Porter CW.
Apoptotic signaling in polyamine analogue-treated SK-MEL-28 human melanoma cells. Cancer Res. 61:6437-6444, 2001.
Comments: N(1),N(11)-Diethylnorspermine (DENSPM) and three of its analogues were tested on SK-MEL-28 melanoma cells for their effects on polyamine depletion and apoptosis. Inhibitors of polyamine biosynthesis resulted in growth inhibition but did not cause apoptosis. Therefore the effects of DENSPM and analogues were considered to be mediated by induction of the polyamine catabolic enzyme spermidine/spermine N(1)-acetyltransferase (SSAT) and related oxidative events and subsequently mediated by the mitochondrial apoptotic signaling pathway as indicated by cytochrome c release and caspase activation

- Cheng HH, Wang HK, Ito J, Bastow KF, Tachibana Y, Nakanishi Y, Xu Z, Luo TY, Lee KH.
Cytotoxic pheophorbide-related compounds from Clerodendrum calamitosum and C. cyrtophyllum. J. Nat. Prod. 64:915-919, 2001.
Comments: Strong cytotoxicity of the above mentioned natural compounds was found towards various tumour cell lines including SK-MEL-2 melanoma cells.

- Demary K, Wong L, Liou JS, Faller DV, Spanjaard RA.
Redox control of retinoic acid receptor activity: a novel mechanism for retinoic acid resistance in melanoma cells. Endocrinology 142:2600-2605, 2001.
Comments: Interesting results are described in this study showing that the response to retinoic acid (RA) among different melanoma cultures depends on the levels of reactive oxygen species (ROS) in the cells. Lowering the ROS levels in RA resistant A375 melanoma cells restored RAReceptor trans-activity. This may be demonstrative for the importance of the redox status of melanocytic cells and its influence on cell signaling leading to growth or differentiation.

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Exploring the mechanisms of action of FB642 at the cellular level. J.Cancer Res. Clin.Oncol. 127:301-313, 2001.

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In vivo antitumour efficacy of MGI-114 (6-hydroxymethylacylfulvene, HMAF) in various human tumour xenograft models including several lung and gastric tumours. Eur. J. Cancer. 37:1419-1428, 2001.

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N-(2-hydroxypropyl)methacrylamide copolymer-6-(3-aminopropyl)-ellipticine conjugates. synthesis, in vitro, and preliminary in vivo evaluation. Bioconjug. Chem. 12:711-718, 2001.
Comments: Ellipticine derivatives have potential as anticancer drugs. Various conjugates of the elepticin derivative (APE) were prepared in order to reduce host toxicity of the compounds. In vitro the cytotoxicity of the conjugates was much less than that of the free APE but in vivo the copolymer conjugate showed somewhat more activity towards B16F10 melanoma in mice.

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Rexpression of HLA class I antigens and restoration of antigen-specific CTL response in melanoma cells following 5-aza-2'-deoxycytidine treatment. Int. J. Cancer. 94:243-251, 2001.
Comments: The MSR3-mel melanoma line shows no expression of HLA class I molecule expression due to hypermethylation of the HLA-A and –B genes. HLA class I expression could be restored by treatment with the demethylating agent 5-aza-2'-deoxycytidine. This also resulted in the recognition of the MSR3-mel cells by MAGE specific cytotoxic T lymphocytes.

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Polymer conjugated bovine seminal ribonuclease inhibits growth of solid tumors and development of metastases in mice. Neoplasma. 48:127-132, 2001.
Comments: In C57B1/6 mice inoculated with B16 melanoma survival was increased after intravenous administration of a polymer conjugated bovine seminal Rnase. Normally the free BS-Rnase was only effective when injected intratumorally.

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Ortho-carboranyl glycosides for the treatment of cancer by boron neutron capture therapy. Bioorg.Med.Chem. 9:1747-1752, 2001.
Comments: Boron uptake for boron neutron capture therapy can be achieved by the clinically used p-boronophenylalanine (BPA). The boron uptake into B16melanoma cells could be significantly increased using carboranyl glycosides.

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Quercetin inhibits the invasion and mobility of murine melanoma B16-BL6 cells through inducing apoptosis via decreasing Bcl-2 expression. Clin. Exp. Metastasis. 18:415-421, 2000.

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Tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of human melanoma is regulated by smac/DIABLO release from mitochondria. Cancer Res. 61:7339-7348, 2001.

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2002 European Conference on Textiles and the Skin

Weimar, April 11-13

Contact: AKM Congress Service GmbH
Hauptstrasse 18
D- 79576 Weil am Rhein
Tel: +49-76 21 –98 66-0
Fax: +49-76 21-7 87 14
E-mail: akmweil@akmcongress.com
Web-site: www.ects2002.de

2002 20th World Congress of Dermatology

Paris, July 1– 5

Contact: Philippe FOURNIER
12, rue de la Croix Faubin
75557 Paris cedex 11 - France
Tel: 33.(0).1.44.64.15.15
Fax: 33.(0).1.44.64.15.16
E-mail : p.fournier@colloquium.fr
Web-site: http://www.derm-wcd-2002.com/

2002 XIXth IUPAC Conference on Photochemistry

Budapest, July 14–19

Contact: Hungarian Chemical Society, (MKE)
Fu u. 68. Hungary
H - 1027 Budapest,
Tel: 36-1-201-6886
Fax: 36-1-201-8056
E-mail: mail.mke@mtesz.hu

2002 XVIIIth International Pigment Cell Conference

Egmond aan Zee, Holland, 9 - 13 September 2002

Contact: Dr. Stan PAVEL
E-mail: SPavel@algemeen.azl.nl
Boerhaave Congress Office
Congress manager Mrs Caroline M. van Battum
P.O. Box 2084, NL-2301 CB Leiden
Telephone:+31(0)715276434
Fax:+31(0)715275262

Email: C.M.van_Battum@lumc.nl
Web-site: http://users.raketnet.nl/ipcc/

2002 Annual ESDR Meeting,
Geneva, September 19 – 21

2003 International Investigative Dermatology Meeting
South Miami Beach, Florida USA , April 30 - May 4
Joint Meeting of the ESDR, JSID, and SID

2003 XIth Annual Meeting of the PASPCR
Wood's Hole, MA, September 3-7
Contact: Dr. Jean BOLOGNIA
E-mail: jean_bolognia@qm.yale.edu

2003 XIth Meeting of the ESPCR
Gent, Belgium
Contact: Prof. JM NAEYAERT
E-mail: JeanMarie.Naeyaert@rug.ac.be

2004 14th International Congress on Photobiology
Jungmoon, Jeju (Cheju), Korea, June 10-15

2004 XIIth Meeting of the ESPCR
Paris, France
Contact: Dr. Lionel LARUE
E-mail: Lionel.Larue@curie.fr

2005 XIVth International Pigment Cell Conference (IPCC)
Bethesda, USA
Contact: Dr. V. HEARING
E-mail: hearingv@nih.gov

The Wellcome Trust Functional Genomics Cell Bank
The Wellcome Trust Pigmentary Mutants Mouse Repository

We would like to inform ESPCR members about two new Functional Genomics resources that are of special interest to those working on pigment cells.

The Wellcome Trust Functional Genomics Cell Bank
The Wellcome Trust has established a mammalian cell bank at St George's Hospital Medical School, London, director Dr Elena Sviderskaya, in association with Dot Bennett's research group. The cell bank specializes in mouse melanocyte and melanoblast lines carrying a variety of single pigmentary mutations. It also carries other cell types including melanoma cell lines, fibroblasts, keratinocytes, mammary epithelial cells and myoblasts. See our web site for further details and a list of cell lines.
http://www.sghms.ac.uk/depts/anatomy/pages/WTFGCB.htm

The Wellcome Trust Pigmentary Mutants Mouse Repository
Affiliated with the Cell Bank is the Pigmentary Mutants Mouse Repository at Texas A&M University (USA), director Dr Lynn Lamoreux. (This facility has already existed for many years, but without specific funding.) It specializes in congenic mice (mostly in C57BL/6J mice,) carrying pigmentary mutations, singly or in combination. For example:

- The Repository has a large collection of congenic mouse mutant alleles at the Tyr locus and related loci affecting the melanosome, also double mutants with deficiencies in more than one such protein, to facilitate studies of interactions between these loci.
- There is also a large collection of mutants at the Mitf locus and others affecting melanocyte development, some maintained in more than one inbred mouse strain which affect their expression.
- Other specialities include mutants at the agouti locus, and possible mouse models for vitiligo. See our web site for further details and mice available,
  http://www.sghms.ac.uk/depts/anatomy/pages/WTFGMPMR.htm
  or contact Lynn Lamoreux: Mllamoreux@hotmail.com

The two resources will be collaborating to produce further congenic mouse melanocyte lines carrying mutations of interest. The Mouse Repository will also be cryopreserving many of its mouse stocks for the first time, after which frozen embryos will be available, and breeding of some mice will be discontinued.

Your views:
We would be very interested to hear the views of any ESPCR member, on which known mouse pigmentary mutants are of greatest current interest and should be available in congenic form as breeding mice (rather than as frozen embryos), or available in the form of cultured melanocytes. Your suggestions may include mice not yet held at this Repository, and transgenic mice. To make comments, please e-mail Lynn Lamoreux (as above), Dot Bennett, or both:
Dbennett@sghms.ac.uk

Congratulations – ESPCR/IFPCS Travel Award Winners, 2001

The ESPCR Council sends its warmest congratulations to the following members who were the winners of **ESPCR Travel Awards** to attend the Rome meeting, out of 10 applications received:

Dr Jo Lambert (Belgium)
Dr Zhuo Li (Germany)
Dr Silvia Martinozzi (France)
Ms Karen Pinder (South Africa)
Ms Elizabeth Waterman (UK)

We are very grateful to **IntegriDerm Inc.** (Alabama, USA) for contributing funding towards these awards for 2001. All younger ESPCR members are reminded that they are eligible to apply for these travel awards to attend ESPCR or IPCC meetings.

Many congratulations likewise to the ESPCR winners of **IFPCS Visiting Scientist Awards** for 2001/2002, who were:

Dr Davinder Parsad (India)
Dr Olga Solovieva (France)

These awards are to fund a short period in a collaborating laboratory in another country, working on pigment cell research.

For further information on both of these, please either contact Dr Friedo Beermann (Chair, ESPCR Travel Awards Committee) (Friedrich.Beermann@isrec.unil.ch) or see the relevant web pages:

Travel Awards:
http://www.ulb.ac.be/medecine/loce/espcr/awards.htm
Visiting Scientist Awards:
http://www.ulb.ac.be/medecine/loce/espcr/awards2.htm

Letter from V. Hearing, Editor of Pigment Cell Research

Dear Members of the ESPCR, JSPCR and PASPCR :

It has now been 2 years since I began my 5 year term as Editor of ***Pigment Cell Research*** and I would like to take this occasion to thank you for the tremendous support that has been given to me on every level. The quality of submissions has improved, the speed and quality of reviewers has improved, the support by the publisher has improved and in my opinion, the journal has become a much more vital resource for all of us as a result. The Journal is widespread in its coverage and it welcomes potential authors from the more peripheral areas of research in pigmentation ranging from comparative biology to chemistry to clinical and applied aspects. The outlook for 2002 and beyond is quite bright and I have summarized below some key points regarding that. I'll look forward to the remaining 3 years of my term confident that our journal will continue to progress significantly in the future. Best regards, /s/ Vince Hearing, Editor, ***Pigment Cell Research*** (email: editor@pigment.org)

- **Web Site** – The PCR Web site (http://www.pigment.org) is being used more and more frequently with more than 10,000 hits in its first 2 years; not only can you access titles and abstracts of all Volumes of papers back through the years, but abstracts and titles of papers now in press can also be accessed. The P*C*R Primer is sent to more than 700 scientists in the field that are in our database – if you don't get that you can sign up from the PCR Web site to receive information about journal publications as they come out.
- **Online Submissions** – Speed is the key, and manuscripts can now be submitted online beginning in 2002. See the 'Authors' page on the Web site for information about this and what types of files can be submitted.
- **Turnaround Time** – Electronic processing has also sped up handling of your submissions; the average time to a decision from the date my office received a manuscript in 2001 was only 24 days; the average total time in my office for accepted manuscripts from receipt to transmission to the Publisher was only 31 days.
- **Impact Factor** – The Impact Factor for PCR rose for a 3rd straight year (to 1.87) in 2001; we will surely break the 2.00 barrier next year, particularly if you take care to cite relevant reviews and research papers in PCR that were published in 2000 and 2001.
- **Circulation** – rose again for the second straight year by about 15%; the Publisher has acknowledged this by increasing our color and page budget (cf below), but we can do a lot better if Institutional subscriptions are increased.
- **Color increase** – the Publisher has doubled our color publication budget for 2002; it is not yet an unlimited amount, but you should notice a further increase in color next year.
- **Page increase** – the Publisher has added 96 pages to our standard printing budget next year; this will allow for timely publication of the increased number of excellent reviews and research articles that are being submitted.
- **Outstanding Reviews** – once again, virtually everyone asked to contribute a review next year has agreed to do so. You can look at the upcoming list of Reviews (Regular, Gene Focus and Innovative Technology) on the 'Forthcoming' page of the Web Site.

Three things you can do to help – **(1)** submit your quality papers to PCR, **(2)** make sure your Institution's Library subscribes to PCR, and **(3)** cite relevant recent PCR articles in your own publications next year. It's that easy.