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DISCUSSION 1

Myosin V : a motor protein involved in the regulation of dendrite formation and melanosome transport in the melanocyte?

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The cytoskeleton is important for determination of cell shape, organelle transport and cell movement. Compared to the vast body of research devoted to the role of cytoskeletal components in determining neuronal shape, the study of melanocytic dendricity received very little attention (for review see Naeyaert, 1993). Recent data suggest that actin microfilaments are implicated in dendrite outgrowth, but not in maintenance, and conversely, that microtubules are involved in dendrite maintenance, but not in formation (Lacour et al., 1992). Even less is known about another crucial characteristic of melanocyte differentiation, the movement of melanosomes.

Motor proteins are closely associated with cytoskeletal components and uptil now have received little attention in the study of melanocytic dendricity and melanosome transport.

Myosins are actin-dependent motor proteins and for readers who have not kept up with literature, it will be surprising to see how much the knowledge on myosins has extended during the last years. Classically, myosins have been divided into two distinct classes: myosin II and myosin I. The myosins II include the *conventional* two-headed, filament-forming dimeric myosins with a coiled-coil tail, found in muscle and virtually all non-muscle cells. The term 'myosin I' was originally used to identify an unusual monomeric, single-headed myosin that was found in an amoebe and typically lacks the coiled-coil tail. Later, it appeared to be present in almost all vertebrate tissues. Given the structural difference with myosin II, myosin I was referred to as an *unconventional* myosin. A large number of recent studies make it clear that myosins I are not the only unconventional myosins at all. Indeed, there is a growing family of unconventional myosins that differ in structure, distribution and function. Therefore, two groups (Cheney et al., 1993; Goodson et al., 1993) suggested a more accurate classification based on phylogenetic comparisons with at least eight classes of (conventional and unconventional) myosins. Roman numerals indicate the order of discovery after myosin I and II.

Class V myosins have characteristics of both myosins I and II and are represented by the products of the mouse *dilute* gene (Mercer et al., 1991), the chicken *p190* gene (Espreafico et al., 1992), the human *"myoxin"* gene (Engle et al., 1994) and the *myo2/myo4* genes in yeast (Johnston et al., 1991).

As is often the case in melanocyte biology, murine mutations have provided further information on the potential function of these myosins. Mice that have the original *dilute* homozygous recessive *mutation (d^v)* at the *dilute* gene located on chromosome 9 have short, stubby dendrites while possessing normal amounts of pigment granules that remain packaged in the perinuclear area. This abnormality disturbs the

transfer of pigment from melanocytes to the surrounding keratinocytes, so that, in vivo, these mice exhibit a washed-out or diluted coat colour. These findings suggest that this class of myosin may perhaps associate with cytoplasmic vesicles, the melanosomes, serving to transport them to outer regions of the melanocyte.
Furthermore, it suggests that the dilute myosin is required for the elaboration and/or maintenance of the cellular processes of melanocytes.
In addition to *d*^{v}, several spontaneous and mutagen-induced dilute alleles have been identified.
Whereas *d*^{v} only affects coat colour, most of these alleles (dilute^{lethal} - dilute^{opisthotonus}) result in severe neurological defects and/or death in the homozygous status, indicating that the dilute gene product has an essential function.
Additionally, mutations in the yeast *myo2*-gene result in large, unbudded cells that accumulate vesicles within their cytoplasm (Johnston et al., 1991). This has been interpreted as further evidence for a role for this member of the myosin V family in intracellular vesicle transport.
Very often, murine genetics have opened the way for unpuzzling the pathogenesis of human genodermatoses concerning cutaneous pigmentation (Ortonne, 1993). The human counterpart of the dilute mutation might be found in the *Griscelli Pruniéras* syndrome, a very rare autosomal recessive disease, of which the genetic locus has not yet been identified. This syndrome is characterized by partial albinism, silver-blond hair discoloration, primary immuno-deficiency and, frequently, neurological deficits. Ultrastructural analysis of skin sections shows a characteristic appearance of melanocytes with short, stubby dendrites. The melanocytes are packaged with mature melanosomes, indicating a transfer block towards the surrounding keratinocytes. These structural alterations are very reminiscent of those seen in melanocytes of *dilute* mice. The *myoxin* locus on chromosome 15 is a good candidate for mutation analysis in
this disease.
The available data clearly suggest an important role of myosins in the elaboration, maintenance or function of melanocytic dendrites and the participation of these motor proteins in the intracellular transport of melanosomes. However, it is likely that unconventional myosins participate in very complex cellular structures that require a multitude of cytoskeletal proteins and that are driven by multiple mechanisms. Cooperation between the actin-based motor myosin and microtubuli-based motors such as kinesin and dynein also remains to be elucidated in melanocytes.

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A NEW APPROACH FOR THE UNDERSTANDING OF MELANOCYTES: STEREOLOGICAL QUANTIFICATION OF MELANIZATION BY IMAGE ANALYSIS

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Pigmentation of human skin results from the transport of melanized melanosomes (MMs) in melanocytes dendrites which developpement is controled by keratinocytes [1,2], their transfer to the surrounding undifferentiated keratinocytes according to a mechanism still debated today [3, 4, 5, 6], and finally from the more or less efficient degradation of melanosomes in keratinocytes [7] which ascend in the epidermis to finish their differentiation as corneocytes fated to desquamation.

The macroscopic cutaneous degree of pigmentation depends on several factors playing a critical role at various levels in this process: phototype (heredity, race), solar exposition, hormonal background [8, 9], etc. However, the initial requirement for variations of skin pigmentation is the potential variations of melanin biosynthesis by melanocytes themselves, which modulation may be reproduced *in vitro* by direct action of artificial ultra violet radiations [10,11,12].

Routine techniques for the quantification of melanin pigments are used for a long time. Spectrophotometrical dosages are the most commonly utilized and consist in the comparison of the optical densities obtained at 415 nm after alkaline extraction, with those of standards (e.g. techniques used in [10,1,13]); Nonetheless, natural (from *Sepia officinalis*), synthetic (chemical oxidation of L-tyrosine) or biosynthetic (oxidation of L-tyrosine or L-DOPA by purified tyrosinase) eumelanin may be used as standard [14].

Physically deposited melanins to be measured are extracted by alkaline solutions, which may induce alterations in the polymer with release of colorless precursors [14] and may be influenced by the percentage of pheomelanic and eumelanic monomers in the case of mixed type melanin [15].

More recently, High Performance Liquid Chromatography (HPLC) procedures have been ellaborated [16,17] and now permit to estimate pheomelanin and eumelanin contents separately, which allows to understand how certain bioactive molecules change the pheomelanin to eumelanin ratio, e.g. α-MSH in mammals [18, 19, 20]. Nevertheless, HPLC methods or like spectrophotometrical methods require to be applied, a great number of melanocytes.

With the aim to understand the fine mechanisms of stimulation and inhibition of pigment production at the organite level, we developped in our laboratory a quantitative method based on the analysis of melanocytes images obtained by transmission electron microscopy [21, 22].

Melanin profiles are automatically extracted from melanocytes images with the aid of an image analysis system which generates melanization primary data. Those primary data are introduced in stereological formula, stereology being the body of mathematical methods relating three-dimensional parameters defining the structure to two-dimensional measurements obtainable on sections of the structure [23,24,25]. Those formulae enables us to estimate various meaningful melanization parameters. Thus, we are able to estimate concomitantly the melanocyte volume, the melanin volume density in the cytoplasm, the mean melanin volume per melanocyte, the numerical density of MMs in the cytoplasm and the mean number of MMs per melanocyte.

Additionally, two parameters, the mean melanin volume per MM and an indicator of melanosomal maturation, i.e. $N_{N(mp,m)}$, the mean number of detected melanin particles per MM [26], can be calculated in order to quantitatively appraise the level of melanosomal maturation.

Only two studies on the melanocyte have used morphometrical techniques, including one relating to the manual morphometrical measurement of melanosomes observed by transmission electron microscopy [19] and the other, dealing with a quantitative image analysis of Fontana Masson-stained 1 μm plastic sections of human skin samples observed by light microscopy [27].

Our stereological computerized tool permits to quantify different aspects of the intracytoplasmic melanization, which have never been performed at the ultrastructural level with automatic methods, and never approached from a stereological angle.

Works are carried out at the present time in our laboratory, with the aim to validate this original method by comparing morphometrical data and results obtained from a classical melanin assay [28].

Moreover, the Inazu's alkali elution method, which permits to dissolve pheomelanin on ultrathin sections with an acceptable specificity [29], is actually applied to our quantitative method in order to measure pheomelanin and eumelanin like only HPLC methods allow.

At least, the last appreciable advantage of our method is that it requires a low number of pigment cells. As a consequence, more complex experiments with human epidermal melanocytes cultured physiologically might be perfomed with small experimental units of short term cultures.

In conclusion, this original approach, by using new type of data will contribute to a better understanding of intracytoplasmic events underlying stimulation or repression of human melanogenesis by physical or chemical agents or in pathological conditions.

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DISCUSSION 3

SERUM S100: A NEW TUMOR MARKER IN METASTATIC MALIGNANT MELANOMA[1]

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1. The complete study will be published in one of the next issues of the European Journal of Cancer (Guo HB, Stoffel-Wagner B, Bierwirth T, Mezger J, Klingmüller D. Clinical significance of serum S100 in metastatic malignant melanoma. *Eur J Cancer*, 1995, in press.)

The incidence of melanoma has increased dramatically in the past decades [1, 2]. As melanoma can metastasize to any organ, follow-up after surgery is difficult. Therefore, a major challenge facing clinical biochemical research is to detect specific and reliable serum markers that are of value in diagnosing and monitoring progression of the disease.
S100 is an acidic calcium-binding protein with a molecular weight of 21 000 found in the nervous system of vertebrates [6].In immunohistochemistry the use of S100 is well established for the diagnosis of malignant melanoma. Recent studies have shown that S100 is excreted from melanoma cells [5]. Lately, it was demonstrated that neuron-specific enolase (NSE) is a useful prognostic factor for metastatic malignant melanoma [3,4].

The aims of this study were to analyse the clinical significance of serum S100 at the time of diagnosis of malignant melanoma, to evaluate the use of serum S100 in the follow-up of patients treated for malignant melanoma and to compare serum levels of S100 with NSE in patients with malignant melanoma.

Serum samples were taken from 126 patients with malignant melanoma. Staging was performed according to the criteria of the American Joint Committee on Cancer (AJCC) based on the tumor-node-metastasis (TNM) system: stage I (T1/T2, N0, M0), stage II (T3/T4, N0, M0), stage III (any T, N1/2, M0) and stage IV (any T, any N, M1) [2]. 80 patients had a stage I or II disease, 23 patients a stage III disease and 23 patients had a stage IV disease. The reference groups consisted of 25 healthy persons, 45 patients with benign skin lesions. Serum S100 was measured by an immunoradiometric assay (Byk-Sangtec Diagnostics, Dietzenbach, Germany). The detection limit of the S100-IRMA was 0.15 μg/l. Serum NSE was determined using a commercially available radioimmunassay (Pharmacia, Uppsala, Sweden). The detection limit was 2 μg/l. Values exceeding 12.5 μg/l were considered as elevated.
In the reference groups consisting of 45 patients with benign skin lesions and 25 healthy persons, serum S100 concentrations were < 0.15 μg/l in all cases. S100 was detectable only in one of 80 patients without metastases (stage I/II). Values exceeding 0.15 μg/l were found in 2 of the 23 patients with stage III (sensitivity: 8.7%) and 17 of the 23 patients with stage IV (sensitivity: 73.9%). The overall sensitivity for metastatic malignant melanoma stage III/IV was 41.3% (19/46). In the reference groups NSE concentrations were below the cut-off value in all cases. In 7 of 80 patients without metastases (stage I/II) serum NSE was elevated (sensitivity: 8.75%). Increased serum concentrations of NSE ($> 12.5\mu$g/l) were found in 3 of 23 patients with stage III (sensitivity: 13.0%) and in 8 of 23 patients with stage IV (sensitivity: 34.8%). The overall sensitivity for metastatic malignant melanoma (stage III/IV) was 23.9% (11/46). The sensitivity of S100 for metastatic malignant melanoma stage IV was significantly higher than that of NSE ($p < 0.05$).

Serum S100 and NSE concentrations were serially measured in 23 patients with malignant melanoma stage III/IV in the course of treatment. Two representative follow-ups are shown in Fig. 1 and 2.

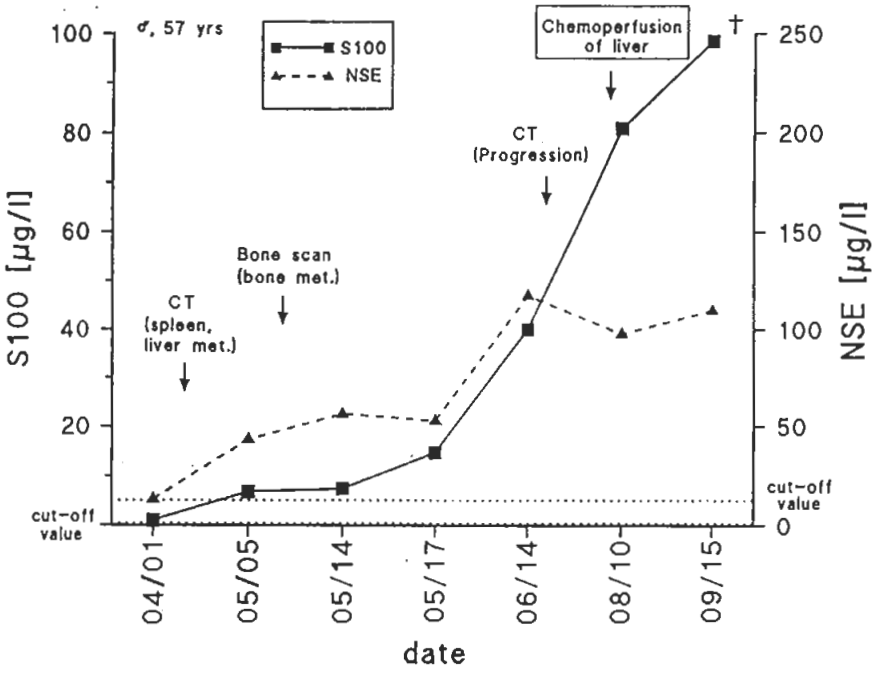

Fig 1: Serum S100 and NSE concentrations in a 57-year-old male patient with stage IV disease (liver, spleen and bone metastases)

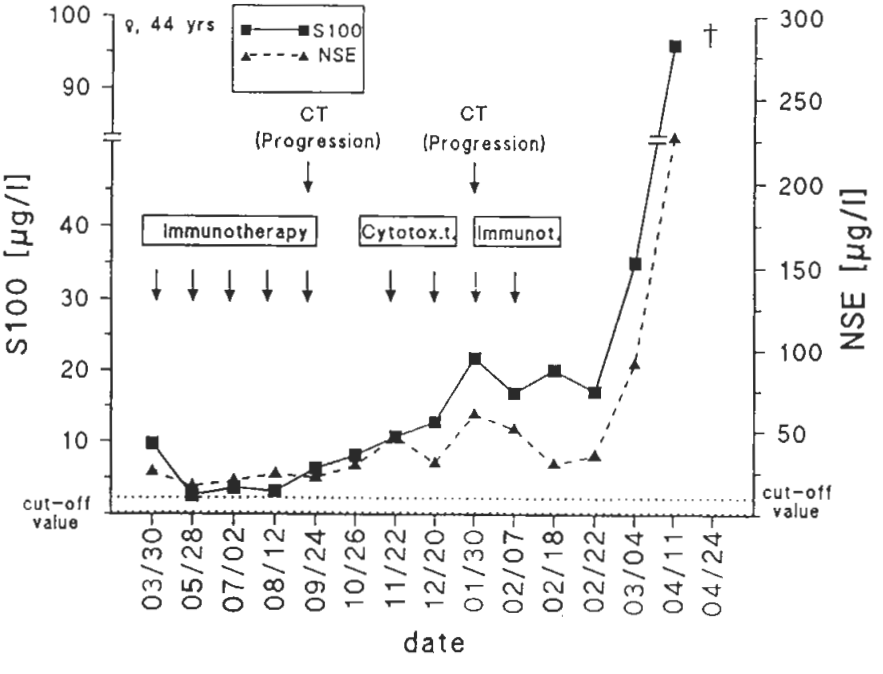

Fig 2: Serum S100 and NSE concentrations in a 44-year-old female patient with stage IV disease (lung and visceral metastases)

In some patients, serum S100 showed a gradual rise accompanying the progression of the disease under treatment. For example, in a 57-year-old man, liver, spleen, and bone metastases were already present at the time of diagnosis (Fig. 1). Serum S100 and NSE were slightly elevated. With progression of liver and spleen metastases, serum S100 and NSE levels began to increase. For example, a representative liver metastasis increased from a diameter of 3 cm to a diameter of 6 cm. When bone and

CT scan indicated even further progression, a chemoperfusion of the liver with α-interferon and interleukin-2 was performed. Serum NSE levels remained between 117.3 and 97.7 μg/l, but S100 persistently rose from 7.4 to 98.4 μg/l corresponding to an enlargement of the liver metastasis to 8 cm and to the clinical deterioration until death.

In other patients a decline of serum S100 concentrations was observed corresponding to response to therapy, whereas serum S100 increased again, when radiological diagnostics showed later on progression of the disease. For example, in a 44-year-old woman with stage IV, the first measurement showed elevated serum S100 and NSE concentrations (Fig. 2). Metastases in the right lower lobe of the lung and visceral metastases were demonstrated by CT scan. During immunotherapy with α-interferon and interleukin-2, stable disease was achieved, and serum S100 remained slightly elevated. Afterwards S100 and NSE levels increased again and a thoracal and abdominal CT indicated progression of lung and visceral metastases. Now, in both, the left and the right lobe of the lung metastases were found. Despite two courses of cytostatic therapy and further immunotherapy, CT scan demonstrated progressive lung metastases and MRI scan showed intraspinal metastases in the level of the 8th and 9th thoracic vertebra. The changes of serum NSE correlated with serum S100 throughout the whole course of the disease.
The data presented here confirm serum S100 to have a interesting sensitivity (41.3%) for advanced disease (stage III/IV). 17 of 20 patients with initially detectable serum S100 had distant metastases, whereas elevated serum S100 concentrations were detected in only 2 patients with involvement of lymph nodes (stage III). In only one of 80 patients without metastases (stage I/II), serum S100 exceeded 0.15 μg/l. The low incidence of elevated serum S100 levels in patients without metastases indicates clearly that serum S100 is not useful for screening or for early diagnosis of the disease.
Serum S100 was serially determined in patients with metastases (stage III/IV). To our knowledge, this study is the first to follow-up on the development of serum S100 in patients with metastatic melanoma. Serum S100 concentrations reflected the course of the disease during therapy. A persistent rise in serum S100 indicated progression of the disease, whereas decline in serum S100 indicated response to therapy. Reflecting the fact that no complete remission was achieved, S100 remained detectable in all patients.
In conclusion, the current study supports the clinical significance of serum S100 in metastatic malignant melanoma. Serum S100 showed a higher sensitivity than serum NSE and correlated with the clinical stage of the tumor. Serial measurements of S100 were helpful to monitor the treatment.

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1. Melanins and other pigments chemistry

(Comments by Prof. M. Peter)

Oxidation of mono- and diphenolic compounds: A kinetic study of the reaction of dopamine with dioxygen at pH 7-9 was carried out by *Herlinger* et al. No metal ion catalysis is necessary. The reaction is first order in [O_2], in [dopamine], and [H+]$^{-1}$. Stoichiometric amounts of H_2O_2 are formed. The rate-determining step is assumed to be hydrogen atom abstraction from the monodeprotonated species by O_2.

Interactions of ascorbic acid (AA) and reduced glutathione (GSH) in the oxidations of 6-hydroxydopa (TOPA) and 6-hydroxydopamine (6-OHDA) were investigated by both HPLC with electrochemical detection and spectrometric methods by *Nappi* and *Vass*. TOPA and 6-OHDA are extremely unstable, with 100% of the trihyroxyphenyls oxidized within 0.5 min at physiological pH in KPO_4 buffer. Though neither AA nor GSH reduce back the oxidation products it is assumed that GSH may influence the neurotoxicity of trihydroxyphenols by interacting with their oxidation products, resulting in the production of pheomelanin. Electrochemical analyses established the formation of two oxidation products, one of them beeing reduced leukochrome, from each trihydroxyphenyl. GSH significantly decreased the levels of both oxidation products.

Oxidation of Tyr-peptides was studied by *Rosei* et al. in relation to melanin formation on various levels have investigated theoxidation of opioid peptides (Tyr-Gly, Tyr-Gly-Gly and Leu-enkephalin) by tyrosinase into melanin-like pigments, named opio-melanins. The peptide moiety is retained in the products. The pigments are soluble at neutral and basic pH. UV and solubility properties as well as pK_a values are reported. Concomitant oxidation of dopa and opioid peptides by tyrosinase produces mixed polymers. The pigments are bleached by H_2O_2, sunlight.

Conclusion: the different initial structure of the precursors accounts for the final diverse polymeric architecture of these pigments with respect to dopa-melanin.

Schraermeyer observed a colour change from clear transparent to dark brown upon exposure of bovine serum albumin (BSA) to hydroxyl radicals generated by the Fenton reaction. A melanin-like absorption spectrum developed. The reaction with ferricyanide and the dark brown BSA solution resulted in the same dark blue-green colour as is typical for melanin. The author suggests that, by the reaction of BSA with hydroxyl radicals, melanin was formed.

Physical methods: The thermal stability of melanin (no source identifiable from abstract) was investigated by *Sawhney* using differential scanning calorimetry (DSC).

Rakoczy and *Panz* reported differences in signal intensities in the ESR spectra of melanin type pigments present in the spore wall as well as black pigment isolated from the spores of *Physarum polycephalum*, *Physarum nudum*, and *Fuligo septica*.

Papers on drug binding: Melanin granules isolated from bovine eye were used in a simple *in vitro* test to model the potential for a range of veterinary drugs to accumulate in melanin-containing tissues (*Sauer* and Anderson). The following drugs showed appreciable binding: Clenbuterol, salmeterol, propranolol, carazolol azaperone, xylazine, 17 beta-estradiol, testosterone, alpha-zeranol, diethylstilbestrol and 19-nortestosterone. Progesterone and 17 alpha-trenbolone bound to a lesser extend. Salbutamol did not show significant binding. To provide a preliminary indication of the validity of the model, animals were treated with clenbuterol for 21 d, to enable the assessment of accumulation. *In vivo*, clenbuterol was detected in choroid/PRE and hair at high concentrations. The studies indicate the potential for pigmented tissue analysis in monitoring for the use of veterinary drugs in food producing animals. Hair analysis may offer particular advantages for on-farm monitoring and in providing historic information.

Howells et al. studied the binding of seven veterinary drugs (clenbuterol, chlorpromazine, diethylstilbestrol, 19-nortestosterone, salbutamol, salicylic acid and trenbolone) to melanin from *Sepia officinalis*. Basic and hydrophobic drugs were the most strongly bound. Basic drug binding involves an ionic component. An interesting novel application was suggested by use of melanin, immobilized on the surface of porous silica. The capacity to bind basic clenbuterol was found to be higher than that for neutral 19-nortestosterone. By virtue of its binding kinetics, high capacity and mechanical-robustness, silica-immobilized melanin offers potential for use in chromatography or solid-phase extraction and may additionally enable modelling of drug-melanin interactions.

- Herlinger E, Jameson RF, Linert W.
  Spontaneous autoxidation of dopamine. J Chem Soc, Perkin Trans.2:259-263, 1995.

- Howells L, Godfrey M, Sauer MJ.
  Melanin as an adsorbent for drug residues. Analyst. 119:2691-2693, 1994.

- Nappi AJ, Vass E.
  The effects of glutathione and ascorbic-acid on the oxidations of 6-hydroxydopa and 6-hydroxydopamine. Biochim Biophys Acta. 1201:498-504, 1994.

- Rakoczy L, Panz T.
  Melanin revealed in spores of the true slime-molds using the electron-spin-resonance method. Acta Protozool. 33:227-

231, 1994.

- Rosei MA, Mosca L, Demarco C.
Spectroscopic features of native and bleached opio-melanins. Biochim Biophys Acta. 1243:71-77, 1995.

- Sauer MJ, Anderson SPL.
In- vitro and in- vivo studies of drug residue accumulation in pigmented tissues. Analyst. 119:2553-2556, 1994.

- Sawhney SS.
Thermal-stability of melanin. Thermochim Acta. 247:377-380, 1994.

- Schraermeyer U.
Transformation of albumin into melanin by hydroxyl radicals. Comp Biochem Physiol. 108 C:281-288, 1994.

2. Biology of pigment cells and pigmentary disorders

Melanocyte cultures
(Comments by Dr N. Smit)

- Abdel-Malek Z, Swope VB, Suzuki I, Akcali C, Harringer MD, Boyce ST, Urabe K, Hearing VJ.
Mitogenic and melanogenic stimulation of normal human melanocytes by melanotropic peptides. Proc Nac Sci USA. 92:1789-1793, 1995.
Commentary: The exact role of melanocyte stimulating hormone (MSH) in the melanogenic process is not completely defined. MSH and its synthetic analogue Nle^{4}DPhe^7 MSH stimulate tyrosinase activity in murine models and can increase tanning when administered in humans. However, human melanocytes, eventhough express MSH receptors, are poorly responsive to the effects of MSH if cultured in the presence of AMPc stimulators. Hunt and co-workers show that, as reported in animal models, treatment of human melanocytes with the synthetic analogue of MSH increase the ratio eumelanin: pheomelanin. Abdel-Malek and co-workers show that MSH and ACTH stimulates, at nanomolar concentrations, the proliferation and melanogenesis of human melanocytes cultured in the absence of AMPc inducers and increase the expression of tyrosinase and of TRP-1 and TRP-2 at post transcriptional step. The results suggest several considerations: a) MSH seems to have a physiological role in vivo. MSH could be synthesised by keratinocytes and could have a local effect. b) MSH and its receptors can mediate the effects of UV radiation of skin, by regulating the epidermal-melanin unit, by increasing eumelanin synthesis and by stimulating skin pigmentation. c) synthetic analogues of MSH may have application as artificial tanning agents in particular in individuals with red skin. Phoemelanin, in contrast to eumelanin, produces free radicals when irradiated and can amplify the damage of UV radiation. Changing the relative proportion between the two melanins may be an important protecting factors in these subjects.

- Bohm M, Moellmann G, Cheng E, Alvarez Franco M, Wagner S, Sassonecorsi P, Halaban R.
Identification of p90 (RSK) as the probable CREB-Ser (133) kinase in human melanocytes. Cell Growth & Differentiation. 6(3):291-302, 1995.

- Buffey JA, Messenger AG, Taylor M, Ashcroft ATT, Westergate GE, Mac Neil S.
Extracellular derived hair and skin fibroblasts stimulate human skin melanocyte tyrosinase activity. Br J Dermatol. 131:836-842, 1994.
Commentary: Different indirect evidences suggests that the activity of both epidermal and hair melanocytes is regulated or at least correlated with the activity of adjacent cells. The hair pigmentation and the morphological modification of melanocytes during the hair cycle is a clear evidence of the relationship between melanocytes and hair bulb epithelium and some papers have been presented on the influence, in vitro, between keratinocytes and melanocytes. In this paper, the authors describe the influence of co-culture of melanocytes and fibroblasts, showing that extracellular matrix produced by both skin and hair fibroblast significantly increases tyrosinase activity without apparently stimulating cell proliferation. This phenomenon could have relevanee in the physiology of pigmentation and possibly in some pigmentary pathologies.

- Cui JA, Arica Y, Bystryn JC.
Characterization of vitiligo antigens. Pigment Cell Research. 8(1):53-59, 1995.

- Hubner B, Eckert K, Garbe C, Maurer HR.
Synergistic interactions between interferon beta and carboplatin on SK-MEL 28 human melanoma cell growth inhibition in vitro. Journal of Cancer Research and Clinical Oncology. 121(2):84-88, 1995.

- Hunt G, Kyne S, Wakamatsu K, Ito S, Thody A.
Nle^{4}DPhe^7 α-melanocyte-stimulating hormone increases the eumelanin pheomelanin ratio in cultured human melanocytes. J Invest Dermatol. 104(1):83-85, 1995.
Abstract: In mammals, melanin exists in two chemically distinct forms: the red-yellow phaeomelanin and the brown-black eumelanin. Although administration of the pigmentary hormone alpha-melanocyte-stimulating hormone (alpha MSH) and

its synthetic analogue Nle4DPhe7 alpha MSH induces skin darkening in man, the increases in melanogenesis in cultured human melanocytes in response to these peptides are relatively small. However, it is possible that MSH affects the eumelanin:phaeomelanin ratio rather than total cellular melanin. Thus, this study examined the specific effects of Nle4DPhe7 alpha MSH on the two melanins in cultured human melanocytes, quantifying eumelanin and phaeomelanin by high performance liquid chromatography. Nle4DPhe7 alpha MSH induced significant increases in the eumelanin content of these cells while having lesser and varied effects on the levels of phaeomelanin. As a consequence, the eumelanin:phaoemelanin ratio was increased in every culture. These results demonstrate that Nle4DPhe7 alpha MSH affects melanin type in human melanocytes and suggest a possible mechanism by which this peptide induces skin darkening in man.

- Krasagakis K, Garbe C, Eberle J, Orfanos CE.
Tumour necrosis factors and several interleukins inhibits the growth and modulate the antigen expression of normal human melanocytes in vitro. Archives of Dermatological Research. 287(3-4):259-265, 1995.

- Smit NPM, Westerhof W, Menko WJ, Verbeek NM, Pavel S.
Stimulation of cultured melanocytes in medium containing a serum substitute: Ultroser-G. Pigment Cell Research 8(1):19-27, 1995.

- Tobin DJ, Colen SR, Bystryn JC.
Isolation and long term culture of human hair follicle melanocytes. J Invest Dermatol. 104:86-9, 1995.
Commentary: Few is known about the mechanisms regulating the modification of melanocytes during the hair cycle and the correlation between the cell populations of hair. Two different populations of melanocytes can be identified in human hair: a) pigmented cells present in the bulb and in the infundibulum; b) amelanotic cells present in the outer root sheath. Now the authors report an apparently simple and reproducible method to obtain long term culture of hair-melanocytes which certainly will provide and useful model to evaluate the biological characteristic of these cells.

3. MSH, MCH, other hormones, differentiation

- Adan RA, Cone RD, Burbach JP, Gispen WH.
Differential effects of melanocortin peptides on neural melanocortin receptors. Mol Pharmacol. 46(6):1182-90, 1994.
Abstract: Melanocortins (MCs) have various physiological actions on the brain. The recent cloning of neural MC receptors opened new avenues to study the effects of these neuropeptides on the nervous system. Here we investigated the structure-activity relationships (SARs) of peptides derived from adrenocorticotropic hormone (ACTH) with cloned MC3 and MC4 receptors in vitro and correlated these with central effects of MCs in vivo. Analysis of the effects of various MC peptides on cAMP accumulation in and binding to cells that expressed either the rat MC3 receptor or the human MC4 receptor demonstrated that ACTH-4-9-NH2 was the core sequence of ACTH able to activate these receptors. Furthermore, gamma-melanocyte-stimulating hormone (MSH) displayed selectivity for the MC3 receptor, whereas [D-Phe7]ACTH-4-10 more efficiently activated the MC4 receptor than the MC3 receptor. The activities of MC fragments that lacked the three carboxyl-terminal amino acids (residues 11-13) of ACTH-1-13 were much lower than that of alpha-MSH, for both receptors. Furthermore, the three amino-terminal amino acids (residues 1-3) of alpha-MSH were more important for full activation of the MC4 receptor, compared with the MC3 receptor. The SAR for the MC4 receptor resembled that for the induction of excessive grooming behavior by MC peptides. Therefore, we suggest that this behavioral response is mediated by MC4 receptors. The SAR for the MC3 receptor did not overlap with that for in vivo effects of MCs. ORG2766, an ACTH-4-9 analog that is very potent in an active avoidance task, did not activate, antagonize, or bind to the MC3 and MC4 receptors. This suggests the presence of still other MC receptors, in addition to the MC3 and MC4 receptors, in the brain. These data identify peptides with selectivity for either the MC3 receptor or the MC4 receptor, which may be used for development of novel MC receptor-specific ligands. Furthermore, this is the first report that discusses behavioral effects of MCs in light of data on cloned MC receptors.

- Clarke BL, Moore DR, Blalock JE.
Adrenocorticotropic hormone stimulates a transient calcium uptake in rat lymphocytes. Endocrinology. 135(5):1780-6, 1994.
Abstract: Freshly isolated rat lymphocytes were tested for corticotropin (ACTH)-dependent calcium uptake. Physiological levels of corticotropin (0.01-1 nM) were found to stimulate both an uptake of 45Ca2+ and a rise in cAMP. The calcium uptake was delayed by 2 min after ACTH addition, but was rapid and transient after the onset of uptake. The extent of calcium uptake was dose dependent on the corticotropin concentration and reached a maximum by 1 nM. Several fragments of corticotropin were tested for activity; both full-length 1-39 and a functional truncated form, 1-25, had equivalent effects on 45Ca influx at 1 nM; however, alpha MSH-(1-13), ACTH-(11-24), or a mixture of alpha MSH and ACTH-(11-24) had no effect on 45Ca influx. Extracellular calcium uptake was blocked by the calcium channel blockers lanthanum, diltiazem, nifedipine, and omega-conotoxin. Splenic lymphocytes that express ACTH receptors had ligand-dependent calcium uptake, but thymocytes that lack ACTH receptors had no ligand-dependent calcium uptake. A mouse adrenal cell line, Y-1, showed the same 45Ca uptake kinetics. These findings demonstrate that both lymphocytes and adrenal cells have a functional ACTH-dependent calcium uptake mechanism.

- Haskell Luevano C, Miwa H, Dickinson C, Hruby VJ, Yamada T, Gantz I.
Binding and cAMP studies of melanotropin peptides with the cloned human peripheral melanocortin receptor, hMC1R. Biochem Biophys Res Commun. 204(3):1137-42, 1994.
Abstract: Binding and stimulation of cAMP by the melanotropin peptides alpha-MSH (alpha-melanocyte-stimulating hormone) and its superpotent analogues [Nle4, DPhe7]alpha-MSH (MT-I) and Ac-[Nle4,[formula: see text]alpha-MSH4-10-NH2 (MT-II) were undertaken to examine their respective properties on the human peripheral melanocyte melanocortin receptor, hMC1R. alpha-MSH was found to possess a binding IC50 value of 6.5 +/- 0.9 x 10(-9) M and cAMP EC50 value of 2.0 +/- 0.6 x 10(-9) M. MT-I possesses a binding IC50 value of 1.2 +/- 0.3 x 10(-9) M and a cAMP EC50 of 0.5 +/- 0.03 x 10(-9) M. MT-II possesses a binding IC50 of 0.57 +/- 0.08 x 10(-9) M and cAMP EC50 value of 0.20 +/- 0.05 x 10(-9) M.

- Hunt G, Donatien PD, Lunec J, Todd C, Kyne S, Thody AJ.
Cultured human melanocytes respond to MSH peptides and ACTH. Pigment Cell Res. 7(4):217-21, 1994.
Abstract: Although the administration of melanocyte-stimulating hormone (MSH) peptides results in skin darkening in man, cultured human melanocytes have been reported to be unresponsive to these peptides. This may be a consequence of the conditions under which the cells were maintained in vitro, particularly the use of phorbol esters and cholera toxin as melanocyte mitogens. By culturing the cells in the absence of these additives, we demonstrate that alpha-MSH and its synthetic analogue Nle4DPhe7 alpha-MSH (NDP-MSH) induce dose-related increases in melanin content and tyrosinase activity and affect cell morphology in the majority of human melanocyte cultures. In addition, NDP-MSH induces increases in tyrosinase mRNA and tyrosinase-related protein-1 (TRP-1) mRNA. The dose-response curves for the MSH peptides are sigmoidal and the two peptides are equipotent in their effects on human melanocytes. Adrenocorticotropic hormone (ACTH) also affects morphology and stimulates melanogenesis and tyrosinase activity in human melanocytes. However, the dose-response curves for ACTH are biphasic, and the melanocytes respond to lower concentrations of ACTH than MSH peptides, similar to those normally present in human plasma. These findings may be important in understanding the role of these pro-opiomelanocortin peptides in human skin pigmentation.

- Magenis RE, Smith L, Nadeau JH, Johnson KR, Mountjoy KG, Cone RD.
Mapping of the ACTH, MSH, and neural (MC3 and MC4) melanocortin receptors in the mouse and human. Mamm Genome. 5(8):503-8, 1994.
Abstract: The melanocortin peptides regulate a wide variety of physiological processes, including pigmentation and glucocorticoid production, and also have several activities in the central and peripheral nervous systems. The melanocortin receptor family includes the melanocyte-stimulating hormone receptor (MSH-R), adrenocorticotropic hormone receptor (ACTH-R), and two neural receptors, MC3-R and MC4-R. In the human these receptors map to 16q24 (MSH-R), 18p11.2 (ACTH-R), 20q13.2 (MC3-R), and 18q22 (MC4-R). The corresponding locations in the mouse are 8, 18, and 2; a variant for mapping MC4-R has not yet been identified. The data reported here also show that the neural MC3 receptor maps close to a disease locus for benign neonatal epilepsy in human and near the E1-2 epilepsy susceptibility locus in the mouse.

- Radinsky R, Beltran PJ, Tsan R, Zhang R, Cone RD, Fidler IJ.
Transcriptional induction of the melanocyte-stimulating hormone receptor in brain metastases of murine K-1735 melanoma. Cancer Res. 55(1):141-8, 1995.
Abstract: Metastatic K-1735 murine melanoma cells are amelanotic in culture or in the subcutis of syngeneic mice. When injected into the internal carotid artery, these cells produce melanotic brain metastases. The production of melanin in tumor cells growing in the brain was directly correlated with induction of melanocyte-stimulating hormone receptor (MSH-R) steady-state mRNA transcripts. K-1735 cells isolated from brain lesions and implanted into the subcutis or grown in culture lose MSH-R transcripts and become amelanotic. In contrast to K-1735 cells, B16-BL6 melanoma cells constitutively produce melanin and express high levels of MSH-R mRNA regardless of the site of growth. Somatic cell hybrids between K-1735 and B16 cells produced melanin and expressed high levels of MSH-R mRNA transcripts, regardless of the site of growth, suggesting the dominance of the B16 phenotype. Treatment with alpha-MSH failed to upregulate MSH-R expression in cultured K-1735 cells or to maintain MSH-R expression in K-1735 cells isolated from brain metastases to be grown in culture. Responsiveness to alpha-MSH as determined by cell proliferation, melanin production, and intracellular accumulation of cyclic AMP directly correlated with MSH-R expression. These data demonstrate that a specific organ environment influences the phenotype of metastatic cells by regulation of specific genes that encode for cell surface receptors.

4. Photobiology and photochemistry

- Boutwell WB.
The Under Cover Skin Cancer Prevention Project. A community-based program in four Texas cities. Cancer. 75(2 Suppl):657-60, 1995.

- Carsberg CJ, Ohanian J, Friedmann PS.
Ultraviolet radiation stimulates a biphasic pattern of 1,2-diacylglycerol formation in cultured human melanocytes and keratinocytes by activation of phospholipases C and D. Biochem J. 305(Pt 2):471-7, 1995.
Abstract: Ultraviolet radiation (UVR) induces melanin synthesis by human epidermal melanocytes, and

phospholipid-derived 1,2-diacylglycerols (DAGs) have been implicated in mediating this response. In previous experiments, addition of the synthetic DAG 1-oleoyl-2-acetylglycerol to cultured pigment cells stimulated melanogenesis. The purpose of the present study was to analyse the effects of UVR on the endogenous generation of DAGs. It was found that in a number of cultured cell types, including human melanocytes and B16 mouse melanoma cells, but also human keratinocytes and Swiss 3T3 fibroblasts, exposure to a single dose of UVR stimulated a biphasic increase in endogenous DAG formation. An early transient rise, over seconds, was followed by a more sustained delayed rise over minutes. The early rise in DAG levels was accompanied by a transient rise in inositol trisphosphate formation, indicating activation of phosphatidylinositol-specific phospholipase C. The delayed rise was accompanied by activation of phospholipase D. This endogenous DAG formation by pigment cells is further evidence for the involvement of DAGs in UVR-induced epidermal melanin synthesis. Since DAG formation is also seen in other cells types, it is possible that DAGs may be involved in an array of UVR-induced responses.

- Coleman K, Baak JP, van Diest PJ, Curran B, Mullaney J, Fenton M, Leader M.
DNA ploidy status in 84 ocular melanomas: a study of DNA quantitation in ocular melanomas by flow cytometry and automatic and interactive static image analysis. Hum Pathol. 26(1):99-105, 1995.

- Gragoudas ES, Egan KM, Walsh SM, Regan S, Munzenrider JE, Taratuta V.
Lens changes after proton beam irradiation for uveal melanoma. Am J Ophthalmol. 119(2):157-64, 1995.

- Hatton DH, Mitchell DL, Strickland PT, Johnson RT.
Enhanced photoproduct repair: its role in the DNA damage-resistance phenotype of human malignant melanoma cells. Cancer Res. 55(1):181-9, 1995.
Abstract: A fundamental issue in understanding melanoma is to seek the basis for the cellular resistance to DNA damaging agents, which is manifested in vivo as pronounced tumor resistance to therapeutic agents. The published consensus on melanoma has been that exaggerated postreplication recovery (PRR), rather than excision repair, underlies the unusual damage-resistance phenotype. We examined the resistance to the model DNA damaging agent, UV-C, of subclones derived from a human metastatic melanoma cell line. The clones essentially fall into two groups: one with normal and the other with enhanced resistance. We exploited this range to investigate the interrelationships between replication, transcription, and repair of DNA after UV irradiation. Subclones resistant to UV killing were indeed found to possess enhanced rates of PRR and were coresistant to cisplatin. However, we now report an overall elevation of photoproduct repair in both melanoma groups compared to nonmelanoma controls and conclude that this accounts for the resistant melanoma phenotype, including that of enhanced PRR. Repair enhancement may explain chemoresistance, while loss of efficiency of certain functions, such as PRR, due to the intrinsic genetic lability of tumor cells, may generate the class of melanoma subclones exhibiting only normal resistance.

- Im S, Hann SK, Kim HI, Kim NS, Park YK.
Biologic characteristics of cultured human vitiligo melanocytes. Int J Dermatol. 33(8):556-62, 1994.

- Kageshita T, Hirai S, Ono T, Ferrone S.
Human high molecular weight-melanoma associated antigen mimicry by mouse anti-idiotypic MAb MK2-23. Immunohistochemical analysis of the reactivity of anti-anti-idiotypic MAb with surgically removed melanoma lesions. Int J Cancer. 60(3):334-40, 1995.

- Karlsson M, Boeryd B, Carstensen J, Kagedal B, Wingren S.
DNA ploidy and S-phase fraction as prognostic factors in patients with uveal melanomas. Br J Cancer. 71(1):177-81, 1995.
Abstract: In 96 patients with uveal malignant melanomas the tumours were investigated by DNA flow cytometry. Thirty-eight per cent of the melanomas were aneuploid. By univariate analysis significant correlations with survival were found for histological type, tumour size, DNA ploidy, evidence of 'blind eye' and S-phase fraction. By multivariate analysis, significant prognostic variables were found to be histological type ($P = 0.0008$), tumour size ($P < 0.0001$) and DNA ploidy ($P = 0.0038$). Evidence of 'blind eye' was not significantly correlated with survival after adjustments for the other variables mentioned above. The S-phase fraction could be estimated in all 60 diploid tumours and in 12 of 36 aneuploid melanomas. By univariate analysis this variable was found to be a significant prognostic factor, but did not remain so after adjustment for ploidy, histological type and tumor size. We further conclude that patients with small DNA diploid uveal melanomas of spindle cell type have a rather favourable prognosis.

- Malorni W, Rivabene R, Straface E, Rainaldi G, Monti D, Salvioli S, Cossarizza A, Franceschi C.
3-Aminobenzamide protects cells from UV-B-induced apoptosis by acting on cytoskeleton and substrate adhesion. Biochem Biophys Res Commun. 207(2):715-24, 1995.
Abstract: 3-aminobenzamide (3-ABA) is an inhibitor of poly-(ADP-ribose)-polymerase, an enzyme involved in numerous subcellular processes, including cell death. Recently, a target effect of the drug on some cytoskeletal elements has also been described (Malorni et al., Biochem. Biophys. Res. Commun. 202: 915-922, 1994). In this study we evaluated the ability of 3-ABA to interfere with UV-B ray-induced apoptosis in cells selected for their cytoskeletal features and their different capability to adhere to the substrate. Human melanoma (M14) and epithelial (A431) cell lines and murine primary fibroblastic cultures (MFC) were studied. Our results indicate that cytoskeleton is indeed an important cellular target of 3-ABA, which can prevent apoptotic cell death by UV-B through a specific effect on the adhesion properties of the cells.

Indeed, an inverse correlation was observed between sensitivity to UV-B-induced apoptosis (M14 > A431 > MFC) and substrate adhesion (MFC > A431 > M14). The potential relevance of these observations to understand the possible relationships among apoptosis, cytoskeletal functions and substrate adhesion is discussed.

- Voigt H, Classen R.
Topodermatographic image analysis for melanoma screening and the quantitative assessment of tumor dimension parameters of the skin. Cancer. 75(4):981-8, 1995.
Abstract: BACKGROUND. The clinical need to identify and evaluate changes of cutaneous lesions in melanoma screening or follow-up of patients with cancer is of paramount importance. Because skin-lesion changes may be small and numerous, clinical assessment alone does not meet the requirements of quantitative assessment. Using the computer as a diagnostic tool for the image analysis of sequentially captured skin surface images has resulted in the technical problem of insufficient registration reproducibility. This paper describes the technical logistics, setup procedure, and clinical evaluation of the novel technique termed "topodermatography," which performs the quantitative videographic image analysis of skin-lesion changes over time. METHODS. Digitized measurements of skin-surface image parameters were performed using a high-speed processor with an onboard coprocessor, a high-resolution video camera, specifically designed image processing software, and a position framework for the adjustment of the patient's standing position. The topodermatographic image analysis was performed on 109 consecutive patients who were at risk for melanoma (N = 98), had lesions from Kaposi's sarcoma (N = 4), had metastatic skin deposits from melanoma (N = 3), and had breast cancer (N = 4). RESULTS. Skin lesion changes over time could be identified reliably within a few millimeters of diametric enlargement. In this series, a 0.51% early melanoma detection rate was assessed in 19 of 98 patients followed for 12 months. By monitoring manifest neoplastic skin lesions, tumor growth kinetics were analyzed quantitatively to determine the total area of skin involvement, thus facilitating precise response assessment. CONCLUSIONS. Topodermatographic image analysis helps to optimize screening and follow-up procedures for patients with melanoma and populations at risk for melanoma. In addition, metastatic tumor lesions on the skin can be monitored dynamically, facilitating the accurate evaluation of the impact of systemic therapy on multiple skin deposits from melanoma and nonmelanoma cancers.

5. Neuromelanins

(Comments by Dr M. d'Ischia)

The nature of the oxidizing system(s) involved in the biogenesis of Substantia nigra neuromelanin is still an open problem. From time to time, a number of hypotheses have been put forward, but none of them has thus far received definite experimental confirmation. We are therefore especially interested in the paper by *Hastings* (J. Neurochem. 1995,64,919-924) suggesting a role of prostaglandin H synthase in the oxidative conversion of dopamine to neuromelanin. Evidence supporting this view comes mainly from in vitro experiments, showing that a purified preparation of the enzyme is capable of promoting the oxidation of dopamine to the corresponding aminochrome in the presence of arachidonic acid or hydrogen peroxide. Should such a mechanism occur in vivo, it would provide an additional important link between oxidative stress and neuromelanin accumulation. Considering, however, the broad range of enzymic and non-enzymic factors that can be envisaged as being capable of promoting neuromelanin formation, and the evident mismatch between the time frames of dopamine oxidation in vitro and in aging brain, it seems likely that neuromelanin formation results from conditions of imbalanced pro-oxidant/antioxidant equilibria within susceptible microenvironments of specific dopaminergic neurons. We would therefore be inclined to await more cogent evidence before ranking prostaglandin H synthase among the major melanogenic enzymes in Substantia nigra.

- Swartz HM.
Biophysics of melanin and informal group on the characterization of neuromelanin. Pigment Cell Research. 7(4):244, 1994.

- Hastings TG.
Enzymatic oxidation of dopamine: the role of prostaglandin H synthase. J. Neurochem. 64(2):919-924, 1995.

6. Genetics, molecular biology

(Comments by Dr F. Beerman)

Microphthalmia (mi): Over the last year, several papers were published dealing with the mouse *microphthalmia* mutation. *microphthalmia* mutations in the mouse, when homozygous or heterozygous, affect the development of a variety of cell types including melanocytes, osteoclasts and mast cells. Therefore, pigmentation of the hair is affected, and, in some mutations, pigmentation of the eye (and even in the RPE). The corresponding gene has been cloned by use of transgenic insertional mutations at the *mi* locus and been shown to represent a member of the basic helix-loop-helix transcription factor familiy (see Jackson and Raymond, 1994; Jackson, 1994; Hemesath et al., 1994; Steingrimsson et al., 1994 for more information and further references). In humans, it has been shown that Waardenburg Syndrome type 2 is caused by mutations in the human gene, called MITF, and heterozygous patients do often show mild pigmentation abnormalities. The Mi transcription factor can bind to DNA as either homodimer or heterodimer with members of the same transcription factor family. It is intriguing that a potential binding site for the microphthalmia transcription factor, the so-called M-box, an 11bp long element, has been

identified in promoters of tyrosinase, TRP-1 and TRP-2 (Jackson et al., Nucleic Acids Research 19, 3799-3804, 1991; Lowings et al., Molecular and Cellular Biology 12, 3653-3662, 1992). It has now been convincingly shown (Bentley et al.,1994; Ganss et al., 1994; Yasumoto et al., 1994; Yavuzer et al., 1995), that the microphthalmia gene product is able to activate transcription of tyrosinase and TRP-1 in cell culture.

- Beermann F, Ganss R, Schytz, G.
  Regulation of pigmentation during mammalian development. Advances in Developmental Biochemistry 3:149-177, 1994.

- Bentley NJ, Eisen T, Goding CR.
  Melanocyte-specific expression of the human tyrosinase promoter: activation by the microphthalmia gene product and role of the initiator. Molecular & Cellular Biology 14(12):7996-8006, 1994.
  Commentary: Identification of elements in the human tyrosinase promoter: (1) A positive element between -185 and -150, (2) a negative element between -150 and -115, (3) the M box, a conserved element found in other melanocyte-specific promoters (4) an Sp1 site (5) a highly evolutionarily conserved element located between -14 and +1 comprising an E-box motif and an overlapping octamer element. They report that as little as 115 bp of the upstream sequence is sufficient to direct tissue-specific expression. Morevover, the microphthalmia gene product can transactivate the tyrosinase promoter via the M box and the conserved E box located close to the initiator.

- Bossler A, Kari C, Humphreys CW, Gyorfi T, Maurer J, Thiel E, Menssen HD.
  Expression of the wt1 Wilms' tumor gene by normal and malignant human melanocytes. International Journal of Cancer 59(1):78-82, 1994.

- Fukai K, Holmes SA, Lucchese NJ, Siu VM, Weleber RG, Schnur RE, Spritz RA.
  Autosomal recessive ocular albinism associated with a functionally significant tyrosinase polymorphism. Nature Genetics 9:92-95, 1995.

- Ganss R, SchŸtz G, Beermann F.
  The mouse tyrosinase gene. Promoter modulation by positive and negative regulatory elements. Journal of Biological Chemistry 269(47):29808-16, 1994.
  Commentary: The mouse tyrosinase promoter contains two positive elements and one negative element. One of the positive elements includes the M-box. Cotransfection experiments provide evidence that a basic helix-loop-helix-zipper protein, encoded at the microphthalmia gene locus, transactivates the tyrosinase promoter, probably by binding to the M-box.

- Ganss R, Schmidt A, Schytz G, Beermann F.
  Analysis of the mouse tyrosinase promoter in vitro and in vivo. Pigment Cell Research 7(5):275-278, 1994.
  Commentary: Transgenic experiments show that the positive promoter elements (see previous paper) are not exclusively required for pigment production in vivo and, therefore, are suggested to rather modulate tyrosinase gene expression.

- Hemesath TJ, Steingrimsson E, McGill G, Hansen MJ, Vaught J, Hodgkinson CA, Arnheiter H, Copeland NG, Jenkins NA, Fisher DE.
  Microphthalmia, a critical factor in melanocyte development, defines a discrete transcription factor family. Genes & Development 8(22):2770-80, 1994.

- Inagaki H, Bessho Y, Koga A, Hori H.
  Expression of the tyrosinase-encoding gene in a colorless melanophore mutant of the medaka fish, Oryzias latipes. Gene 150(2):319-324, 1994.

- Jackson IJ.
  Molecular and developmental genetics of mouse coat color. Annual Review of Genetics 28:189-217, 1994.

- Jackson IJ, Raymond S.
  Manifestations of microphthalmia. Nature Genetics 8(11):209-210. (Minireview)

- Kobayashi T, Urabe K, Orlow SJ, Higashi K, Imokawa G, Kwon BS, Potter B, Hearing VJ.
  The Pmel 17/silver locus protein. Characterization and investigation of its melanogenic function. Journal of Biological Chemistry. 269(46):29198-205, 1994.
  Commentary: With help of an antibody (aPEP13) generated against the C-terminal part of mouse Pmel17, the authors isolated the Pmel17 protein. Apparently, the protein did not show any of the melanogenic catalytic activities described for tyrosinase-related proteins. The silver protein was rich in the melanosome fraction but was absent from coated vesicles which deliver TRPs to melanosomes. These results suggest that the silver locus product is a melanosomal matrix protein which may contribute to melanogenesis as a structural protein, although the possibility remains that it also has a novel catalytic function in melanogenesis.

- Kwon BS, Halaban R, Ponnazhagan S, Kim K, Chintamaneni C, Bennett D, Pickard RT.
  Mouse silver mutation is caused by a single base insertion in the putative cytoplasmic domain of Pmel 17. Nucleic

Acids Research 23(1):154-158, 1995.
Commentary: A single nucleotide (A) insertion was found in the putative cytoplasmic tail of the si/si Pmel 17 cDNA clone. This insertion is predicted to alter the last 24 amino acids at the C-terminus and extends the Pmel 17 protein by 12 residues. Therefore, the silver Pmel 17 protein has a major defect at the carboxyl terminus.

- Kwon BS, Kim KK, Halaban R, Pickard RT.
Characterization of mouse Pmel 17 gene and silver locus. Pigment Cell Research 17(6):394-397, 1994.

- Maresh GA, Marken JS, Neubauer M, Aruffo A, Hellstrom I, Hellstrom KE, Marquardt H.
Cloning and expression of the gene for the melanoma-associated me20 antigen. DNA & Cell Biology 13(2):87-95, 1994.
Commentary: Human melanoma cells, but not tumor cells of other histological origin, express a unique membrane-associated glycoprotein, designated ME20-M, and secrete a soluble glycoprotein, designated ME20-S, defined by monoclonal antibody ME20. The amino acid sequence of the ME20 antigen deduced from the cDNA differs from the human Pmel 17 gene product by a single amino acid substitution and deletion of 7 amino acid residues. The observed differences in the sequences, of the melanoma-derived and normal melanocyte-derived gene products may suggest that ME20-M is a unique tumor antigen or, alternatively, an oncofetal self-antigen.

- Michaud EJ, van Vugt MJ, Bultman SJ, Sweet HO, Davisson MT, Woychik RP.
Differential expression of a new dominant agouti allele (Aiapy) is correlated with methylation state and is influenced by parental lineage. Genes & Development 8(12):1463-72, 1994.

- Moore KJ, Swing DA, Copeland NG, Jenkins NA.
The murine dilute suppressor gene encodes a cell autonomous suppressor. Genetics 138(2):491-7, 1994.

- Oetting WS, King RA.
Analysis of tyrosinase mutations associated with tyrosinase-related oculocutaneous albinism (OCA1). Pigment Cell Research 7(5):285-290, 1994.

- Porter SD, Meyer CJ.
A distal tyrosinase upstream element stimulates gene expression in neural-crest-derived melanocytes of transgenic mice: position-independent and mosaic expression. Development 120(8):2103-11, 1994.
Commentary: As shown also by Ganss et al. (EMBO J. 13, 3083-93, 1994), a melanocyte-specific DNAse I hypersensitive site situated at about -12 to -15 kb upstream of the mouse tyrosinase gene is able to confer position-independent, high level and melanocyte-specific expression in transgenic mice.

- Sato S, Miura H, Yamamoto H, Takeuchi T.
Identification of nuclear factors that bind to the mouse tyrosinase gene regulatory region. Pigment Cell Research 7(5):279-284, 1994.

- Steingrimsson E, Moore KJ, Lamoreux ML, Ferré-D'Amaré AR, Burley SK, Sanders Zimring DC, Skow LC, Hodgkinson CA, Arnheiter H, Copeland NG, Jenkins NA.
Molecular basis of mouse microphthalmia (mi) mutations helps explain their developmental and phenotypic consequences. Nature Genetics 8(11):256-263, 1994.

- Stevens G, Vanbeukering J, Jenkins T, Ramsay M.
An intragenic deletion of the P gene is the common mutation causing tyrosinase-positive oculocutaneous albinism in southern african negroids. American Journal of Human Genetics 56(3):586-591, 1995.
Commentary: This study shows that the intragenic deletion in the OCA2 gene, P, is a common cause of tyrosinase-positive albinism (OCA2) in southern African Negroids and is of African origin. On the basis of haplotype data, it appears that at least seven additional, less frequent OCA2 mutations occur in this population.

- Sturm RA, Osullivan BJ, Thomson JAF, Jamshidi N, Pedley J, Parsons PG.
Expression studies of pigmentation and pou-domain genes in human melanoma cells. Pigment Cell Research 7(4):235-240, 1994.

- Suzuki H, Takahashi K, Yasumoto K, Shibahara S.
Activation of the tyrosinase gene promoter by neurofibromin. Biochemical & Biophysical Research Communications 205(3):1984-1991, 1994.

- Wildenberg SC, King RA, Oetting WS.
Detection of a tsp5091 polymorphism in the 3' utr of the human tyrosinase related protein-1 (tyrp) gene. Human Genetics 95(2):247, 1995.

- Wilson BD, Ollmann MM, Kang L, Stoffel M, Bell GI, Barsh GS.
Structure and function of asp, the human homolog of the mouse agouti gene. Human Molecular Genetics 4(2):223-230,

1995.
Commentary: The human agouti gene, named Agouti Signaling Protein (ASP), encodes a 132 amino acid protein, the mRNA for which is expressed in testis, ovary, and heart, and at lower levels in liver, kidney, and foreskin. Expression of ASP in transgenic mice produces a yellow coat, and expression of ASP in cell culture blocks the alpha-MSH-stimulated accumulation of cAMP in mouse melanoma cells. The expression of ASP in human tissues suggests a function for agouti homologs in species that do not exhibit the characteristic phenotype of banded hairs.

- Yasumoto K, Yokoyama K,Shibata K,Tomita Y,Shibahara S.
Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene. Molecular & Cellular Biology 14(12):8058-70, 1994.
Commentary: Identification of a tyrosinase distal element (TDE) (positions -1861 to -1842) responsible for pigment cell-specific transcription This element can be bound by the human microphthalmia gene product (MITF). Transient coexpression assays showed that MITF specifically transactivates the promoter activity of the tyrosinase gene through the CATGTG motif of TDE but not the promoter of the ubiquitously expressed heme oxygenase gene. These results indicate that MITF is a cell-type-specific factor that is capable of activating transcription of the tyrosinase gene.

- Yavuzer U, Keenan E, Lowings P, Vachtenheim J, Currie G, Goding CR
The Microphthalmia gene product interacts with the retinoblastoma protein in vitro and is a target for deregulation of melanocyte-specific transcription. Oncogene 10(1):123-34, 1995.

- Yokoyama K, Yasumoto K, Suzuki H, Shibahara S.
Cloning of the human DOPAchrome tautomerase/tyrosinase-related protein 2 gene and identification of two regulatory regions required for its pigment cell-specific expression. Journal of Biological Chemistry 269(43):27080-7, 1994.
Commentary: A first analysis on the promoter of the TRP-2/DT gene (human) reveals two regulatory regions, a 32-bp element and the proximal region, which are required for pigment cell-specific expression of the DT gene. Both regulatory regions contain a CANNTG motif, a well known binding site for a large family of transcription factors possessing a basic helix-loop-helix structure.

(Comments by Dr M. Picardo)

- Battayani Z, Grob JJ, Xerri L, Noe C, Zarour H et al.
Polymerase chain reaction detection of circulating melanocytes as prognostic marker in patients with melanoma. Arch Dermatol. 131:443-447, 1995.
Commentary: The identification of specific markers for disease progression in melanoma patients is an important aspect of both the biological and therapeutic approach to malignant melanoma. The identification of tyrosinase gene has opened the way to use polymerase chain reaction, a specific and sensible methods to detect circulating melanocytes in patients with melanoma. The authors have conducted an interesting study to correlate the presence of circulating melanocytes with the prognosis of the patients. Their results strongly suggest that PCR can be used as a specific marker in high risk disease free patients and in patients with regional node or systemic metastasis and that the test may have a crucial role in the treatment and prognosis of melanoma patients.

7. Tyrosinase, TRP1, TRP2 and other enzymes

(Comments by Prof. J.C. Garcia-Borron)

The paper by *Kobayashi et al.* (J. Biol. Chem. 269:29198-205) is the first thourough characterization of a melanosomal matrix protein reported so far. Although a still undetermined enzymatic activity for the *silver* protein cannot yet be definitively ruled out, the paper presents strong evidence pointing to a structural role for the *silver* gene product. Since mutations at the *silver* locus in mice cause the progressive loss of functional melanocytes, this work proves, for the first time, that the structural melanosomal defects, in addition to enzymatic deficits, can lead to impaired pigmentation.

Solano et al. (Biochem. Biophys. Res. Commun. 204:1243-1250) report the reconstitution of dopachrome tautomerase activity with Zn rather than with Cu, thus pointing to the possibility that tautomerase could be a zinc rather than a copper containing metalloenzyme. In fact, the involvement of copper at the active site of DCT has never been shown, although it was generally assumed by homology to tyrosinase. As opposed to Cu, Zn does not participate in redox reactions, and this might account for the different enzymatic activity of DCT as compared to tyrosinase, in spite of the high degree of homology of the two proteins. If the cofactor role of Zn for DCT activity is confirmed, this will rise the interesting question of the determinants of metal ion binding to the protein, accounting for the incorporation of different ions to highly homologous polypeptide chains. The melanogenic proteins might provide a convenient system to study the structural motifs involved in the selective binding of a specific metal ion to the polypeptide.

Naish-Byfield et al. (Biochem. J., 304:155-162) show that, in addition to the known inhibitory effect on tyrosinase activity exerted by thiol compounds at high concentrations, low concentrations of dithiothreitol can activate the enzyme by abolishing the lag phase for the monophenolase activity. This dual role of the low molecular weight thiol compounds might confer them important regulatory properties.

- Fukazawa K, Sakagami M, Umemoto M, Senda T.
Development of melanosomes and cytochemical observation of tyrosinase activity in the inner ear. ORL J Otorhinolaryngol Relat Spec. 56(5):247-252, 1994.
Abstract: The ultrastructure and tyrosinase activity of melanocytes in the inner ear of pigmented guinea pigs were observed. Melanocytes/melanocyte-like cells were seen in the stria vascularis, the vestibular dark cell area and the endolymphatic sac. In the stria vascularis, melanosomes in several stages of maturation were seen in the cytoplasm of the intermediate cells and melanin-laden endosomes existed in the basal cells. Only the intermediate cells contained tyrosinase-positive cytoorganelles; Golgi sacs and neighboring small vesicles. Numerous melanocytes containing many melanosomes were observed under the epithelium of the vestibular dark cell area, and they showed tyrosinase activity. Melanocytes were seen in the endolymphatic sac, and they also showed tyrosinase activity. However, the epithelial cells of the endolymphatic sac, which had vacuoles containing melanin, did not show tyrosinase activity. Based on these findings, it can be said that (1) most of the intermediate cells of the stria vascularis must be melanocytes, (2) melanogenesis is vigorous in melanocytes of the inner ear, and (3) melanin in the epithelial cells of the endolymphatic sac is transferred in from melanocytes and is never synthesized in the epithelial cells.

- Kobayashi T, Urabe K, Orlow SJ, Higashi K, Imokawa G, Kwon BS, Potterf B, Hearing VJ.
The Pmel 17/silver locus protein. Character. & investigation of its melanogenic function. J Biol Chem. 269(46):29198-205, 1994.

- Kobayashi T, Urabe K, Winder A, Jimenez-Cervantes C, Imokawa G, Brewington T, Solano F, Garcia-Borron JC, Hearing VJ.
Tyrosinase related protein 1 (TRP1) functions as a DHICA oxidase in melanin biosynthesis. EMBO J. 13(24):5818-25, 1994.
Abstract: Several genes critical to the enzymatic regulation of melanin production in mammals have recently been cloned and mapped to the albino, brown and slaty loci in mice. All three genes encode proteins with similar structures and features, but with distinct catalytic capacities; the functions of two of those gene products have previously been identified. The albino locus encodes tyrosinase, an enzyme with three distinct melanogenic functions, while the slaty locus encodes tyrosinase-related protein 2 (TRP2), an enzyme with a single specific, but distinct, function as DOPAchrome tautomerase. Although the brown locus, encoding TRP1, was actually the first member of the tyrosinase gene family to be cloned, its catalytic function (which results in the production of black rather than brown melanin) has been in general dispute. In this study we have used two different techniques (expression of TRP1 in transfected fibroblasts and immunoaffinity purification of TRP1 from melanocytes) to examine the enzymatic function(s) of TRP1. The data demonstrate that the specific melanogenic function of TRP1 is the oxidation of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) to a carboxylated indole-quinone at a down-stream point in the melanin biosynthetic pathway. This enzyme activity appears to be essential to the further metabolism of DHICA to a high molecular weight pigmented biopolymer.

- Naish-Byfield S, Cooksey CJ, Riley PA.
Oxidation of monohydric phenol substrates by tyrosinase: effect of dithiothreitol on kinetics. Biochem J. 304(Pt1):155-162, 1994.

- Schallreuter KU, Lemke KR, Hill HZ, Wood JM.
Thioredoxin reductase induction coincides with melanin biosynthesis in brown and black guinea pigs and in murine melanoma cells. J Invest Dermatol. 103(6):820-824, 1994.
Abstract: X-rays were used to induce melanin biosynthesis in brown and black guinea pigs in vivo. During the course of pigmentation, the expression of thioredoxin reductase was increased, whereas for the other antioxidant enzymes, superoxide dismutase (cytosol Cu/Zn-enzyme), catalase, and glutathione reductase, levels and activities decreased. Isobutylmethylxanthine induced eumelanin biosynthesis in murine melanoma cells (Cloudman S-91). In these cells, thioredoxin reductase levels coincided with melanogenesis. Our results suggest that both tyrosinase and thioredoxin reductase respond to oxidative stress in the epidermis as well as in melanoma cells and react with superoxide anion radicals to stimulate melanogenesis and to prevent peroxidative damage, respectively.

- Solano F, Martinez-Liarte JH, Jimenez-Cervantes C, Garcia-Borron JC, Lozano JA.
Dopachrome tautomerase is a zinc-containing enzyme. Biochem Biophys Res Commun. 204(3):1243-50, 1994.
Abstract: Dopachrome tautomerase (DCT) catalyzes the conversion of L-dopachrome into 5,6-dihydroxyindole-2-carboxylic acid through the melanogenic biosynthetic pathway. This enzyme, also named TRP2, belongs to the family of the tyrosinase related proteins. The three members of the family contain two highly conserved metal-binding sites with three histidines on each. Tyrosinase has copper at its active site. It was assumed that although DCT might have copper in those metal binding sites, its active site could be related to other two putative iron-binding sites located in different positions. Based on apoDCT preparation with cyanide and reconstitution experiments, we propose that DCT have zinc instead of copper at the two metal-binding sites and that those sites actually correspond to the active site. The involvement of zinc, which cannot undergo redox reactions, accounts for the reaction that DCT catalyzes, a tautomerization versus the copper-mediated oxidations catalyzed by tyrosinase.

- Sugumaran M, Ricketts D.
Model sclerotization studies. 3. Cuticular enzyme catalyzed oxidation of peptidyl model tyrosine and dopa derivatives. Arch Insect Biochem Physiol. 28(1):17-32, 1995.

Abstract: Incubation of N-acetyltyrosine methyl ester with cuticular enzymes, isolated from the wandering stages of Calliphora sp larvae, resulted in the generation of N-acetyldopa methyl ester when the reaction was carried out in the presence of ascorbate which prevented further oxidation of the o-diphenolic product. Enzymatic oxidation of N-acetyldopa methyl ester ultimately generated dehydro N-acetyldopa methyl ester. The identity of enzymatically produced N-acetyldopa methyl ester and dehydro N-acetyldopa methyl ester has been confirmed by comparison of the ultraviolet and infrared spectral and chromatographic properties with those of authentic samples as well as by nuclear magnetic resonance studies. Since N-acetyldopaquinone methyl ester was also converted to dehydro N-acetyldopa methyl ester and tyrosinase was responsible for the oxidation of N-acetyldopa methyl ester, a scheme for the cuticular phenoloxidase catalyzed conversion of N-acetyltyrosine methyl ester to dehydro N-acetyldopa methyl ester involving the intermediary formation of the quinone and the quinone methide is proposed to account for the observed results. The conversion of N-acetyldopa methyl ester to dehydro derivative remarkably resembles the conversion of the sclerotizing precursor, N-acetyldopamine, to dehydro-N-acetyldopamine observed in the insect cuticle. Based on these comparative studies, it is proposed that peptidyl dopa derivatives could also serve as the sclerotizing precursors for the sclerotization of the insect cuticle.

- Tobin D, Thody AJ.
The superoxide anion may mediate short- but not long-term effects of ultraviolet radiation on melanogenesis. Exp Dermatol. 3(3):99-105, 1994.
Abstract: The present study was carried out to examine the role of reactive oxygen species in mediating the melanogenic effects of UVR. B16 mouse melanoma cells responded to a single dose of UVR by showing increases in their melanin content. Although there was a small increase in melanin at 48-72 hours, which was associated with a rise in tyrosinase activity at 48 h, the greatest change occurred at 3 h and this was not associated with an increase in tyrosinase activity. This short-term response, unlike the more delayed melanogenic response, was reduced by superoxide dismutase (SOD). Xanthine oxidase (XO), which generates the superoxide anion (O2-), also increased the melanin content of B16 melanoma cells with effects at 3 h and 48 h. As with UVR, the delayed response was accompanied by an increase in tyrosinase activity but no such association was evident at 3 h. In addition, the short-term effect, like that seen with UVR, was reduced with SOD and to a lesser extent with catalase. In contrast to the effects found with XO, glucose oxidase, which generates hydrogen peroxide, had no effect on the melanin content or tyrosinase activity of the B16 cells. These results confirm previous observations that UVR is able to act directly on cells to bring about delayed increases in melanogenesis. They further demonstrate that UVR also stimulates melanogenesis through a more rapid action that is not associated with an activation of tyrosinase. This effect could be mediated by the O2- which, rather than activating tyrosinase, could act by serving as a substrate for the enzyme.

- Wood JM, Schallreuter-Wood KU, Lindsey NJ, Callaghan S, Gardner ML.
A specific tetrahydrobiopterin binding domain on tyrosinase controls melanogenesis. Biochem Biophys Res Commun. 206(2):480-485, 1995.
Abstract: (6R)5,6,7,8-tetrahydrobiopterin(6-BH4) directly regulates tyrosinase activity by specifically binding to a putative 13 amino acid domain. This domain has sequence homology to 6-BH4 binding sites already identified on phenylalanine hydroxylase and 4a-carbinolamine dehydratase. Furthermore, this binding sequence appears to have been conserved during the evolution of tyrosinase as it has also been identified in the frog, mouse and human enzymes. 6-BH4 controls tyrosinase activity by an uncompetitive mechanism requiring the presence of L-tyrosine for effective down-regulation. When L-dopa is substrate, 6-BH4 does not inhibit the enzyme implicating separate binding sites for L-dopa and L-tyrosine on tyrosinase. Dihydropterin and 6-biopterin, the oxidation products of 6-BH4, do not inhibit tyrosinase significantly, indicating that melanin biosynthesis is controlled by a 6-BH4/6-biopterin redox-switch mechanism which can be initiated by photo-oxidation of 6-BH4.

8. Melanoma and other pigmented tumours

- Barnhill RL, Aguiar M, Cohen C, Kang S, Kennedy J, Schmidt B, Sober AJ, Solomon AR.
Congenital melanocytic nevi and DNA content. An analysis by flow and image cytometry. Cancer. 74(11):2935-43, 1994.
Abstract: BACKGROUND. Potential risk factors for the development of melanoma in congenital melanocytic nevi (CMN) are not well established. DNA aneuploidy may constitute such a risk factor but has not been sufficiently studied in CMN. METHODS. In the present study, DNA analysis of eight giant CMN, nine medium CMN (1.5-20 cm), and eight small CMN (< 1.5 cm) was assessed by flow cytometry and selected lesions (six nevi) by DNA image cytometry. DNA content was correlated with patient age, nevus size, and degree of cytologic atypia. RESULTS. DNA aneuploidy was detected by flow cytometry in two giant CMN from adult patients and in a small CMN from a child. DNA aneuploidy was not observed in any of the six CMN studied by image cytometry, although an increased S-phase was noted in a markedly atypical giant CMN. No DNA aneuploidy was detected in medium-sized CMN or in the CMN of nine patients 1 year of age or younger. CONCLUSION. In contrast to previous studies, it was observed that abnormal DNA content does tend to correlate with cytologic atypia, particularly in giant CMN with atypia or melanoma, in adults. Conversely, frank DNA aneuploidy in any CMN in children younger than 1 year of age, irrespective of histologic findings, was not detected. Finally, based on these limited studies, greater sensitivity of image over flow cytometry for detection of DNA aneuploidy cannot be verified.

- Cascinelli N, Zurrida S, Galimberti V, Bartoli C, Bufalino R, Del Prato I, Mascheroni L, Testori A, Clemente C.
Acral lentiginous melanoma. A histological type without prognostic significance. J Dermatol Surg Oncol. 20(12):817-22, 1994.
Abstract: BACKGROUND. Acral lentiginous melanoma (ALM) is reported to have a poorer prognosis than melanomas of other histotypes. OBJECTIVE. The purpose of this paper is to verify if ALMs, per se, have a more aggressive behavior or if the poorer prognosis of these patients is due to a later diagnosis. METHODS. Clinical charts of 165 patients with acrally located melanoma were reviewed as well as all histological slides. In all patients, histotype, Clark's level, Breslow thickness, pigmentation, presence of ulceration, and regression were reported. Mitotic index, vascular invasion, and microscopic satellitosis were also determined. Survival of the 64 patients with ALM was compared with survival observed in the remaining 101 acral (nonlentiginous) melanomas. RESULTS. Melanoma localized at acral sites constitute 8.9% of the 1845 patients with melanoma treated at our Institute from 1975 to 1990. All were Caucasian, 57 were males, and 108 females. Out of the 165 patients with this localization, 64 (38.8%) were classified as ALM. Disease-free and overall survival of the 64 ALM patients were not different at a statistically significant level compared with the areas observed in the other acral nonlentiginous melanoma. CONCLUSION. The survival difference between ALM and other histological types is due to a later diagnosis and not to a more aggressive behavior of ALM.

- Cui J, Bystryn JC.
Melanoma and vitiligo are associated with antibody responses to similar antigens on pigment cells. Archives of Dermatology. 131(3):314-318, 1995.
Abstract: Background and Design: Several clinical observations suggest that there is a link between vitiligo and melanoma. We examined whether an immune response to similar antigens on pigment cells could account for this association. We tested 30 patients with melanoma, 29 patients with vitiligo, and 28 patients with unrelated conditions for antibodies to human melanocyte antigens using an immunoprecipitation sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis assay.
Results: Antibodies to melanocytes were present in 24 (80%) patients from the melanoma group, 24 (83%) patients from the vitiligo group, and in two (7%) patients from the control group. The antibodies in patients with melanoma or vitiligo were directed to similar antigens with molecular weights of approximately 40 to 45, 75, and 90 kd. The frequency of antibody responses to each of these antigens was similar in both diseases. By sequential immunodepletion, the antigens defined by antibodies in both diseases were similar. These antigens were also expressed on melanoma cells.
Conclusions: Most patients with melanoma or with vitiligo develop antibodies to similar antigens that are present both on melanocytes and on melanoma cells. These findings support the hypothesis that the clinical link between the two diseases results from immune responses to antigens shared by normal and malignant pigment cells.

- Iyengar B.
Expression of proliferating cell nuclear antigen (PCNA): proliferative phase functions and malignant transformation of melanocytes. Melanoma Res. 4(5):293-5, 1994.

- Kobayashi T, Urabe K, Winder A, Jimenez-Cervantes C, Imokawa G, Brewington T, Solano F, Garcia-Borron JC, Hearing VJ.
Tyrosinase related protein 1 (TRP1) functions as a DHICA oxidase in melanin biosynthesis. EMBO J. 13(24):5818-25, 1994.
Abstract: Several genes critical to the enzymatic regulation of melanin production in mammals have recently been cloned and mapped to the albino, brown and slaty loci in mice. All three genes encode proteins with similar structures and features, but with distinct catalytic capacities; the functions of two of those gene products have previously been identified. The albino locus encodes tyrosinase, an enzyme with three distinct melanogenic functions, while the slaty locus encodes tyrosinase-related protein 2 (TRP2), an enzyme with a single specific, but distinct, function as DOPAchrome tautomerase. Although the brown locus, encoding TRP1, was actually the first member of the tyrosinase gene family to be cloned, its catalytic function (which results in the production of black rather than brown melanin) has been in general dispute. In this study we have used two different techniques (expression of TRP1 in transfected fibroblasts and immunoaffinity purification of TRP1 from melanocytes) to examine the enzymatic function(s) of TRP1. The data demonstrate that the specific melanogenic function of TRP1 is the oxidation of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) to a carboxylated indole-quinone at a down-stream point in the melanin biosynthetic pathway. This enzyme activity appears to be essential to the further metabolism of DHICA to a high molecular weight pigmented biopolymer.

- Piepkorn M.
On the nature of histologic observations: the case of the Spitz nevus. J Am Acad Dermatol. 32(2 Pt 1):248-54, 1995.

- Podhajcer OL, Bover L, Bravo AI, Ledda MF, Kairiyama C, Calb I, Guerra L, Capony F, Mordoh J.
Expression of cathepsin D in primary and metastatic human melanoma and dysplastic nevi. J Invest Dermatol. 104(3):340-4, 1995.
Abstract: High levels of cytosolic cathepsin D expression have been associated with poor prognosis in breast cancer node-negative patients. In this work, we provide evidence that three cell lines established from human metastatic melanomas--IIB-MEL-J, IIB-MEL-LES, and IIB-MEL-IAN--express high levels of procathepsin D mRNA. IIB-MEL-J cells secreted into the conditioned media about 30% of the newly synthesized protein, which was active at acidic pH. Melanoma tumors arising in nude mice after injection of the three different cell lines expressed high levels of procathepsin D mRNA. Moreover, 13 human metastatic melanomas expressed variable levels of procathepsin D mRNA. To study the

possible association between cathepsin D expression and melanoma development, samples corresponding to 10 primary tumors, 11 metastatic melanomas, 10 dysplastic nevi, 27 nevocellular nevi, and normal melanocytes were studied by immunohistochemistry for cathepsin D-specific staining. We found that cathepsin D was expressed in all of the dysplastic nevi and primary and metastatic melanomas tested but in only 18% of nevocellular nevi (five of 27), whereas normal melanocytes showed no cathepsin D expression. The overall data indicate that cathepsin D is expressed at a high level by melanoma cells, and because of its expression in preneoplastic lesions, it may be associated with melanoma development.

- Quong RY, Bickford ST, Ing YL, Terman B, Herlyn M, Lassam NJ.
Protein kinases in normal and transformed melanocytes. Melanoma Res. 4(5): 313-9, 1994.

- Soballe PW, Maloy WL, Myrga ML, Jacob LS, Herlyn M.
Experimental local therapy of human melanoma with lytic magainin peptides. Int J Cancer. 60(2):280-4, 1995.
Abstract: Magainin peptides and model amphipathic peptides exhibit antibiotic activity and are also cytolytic for transformed human cells. Here we demonstrate in vitro that MSI-511 (an all-D amino-acid model magainin peptide) and MSI-130 (a margainin analogue) were more lytic for 17 human melanomas than for normal melanocytes. Melanomas established s.c. in athymic nude mice and then injected once with the peptide MSI-511 completely disappeared in 6 out of 9 animals, whereas a control peptide had no effect. Murine skin at the tumor injection site was initially affected, but healed within 2 weeks with minimal scarring. Similarly, accelerated healing was seen in human skin grafted to SCID mice and injected with MSI-511. Our results indicate that lytic magainin peptides can be used for local tumor therapy with minimal long-term damage to normal tissues.

- Takahashi T, Chapman PB, Yang SY, Hara I, Vijayasaradhi S, Houghton AN.
Reactivity of autologous CD4+ T lymphocytes against human melanoma. Evidence for a shared melanoma antigen presented by HLA-DR15. J Immunol. 154(2):772-9, 1995.
Abstract: Reactivity of CD8+ T lymphocytes against human melanoma has been extensively characterized, but little is known about melanoma Ags recognized by CD4+ lymphocytes. We have identified CD4+ CTL that recognize shared melanoma Ag(s) expressed by autologous melanoma cells and a subset of allogeneic melanomas. The same Ag(s) was shared by autologous and positive allogeneic melanomas by cross-blocking experiments. Cytotoxicity was directed against epitopes presented by HLA-DR on target melanoma cells, and allelic typing revealed that cytotoxicity was restricted through HLA-DR15. These CD4+ T cells released IFN-gamma, IL-4, and TNF-alpha, but not IL-2, in response to HLA-DR15+ target cells. CD4+ T cells did not lyse DR15+ nonmelanoma cell types, including melanocytes or fibroblasts (induced to express HLA-DR by IFN-gamma). Thus, by cytotoxicity assays, shared Ags were only recognized on melanoma cells but not on normal melanocytes. In summary, this analysis shows that melanoma cells share an Ag that is presented by HLA-DR15.

- Yang TH, Aosai F, Norose K, Ueda M, Yano A.
Enhanced cytotoxicity of IFN-gamma-producing CD4+ cytotoxic T lymphocytes specific for T. gondii-infected human melanoma cells. J Immunol. 154(1):290-8, 1995.
Abstract: CD4+ lines specific for Toxoplasma gondii-infected human melanoma P36 cells were established from PBL of a patient with chronic toxoplasmosis. CD4+ CTL lines were obtained by weekly in vitro stimulation with T. gondii-infected P36 cells that shared HLA-DR4 molecules with the patient. The lytic activity of CD4+ CTL lines against T. gondii-infected P36 or T. gondii-infected autologous EBV-transformed B lymphoma (EBV-Ya) was inhibited by anti-HLA-DR mAb, whereas anti-HLA-A, B, C mAb failed to block the lytic activity. Thus, the cytotoxicity of CD4+ CTL lines against T. gondii-infected P36 was restricted by HLA-DR molecules. In response to Ag-specific stimulation, CD4+ CTL lines produced significant levels of IFN-gamma. Exogenously added IFN-gamma up-regulated the surface expression of MHC class II, but not of class I in T. gondii-infected P36 cells. In addition, the CTL activity against T. gondii-infected P36 cells was augmented when target cells were co-cultured with IFN-gamma. These data indicate that CD4+ CTL-mediated cytotoxicity against T. gondii-infected melanocytes is enhanced by the autocrine production of IFN-gamma. Further, CD4+ CTL may play a role in the manifestation of toxoplasmic retinochoroiditis by killing T. gondii-infected melanocytes.

ANNOUNCEMENTS & RELATED ACTIVITIES

6th Meeting of the European Society for Pigment Cell Research

Centre Hospitalier Universitaire Vaudois
Lausanne, Switzerland
October 19-21, 1995

SCIENTIFIC PROGRAM

Guest lectures:
Dr. H.T. Vaudry, Rouen, France: **"Multifactorial control of melanotropic hormone secretion."**
Prof. L. Bito, Comlumbia, USA:**"The pigmented cells of the eye: a multiplicity of function, with a common denominator"**
Prof. M. Peter, Postdam, Germany: **"The Bio-organic Chemistry of melanogenesis".**
Prof. R.A. Spritz, Madison, USA: **"Molecular basis of genetic hypopigmentary disorders."**
Prof. A. Knuth, Frankfurt, Germany: **"New immunotherapeutical approaches in melanoma."**

SOCIAL EVENTS

- Welcome reception at the CHUV on Thursday 19 Oct., at 18.30 PM
- Friday 20 Oct., official banquet in the *"Château d'Aigle"*, in the middle of vineyards. Participation fee, including bus: 100,-SFR. Limited numbers of participants: 150.
- Half and full day tour during and after the Conference can be booked through the "Office du Tourisme Lausanne" which will be present at the meeting.

CONTACT
ESPCR '95
Centre Pluridisciplinaire d'Oncologie - CHUV - BH 06
CH-1011 Lausanne - Switzerland
Tel: 41/21/314.39.58 - Fax: 41/21/314.39.57

REGISTRATION

During the meeting, registration will take place at the hospital, level 08 on Thursday 19 October 95 between 08.00 and 09.30, and will continue to Saturday.

REGISTRATION FEES

All figures in CHF:	**Before Sept. 1st**	**After Sept. 1st**
ESPCR, PASPCR, JSPCR members, medical	300,-	400,-
residents in training and post-graduate students	300,-	400,-
Non-members	400,-	500,-

LUNCHES

Lunches will be served at the congress site.
Cost for the meals: 15,-/lunch

XVI INTERNATIONAL PIGMENT CELL CONFERENCE

DISNEYLAND HOTEL
ANAHEIM, CALIFORNIA

OCTOBER 29 - NOVEMBER 3, 1996

Important Deadlines

Call for Abstracts - To be considered for presentation, all abstracts/posters must be received by the committee no later than May 1, 1996.

Exhibitors - Companies are invited to participate as either on-site exhibitors or financial supporters, or both. To exhibit on-site, the fee is $600. Companies donating $2,000 or more will be given complimentary exhibit space (6-foot table top booth space), and acknowledgement in the course publications. Inquiries should be directed to the Memorial/UCI Center for Health Education (CHE), and confirmation of support must be received by the CHE no later than April 1, 1996, to be listed in the course brouchure.

Tuition Fees

Tuition fees are as follows:

	Prior to 8/1/96	After 8/1/96
IPCC Member	$300.00	$400.00
Non-Member	$350.00	$450.00
Student/Post Doc	$200.00	$300.00
Single Day	$125.00	$125.00
Spouse/Guest	50% of Registrant Fee	

Tuition fees include refreshments, the Welcome Reception, Fashion Show, Banquet and Farewell Reception. Early registration is advised!

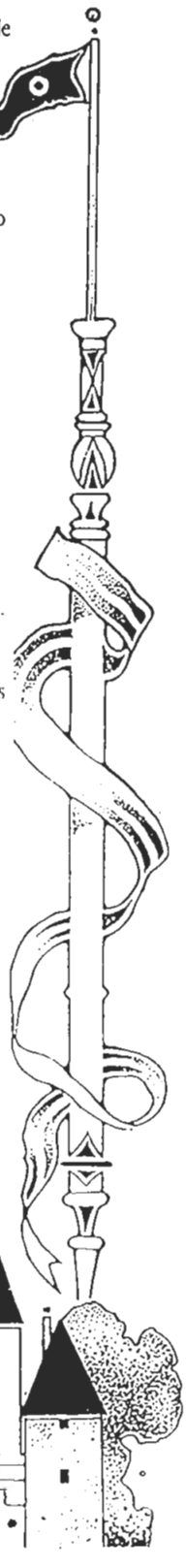
Accommodations

The Disneyland Hotel offers one of the most unique environments in the West. Here you will discover a myriad of pleasurable activities throughout 60 acres of meticulous grounds.

Dining - There are six superb restaurants including Granville's Steak House, seafood at the Shipyard Inn, Italian cuisine at Caffe Villa Verde, or a quick bite at the Monorail Cafe or Mazie's. You can even meet the real Disney Characters at Chef's Kitchen all-you-can-eat buffet.

Entertainment - As a guest at the Disneyland Hotel, course participants and their family members have the option of purchasing a "Length of Stay Passport" to Disneyland Park. This one ticket is valid from October 28-November 4, 1996 and can be used each and every day, for the same price you would pay for a one-day passport (approximately $40 per person). The Disneyland Hotel also offers their guests a variety of activites including country music at the Wharf Bar, a musical water show nightly, dancing at Neon Cactus, as well as lounges featuring soft piano music. In addition, the Hotel has 35 specialty shops, three swimming pools and a spa, tropical Papeete Beach, business support services and plenty of parking.

Room Rate - The special conference room rate for the XVIth International Pigment Cell Conference, will be $115.00 single/double occupancy, per night, $125.00 triple occupancy, or $135.00 quadruple occupancy. Children seventeen years and younger are accommodated free when staying in the same room with their parents. Cribs are complimentary. "Club Disneyland" accommodations (concierge floors) are offered at a special rate of $160.00 single occupancy or $175.00 double occupancy. All reservations must be received by the Disneyland Hotel no later than September 29, 1996.



Travel

Sundance Travel, Inc. can assist you with all your travel needs. Whether you are looking for the best airfare for your travel to the **XVIth International Pigment Cell Conference**, or arranging your ground transportation, Sundance can do it all. Special airfares have been negotiated - 5% discount off the lowest applicable airfare and 10% discount off any full coach fare (non-restricted) are available for most meetings. Registrants booking their air travel through Sundance Travel can also arrange for ground transfers and are eligible to receive discounts on shuttle services to and from any southern California airport. Call **Sundance Travel, Inc.** at their nationwide toll free number (800) 424-3434 or (714) 752-5456 and ask for the Group Desk, Extension 195. Be sure to identify yourself as attending the XVIth International Pigment Cell Conference. While still guaranteeing you the best airfares, your reservation through Sundance will help support our educational programs.

Additional Information

For additional information concerning this program please call the Memorial/UCI Center for Health Education at (310) 933-3811. The final course brochure will be mailed after January 15, 1996. To ensure receipt of the course brochure, please complete the form below and return by mail or FAX to:

Memorial/UCI Center for Health Education
2801 Atlantic Avenue (P.O. Box 1428)
Long Beach, California 90801-1428
FAX - (310) 933-2012

<u>Contact:</u> XVI INTERNATIONAL PIGMENT CELL CONFERENCE
MMC/UCI Centre for Health Education
P.O. Box 1428, Long Beach, California 90801-1428

Registration Application

XVIth International Pigment Cell Conference
October 29-November 3, 1996

Name _____

Degree _____ SS# _____

Mailing Address _____

City _____ State _____ Zip _____

Country _____

Telephone - Office ( ) _____

Fax ( ) _____

Home ( ) _____

Please assist us by completing the following questionnaire:

☐ Yes, I am planning to attend the IPCC Meeting in 1996 in the following category

☐ IPCC Member ☐ Student/Post Doc
☐ Non-Member ☐ Spouse/Guest

☐ No, I will be unable to attend the IPCC Meeting in 1996

As an attendee I will be:

☐ Coming to the meeting alone

☐ Bringing a total of _____ guests

☐ Will be staying at the Disneyland Hotel
I will need a total of _____ rooms

☐ Submitting a
☐ poster and/or ☐ abstract for presentation

☐ I am interested in conducting a Satellite Meeting on the following subject: _____

_____

My accompanying guest(s) would be interested in the following outside activities (please rate in order of preference, 1 being first choice):

___ Getty Museum and Rodeo Drive Excursion
___ Old Pasadena, Huntington Gardens & Library Excursion
___ Shopping at South Coast Plaza
___ Southern California Beach Excursion
___ Disneyland

Mail completed form to:

Memorial/UCI Center for Health Education
Long Beach Memorial Medical Center
2801 Atlantic Avenue (P.O. Box 1428)
Long Beach, CA 90801-1428

Information about the XVI[th] IPCC

1. **Satellite Conferences:** No satellite conference will be supported by the local organizing committee that are held within the time frame of the XVI[th] International Pigment Cell Conference, Tuesday, October 29, 1996; 6:00 p.m. to Sunday, November 3, 1996; 8:00 a.m. There are a wide number of venues possible to hold small or large satellite conferences either before or after the main pigment cell meeting. Our Memorial/UCI Educational Foundations will be happy to work with you in planning, for a small fee, and we request that we be notified of the intent of any satellite conference no later than June 1, 1995. If we are notified later than this dat, accommodations and planning availability cannot be guaranteed.

2. **Competitive Stipend for Travel Support:** The Organizing Committee will provide funds in a competitive manner for graduate students, post doctoral fellows and those within five years of formal academic appointment. The number of stipends will depend on the availability of funds and further information will become available during the second and subsequent informational mailings.

Frank L. MEYSKENS, Jr., M.D., F.A.C.P.
President, XVI[th] International Pigment Cell Conference
Clinical Cancer Center
University of California Irvine Medical Centre
101 The City Drive, R1. 81, Bldg. 23
Orange, California 92668

Tel: 714 456 5081
Fax: 714 456 5039

NEWS FROM THE ESPCR

Leiden, May 29, 1995.

Dear colleagues,

This year, our Society enters the second decade of its existence. For many of us the ESPCR has become a part of our scientific and even social life.

The advantage of the societies like ours is that, next to the international structure (our members are from more than 20 countries), it has a multidisciplinary character connected with one common denominator - pigment, in our situation. The multidisciplinary character makes it possible for us to learn from each others specialty.

The regular attendance of the ESPCR meetings has led to numerous scientific collaborations and personal friendships. New Officers and Council members will do their best to keep the friendly spirit of our society alive.

Personally, I have an impression that there has been some increase in passivity between the members during the last several years. This was also reflected by very low presence and activity of the members during the last General Assembly in Vienna. Our Society needs new members, new ideas, new undertakings. Please, bear this in mind when you are going to nominate new candidates for the ESPCR Council.

CALL FOR NOMINATIONS

As the term of several Council members expires this year, it is necessary to hold new elections According to ESPCR Constitution the members of the Council are elected for 4 years and are eligible for the following 4-years period. Nominations for Council members must be in writing and countersigned by at least 5 members.

The office term expires for the following Council members: Dr. F.J. Lejeune, Dr. R. MacKie, Dr. W. Westerhof. The following Council members are eligible for re-election, if nominated: Dr. J.F. Doré, Dr. P.A. Riley and Dr. P.G. Parsons.

I would like to ask you to send me your nominations with an accompanying statement of willingness to stand from the nominee. All nominations should be present in my office by August 15, 1995. Shortly after that a ballot form will be sent to you. The elections will be completed in September and their results will be made public during the ESPCR Meeting in Lausanne.

With kind regards,
Yours,

Dr. S. Pavel, ESPCR secretary

Leiden, May 29, 1995.

Dear ESPCR member,

After a certain delay caused by the transfer of our bank account to Germany we would like to ask you to pay your membership fee for 1995. Please note that it is essential to return the *Pro Forma Invoice* to the treasurer for his record purposes.

The ESPCR subscription is now DM 95,00 and for the Ph.D. students and medical residents DM 48,00. Those who pay the reduced fee must send a letter signed by their supervisor confirming their in-training status.

The members (and non-members) willing to give an extra financial donation to our Society, will become 1995 Patron Members and their names will be published in the ESPCR Bulletin.

Benefits of ESPCR membership include:

* Regular literature surveys and information exchange through ESPCR Bulletin issued free to members.
* Forty percent reduction in subscription to Pigment Cell Research.
* Forty percent reduction in subscription to Melanoma Research.
* Reduced registration fees for the annual ESPCR Meetings
* Membership of the International Federation of the Pigment Cell Societies (IFPCS).

With best wishes,
yours,

Dr. Stan Pavel, ESPCR Secretary

PRO FORMA INVOICE
ESPCR Annual Subscription 1995

Please, use your credit card, if possible by

☐ I am paying DM
☐ VISA ☐ American Express ☐ EuroCard/Masters/Access

Card number .... Expiry ..... / .....

☐ I have made arrangement for transfer of the net equivalent of DM ..... to the Account of the „European Society for Pigment Cell Research" No. 0494989, c/o Deutsche Bank, Bonn (Bank Sorting Code: 380 700 59), Postfach 1406, D-53113 Bonn, Germany (for cheques, other than EUROCHEQUES, please add DM 20.-).

☐ I enclose herewith an Eurocheque for the amount of DM .....

Signature..... Date..... PHONE:

Name (in block letters) ..... FAX:

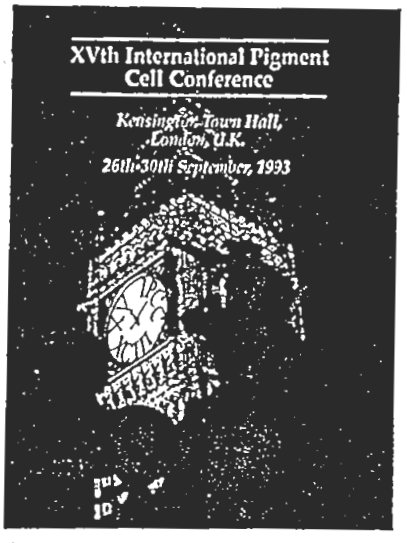
Please returtn this form to:
Prof. Dr. Martin G. Peter
Treasurer ESPCR
Universität Potsdam
Institut für Organische Chemie und Strukturanalytik
Am Neuen Palais 10
D - 14469 POTSDAM
FAX (+49-331) 977 1131

Sent by

At last!
The perfect gift for the pigment cell researcher who has everything. Bring a flush of excitement and a glow of pleasure to the lab.

Collected Papers from the Proceedings of the XVth IPCC

Hot off the press: the Collected Papers selected from the Proceedings of the XVth IPCC held at the Kensington Town Hall, London, September 26–30, 1993, are offered as a valuable limited edition. This is a unique collector's item that will grace any research library. The volume is available now (one size only) in an attractive phaeomelanic softback cover at the eminently affordable price of £36.00 (Sterling) (US $55.00) each. The price includes postage.



Rush now to buy your copy while stocks last!

Send your order to:
International Pigment Cell Conference (Publications)
Department of Molecular Pathology
UCL Medical School
46 Cleveland Street
London W1P 6DB, U.K.

☐ *Yes!* Please send me _____ copy(ies) of
Collected Papers from the Proceedings of the XVth IPCC

☐ I enclose a check for _____ made payable to *The International Pigment Cell Conference*

NAME _____

ADDRESS _____

_____

International Federation of Pigment Cell Societies DataBase

As one of the new initiatives of the IFPCS, a Committee has been established to collect data on resources available to the pigment cell community; that Committee includes a member from each constituent Society of the IFPCS and consists of V.J. Hearing (chair), P.A. Riley and T. Takeuchi. The Committee is establishing an interactive data base of available resources (from research and commercial sources), to be known as the "International DataBank of Pigment Cell Research Resources" (to be abbreviated **INTERPIG** on the InterNet). This DataBank will be available to all researchers, especially to members of the IFPCS. Listed in the DataBase will be all reagents, probes and other materials available and useful in research targeted towards pigment cells, including those for chemical, biochemical, immunological, molecular biology and other types of studies. The data collection form is attached (cf instructions below and the attached sample) and we encourage everyone to complete these forms and return them as soon as possible. Listing of resources will be free for nonprofit research laboratories; commercial suppliers must enclose a check for $25 USD for each item (made payable to the IFPCS). We will attempt to have the IFPCS **INTERPIG** DataBase available within the next year and IFPCS members will be advised as soon as it is ready; it will be accessible on the InterNet, or by request on computer diskette in a number of different database and word processing formats. Of course, the ultimate success of the IFPCS **INTERPIG** DataBase will depend on our members to complete the forms as thoroughly and clearly as possible and to return them in as timely a fashion as feasible. We thank you in advance for your help and cooperation and we feel that the **INTERPIG** DataBase will be a valuable resource for all of us within the near future.

Instructions for Completing the IFPCS 'INTERPIG' DataBase Entry Form:

* * * * * Please Type or Print Clearly * * * * *

The bottom part of the DataBase form contains information specific to each contributor. Please fill out the bottom part of the form first with your name and the other relevant information requested, as completely as possible, and then make as many photocopies of that completed form as necessary (a separate copy will be required for each item to be listed in the DataBase).

Complete a separate form for each distinct item to be entered into the IFPCS DataBase.

'Item' - enter the specific name of the reagent, antibody, probe, etc.

'Description' - enter a brief description of the material.

'Method' - list the most relevant method for the material - please choose from one of the following categories:

Biochemistry	Dermatology	Molecular Biology
Cell Biology	Electron Microscopy	Physics
Cell Culture	Immunology	
Chemistry	Melanoma	Other: (specify)

'Quantity' - indicate the quantity of the reagent supplied in a single request.

'Reference' - list the relevant citation for the reagent (if any).

'Comment' - list any further comment on the reagent.

Please return all forms to: Dr. Vincent J Hearing, Laboratory of Cell Biology, Bldg 37 Rm 1B25, National Institutes of Health, Bethesda, MD 20892 USA or FAX to: 1-301-402-8787

INTERPIG DataBase

Please Type or Print Clearly

Item:

Description:

Method: Quantity:

Reference:

Comment:

Full Name:

Institution:

Department:

Building/Room:

Street Address:

City: State/Prov:

Postal Code: Country:

Membership:

ESPCR:

JSPCR:

PASPCR:

Nonmember:

Work Phone: Fax Number:

Email Address:

Nonprofit: Commercial:

If Commerical, please include US $25 fee for each item

Please Return All Forms to :

Dr. Vincent J. Hearing
Laboratory of Cell Biology
Building 37 Room 1B25
National Institutes of Health
Bethesda, MD 20892 U.S.A.
FAX: (301) 402-8787

SURVEY OF CURRENT PIGMENT CELL RESEARCH IN EUROPE
1994-1995

1. FIELD OF STUDY :

2. TITLE / DESCRIPTION :

3. INVESTIGATOR(S) :

4. INSTITUTION :

5. ADDRESS :

6. TELEPHONE :

7. FAX :

8. COMMENTS :

9. Key Words :

Please
Send back to :

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