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Announcements and related activities
In Memoriam
Giuseppe PROTA (1938-2003)

Giuseppe Prota was an important influence in mobilising and inspiring scientific advance in the area of pigmentation. His energetic espousal of new areas of research and his organizational abilities made him a key figure in 20th century pigment cell research.

Giuseppe Prota was born in Naples on 24th January 1938 and grew up in war-torn Italy and the difficult post-war years. His early education gave no hint of his later scientific eminence and he completed his Liceo specialising in classical studies. He entered the University of Naples where, under the inspiring tutelage of his eventual mentor, Rodolfo Nicolaus, he took up studies in science and graduated in 1962 with a degree in Chemistry. He must have shown great promise, because four years later he was appointed lecturer in the Department of Organic and Biological Chemistry at the University of Naples and by 1969 had obtained his 'libera docenza' in the Chemistry of Natural Products. In 1972 he was made an Associate Professor and he was appointed Professor of Natural Products in 1975. During this time he was deeply involved in the research that led to the elucidation of the structure of the phaeomelanins and trichochromes and rapidly gained international recognition. His demonstration of a pathway of thiol incorporation into benzothiazine precursors of phaeomelanin and his work on the biogenesis of phaeomelanins was quickly appreciated as highly significant and officially recognised by the Myron Gordon Award presented to him at the IPCC in 1977.

This was a busy and highly productive time during which he divided his attention between teaching and research at the University, and extensive collaborations with groups at the Zoological Station in Naples. This included work on melanogenesis in the ink sac of Sepia officinalis with, among others, his devoted wife, Giovanna Misuraca, and Anna Palumbo.

Most of his work was conducted in Naples but Giuseppe Prota spent some time as a post-doctoral student in the Chemistry Department at Manchester University and brief collaborative sojourns in France and Sweden. In later years he travelled extensively to other laboratories in Europe, Japan and the USA and also acted as host in his Department to many prominent pigment cell scientists.

He was the recipient of many awards and prizes including the Myron Gordon Award (1977), the Seiji Lectureship (1986), the Raper Medal (1996) and the WHO Melanoma Award (2001). He was elected an honorary member of the ESPCR, the Italian Dermatological Society, and the Italian Society of Chemistry and Cosmetic Sciences. His wisdom and expertise were widely in demand and he was for many years an advisor to the Italian Ministry of Education. He was on the Advisory Boards of several national and international bodies including the Board of Directors of the International Marine Research Institute 'A. Dohrn' and was appointed to the Interdisciplinary Committee of the World Cultural Council.

Giuseppe Prota was very largely responsible for the establishment of the European Society for Pigment Cell Research. The political background to this and the events leading to the setting up of the ESPCR have previously been alluded to, and a definitive version of events is best left in the hands of the Society's resident historian, Jan Borovansky. Suffice it to say here that, without the enthusiasm and determination of Guiseppe Prota, the ESPCR, and the subsequent establishment of the other regional...
Giuseppe Prota's research interests spanned a wide field and his quick intellect and natural inquisitiveness led him to explore many areas. Many of his special contributions to this area of science are described in his biographical review published in Pigment Cell Research, 2000; 13: 283) and the scope of his work embodied in his book 'Melanins and Melanogenesis' (Academic Press: San Diego).

One of his initial goals was to understand the process of polymerization considered to be a central element of melanogenesis. The mechanism of addition of DHICA residues had been examined by several leading organic chemists, such as Longuet-Higgins, Cromartie, Harley-Mason, de Beer, and others, but no satisfactory resolution to the problem had been found. Prota was able to isolate and characterize some dimers and trimers considered typical of the oligomeric precursors of melanin under conditions which suggested a free radical polymerization reaction, although proof of this mechanism remains to be demonstrated. His important involvement in the elucidation of the phaeomelanotic pathway is, of course, well known and he was largely responsible for the idea of a balance between the eu- and phaeo-melanogenic processes - an idea for which he coined the fashionable term 'intermeshing'. It is a measure of his broad insight that this balance is universally recognised as the natural outcome of many regulatory factors acting at differing points in the biogenesis of pigment.

Giuseppe Prota showed an interest in the biophysics of melanin and, with Raymondo Crippa, organised a meeting in Parma devoted to what, at the time, were esoteric aspects of pigmentation. The possibility that melanin might act as an efficient acoustic buffer, which was one of the findings emphasized at this meeting, led to a natural extension of Giuseppe's ambit to extracutaneous melanin. He was also interested at an early stage of the research in the possibility of employing the melanogenic pathway as a targeting strategy for chemotherapy of melanoma and, with his collaborators, synthesized a number of potentially cytotoxic pro-drugs with melanocyte specificity. His enthusiasm for using melanoma as a general model of cancer biology was partly instrumental in the launching of the journal Melanoma Research.

The enduring puzzle of the role of neuromelanin also attracted Giuseppe's attention, especially the apparent link with the development of Parkinson's disease that were highlighted by the drug-induced models of extrastriatal degeneration. There were intriguing suggestions of the involvement of transition metals, such as iron and nickel, in the induction of neurodegeneration in the pigmented cells of the substantia nigra and Giuseppe was much drawn to the notion that metals play an important part in pigmentation. Also there was much evidence that neuromelanin comprised a special class of phaeomelanins, probably including glutathionyl residues in their structure. Since this implied possible depletion of GSH in pigmented neurones this seemed to suggest a way in which such cells might be rendered vulnerable to oxidative stress. The time seemed ripe to explore the possibilities and Giuseppe set about organizing an International Colloquium on Neuromelanin and Parkinson's Disease. This event was held in Sorrento in 1993 and proved not only an enjoyable occasion but also scientifically highly productive.

Always quick to see novel possibilities, Giuseppe was also briefly involved with the notion of cytoprotection by melanin precursor molecules with potential anti-oxidant properties. But photoprotection by melanin, rather than the monomers that comprise the pigment, remained his main biological preoccupation. He was for many years a technical consultant to a number of Cosmetic...
companies and in recent years he devoted much energy to developing quantitative measures of photoprotection of individuals based on sensitive new techniques applied to hair samples.

Although he kept up his effort and scientific input to the end, he was very deeply affected by the death of Giovanna. She had been his intellectual partner as well as the calm sustainer of his happiness and optimism; life was never the same again.

Of course, we recognise Giuseppe Prota as a major contributor to our current understanding of melanins and melanogenesis and as a much-honoured and respected scientist of international stature. He was also a complex mixture of optimistic assertiveness and exceptional sensitivity and kindliness. Whilst, as Marco d'Ischia has eloquently put it in his obituary (published in Pigment Cell Research), Giuseppe Prota "suffered rules badly", we shall not remember him as a rebel, but rather as an energetic, imaginative and generous free spirit. His enduring legacy is a better and wiser world.

Patrick Riley
1. Biology of pigment cells and pigmented disorders

(Dr. M. Picardo)

New fragments of melanocyte puzzle
The intracellular pathway leading to melanine synthesis is under constant revision with the aim to better understand the physiological and pathological mechanisms of the melanocyte cell biology. A consistent number of researchers studied the factors influencing the level and activity of the transcription factors such as Mitf, Sox-10, Pax3. The role of the intracellular check-point involved in the melanogenesis was investigated by CB Lin. He focused the attention to Mitf and confirms the ability, upon the induction by several agents (UVB, Lipoic Acid, Resveratrol), of this transcription factor to regulate the melanogenesis by mean of an association with the tyrosinase, TRP1/2 proteins promoter M-box. These studies were performed using a luciferase reporter construct and western blot analysis. On the basis of the results obtained, the author shows that UVB can induce Mitf promoter activity in a time- and concentration-dependent manner, and that the inhibition of the tyrosinase promoter by the antioxidants (Dihydrolipoic Acid, Lipoic Acid, Resveratrol) is due to a cAMP relocation-mediated down regulation of Mitf gene expression, without change in the cAMP total level. S Prince evaluated the relation between TPA (tetradecanoyl 13-cetate) administration, Mitf/Tyr/Trp1 expression and melanogenesis. Mitf appears to be expressed, even if at very low level, as well in non-melanogenic cells. Consequently, other intrinsic factors must be involved in the melanogenic specific induction of Mitf. RT-PCR and western blot analysis here show that TPA mimics (acting as DAG) endothelin pathway through PKC and MAPK activation. TPA up regulates both mRNA and protein level of Mitf, Tyr and Trp1. Probably, the up regulation of Mitf gene in melanogenic cells by TPA is dependent on a preparatory steps of Pax3/Sox10/Lef1 expression. Significantly, Pax3 is not induced by TPA but its expression is required to the Mitf induction by TPA. KU Schallreuter investigates (methods of RT-PCR, immunofluorescence, HPLC, enzyme activity assay) the distribution and the function of the tyrosine hydroxylase enzyme. She found in epidermal melanocytes only the isoform 1 (hTH1)(the four isoenzyme were obtained by alternative splicing starting from one gene). Moreover, the hTH1 was detected in melanosomes strictly associated with tyrosinase, indicating a relevant role in melanogenesis (TH provides DOPA, substrate for Tyr). Among the drugs interfering with pigmentation, HY Kang studied the intracellular pathway involved in the lithium-induced hyper-pigmentation. This study can open a way from bipolar disorder therapy to pigmentation defect? The data (based on melanoma and melanocyte cell lines melanin content, tyrosinase activity and western blot assay) are suggestive of an induction of tyrosinase activity through the increased level of IP3. However, further studies must be performed before of an effectual use of the LiCl in the treatment of the pigmented disorders. K Hiramoto studied the pathway of the skin pigmentation induced by eye UVB irradiation, suggesting a nitric oxide-dependent induction of the hypothalamopituitary system with a subsequent secretion of -MSH.

The role and the extent of the apoptotic process is another area of interest in melanocyte biology. The pattern of pro/anti-apoptotic proteins expressed by melanocytes, keratinocytes and melanoma cells were analyzed by AR Bowen. The cells, treated with UVB, cisplatin, etoposide, staurosporine, or 4-tert-butylphenol, show a different apoptosis threshold of sensitivity to the different agents. Moreover, the pattern of factors affecting the executive phase of apoptosis appears to be different in the different cell types suggesting a biological basis for a lower sensitivity of melanoma cells to apoptosis. Thus, the low level of apoptotic inhibitors in keratinocytes could allow the rapid cell turnover and the elimination of damaged cells. However, it is not evident a reduced susceptibility to apoptosis correlated with the progression from melanocyte to melanoma, but rather the melanoma apoptosis resistance could reflect a characteristic of the precursor melanocyte.

Hypo-pigmentation and....
The pigmented disorders were evaluated by genetic, molecular and cell biology approach. Q Zhang presented an animal model for Hermansky-Pudlak syndrome (HPS) characterized by two mutated genes (ruby-eye 2 and ruby-eye) orthologs of the human genes mutated in HPS types 5 and 6. The definition of the molecular pathways underlying pigmentation and involving transcription factors, intracellular messengers, is relevant also in disease such as vitiligo. A relevant contribute to the knowledge of the mechanism of action of a common therapy (PUVA) for vitiligo was offered by T Chi Lei. The pathogenesis of this disease is still unclear and also the molecular basis of the common therapeutic approaches must be still defined. Several different pathways could be involved in the 8MOP-mediated repigmentation: direct stimulation of melanosomal tyrosinase, Mitf induction, protein kinase A and/or C (PKA, PKC) activation, proteasome-mediated tyrosinase degradation. RT-PCR, western blot and tyrosinase radiometric activity assay indicated that (1)8MOP induced pigmentation through PKA pathway (the specific PKC activator fails to increase tyrosinase activity) without significantly affecting the proliferative rate, (2) 8MOP increased the tyrosinase synthesis, (3)Mitf appears to be implicated in the tyrosinase synthesis mediated by 8MOP.
P Wentzel studied the answer of the iridial melanocytes to endogenous and exogenous prostaglandins. The observed hyperpigmentation could be the consequence of an activation of the prostaglandin FP receptors and of the cyclooxygenase-2 enzyme gene.

C Stoll reported a case of hyperpigmentation associated with a normal karyotype (peripheral blood) and a mosaicism 12q;14q translocation (fibroblasts). Another aspect recently considered is the role of the mitochondria in the pathogenesis of several syndromes associated with pigmentary pathologies. At this regard, V Bioussse presents an intriguing review about the mitochondrial disorders and the neuro-ophtalmic abnormalities with pigment defect. Even if the definitive mechanism underlying the disappearance of melanocyte in course of vitiligo has been to clarified, an autoimmune destruction could be in any case implicated. A complete and remarkable review was proposed by K Ongenae who, still giving open the possibility of distinct primary defect, focused on the role of cellular and humoral immunity with an evaluation also of the new identified antigens (ie,Sox10 and MCHR1). A further clinical proof of the immune system involvement in vitiligo was supplied by the EA Tanghetti study of the effectiveness of the topical tacrolimus application. In agreement with the indirect demonstration of the PGE2-induced pigmentation (see above), D Parsad suggests a possible employ of a topical PGE2 analog in vitiligo. Briefly, the author reports the promising results of a clinical trial, at the basis of which there is the know immunomo-dulating and proliferative action of the PGE2 on Langerhans cells, monocytes and melanocytes. The association of the vitiligo with some autoimmune diseases (Hashimoto's thyroiditis and insulin-dependent diabetes mellitus), where an increased level of some inflammatory factors was reported, supports the potential role of the cytokines in the vit. At this regard, CX Tu evaluated the sera level of IL-1?, IL-6, TNF-?, IL-8, and GM-CSF correlating it to the disease phase. Even if the standard deviations were too large, the levels of the different cytokines analyzed indicate an increase stage-dependent of the IL-1?, IL-6, and GM-CSF without significant change of the other. Several practical problems conditioned the vitiligo skin biopsies availability. Thus, the LF Montes work about the cytokines analyzed indicate an increase stage-dependent of the IL-1?, IL-6, and GM-CSF correlating it to the disease phase. Even if the standard deviations were too large, the levels of the different cytokines analyzed indicate an increase stage-dependent of the IL-1?, IL-6, and GM-CSF without significant change of the other. Several practical problems conditioned the vitiligo skin biopsies availability. Thus, the LF Montes work about the histological characteristics of the vitiligo lesional/non lesional skin raises particular relevance. Briefly, the microscopic analysis (light and electronic microscopy) shows an inflammatory alteration at the borders of the new lesions. Another ultrastructural study is presented by AL Panuncio which, analyzing long-standing stable segmental vitiligo lesions, evidenced alterations of keratinocytes, melanocytes, and of Langerhans cells and the presence of infiltrating lymphocytes. All these data led us to reconsider the definition of "stable" vitiligo. A further study regarding the loss of melanocyte in course of vitiligo was carry out by Y Gauthier by means of electron microscopy and immunohystochemistry. The Koebner phenomenon appears to be the manifestation of a detachment of melanocytes probably previously affected by several injuries. As regard to other side, the hyper-pigmentation, a broad assortment of drugs inducing hypo-pigmentation was introduced by the cosmetic business. Among these, the hydrocoumarins derivatives have been considered by T Yamamura, which, by means of measure of melanogenic activity and GSH level, indicates that these compounds act as well as ?-tocopherol by inducing GSH synthesis (up regulation of ?-glutamylcysteine synthetase) and by acting as free radicals scavenger. Moreover, he found a correlation between antimelanogenic activity of hydrocoumarins and their substituent groups. An animal model provides an interesting model of the mechanism of action of chemotherapy-induced hair loss and altered pigmentation. Indeed, AA Sharov shows that cyclophosphamide (CYP) affected hair follicle melanocytes acting on Fas pathway and, consequently, a pharmacological manipulation of Fas-induced apoptosis and c-kit signaling could be useful in the hair loss associated with CYP therapy.


- **Lee DY, Lee JH, Lee ES, Cho KH, Yang JM.** Fibroblasts play a stimulatory role in keratinocyte proliferation but an inhibitory role in melanocyte growth and pigmentation in a skin equivalent system from skin type IV. Arch Dermatol Res 294: 444-6, 2003.


Yamamura T, Onishi J, Nishiyama T. 

Wentzel P, Bergh K, Wallin O, Niemela P, Stjernschantz J. 

Zetting G, Tanew A, Fischer G, Mayr W, Dudczak R, Weissel M. 


### 2. MSH, MCH, other hormones, differentiation

(Dr. R. Morandini)

**Regulation and signal transduction**

Bellemere G, Morain P, Vaudry H, Jegou S. 

Moustafa M, Szabo M, Ghanem GE, Morandini R, Kemp EH, MacNeil S, Haycock JW. 

**Global effect on cell in vitro**

Cornish J, Callon KE, Mountjoy KG, Bava U, Lin JM, Myers DE, Naot D, Reid IR. 
**[alpha]-melanocyte stimulating hormone ([alpha]-msh) is a novel regulator of bone.** Am J Physiol Endocrinol Metab. 2003 Mar 4.

Namba K, Kitaichi N, Nishida T, Taylor AW. 


Scholzen TE, Sunderkotter C, Kalden DH, Brzoska T, Fastrich M, Fisbeck T, Armstrong CA, Ansel JC, Luger TA. 
**Alpha-melanocyte stimulating hormone prevents lipopolysaccharide-induced vasculitis by down-regulating endothelial cell adhesion molecule expression.** Endocrinology.144(1):360-70, 2003.


**Clinical investigations**

Fetissov SO, Hallman J, Oreland L, Af Klinteberg B, Grenback E, Hulting AL, Hokfelt T. 


Others - Not classified


3. Photobiology
(Dr. E. Wenczl)


4. Neuromelanins
(Prof. M. d'Ischia)

Three papers and a patent that appeared in the late 2002-early 2003 centred on the role of neuromelanin in the etiopathology of Parkinson’s disease and as a possible target for innovative therapeutic interventions. Wilms et al. (2003) investigated the effects of human neuromelanin (NM) on the release of neurotoxic mediators and the underlying signaling pathways from rat microglia in vitro, to gain an insight into the nature of endogenous factors initiating microglial activation during progression of Parkinson's disease (PD). The results indicated activation of nuclear factor B (NF- B) via phosphorylation and degradation of the inhibitor protein B (I B), and an up-regulation of tumor necrosis factor alpha, interleukin-6, and nitric oxide, supporting a crucial role of NM in the pathogenesis of PD by augmentation of microglial activation.

Fasano et al. (2002) reported data suggesting that -synuclein, the main component of Lewy bodies and dystrophic neurites in Parkinson's disease and Lewy body dementia, accumulates within substantia nigra neurons and is entrapped in pigment granules during neuromelanin biosynthesis, i.e. before melanin depletion.

Using synchrotron radiation (SR) micro beam, Yoshida et al. (2003) investigated the distribution and chemical states of Fe within a single neuron of two disease cases, Parkinson's disease (PD) and parkinsonism-dementia complex (PDC). The results added to the massive body of evidence suggesting a crucial role of changes in distribution and chemical states of Fe in the oxidative damage of the melanized neurons in PD. Finally, a patent by Nelson (2002) described the potential of neuromelanin-binding compounds having a quinoline ring, e.g. (-)-chloroquine, in a suitable pharmaceutical carrier as a means for increasing cellular respiration of melanized catecholamine neurons and for alleviating symptoms of Parkinson's disease.
Coat color mutations: Several genes underlying mutations affecting coat color have been identified recently. Grey lethal (gl) is responsible for a coat color defect and for development of osteopetrosis, and encodes for a type I transmembrane protein (Chalhoub et al., 2003). Ruby eye (ru) and ruby-eye 2 (ru2) encode for genes involved in synthesis of specialized lysosome-like organelles. Both genes are mutated in human in Hermansky-Pudlak syndrome (HPS types 5 and 6) (Zhang et al., 2003). Buff (bf) results from a mutation in Vps33, which is a gene homologous to the yeast vacuolar sorting mutant vps33 and Drosophila carnation. Buff is a candidate gene for Hermansky-Pudlak syndrome in human (Suzuki et al., 2003).


Shortened abstract: To study the role of beta-catenin in neural crest development, we used the Cre/loxP system to ablate beta-catenin specifically in neural crest stem cells. Although several neural crest-derived structures develop normally, mutant animals lack melanocytes and dorsal root ganglia (DRG). We propose that the requirement of beta-catenin for the specification of melanocytes and sensory neuronal lineages reflects roles of beta-catenin both in Wnt signaling and in mediating cell-cell interactions.


Abstract: In the mouse, more than 16 loci are associated with mutant phenotypes that include defective pigmentation, aberrant targeting of lysosomal enzymes, prolonged bleeding, and immunodeficiency, the result of defective biogenesis of cytoplasmic organelles: melanosomes, lysosomes, and various storage granules. Many of these mouse mutants are homologous to the human Hermansky-Pudlak syndrome (HPS), Chediak-Higashi syndrome, and Griscelli syndrome. We have mapped and positionally cloned one of these mouse loci, buff (bf), which has a mutant phenotype similar to that of human HPS. Mouse bf results from a mutation in Vps33a and thus is homologous to the yeast vacuolar protein-sorting mutant vps33 and Drosophila carnation (car). This is the first found defect of the class C vacuole/prevacuole-associated target soluble N-ethylmaleimide-sensitive factor attachment protein receptor (t-SNARE) complex in mammals and the first mammalian mutant found that is directly homologous to a vps mutation of yeast. VPS33A thus is a good candidate gene for a previously uncharacterized form of human HPS.


Abstract: Hermansky-Pudlak syndrome (HPS) is a genetically heterogeneous disease involving abnormalities of melanosomes, platelet dense granules and lysosomes. Here we have used positional candidate and transgenic rescue approaches to identify the genes mutated in ruby-eye 2 and ruby-eye mice (ru2 and ru, respectively), two 'mimic' mouse models of HPS. We also show that these genes are orthologs of the genes mutated in individuals with HPS types 5 and 6, respectively, and that their protein products directly interact. Both genes are previously unknown and are found only in higher eukaryotes, and together represent a new class of genes that have evolved in higher organisms to govern the synthesis of highly specialized lysosome-related organelles.

6. Tyrosinase, TRPs, other enzymes
(Prof. J.C. Garcia-Borron)

Comments
Two papers by P.E. Siegbahn (Mechanisms of metalloenzymes studied by quantum chemical methods, Q Rev Biophys 2003 Feb;36(1):91-145 and The catalytic cycle of tyrosinase: peroxide attack on the phenolate ring followed by O-O bond cleavage, J Biol Inorg Chem 2003 Mar 13; [epub ahead of print]) report theoretical studies on the tyrosinase mechanism of action. One of them proposes a new catalytic cycle for the phenolase activity, where tyrosine is bound to the enzyme and converted into dopaquinone without the formation of an o-diphenol intermediate. The application of theoretical quantum chemical methods and thermodynamic concepts to the study of enzymatic mechanisms of action is obviously promising, but these studies must take into account, and be consistent with, the available experimental evidence. The reaction cycle proposed by Siegbahn does not account for the well-known occurrence of a lag period in the phenolase activity, that can be shortened or abrogated by catalytic concentrations of a diphenol. Therefore, this cycle is necessarily incomplete and must be considered with caution, for the moment.

The first two issues of Pigment Cell Research volume 16 contain a wealth of interesting and relevant information. In issue number 1, Alaluf et al. (Alaluf S, Barrett K, Blount M, Carter N Ethnic Variation in Tyrosinase and TYR1 Expression in Photorexposed and Photoprotected Human Skin, Pigment Cell Res 2003 Feb;16(1):35-42) report on ethnic variations in melanogenic enzymes, and point to an important role of TYR1 in mediating ethnic differences. Previous studies have also established an association of TYR1 with eumelanogenesis as opposed to pheomelanogenesis. These aspects of TYR1 function will be better understood once the catalytic activity of the human protein is definitely established. In this same issue, Prince et al. report on the effects of TPA in melanocytes and neural crest cells (Prince S, Wiggins T, Hulley PA, Kidson SH Stimulation of Melanogenesis by Tetradecanoylphorbol 13-acetate (TPA) in Mouse Melanocytes and Neural Crest Cells Pigment Cell Res 2003 Feb;16(1):26-34). The second issue of PCR volume 16 also contains several very interesting papers, including a review by Briganti, Camera and Picardo on depigmenting approaches, and papers from Lionel Larue's and Seth Orlow's laboratories, among others. The paper by Seth Orlow and co-workers (Hall AM, Krishnamoorthy L, Orlow SJ Accumulation of Tyrosinase in the Endolysosomal Compartment is Induced by U18666A Pigment Cell Res 2003 Apr;16(2):149-58) identifies several steroid hormones as agents interfering with tyrosinase...
processing to the melanosomes. These compounds may become useful tools to study the intracellular traffic of melanogenic proteins, a very complex pathway with important pathological implications.

A very interesting work by Stevenson et al. (Stevenson TC, Ciccotosto GD, Ma XM, Mueller GP, Mains RE, Eipper BA. Menkes protein contributes to the function of peptidylglycine alpha-amidating monoxygenase Endocrinology 2003 Jan;144(1):188-200) provides evidence of several significant similarities between to apparently unrelated proteins, namely peptidylglycine alpha-amidating monoxygenase (PAM) and tyrosinase. Both are copper containing enzymes, with complex intracellular sorting processes related to the secretory pathway. Interestingly, the Menkes protein seems important for the function of both enzymes, an observation very probably related to copper delivery in the trans-Golgi network. The delivery of copper to tyrosinase after the exit of the enzyme from the endoplasmic reticulum is also strongly suggested by recent work from our laboratory (Olivares C, Solano F, Garcia-Borron JC. Conformation-dependent post-translational glycosylation of tyrosinase. Requirement of a specific interaction involving the CuB metal binding site. J Biol Chem 2003 Feb 20; [epub ahead of print]).

Two papers published side by side in the same issue of J. Invest. Dermatol. provide new and relevant information on the role of microphthalmia-associated transcription factor as a key master gene governing the biology of melanocytes. Here, I will only recommend strongly to have a look at them and will not go on further comments, since the issue of JID already contains a specific comment on both papers.

- Haghbeen K, Wue Tan E. Direct spectrophotometric assay of monoxygenase and oxidase activities of mushroom tyrosinase in the presence of synthetic and natural substrates. Anal Biochem 312(1):23-32, 2003. Alternative substrates were synthesized to allow direct and continuous spectrophotometric assay of both monoxygenase (cresolase) and oxidase (catecholase) activities of mushroom tyrosinase (MT). Using diazo derivatives of phenol, 4-[(4-methoxybenzo)azo]-phenol, 4-[(4-methylphenyl)azo]-phenol, 4-(phenylazo)-phenol, and 4-[(4-hydroxyphenyl)azo]-benzenesulfonamide, and diazo derivatives of catechol 4-[(4-methylbenzo)azo]-1,2-benzenedioli, 4-(phenylazo)-1,2-benzenedioli, and 4-[(4-sulfonamido)azo]-1,2 benzenedioli (SACat), as substrates allows measurement of the rates of the corresponding enzymatic reactions through recording of the depletion rates of substrates at their lambda(max)(s) with the least interference of the intermediates' or products' absorption. Parallel attempts using natural compounds, p-coumaric acid and caffeic acid, as substrates for assaying both activities of MT were comparable approaches. Based on the ensuing data, the electronic effect of the substituent on the substrate activity and the affinity of the enzyme for the substrate are reviewed. Kinetic parameters extracted from the corresponding Lineweaver-Burk plots and advantages of these substrates over the previously used substrates in similar assays of tyrosinases are also presented.

- Alaluf S, Barrett K, Blount M, Carter N. Ethnic Variation in Tyrosinase and TYRP1 Expression in Photoexposed and Photoprotected Human Skin. Pigment Cell Res 16(1):35-42, 2003. The relative expression of a number of key mediators of human pigmentation including tyrosinase, tyrosinase related protein-1 (TYRP1), endothelin-1 and adrenocorticotropic hormone (ACTH) proteins were analysed and quantified in immunohistochemically stained skin sections using semiquantitative computer assisted image analysis. Comparisons were made between a range of different ethnic skin types including European, Chinese, Mexican, Indian and African at both chronically photoexposed and photoprotected sites. Melanocyte number varied little with ethnicity except in the European group which had 60-80% more melanocytes than other skin types (P < 0.01, n = 10; Student Neuman-Keuls). However, melanocyte number was increased approximately twofold in chronically photoexposed skin of all ethnic groups (P < 0.001, n = 48; paired t-test). Tyrosinase protein expression in melanocytes did not vary with ethnicity, but TYRP1 protein was significantly elevated (approximately 2.6-fold) in darkly pigmented African and Indian skin types compared with lightly pigmented Mexican, Chinese and European skin types. In melanocytes from chronically photoexposed skin, there was a modest but significant increase in the expression of tyrosinase protein (approximately 1.2-fold, P < 0.001, n = 48; paired t-test), together with a significant and slightly larger increase in the expression of TYRP1 protein (approximately 1.6-fold, P < 0.005, n = 48; paired t-test). In contrast, the expression of endothelin-1 and ACTH showed no significant variation with either ethnicity or photoexposure. These data are consistent with the view that maintenance of a chronically hyperpigmented phenotype in chronically photoexposed human skin is largely the result of a stable increase in the number of tyrosinase positive melanocytes at these sites. Moreover, the observed ethnic variation in TYRP1 protein expression suggests that TYRP1 may play a significant role in mediating ethnic differences in melanogenesis and constitutive skin pigmentation in vivo.

- Battaini G, Monzani E, Casella L, Lonardi E, Tepper AW, Canters GW, Bubacco L. Tyrosinase-catalyzed oxidation of fluorophenols. J Biol Chem 277(47):44606-12, 2002. The activity of the type 3 copper enzyme tyrosinase toward 2-, 3-, and 4-fluorophenol was studied by kinetic methods and (1)H and (19)F NMR spectroscopy. Whereas 3- and 4-fluorophenol react with tyrosinase to give products that undergo a rapid polymerization process, 2-fluorophenol is not reactive and actually acts as a competitive inhibitor in the enzymatic oxidation of 3,4-dihydroxyphenylalanine (L-dopa). The tyrosinase-mediated polymerisation of 3- and 4-fluorophenols has been studied in detail. It proceeds through a phenolic coupling pathway in which the common reactive fluoroquinone, produced stereospecifically by tyrosinase,
eliminates an inorganic fluoride ion. The enzymatic reaction studied as a function of substrate concentration shows a prominent lag that is completely depleted in the presence of L-dopa. The kinetic parameters of the reactions can be correlated to the electronic and steric effects of the fluoride substituent position. Whereas the fluoride electron withdrawing effect appears to control the binding of the substrates (K(m) for 3-and 4-fluorophenols and K(I) for 2-fluorophenol), the k(cat) parameters do not follow the expected trend, indicating that in the transition state some additional steric effect rules the reactivity.

- **Biet E, Alberti C, Faccella P, Sun JS, Dutreix M, Larue L.**


Gene therapy and production of mutated cell lines or animal models should be improved significantly once efficient controlled gene targeting strategies are developed. We used short single-stranded oligodeoxynucleotides (ODN), in some cases coupled to the fluorescent dye fluorescein isothiocyanate (FITC), to correct an endogenic natural point mutation in melanocytes in culture. The addition of the FITC molecule to the 5′ extremity of the ODN did not interfere with the efficiency of the reversion of the mutation and did not have any deleterious side-effects. The use of fluorescent ODN could lead to great improvement in the technique. In particular, it may facilitate sorting of the transfected cells in the treated population, and thereby significantly increase the percentage of corrected cells.

- **Briganti S, Camera E, Picardo M.**

**Chemical and instrumental approaches to treat hyperpigmentation.** Pigment Cell 16(2):101-10, 2003.

Many modalities of treatment for acquired skin hyperpigmentation are available including chemical agents or physical therapies, but none are completely satisfactory. Depigmenting compounds should act selectively on hyperactivated melanocytes, without short- or long-term side-effects, and induce a permanent removal of undesired pigment. Since 1961 hydroquinone, a tyrosinase inhibitor, has been introduced and its therapeutic efficacy demonstrated, and other whitening agents specifically acting on tyrosinase by different mechanisms have been proposed. Compounds with depigmenting activity are now numerous and the classification of molecules, based on their mechanism of action, has become difficult. Systematic studies to assess both the efficacy and the safety of such molecules are necessary. Moreover, the evidence that bleaching compounds are fairly ineffective on dermal accumulation of melanin has prompted investigations on the effectiveness of physical therapies, such as lasers. This review which describes the different approaches to obtain depigmentation, suggests a classification of whitening molecules on the basis of the mechanism by which they interfere with melanogenesis, and confirms the necessity to apply standardized protocols to evaluate depigmenting treatments.

- **Bubacco L, Van Gastel M, Groenen EJ, Vijgenboom E, Canters GW.**

**Spectroscopic characterization of the electronic changes in the active site of Streptomyces antibioticus tyrosinase upon binding of transition state analogue inhibitors.** J Biol Chem 278(9):7381-9, 2003.

The dinuclear copper enzyme tyrosinase (Ty) from genetically engineered Streptomyces antibioticus has been investigated in its paramagnetic half-met form [Cu(I)-Cu(II)]. The cw EPR, pulsed EPR, and hyperfine sublevel correlation spectroscopy (HYSCORE) experiments on the half-met-Ty and on its complexes with three different types of competitive inhibitor are reported. The first type includes p-nitrophenol, a very poor substrate for the monooxygenase activity of Ty. The second type comprises hydroxyquinones, such as kojic acid and l-mimosine, and the third type of inhibitor is represented by toluic acid. The electronic and structural differences of the half-met-Ty form induced at the cupric site by the different inhibitors have been determined. Probes of structural effects are the hyperfine coupling constants of the non coordinating Ndelta histidyl nitrogens. By using the available crystal structures of hemocyanin as a template in combination with the spectroscopic results, a structural model for the active site of half-met-Ty is obtained and a model for the binding modes of both mono- and diphenols could be proposed.

- **Hall AM, Krishnamoorthy L, Orlow SJ.**

**Accumulation of Tyrosinase in the Endolysosomal Compartment is Induced by U18666A.** Pigment Cell Res 16(2):149-58, 2003.

The 3beta-(2-diethylaminoethoxy)-androstenedione HCl (U18666A), progesterone and several cationic amphiphilic drugs have been shown to alter the trafficking of a number of intracellular membrane proteins including CD63/Lamp-3, insulin growth factor 2/mannose 6-phosphate receptor (IGF2/MPR), and the Niemann-Pick C1 gene product (NPC1) as well as ganglioside GM1. We have examined the effects of these compounds on cultured melanocytes at concentrations that have been shown to effectively alter intracellular trafficking. Treatment of melanocytes with U18666A (2.5 micro M) or progesterone (15 micro M) for 96 h decreased melanin content an average of 67% as compared with control without lowering the total cellular tyrosinase activity. Steroidal alkaloids that preferentially act on the Sonic Hedgehog signaling pathway showed no related specificity in their ability to decrease pigmentation. In melanocytes treated with U18666A, tyrosinase accumulates in a compartment that contains both lysosome-associated membrane protein-1 (Lamp 1) and MPR, and stains with filipin, consistent with cholesterol-laden late endosomes/lysosomes. Our results suggest that tyrosinase, like the NPC1 gene product, traverses a U18666A-sensitive trafficking pathway.
Lei TC, Virador V, Yasumoto K, Vieira WD, Toyofuku K, Hearing VJ.

In this study, we used melb-a melanoblasts as a model to study mechanisms involved in stimulating melanocyte function in vitiliginous skin following exposure to 8-methoxypsoralen (8MOP). Melanin content and tyrosinase activity increased 3- and 7-fold, respectively, in melanoblasts treated with 8MOP for 6 d compared with untreated controls. The intracellular signal pathways involved in 8MOP-induced effects on melanoblasts were investigated, particularly the roles of protein kinase A and protein kinase C. Forskolin, a protein kinase A activator, mimicked and enhanced the 8MOP stimulation of melanoblast pigmentation whereas a protein kinase C activator, 1-oleoyl-2-acetylglycerol, had no effect, indicating that the protein kinase A pathway is involved rather than the protein kinase C pathway. Those observations were confirmed using inhibitors of the protein kinase A or protein kinase C pathways. Western blot and semiquantitative reverse transcriptase polymerase chain reaction were performed to assess the protein and mRNA expression levels of microphthalmia-associated transcription factor and tyrosinase in melanoblasts treated with 8MOP for 3 h, 6 h, 1 d, 3 d, or 6 d. Incubation with 8MOP stimulated microphthalmia-associated transcription factor protein and mRNA levels within 3 h, but, in contrast, tyrosinase mRNA and protein levels did not increase following 8MOP treatment until 1 d after treatment. The proteasome inhibitor lactacystin blocked the proteasome-mediated proteolysis of tyrosinase, and its effect on proteasomal function was enhanced by 8MOP. Taken together, these results show that 8MOP functions by initially stimulating levels of microphthalmia-associated transcription factor expression via activation of the protein kinase A pathway, which thereby stimulates tyrosinase expression and function and eventually leads to dramatic increases in melanin production by melanoblasts.

The microphthalmia-associated transcription factor is implicated in melanocytes development and in the regulation of melanogenesis. Microphthalmia-associated transcription factor is thought to bind to the M-box promoter elements of tyrosinase, tyrosinase-related protein-1 and dopachrome tautomerase/tyrosinase-related protein-2 and transactivate these genes, resulting in increased pigmentation. Using a luciferase reporter construct driven by the microphthalmia-associated transcription factor promoter, we identified agents that modulate microphthalmia-associated transcription factor promoter activity. Changes in endogenous microphthalmia-associated transcription factor expression levels upon treatment with these agents were confirmed using northern and western blots, and their pigmentary modulating activities were demonstrated. Ultraviolet B irradiation and traditional Chinese medicine-1, a natural extract used in traditional Chinese medicine, upregulated microphthalmia-associated transcription factor gene expression and enhanced tyrosinase activity in vitro. Dihydrolipoic acid, lipoic acid, and resveratrol reduced microphthalmia-associated transcription factor and tyrosinase promoter activities. These agents also inhibited the forskolin- and ultraviolet B-stimulated promoter activities of these genes and significantly reduced tyrosinase activity in melanocyte cultures, resulting in depigmentation. Overexpressed microphthalmia-associated transcription factor was capable of rescuing the repressive effects of these compounds on the cotransfected tyrosinase promoter. Dark-skinned Yucatan swine treated with these agents showed visible skin lightening, which was confirmed histologically, whereas ultraviolet B-induced tanning of light-skinned swine was inhibited using these agents. Our findings suggest that modulation of microphthalmia-associated transcription factor expression can alter skin pigmentation and further confirm the central role of microphthalmia-associated transcription factor in melanogenesis.

Libby RT, Smith RS, Savinova OV, Zabaleta A, Martin JE, Gonzalez FJ, John SW.
Mutations in the cytochrome P450 family 1, subfamily B, polypeptide 1 (CYP1B1) gene are a common cause of human primary congenital glaucoma (PCG). Here we show that Cyp1b1-/- mice have ocular drainage structure abnormalities resembling those reported in human PCG patients. Using Cyp1b1-/- mice, we identified the tyrosinase gene (Tyr) as a modifier of the drainage structure phenotype, with Tyr deficiency increasing the magnitude of dysgenesis. The severe dysgenesis in eyes lacking both CYP1B1 and TYR was alleviated by administration of the tyrosinase product dihydroxyphenylalanine (L-dopa). Tyr also modified the drainage structure dysgenesis in mice with a mutant Foxc1 gene, which is also involved in PCG. These experiments raise the possibility that a tyrosinase/L-dopa pathway modifies human PCG, which could open new therapeutic avenues.

Marles LK, Peters EM, Tobin DJ, Hibberts NA, Schallreuter KU.
Both human epidermal melanocytes and keratinocytes have the full capacity for de novo synthesis of 6(R) L-erythro 5,6,7,8, tetrahydrobiopterin, the essential cofactor for the rate limiting step in catecholamine synthesis, via tyrosine hydroxylase. Catecholamine synthesis has been demonstrated in proliferating keratinocytes of the
epidermis in human skin. This study presented herein identified for the first time the expression of tyrosine hydroxylase isozyme I mRNA within the melanocyte. The location of the enzyme was demonstrated in both the cytosol and melanosomes of human epidermal melanocytes, using immunohistochemistry and immunofluorescence double staining as well as immunogold electron microscopy. High-performance liquid chromatography (HPLC) analysis of pure melanosomal extracts from the human melanoma cell line, FM94, confirmed the production of L-Dopa within these organelles. In addition, enzyme activities for both tyrosine hydroxylase and tyrosinase were measured in the same preparations, by following the catalytic release of tritiated water from L-[3,5-3H]tyrosine. The melanosomal membrane location of tyrosine hydroxylase together with tyrosinase implies a coupled interaction, where L-Dopa production facilitates the activation of tyrosinase. Our results support a direct function for tyrosine hydroxylase in the melanosome via a concerted action with tyrosinase to promote pigmentation.


Tyrosinase, the rate-limiting enzyme in mammalian melanogenesis, is a copper-containing transmembrane glycoprotein. Tyrosinase undergoes a complex post-translational processing before reaching the melanosomal membrane. This processing involves N-glycosylation in several sites, including one located in the CuB copper binding site, movement from the endoplasmic reticulum (ER) to the Golgi, copper binding, and sorting to the melanosome. Aberrant processing is causally related to the depigmented phenotype of human melanomas. Moreover, some forms of albinism and several other pigmentary syndromes are considered ER retention diseases or trafficking defects. A critical step in tyrosinase maturation is the acquisition of an ER export-competent conformation recognized positively by the ER quality control system. However, the minimal structural requirements allowing exit from the ER to the Golgi have not yet been identified for tyrosinase or other melanosomal proteins. We addressed this question by analysing the enzymatic activity and glycosylation pattern of mouse tyrosinase point mutants and chimeric constructs where selected portions of tyrosinase were replaced by the homologous fragments of the highly similar tyrosinase-related protein 1. We show that a completely inactive tyrosinase point mutant lacking a critical histidine residue involved in copper binding is nevertheless able to exit from the ER and undergo further processing. Moreover, we demonstrate that tyrosinase displays at least two sites whose glycosylation is post-translational and most likely conformation-dependent, and that a highly specific interaction involving the CuB site is essential not only for correct glycosylation but also for exit from the ER and enzymatic activity.


N-terminal amino acid sequences for the two hemocyanin subunits from the deep-sea crustacean Bathynomus giganteus have been determined by Edman degradation, providing the first sequence information for a hemocyanin from an isopod. In addition, purified hemocyanin from B. giganteus exhibited phenoloxidase activity in the presence of sodium dodecyl sulfate. Although a natural activator has not yet been identified, a preliminary study of the enzyme indicated a K(m) of 5mM for dopamine and an initial rate of 0.1 micromol per min per mg protein, values consistent with a significant role for this enzyme in the innate immune system of B. giganteus. Moreover, after separation of hemolymph by alkaline polyacrylamide gel electrophoresis, the only detectable phenoloxidase activity coincided with the two hemocyanin subunits. The hemocyanin of this primitive crustacean may fulfill dual functions, both as oxygen carrier and as the phenoloxidase crucial for host defense.


In vitro studies have shown that the phorbol ester, 12-tetradecanoylphorbol 13-acetate (TPA) induces neural crest cell differentiation into melanocytes, and stimulates proliferation and differentiation of normal melanocytes. As TPA is not a physiological agent, its action is clearly mimicking some in vivo pathway involved in these processes. An understanding of the effect of TPA on the expression of melanogenenic genes will therefore provide valuable insight into the molecular mechanisms regulating melanocyte differentiation. In this study, we utilized primary cultures of neural crest cells and an immortalized melanocytes cell line (DMEL-2) which proliferates in the absence of TPA, to explore the effects of TPA on key melanogenic effectors. In neural crest cells, TPA was found to be necessary for both microphthalmia associated transcription factor (Mitf) up-regulation and for melanin synthesis. Using northern blots, we show that in DMEL-2 cells, TPA significantly increases the messenger ribonucleic acid (mRNA) levels of the tyrosinase gene family (tyrosinase, Tyrp1 and Dct) and the expression of Mitf. Western blots demonstrate that in these TPA-treated cells there is a concomitant increase in Tyr, Tyrp1 and glycosylated Dct protein levels. Pax3, a known Mitf regulator, is unaltered by TPA treatment. This study demonstrates the utility of a novel cell line for investigating the long-term effects of TPA on melanogenesis and provides an understanding of how TPA enhances mouse melanocyte differentiation.
The study of metalloenzymes using quantum chemical methods of high accuracy is a relatively new field. During the past five years a quite good understanding has been reached concerning the methods and models to be used for these systems. For systems containing transition metals hybrid density functional methods have proven both accurate and computationally efficient. A background on these methods and the accuracy achieved in benchmark tests are given first in this review. The rest of the review describes examples of studies on different metalloenzymes. Most of these examples describe mechanisms where dioxygen is either formed, as in photosystem II, or cleaved as in many other enzymes like cytochrome c oxidase, ribonucleotide reductase, methane mono-oxigenase and tyrosinase. In the descriptions below high emphasis is put on the actual determination of the transition states, which are the key points determining the mechanisms.


The enzymatic and bioelectrocatalytic activity of tyrosinase from mushrooms was studied in a system of reversed micelles formed by Aerosol OT (AOT) in hexane. The optimal catechol oxidising activity of tyrosinase incorporated in reversed micelles was found at a hydration degree of w(0)=25. The catalytic activity was comparable with tyrosinase activity in aqueous media. When immobilized at an Au electrode, either directly or in reversed micelles, tyrosinase exhibited a similar efficiency of the bioelectrocatalytic reduction of O(2) mediated by catechol; however, a rapid decrease in the activity correlated with the destruction of reversed micelles and/or the removal of tyrosinase from the electrode surface. The system containing tyrosinase in reversed micelles with caoutchouk, spread on the surface of the Au electrode and successively covered with a Nafion(R) membrane layer, was found to result in stable tyrosinase-modified electrodes, which were resistant to inactivation in dry acetonitrile. The proposed technique offers possibilities for further development of highly active and stable surfactant/enzyme-modified electrodes for measurements carried out in organic solvents.


Menkes protein (ATP7A) is a P-type ATPase involved in copper uptake and homeostasis. Disturbed copper homeostasis occurs in patients with Menkes disease, an X-linked disorder characterized by mental retardation, neurodegeneration, connective tissue disorders, and early childhood death. Mutations in ATP7A result in malfunction of copper-requiring enzymes, such as tyrosinase and copper/zinc superoxide dismutase. The first step of the two-step amidation reaction carried out by peptidylglycine alpha-amidating monoxygenase (PAM) also requires copper. We used tissue from wild-type rats and mice and an ATP7A-specific antibody to determine that ATP7A is expressed at high levels in tissues expressing high levels of PAM. ATP7A is largely localized to the trans Golgi network in pituitary endocrine cells. The Atp7a mouse, bearing a mutation in the Atp7a gene, is an excellent model system for examining the consequences of ATP7A malfunction. Despite normal levels of PAM protein, levels of several amidated peptides were reduced in pituitary and brain extracts of Atp7a mice, demonstrating that PAM function is compromised when ATP7A is inactive. Based on these results, we conclude that a reduction in the ability of PAM to produce bioactive end-products involved in neuronal growth and development could contribute to many of the biological effects associated with Menkes disease.

Based on its monophenolic structure and given its pharmacological and toxicological importance, the ability of tyrosinase to oxidize acetaminophen was studied for the first time. Progress curves showed a transient phase characteristic of the monophenolase activity of tyrosinase prior to attaining the steady-state. The duration of this transient phase strongly increased with the drug concentration, which would partly explain why paracetamol oxidation by tyrosinase has not been studied hitherto. The pathway is enhanced by the presence of minute amounts of L-dopa, which shortens the length of the lag period. Acetaminophen oxidation was inhibited by tropolone, a selective inhibitor of tyrosinase. The presence of the corresponding o-diphenol as intermediate was demonstrated with ascorbic acid by chemical oxidation using NaI04 and by HPLC analysis, indicating that acetaminophen is oxidized by the monophenolase activity of tyrosinase to its corresponding o-quinone. These results contribute to our knowledge of the oxidation mechanisms of acetaminophen.

- Voisey J, Kelly G, Van Daal A. 

Production of the pigment eumelanin is controlled by alpha-melanocyte stimulating hormone (alpha-MSH) stimulation of melanocortin 1 receptor (Mc1r), whereas production of phaeomelanin results from agouti antagonism of alpha-MSH signalling through Mc1r. The role of agouti in mouse pigmentaton has been extensively investigated but a role for agouti signalling protein (ASIP) in human pigmentation has not been determined. To determine whether ASIP regulates known melanogenic genes in humans, ASIP was over-expressed in a human melanoma cell line. Levels of mRNA and protein were measured in genes known to be up or down-regulated by agouti in the mouse, namely microphthalmia (Mitf), tyrosinase (Tyr), tyrosinase-related protein 1 (Tyrp1), dopachrome tautomerase (Dct), Mc1r, silver, initiation transcription factor 2 (Ift2) and mini chromosome maintenance protein 6 (Mcm6). These melanogenic genes were not found to be significantly up or down-regulated by ASIP at the transcriptional level in human melanoma cells. However, ASIP down-regulation of tyrp1 was observed at the translational level. To identify novel genes that may be regulated by ASIP in melanoma cells, microarrays were used to determine differences in gene expression between the control and ASIP transfected melanoma cells. The expression level of human RNAs were determined by microarray analysis using a 19 200 cDNA and a 19 200 oligonucleotide array representing 13 000 and 18 864 individual genes, respectively. Genes observed to be modulated by ASIP were confirmed by quantitative real-time polymerase chain reaction. Results identify five genes, namely PPARbeta, elf-4B, RRM2, MINOR and EVI2B that are down-regulated by ASIP, indicating a likely role for ASIP in human melanogenesis.

- Zarivi O, Bonfigli A, Cesare P, Amicarelli F, Pacioni G, Miranda M. 

Tyrosinase is an enzyme having two copper atoms at the reactive site occurring in prokaryotic and eukaryotic organisms. In animals tyrosinase is responsible for pigmentation, in plants for protection of injured tissues or, as in fungi, to harden cell walls. Some of us have previously shown that tyrosinase is involved in truffle development and differentiation. Here we present the purification, the molecular properties and the reversible inhibition of Tuber melanosporum tyrosinase by dimethyl-sulfide and bis[methylthio]methane, the main flavour compounds of black and whitish truffles. The MW(r) is 39000. L-3,4-dihydroxyphenylalanine and L-tyrosine stain corresponding bands as expected for a true tyrosinase. Phenylthiourea, diethyldithiocarbamate and mimosine inhibit L-tyrosine and L-3,4-dihydroxyphenylalanine oxidation.

### 7. Melanosomes

(Dr. J. Borovansky)

Papers devoted to organelle transport and molecular characterization of intracellular motor proteins were dominant in melanosome literature last year. This trend seems to continue also in 2003 (Deacon et al., Dell, Langford, Westbroek et al). Four papers have recently dealt with the ultrastructure of melanosomes and melanin granules from various points of view (Liu & Simon, Nguyen et al, Sarna et al, Wolff). An excellent review on the lysosome-related organelles was written by Raposo et al. Basrur et al have opened a new stage in melanosome proteome analysis. Sarna et al have brought an evidence that oxidative degradation of melanin in vivo might be possible.


**Comments:** Authors used mass spectrometry and subcellular fractionation to identify protein components of early melanosomes. They identified the six known melanosome-specific proteins, 56 proteins shared with other organelles and confirmed the presence of 6 novel melanosomal proteins both by Western blotting and immunohistochemistry. Thirty one years after the pioneer paper demonstrating the presence of several proteins in isolated melanosomes, the present paper opens a new era towards a complete characterization of proteome of the melanosome.

**Comments:** Dynactin complex, known as an anchor for cytoplasmic dynein, was found to link kinesin II to organelles. Putative cargo-binding subunit of Xenopus kinesin II = Xenopus kinesin II-associated protein binds directly to the p150 subunit of dynactin. These results revealed that dynactin was required for transport activity of microtubule motors of opposite polarity, cytoplasmic dynein and kinesin II.


**Comments:** Review discussing the possibility that dynactin may be a key player in coordinating melanosomal traffic in Xenopus because it can bind not only dynein but also kinesin II.


**Comments:** Review with beautiful 3D colour pictures showing modules of myosin Va and structure of the kinesin/myosin V heteromotor complex attached to a vesicle. Special attention was paid to: a) Modular design of myosin-V; b) myosin-V/kinesin interactions; c) vesicle transport by myosin-V; d) types of vesicles transported by myosin-V in neurons and other cell types; e) recruitment of myosin-V to vesicles by the Rab/Rabphilin complex; f) myosin-V and fast axonal/dendritic transport.


**Comments:** Pigment in the ink sac of Sepia should be referred as melanin granules not melanosomes /see also Schraermeyer-Pigment Cell Res 7, 1994, 52-60/ because one Sepia melanosome produces up to 30 such granules. Unique images obtained by means of ultrahigh resolution SEM and atomic force microscopy demonstrated that a) dominant constituents of Sepia melanin are ~150nm spherical granules; b) melanin granules are easily deformed and are comprised of smaller constituents; c) morphology of pigment (as well as its surface area) was strongly influenced by the preparation procedure.


**Comments:** The morphology of melanosomes in the skin of ten Hermansky-Pudlak syndrome murine strains was examined by electron microscopy. The mutant strains were classified into morphologic groups characterized by the step at which melanosome biogenesis or transfer to keratinocytes was inhibited. All the Hermansky-Pudlak syndrome mutant strains studied had an increase in un- or hypopigmented immature melanosomal forms except gunmetal strain in which retention of melanosomes and failing transfer into keratinocytes were observed.


**Comments:** Excellent review highlighting adaptations and malfunction of the endosomal/lysosomal system in normal and pathological situations with a special focus on MHC class II compartments in antigen presenting cells and melanosomes in pigment cells.


**Comments:** Electron spin resonance spectroscopy study demonstrated a substantial loss of RPE melanin in human eyes with aging. A pronounced photobleaching of human and bovine RPE melanosomes was induced by irradiating cells with visible light /see also Elleder&Borovansky – Histochem J 33, 2001,273-281/. Loss of melanin due to photobleaching in isolated RPE melanosomes was demonstrated by ESR spectroscopy. The data suggest that the photodegradation of melanin in vivo might be possible.


**Comments:** Myosin Va isoforms containing exon F are able to colocalize with and influence melanosome distribution by indirect interaction with rab27a and direct interaction with melanophilin. Myosin Va medial tail domain seems to provide globular tail domain with organelle-interacting specificity.

Comments: Review containing a chapter „Structure of pheo- and eumelanic melanosomes“. The chapter based on only 4 papers is incomplete lacking important facts.

- Yamada T, Fujii R

8. Melanoma experimental, Cell culture
(Dr. N. Smit)

Abdel-Naser describes the culture of normal human melanocytes in the early phase of growth in a serum free medium. The importance of bFGF, insulin and bovine pituitary extract for melanocyte proliferation is demonstrated once more. Barbucci et al studied melanocyte cell behaviour on micropatterned surfaces prepared with hyaluronan and its sulphated derivative. The melanocytes adhered to the patterns formed by the sulphated derivative. Alexeev et al have described before the correction of mutations in the tyrosinase gene using chimeric RNA-DNA oligonucleotide (RDO). Now they used single stranded oligodeoxynucleotide (ODN) for correction of the inactivating mutation in tyrosinase and they introduced an activating mutation into the c-kit gene. ODN gave more reproducible results in gene correction than RDO. Biet et al used ODN as well for gene targeting in melanocyte cultures and the oligodeoxynucleotides were coupled to FITC. Use of this fluorescent dye could be very useful for sorting of the transfected cells.

Duval et al nicely show that the ratio of eumelanin and pheomelanin is strongly influenced by the presence of keratinocytes in co-cultures and reconstructed epidermis. The importance of the keratinocyte-melanocyte interactions for eumelanin and pheomelanin ratio is also demonstrated by using different media that influence the contact between both cell types. Yoon and Hearing now also describe a co-culture system for mouse epidermal cells. As stated by the authors the co-culture of murine melanocytes and keratinocytes is difficult compared to their human counterpart. Since many mutated and knock-out mice are available many different melanocyte cultures can be studied for their interaction with keratinocytes. In 1988 Tomita et al already described that histamine can influence melanocytes and melanogenesis. Later this was also reported by Yoshida et al (2000). Lasalle et al now report that nitric oxide and histamine can increase the eumelanin to pheomelanin ratio in cultured human melanocytes. Scott et al studied the regulation of MC1R expression in normal human melanocytes with low and high melanin content. They showed that MC1R mRNA levels can be influenced by paracrine factors, e.g. MSH itself, and specific endocrine sex hormones. Hoogduijn et al used dark and light melanocyte cultures as well and studied the regulation of Ca²⁺ homeostasis. Making use of videomicroscopy it was found that in the same culture strongly heterogeneous response for different melanocytes could be observed. When extracellular Ca²⁺ was added to the cells the melanin content of the cells seemed to be of importance for increases in cytoplasmic Ca²⁺.

- Abdel-Naser MB.

- Alexeev V, Igoucheva O, Yoon K.

- Barbucci R, Magnani A, Lamponi S, Pasqui D, Bryan S.

- Biet E, Alberti C, Facella P, Sun JS, Dutreix M, Larue L.

- Deacon SW, Serpinskaya AS, Vaughan PS, Lopez FM, Vernos I, Vaughan KT, Gelfand VI.

- Deng Y, Yang L.

- Duval C, Smit NP, Kolb AM, Regnier M, Pavel S, Schmidt R.


Calendar of events

New Members

ESPCR Travel Awards

From the Editor of Pigment Cell Research

IPCC 2005

Calendar of events

2003 Clinical Dermatology
Vienna, Austria, April 10 - 12
Tel : +44 207 407 9731 Fax : +44 207 378 9268
E-mail : d2000@cctltd.u-unet.com

2003 30th Annual Meeting of the Society for Cutaneous Ultrastructure Research (SCUR)
Salzburg, Austria , April 24 – 26
Tel : +43 662 4482 Fax : +43 662 4482
E-mail : w.muss@lks.at
Web site : www.dermatologie-lks.at

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

2003 XIth 1st Mediterranean Melanoma Meeting
Mediterranean Sea – Aegan, 2-5 May 2003
Contact: Panos Travel Ltd.
Mrs Anna ANTONOPOULOU and Mrs Anthoula KATSIMPARDI
4, Filellinon Street
GR- 105 57 Athens – Greece
Tel: +30/210/3230380 Fax: +30/201/3245049
Web site: www.panos-travel.gr
E-mail: info@panos-travel.gr
Web site of the meeting: www.panos-travel.gr/mmm

2003 24ème Congrès International de l’Association des Dermatologistes Francophones
Sfax – Tunisia, May 3
Tel : +216 74 242 627
Fax : +216 74 246 217
E-mail : abdelmajid.zahaf@rns.tn
Web site : www.dermato-tunisie.com
2003 1st Mediterranean Melanoma Meeting
Greece, May 2 - 5
Tel : +3 010 323 0380  Fax : +3 010 324 5049
E-mail : info@panos-travel.gr
Web-site : www.panos-travel.gr

2003 9th World Congress on Cancer of the Skin
Seville, Spain, May 7 - 10
Tel : +34 9 212 725 5176  Fax : +34 9 212 725 5751
E-mail : info@skincancer.org
Web-site : www.sks2003.com

2003 10th international Congress on dermatology and Psychiatry
Brussels, Belgium, May 8 - 10
Tel : +32 2 555 49 69
E-mail : dermapsy@ulb.ac.be
Web-site : www.hopitalerasme.org/dermapsy.htm

2003 The 3rd World Congress of the International Academy of Cosmetic Dermatology
Beijing, China, May 15 - 17
Tel : +86 10 652 50394  Fax : +86 10 651 23754
E-mail : iacd2003@chinamed.com.cn
Web-site : www.chinamed.com.cn/iacd/index.html

2003 International Symposium on Vitiligo
London, UK, May 16-17
Venue: The Royal College of Physicians, London
Topics will include: The clinical nature of Vitiligo, Genetic Research into Vitiligo, understanding Melanocytes, psychology of vitiligo, autoimmunity and vitiligo.
Contact: Joanna Prendergast
Conference Executive
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E-mail: jprendergast@hamptonmedical.co.uk

2003 Australasian College of Dermatologists – Annual Scientific Meeting
Sydney, Australia, May 18-21
Tel : +61 2 9816 1174

2003 11st Joint Meeting: 14th International Congress for Bioengineering and the Skin and 8th Congress of the International Society for Skin Imaging
Hamburg, Germany, May 21-23
Tel : +49 4083 9358 17  Fax : +49 4083 9358 39
E-mail : kpw@proDerm.de
Web-site : www.akm.ch/ISBS/ISSi2003/
2003 Third Research Meeting on Melanoma
Milan, Italy, 29-30 May
Contact: Dr Alessandro TESTORI
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E-mail: melanoma2003@mafservizi.it or Info@mafservizi.it

2003 XIth First Annual Melanoma Research Congress
Philadelphia, 21-24 June
Conference Co-Chairs: M. HERLYN and D. GUERRY
Contact: Sandy PARSONS
The Wistar Institute
3601 Spruce Street
Philadelphia PA 19104
E-mail: parsons@wistars.upenn.edu

2003 British Association of Dermatologists 83rd Annual Meeting
Brighton, UK, July 1 - 5
Tel: +44 207 383 0266
E-mail: enmac@bad.org.uk
Web site: www.bad.org.uk

2003 12th Forum: Human Skin and Company
Lyon, France, July 4
Tel: +33 4 72 11 02 92 Fax: +33 4 72 11 02 90
Web site: www.sfdermato.org

2003 XIth Annual Meeting of the PASPCR
Wood's Hole, Cape Cod, MA, USA MA, September 3-7
Contact: Dr. Jean BOLOGNIA
Tel: +1 513 558 0198
E-mail: jean_bolognia@qm.yale.edu
Web site: www.cbc.umn.edu/ifpcs

2003 10th Congress of the European Society of Photobiology (ESP)
Vienna, Austria, September 6 - 11
Tel: +43 67 16 2500 Fax: +43 22 46 1304
E-mail: office@esp2003.org
Web site: www.esp2003.org
2003 XI<sup>th</sup> Meeting of the ESPCR
Ghent, Belgium, 17-20 September

Contact: MEDISCON
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5660 AC Geldrop
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Web site: www.espcrgent2003.org

ABSTRACT DEADLINE: 12 may 2003

2003 24<sup>th</sup> Symposium of the International Society of Dermatopathology
Istanbul, Turkey, 17-21 September
Tel : +90 312 324 5724  Fax : +90 312 310 5800
E-mail : rana@isdp2003.org
Web site : www.ilds.org

2003 Australasian College of Dermatologists and the Japanese Dermatological Association Combined Conference
Ayers Rock, Australia, 18-21 September
Tel : +61 2 9879 6177  Fax : +61 2 9816 1174
E-mail : admin@dermcoll.asn.au
Web site : www.dermcoll.asn.au

2003 14th European Study Group of Lysosomal Diseases - (ESGLD) Workshop
Podebrady, Czech Republic, September 18-21
PLANNED TOPICS OF THE WORKSHOP
Contact: Ms. Barbora VINSOVA
GUARANT Ltd.,
Opletalova 22,
CZ - 110 00 Praha 1
Tel: +420 2 8400 1444  Fax: +420 2 8400 1448
E-mail: esgld@guarant.cz
Web : http://www.ESGLD2003.CZ/

2003 12<sup>th</sup> Congress of the European Academy of Dermatology and Venereology (EADV)
Barcelona, Spain, October 15-18
Tel : +34 93 200 7083  Fax : +34 93 209 3152
E-mail : congresos@atlantaviajes.es
Web site : www.eadv.org

2003 3<sup>rd</sup> European Symposium on Teledermatology, Chirurgie Dermatologique
Las Vegas, USA, September 18-21
Tel : +1 312 998 7700  Fax : +1 312 988 7759
NEW MEMBERS

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

**BIRLEA S.**
Hospital of Zalau
Univ of Med and Farmacy Cluj
Corneliu Coposu nr 17
R - 4700 ZALAU

**CHAMPEVAL D.**
Institut Curie
UMR146 CNRS
Batiment 110
F - 91405 ORSAY, Cedex

**DELMAS V.**
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UMR146 CNRS
Batiment 110
F - 91405 ORSAY, Cedex

**DIERICKX K.**
L.O.C.E.- Institut J. Bordet
Université Libre de Bruxelles
Rue Héger-Bordet 1
B - 1000 BRUSSELS

**MAGYARY I.**
University of Kaposvar
Dept of Fish and Pet animal breeding
Guba S. u. 40
H - 7400 KAPOSVAR

**YATES P.**
Unilever Research
Dept Biosciences
Sharnbrook
UK - MK44 1LQ, BEDFORD
ESPCR TRAVEL AWARDS
For attendance at the 11th Annual ESPCR Meeting
CALL FOR APPLICATIONS

The European Society for Pigment Cell Research will provide a limited number of travel awards for attendance at its 11th Annual Meeting, to be held in Ghent, September 17-20, 2003. Depending on the number of applicants selected, awards may cover travel (economy return air, rail fare or car fuel costs), conference registration, and in some cases accommodation (economy class). Awards will be made by the ESPCR Travel Awards Committee on a competitive basis.

Applicants must:
- Be a PhD student or junior scientist (i.e. postdoctoral or medical resident).
- Be an ESPCR member in good standing (subscription paid).
- Make a contribution (oral or poster) to the conference.
- Have no other source of funds for this purpose. If funds from elsewhere are subsequently obtained, ESPCR should be informed immediately and the application for ESPCR funding withdrawn, or the ESPCR award declined/returned if already made, so that another applicant can be funded.

Deadline for applications: May 12 2003

Please send an informal letter of application (e-mail or ordinary mail) to:

Dr Friedrich Beermann, Chair, ESPCR Travel Awards Committee
e-mail: Friedrich.Beermann@isrec.unil.ch
ISREC (Swiss Institute for Experimental Cancer Research)
Chemin des Boveresses 155
CH-1066 Epalinges
Switzerland

enclosing:
- Proof of status (usually a short statement from the supervisor or Head of Department), including the date or expected date of completion of PhD or medical qualification.
- Evidence of non-availability of other funds (usually part of the statement from the supervisor or Head of Department). Please state if other applications for funding are being made (this has a positive effect on your application).
- Submitted abstract of the oral or poster contribution.
- Estimates of the costs of travel, accommodation and conference registration.
- Applicant's full address, phone and fax numbers, and e-mail address where available.
From the Editor of Pigment Cell Research

Dear Members of the ESPCR, JSPCR and PASPCR:

It has now been almost 3.5 years since I began my 5 year term as Editor of *Pigment Cell Research* and I would like to take this occasion to thank all of you for the continued support that has been given to me at every level. The quality of submissions has continued to improve and their quantity has almost doubled over the past year. The speed of reviews and decisions has been maintained, the support by the publisher has improved and in my opinion, the journal has become a much more vital resource for all of us. *Pigment Cell Research* is widespread in its coverage of pigment-related topics, and it welcomes potential authors from all areas of research in pigmentation ranging from comparative biology to chemistry to genetics to molecular biology to clinical and applied aspects. The outlook for 2003 and beyond is quite bright and I have summarized below some key points regarding that. I’ll look forward to the remaining year+ of my term confident that our journal will continue to progress significantly in the future.

- **Web Site** – The PCR Web site (www.pigment.org) is being accessed more and more frequently with more than 20,000 hits in its first 3+ years; not only can you access titles and abstracts of papers from all Volumes back through the years, but abstracts and titles of papers now ‘in press’ can also be accessed. The P*C*R Primer is sent to more than 1,000 scientists in the field that are in our database – if you don’t get that you can sign up from the PCR Web site to receive information about journal publications as they come out. Blackwell/Munksgaard has an excellent online web site for PCR (http://www.blackwell-synergy.com/servlet/useragent?func=showIssues&code=pcr) and member subscribers have free access to full manuscripts published from 2000 on at that site (you will need to contact them online the first time to receive your activation information). If you haven’t discovered this yet, articles are typically available online about 2 weeks before the hard copies are mailed and received.

- **Online Submissions and Turnaround Time** – Speed is the key, and manuscripts are submitted online almost exclusively now. See the ‘Authors’ page on the Web site for information about this and what types of files can be submitted. Electronic processing has also sped up handling and review of your submissions; the average time to a decision from the date my office received a manuscript in 2002 was only 27 days; the average total time in my office for accepted manuscripts from receipt to acceptance to transmission to the Publisher was only 36 days.

- **Impact Factor** – The Impact Factor for PCR rose for a 4th straight year (to 2.10) in 2002 which has promoted us into the top half of the highly competitive Cell Biology category. Whether we continue our rise will depend completely upon YOU, since the impact factor is determined by YOUR citations of papers published in PCR over the past 2 years (e.g. our 2003 Impact Factor will be calculated on citations published this year to articles published in PCR in 2001 and 2002). Does it count if you cite your own papers in PCR? Absolutely yes.

- **Expanded Features and Publisher Support** – the Publisher has further increased our color publication budget for 2003; it is not yet an unlimited amount, but you should have noticed the progressive increase in color in PCR. The Publisher has also increased our allotment of pages per issue from 64 to 80; in addition to those, the Programs and Abstracts of the annual meetings of the regional Societies are published as supplementary materials (as are the IPCC Program and Abstracts when that occurs). The PASPCR meeting will be found in Issue #4, the ESPCR meeting in Issue #5 and the JSPCR meeting in Issue #6. Can’t attend all of those meetings? Accessing PCR is your best way to see what is being done in our field. In 2003, subscribers will also receive the IPCC Proceedings as Supplementary material in Issue #3, and a special issue dedicated to the memory of Prof. Giuseppe Prota, which will be published in Issue #5.
• **Outstanding Reviews** – the quality of the Reviews published has continued to be steadily high and I’d like to thank all of our Review writers. Once again, virtually everyone asked to contribute a review agreed to do so, and some unsolicited Reviews have also been submitted and a few of those have been accepted and published. You can look at the upcoming list of Reviews (Regular, Gene Focus and Innovative Technology) for the next year on the ‘In Press’ page of the PCR Web Site.

• **The Bad News** – I assume you noticed that our reduced member subscription fees increased this year. Although that wasn’t too big an increase (about the equivalent of $15 per year) in light of the previous relatively low rate which had remained constant for more than 5 years, it may portend more increases in the future. Why? Well, costs are going up everywhere, including publishing and postage expenses. The solution is simple. The publisher makes very little profit on our discounted subscription fees but makes considerably more on Institutional subscriptions. That being the case, one way to keep your own subscription cost low is to have your library subscribe at the Institutional cost. A few dozen new Institutional subscriptions would probably keep our own personal fees low for the foreseeable future. The solution is in your hands.

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

Best regards,

[Signature]

Editor, *Pigment Cell Research*
IPCC 2005

Dear Members of the ESPCR, JSPCR and PASPCR:

Time has a way of moving on and although it has only been about 6 months since the highly successful International Pigment Cell Conference (IPCC) held in the Netherlands last year, it won’t be that long before it’s time to start thinking about planning to attend the next one. Each IPCC has tended to become more and more useful and important to attend for active scientists in the field (or those thinking of becoming so) and there is great pressure on us to continue that tradition at the 19th IPCC. We fully intend to do our best to meet that expectation.

The 19th IPCC will be held at the Natcher Conference Center at the National Institutes of Health in Bethesda, MD from September 18 - 23, 2005. This area is slightly north of Washington DC (by about 20 km) and is readily accessible by any of our 3 local airports. You will find that the Natcher Conference Center is a spectacular venue to hold such a meeting, and with NIH being the current home of at least a dozen independent research groups studying pigmentation at various levels, you can imagine that we are all excited about this opportunity to showcase NIH as an exciting place to visit and an even better place to call your research home.

We have established an active Local Organizing Committee and an International Scientific Program Committee, both of which have met as a group once and by email on many occasions. We are working from all angles to make this meeting as affordable, as interesting and as productive as possible for all scientists in the field, senior and junior alike.

We have established a Web Site (www.ipcc.info) that is in its infancy but which will be developing quickly. Please bookmark that site in your Web browser and revisit it from time to time to see the meeting mature. We already have there a summary of the meeting, contact information, some information about the Conference center (with photos), preliminary travel information about how best to get here and how best to sneak out of the meeting to visit the Smithsonian and Art Museums when you have time (along with maps of how to do that), the members of our Scientific Committees, and even an overview of the meeting program.

I would like to take this occasion to invite each of you to plan to attend the 19th IPCC and we are always open for useful suggestions on what topics and information you would like to see on the Web site or featured in the scientific or social program. I’ll look forward to welcoming you to the 19th IPCC in mid-September, 2005.

Best regards,

[Signature]

Organizer, 19th IPCC - 2005