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Review of the literature

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Announcements and related activities
The Transcription Network Regulating Melanocyte Development and Melanoma
Review by Keith W. Vance and Colin R. Goding
PCR 17(4), 318 - 2004
The enormous variety of pigmentation phenotypes in nature reflects a series of remarkable events that begin in the neural crest and end with the manufacture and distribution of pigment by mature melanocytes located in the epidermis and hair follicles. While the origins of melanoblasts from multipotent precursors in the neural crest is striking in itself, yet more so is the fact that these pioneer melanoblasts manage to undertake and survive their long migration, and in doing so proliferate and maintain their identity before ultimately arriving at their destination and undergoing differentiation. With the application of the powerful combination of genetics and molecular and cell biology the mystery surrounding the genesis of the melanocyte lineage is slowly being unravelled. At its heart is the powerful alliance between signal transduction and transcription that coordinates the program of gene expression that confers on a cell its identity, provides its passport for migration, and instructs it in the arts of survival and timely reproduction. The realization that the proliferation and migration of melanoblasts during development resembles closely the proliferation and metastasis of melanoma, a highly dangerous and increasingly common cancer, serves to highlight the value of the melanocyte system as a model for addressing key issues of general significance in both development and cancer.

Human Melanocytes Do Not Express EGF Receptors
by Jennifer C. Grahn and R. Rivkah Isseroff
JID 123(1), 244 - 2004

Endothelial cell integrins and COX-2: mediators and therapeutic targets of tumor angiogenesis.
Review by Ruegg C, Dormond O, Mariotti A.
Vascular integrins are essential regulators and mediators of physiological and pathological angiogenesis, including tumor angiogenesis. Integrins provide the physical interaction with the extracellular matrix (ECM) necessary for cell adhesion, migration and positioning, and induce signaling events essential for cell survival, proliferation and differentiation. Integrins preferentially expressed on neovascular endothelial cells, such as alphaVbeta3 and alpha5beta1, are considered as relevant targets for anti-angiogenic therapies. Anti-integrin antibodies and small molecular integrin inhibitors suppress angiogenesis and tumor progression in many animal models, and are currently tested in clinical trials as anti-angiogenic agents. Cyclooxygenase-2 (COX-2), a key enzyme in the synthesis of prostaglandins and thromboxans, is highly up-regulated in tumor cells, stromal cells and angiogenic endothelial cells during tumor progression. Recent experiments have demonstrated that COX-2 promotes tumor angiogenesis. Chronic intake of nonsteroidal anti-inflammatory drugs and COX-2 inhibitors significantly reduces the risk of cancer development, and this effect may be due, at least in part, to the inhibition of tumor angiogenesis. Endothelial cell COX-2 promotes integrin alphaVbeta3-mediated endothelial cell adhesion, spreading, migration and angiogenesis through the prostaglandin-cAMP-PKA-dependent activation of the small GTPase Rac. In this article, we review the role of integrins and COX-2 in angiogenesis, their cross talk, and discuss implications relevant to their targeting to suppress tumor angiogenesis.
The binding properties of melanin remain an issue of continuous interest. The high affinity of synthetic and natural pigments to different drugs including antidepressants (Higashi et al.) antibiotics (Tanaka et al.) the mutagen phenylimidazopyridine (PhIP) (Hashimoto et al.) and the toxic saponin hederacolchiside A1 (Debiton et al.) was considered in different papers also as an approach to the development of analytical methods of diagnostic value.

Liu et al report a systematic investigation on the metal binding capacity of sepiomelanin samples, determination of the binding constants for various metal cations at pH 5.8 relative to EDTA, and discrimination of the different binding sites within the polymer. It is concluded that while Ca (II) and Mg(II) bind to carboxylic groups, Fe(III) is coordinated to the \( o^- \)diphenolic moiety of the pigment monomer units.

Another group of studies deals with the photophysical properties of melanins. Determination of radiative relaxation quantum yields and characterization of the photoluminescence emission of synthetic eumelanins provided evidence for an oligomeric rather than heteropolymeric nature of the pigments (Meredith and Riesz). The surface characteristics of melanin particles were investigated by Crippa and associates taking advantage of the photophysical behavior of pyrene. The fluorescence study allowed determination of the microenvironmental polarity of the melanin surface, the excimer formation, the lifetimes of the emissions and the kinetics of quenching by Cu\(^{2+} \). Thin films of synthetic melanins obtained from solutions in organic solvents were fully characterized by several techniques, including atomic force microscopy, which revealed graphitic-like planar structures (Deziderio et al).

Of interest in this connection is also an hypothesis on structure of melanin presented by W.L. Cheun from St John University at New York in a letter to the editor of Pigment Cell Research. In this a model consisting of stacked layers of plane polymerized units is considered for melanin pigment and is proposed that the interlayer connection is warranted by covalent bonds of the functional groups of the monomers units belonging to adjacent layers. This is claimed to account for the properties of melanin ranging from the chemical reactivity to the light absorption characteristics. Such hypothesis is challenged in a reply by Ito and Simon overviewing the recent literature data on the structural/ultrastructural properties of natural melanins especially the pigment from Sepia Officinalis.

A series of papers address the inhibitory/promoting effects of different compounds on the production of melanin in vitro and in vivo. Among these a further paper by Di and Bi on the effects of alluminium in the iron-mediated oxidation of dopa to melanin. The possibility that some tryptophan metabolites may be incorporated in the growing melanin pigment by covalent linking with melanin intermediates was suggested by Soddu et al. The properties of melanin like pigments formed by 5-hydroxytryptophan by action of tyrosinase or peroxidase were investigated by Allegri et al by mass spectrometric methods (MALDI –MS).

As to melanin analysis of particular interest is the paper by Zoccola et al. testing the use of near IR as a methodology alternative and more expedient than currently used chemical degradation/HPLC procedures for eumelanin determination in hair of potential value for the screening of large populations.

The main reaction pathways of quinones and particularly the \( o^- \)-quinones involved in melanogenesis was reviewed by Land, Ramsden and Riley.

Finally a bibliographic review by Hans Rorsman is an interesting and brilliant story of the continuing and passionate efforts of the group at Lund to unravel some intriguing and most interesting issues in pigment cell research starting from cysteinyldopa (the complete story is tracked from its discovery to its use as tumor marker in melanoma), till neumelanin and melanogenic enzymes.

**MELANIN REACTIVITY AND PROPERTIES:**

- Cheun WL. 

- Crippa PR, Fornes JA, Ito AS. 

- Debiton E, Borel M, Communal Y, Mshvildadze V, Barthomeuf C. 


Biosynthesis:


- Sugimoto K, Nishimura T, Nomura K, Sugimoto K, Kuriki T.

MELANIN ANALYSIS:


MELANIN PRECURSORS:


OTHER PIGMENTS:


2. Biology of pigment cells and pigmentation disorders

(Dr. M. Picardo)

During the last period some relevant papers on in vitro epidermal cell management, melanoma cell pathways, vitiligo pathogenesis and therapy were published. The cultivation of epidermal cells is required for in vitro studies and for clinical application. However, the repeated passages modify the cell morphology and some metabolic pathways allowing a different behaviour. The telomer length has been proposed by Uceda group as possible marker of the quality of the cultured epidermal keratinocytes.

A study on melanocytes migration during fetal development was proposed by E Suder. She performed on skin specimens from fetus a staining with anti-HMB45 antibody and she suggested that the melanocytes, during the migration from neural crest to epidermal basal layer and hair follicles, can locate in the dermal layer.

A lot of researcher groups aim to understand the link between apoptosis, TNF-mediated signals and melanoma development. C Bertolotto by means of a complete and clear in vitro model indicates that TRAIL, counteracted by SCF, promotes the apoptotic process through caspasases 8,9, and 3 activation in primary human melanocytes. The molecular pathway involves PI3K and AKT (and ERK at least) and a sustained PI3K activation protects from apoptosis. These data can provide a further help to understand the resistance of melanoma cells to apoptosis.

As regard to vitiligo pathogenesis and treatment further studies were published. After the paper of Taieb on “melanocytorrhagy” in vitiligo skin, other authors focused the attention on possible adhesion defect in vitiligo melanocytes and on consequent new therapeutic approaches. Mou and coworkers evaluated the in vitro effects of Malytea fruit on adhesion and migration suggesting a possible use the extract to regulate vitiligo melanocyte function, even if he did not analysed the in vitro response of vitiligo melanocytes. Another relevant aspect evaluated was the role of MITF in the loss and impairment of melanocytes. By means of immunohistochemical analysis, R Kitamura shows a low KIT and MITF expression in lesional areas possibly leading to a reduced melanine synthesis and melanocyte death. However, although a high number of works were carried out it is still ambiguous the cause of melanocyte disappearance. In an animal model (Smith line chickens) Wang investigated the occurrence of the apoptotic process and the possible link with an immunological pathogenesis. The immunohistochemical analysis suggests, at least in this animal model, a loss of melanocytes through an apoptotic mechanism (cells TUNEL+) probably due to the infiltrating TCD8’. Hartmann proposed a review about the current therapeutic options for the acquired hypopigmentary disorders, with particular attention to vitiligo. The relevance of the work is determined also by the evaluation of disorders different from vitiligo and by the overall look to therapies. An interesting paper was published by JAAD on the topical application of tacrolimus for the treatment of vitiligo. Whereas the most part of papers regarding the therapies are exclusively clinical trials, this work correlates the clinical improvement with the modification of the epidermal cytokine pattern. Indeed, Grimes shows a reduction of mRNA for TNF- in the lesional areas after the tacrolimus application. Finally, the clarity and the complexity of the arguments analysed render notable the review of Barbara Gilchrest on melanocyte biology.

Among the pigmentation disorders associated with other diseases, AM Hughes reports on increased risk for non-Hodgkin lymphoma in subjects with high sun sensitivity.

Two different groups suggested the use of statins for the treatment of vitiligo. M Noel and MR Namazi proposed a clinical study and the in vitro biological effects, respectively, considering the antinflammaotry properties of these molecules and the recognized association between vitiligo and autoimmune diseases.


3. MSH, MCH, other hormones, differentiation
(Dr. R. Morandini)

Most papers of this period were devoted to the study of the effect of MSH on obesity (Balthasar, Chagnon, Gibson, Hoggard and Martin). An interesting paper by Nicolaou et al focuses on the inhibition of prostaglandin production by MSH in melanocytes. A new mechanism for the antymycotic agent Miconazol explaining its depigmenting effect involves inhibition of tyrosinase activity (Mun). An exciting review by Steinman et al focuses on the relationship between the immune and the nervous systems: MSH can affect the two systems by regulating the cytokine balance. Zhu et al demonstrated that MSH can inhibit TNF-stimulated integrin expression, cell attachment and invasion.

1. Regulation and signal transduction


2. Global effect on cell in vitro


3. Clinical investigations


- Chagnon YC, Merette C, Bouchard RH, Emond C, Roy MA, Maziade M.


- Gibson WT, Pissios P, Trombly DJ, Luan J, Keogh J, Wareham NJ, Maratos-Flier E, O'Rahilly S, Farooqi IS.


- Steinman L.

4. Others - Not classified


- Bai F, Rankinen T, Charbonneau C, Belsham DD, Rao DC, Bouchard C, Argyropoulos G.

- Bouret SG, Draper SJ, Simerly RB. 


- Fukuzawa T. 

- Grandi G, Chicca M. 

**Cloning, tissue distribution, pharmacology and three-dimensional modelling of melanocortin receptors 4 and 5 in rainbow trout suggest close evolutionary relationship of these subtypes.** Biochem J. 380(Pt 2):475-86, 2004.

- He J, Varma A, Weissfeld LA, Devaskar SU. 

- Kuklin AI, Mynatt RL, Klebig ML, Kiefer LL, Wilkison WO, Woychik RP, Michaud EJ. 

- Martin NM, Small CJ, Sajedi A, Patterson M, Ghatel MA, Bloom SR. 
**Pre-obese and obese agouti mice are sensitive to the anorectic effects of peptide YY(3-36) but resistant to ghrelin.** Int J Obes Relat Metab Disord. 28(7):886-93, 2004.

- Pfau JG, Shadiack A, Van Soest T, Tse M, Molinoff P. 

- van den Pol AN, Acuna-Goycolea C, Clark KR, Ghosh PK. 

- Ramos EJ, Suzuki S, Marks D, Inui A, Asakawa A, Meguid MM. 


- Steininger TL, Kilduff TS, Behan M, Benca RM, Landry CF. 

- Steinman L. 

- Volgin DV, Swan J, Kubin L. 

A large number of abstracts from the 12th Annual Meeting of the PASPCR can be read in Pigment Cell Research 2004; 17:426-452, dealing with photobiology of melanocytes. Many interesting contributions can be found there and hopefully full papers on some of the topics will appear soon. Guzman et al investigated the role of Mitf in melanocytes and UV induced apoptosis. A correlation between Mitf expression and resistance to apoptosis was found. Abdel-Malek and coworkers describe a role for MC1R in DNA repair and maintenance of genomic stability. A Role for alpha-MSH and analogs in survival of human melanocytes with functional MC1R is proposed (PCR abstracts p 436, 438 and 449). Induction of DNA photoproducts was reported to inversely correlate with melanin content of the cultured melanocytes. The melanin production could be regarded as a latent response to UV that offers protection against further exposures, whereas the immediate response is directed towards DNA repair to insure the genomic stability. Bohm and Luger describe the plethora of biological actions of alpha-MSH and also suggest the use of MSH peptides for use in the treatment of various skin diseases.

Hussein and Wood described the importance of the DNA repair genes, hMLH1 and hMLH2. Gene mutations in these genes were shown in melanoma cells and could be induced by UVB.

A recent literature search also showed a series of papers in Mar. Biotechnol. (NY) vol 3: from the year 2001. Nevertheless some of the papers (mostly on the Xiphophorus fish model) are still relevant. In the papers by Nairn et al and Setlow et al an involvement of (enhanced) pigmentation in susceptibility to spontaneous and UV induced melanoma formation (Nairn) and DNA damage (Setlow) is suggested. In this respect, the type of melanin may be important. In our recent paper (van Nieuwpoort et al) we show at the melanosomal level that cultured melanocytes of light skin type increase especially their pheomelanin content upon tyrosine-induced melanogenesis. We also find some important differences for the melanosomal melanin in normal melanocytes compared to that in atypical naevi (Pavel et al). Higher calcium levels were demonstrated in the melanosomal compartment and also in the cytosol. Hoogduijn and colleagues from Bradford, UK showed that melanin may be an important chelator of Ca(2+) thus protecting the cells against H(2)O(2) induced DNA damage. The question arises whether the increased melanosomal calcium in atypical naevi should be regarded as a sign of the protection against damage in these cells or is it merely a reflection of a chronically elevated oxidative stress and the nevus cells trying to limit the oxidative damage. The role of melanin in photoprotection was also topic of the paper by Hearing et al. Tanning capacity between different ethnic groups was described to be strongly dependent on the interactions of melanocytes and keratinocytes and their capacity to transfer and redistribute the already existing melanin in the skin.

Hallberg and Johansson indicate that melanoma may not be just a sunshine and UV phototoxicity story. Their study suggests that influences of full-body resonant frequencies caused by FM and TV broadcasting can amplify the carcinogenic effects from (e.g. UV induced) cell damage.

- Abdel-Malek Z, Kadekarao AL, Terzieva S, Kavanagh R, Hauser J. 


- Bohm M, Luger TA. 


- Fletcher O, Easton D, Anderson K, Gilham C, Jay M, Peto J. 

- Ghiorzo P, Bianchi Scarr GB. 

- Grimbaldeston MA, Pearce AL, Robertson BO, Coventry BJ, Marshman G, Finlay-Jones JJ, Hart PH. 

- Guzman E, Tuchinda C, Hornyak TJ, Strickland FM. 


- Hussein MR, Wood GS. hMLH1 and hMSH2 gene mutations are present in radial growth-phase cutaneous malignant melanoma cell lines and can be induced further by ultraviolet-B irradiation. Exp.Dermatol. 12:872-875, 2003.


- Pavel S, van Nieuwpoort F, Van Der MH, Out C, Pizinger K, Cetkovska P, Smit NP, Koerten HK. 
  Disturbed melanin synthesis and chronic oxidative stress in dysplastic naevi. Eur.J.Cancer 40:1423-1430, 
  2004.


- Rouzaud F, Costin GE, Yamaguchi Y, Valencia JC, Chen K, Bohm M, Abdel-Malek AA, Hearing VJ. 
  Expression patterns of two MC1R Isoforms in human Melanocytes and skin of various Pigmentation/Ethnic 

- Sander CS, Chang H, Hamm F, Elsner P, Thiele JJ. 
  Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. Int.J.Dermatol. 43:326-335, 
  2004.

- Setlow RB, Woodhead AD. 
  Three unique experimental fish stories: Poecilia (the Past), Xiphophorus (the Present), and Medaka (the 

- Shigeta Y, Imanaka H, Yonezawa S, Oku N, Baba N, Makino T. 
  Suppressed permeation of linoleic acid in a liposomal formulation through reconstructed skin tissue. 

- Tsoncheva VL, Milchev GI. 
  Delayed reproductive death and ROS levels in the progeny of irradiated melanoma cells. Z.Naturforsch.[C.] 

- van Nieuwpoort F, Smit NP, Kolb R, van der MH, Koerten H, Pavel S. 
  Tyrosine-induced melanogenesis shows differences in morphologic and melanogenic preferences of 

- Wang Z, Dooley TP, Curto EV, Davis RL, VandeBerg JL. 
  Cross-species application of cDNA microarrays to profile gene expression using UV-induced melanoma in 

  GH, Mason RS. 
  1,25-Dihydroxyvitamin D and three low-calcemic analogs decrease UV-induced DNA damage via the rapid 

- Wu C, Marks S, Park HY, Howard C, Gilchrest BA. 
  T-Oligos induce mitf, tyrosinase and pIk-beta in cultured human melanocytes. Pigment Cell Res. 17:452, 
  2004.

  XPA gene-deficient, SCF-transgenic mice with epidermal melanin are resistant to UV-induced 
It has been suggested that neuromelanin pigment is formed within substantia nigra neurons from the excess cytosolic catecholamine that is not accumulated into synaptic vesicles by the vesicular monoamine transporter-2 (VMAT2). In an interesting paper Liang et al. (2004) verified this concept by using quantitative immunohistochemical methods in human postmortem brain, to examine the relative contents of VMAT2 within neurons that contain different amounts of neuromelanin pigment. The result concurred to reinforce the view that the ventral Substantia nigra neurons accumulate the most neuromelanin pigment, in part because they have the least VMAT2 protein.

The metabolic fate and relevance of iron to Parkinson’ s disease are the focus of two papers by Goetz et al., 2004; Moos et al., 2004). The increase in iron levels with the severity of neuropathological changes in PD; the different localization of iron in glial cells (ferric iron in ferritin) and in neurons (predominantly bound to neuromelanin); the presence of nontransferrin-bound iron in brain extracellular fluids; the apparent lack of ferritin in neurons of the substantia nigra pars compacta, have all be taken to support a central role of iron overload in progressive degeneration of nigrostriatal neurons via formation of reactive oxygen species and cytotoxic protein aggregates. It is proposed that pharmacological control over the transferrin receptor/ divalent metal transporter I (DMT1)- mediated uptake may be a viable strategy to arrest progression of the disease.

Connor et al. (2004) showed that in restless legs syndrome (RLS) neuromelanin cells ferritin, divalent metal transporter 1, ferroportin, and transferrin receptor (TIR) were decreased compared with control, but transferrin was increased. They suggested that RLS may result from a defect in iron regulatory protein 1 in neuromelanin cells that promotes destabilization of the transferrin receptor mRNA, leading to cellular iron deficiency.

Dzierzega-Lecznar et al. (2004) used pyrolysis-gas chromatography/mass spectrometry for structural investigation of neuromelanin isolated from human substantia nigra. The results indicated that the nigral pigment from normal brain tissue does not contain benzothiazine-type monomer units, but has high levels of straight-chain C14-C18 fatty acids and is tightly associated with a lipid component. Finally, based on in vitro experiments, Kimura et al (2004) suggested that metal binding dopamine may posses superoxide dismutase (SOD) like activity: Cu- or Fe-binding dopamine melanin in particular displayed higher SOD-like activity as compared to Zn-binding and metal-free dopamine melanins.
6. Genetics, molecular and developmental biology
(Dr. F. Beermann)


- Guyonneau L, Murisier F, Rossier A, Moulin A, Beermann F. Melanocytes and pigmentation are affected in dopachrome tautomerase knockout mice. Mol Cell Biol 24(8):3396-3403, 2004. Comment: Mice lacking dopachrome tautomerase (Tyrp-2, TRP2) have a coat color phenotype (similar to the Dct-mutant slaty), and a reduction in melanin content.


- Torlakovic EE, Bilalovic N, Nesland JM, Torlakovic G, Florenes VA. Ets-1 transcription factor is widely expressed in benign and malignant melanocytes and its expression has no significant association with prognosis. Mod Pathol 18(18, 2004.


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

- Allegri G, Vogliardi S, Bertazzo A, Costa CV, Seraglia R, Traldi P.
  Tyrosine is generally considered to be the physiological precursor of melanins and tyrosinase the enzyme responsible. However, recent studies have shown that also peroxidases are involved in the biosynthesis of melanins. These enzymes use hydrogen peroxide to oxidise various phenol substrates. In this paper, we used a substrate other than tyrosine, i.e. 5-hydroxytryptophan, to verify if its peroxidase/H2O2-mediated oxidation gave rise to the formation of melanin. We also subjected 5-hydroxytryptophan to the action of tyrosinase, for comparison purposes. We observed that both enzymes converted this substrate to melanin and that peroxidase, in the presence of hydrogen peroxide, was much more effective than tyrosinase in catalysing the oxidative polymerization of 5-hydroxytryptophan, with the formation of insoluble black melanin-like pigments. Samples deriving from the reaction-substrate enzyme were ultrafiltered at different times through an Amicon ultrafiltration cell equipped with an Amicon Diaflo XM-50 membrane, in order to remove the enzyme, and immediately lyophilised. The resulting samples were analysed by matrix assisted laser desorption/ionisation (MALDI) mass spectrometry, which clearly identified several oligomer species in the reaction mixture. This work was undertaken to investigate the possible precursors of neuromelanin and the enzyme responsible for melanogenesis in brain, since although the central nervous system does not contain tyrosinase, it is rich in peroxidase and hydrogen peroxide.

  Fatty acids are common components of biological membranes that are known to play important roles in intracellular signaling. We report here a novel mechanism by which fatty acids regulate the degradation of tyrosinase, a critical enzyme associated with melanin biosynthesis in melanocytes and melanoma cells. Linoleic acid (unsaturated fatty acid, C18:2) accelerated the spontaneous degradation of tyrosinase, whereas palmitic acid (saturated fatty acid, C16:0) retarded the proteolysis. The linoleic acid-induced acceleration of tyrosinase degradation could be abrogated by inhibitors of proteasomes, the multicatalytic proteinase complexes that selectively degrade intracellular ubiquitinated proteins. Linoleic acid increased the ubiquitination of many cellular proteins, whereas palmitic acid decreased such ubiquitination, as compared with untreated controls, when a proteasome inhibitor was used to stabilize ubiquitinated proteins. Immunoprecipitation analysis also revealed that treatment with fatty acids modulated the ubiquitination of tyrosinase, i.e. linoleic acid increased the amount of ubiquitinated tyrosinase whereas, in contrast, palmitic acid decreased it. Furthermore, confocal immunomicroscopy showed that the colocalization of ubiquitin and tyrosinase was facilitated by linoleic acid and diminished by palmitic acid. Taken together, these data support the view that fatty acids regulate the ubiquitination of tyrosinase and are responsible for modulating the proteasomal degradation of tyrosinase. In broader terms, the function of the ubiquitin-proteasome pathway might be regulated physiologically, at least in part, by fatty acids within cellular membranes.

- Boonanuntanasarn S, Yoshizaki G, Iwai K, Takeuchi T.
  Tyrosinase has a role in melanin synthesis and several defects of the tyrosinase gene lead to albinism. Here, we cloned and characterized rainbow trout tyrosinase cDNAs and carried out the molecular and biochemical characterization of albino mutants. Two types of cDNA were cloned: tyrosinase-1 (Tyr-1) and tyrosinase-2 (Tyr-2). Both contained regions predicted to encode structural features of tyrosinase, and phylogenetic analysis confirmed that Tyr-1 and Tyr-2 were members of the tyrosinase family. Tyr-1 transcripts were first detected in embryos at 5 d post-fertilization (dpf) and Tyr-2 transcripts at 15 dpf. 3,4-dihydroxyphenylalanine assays revealed significantly reduced tyrosinase activities in dominant and recessive albino mutants compared with wild-type embryos. However, reverse-transcription PCR showed no differences in the amounts or lengths of the coding regions of Tyr-1 and Tyr-2 transcripts between wild-type embryos and albino mutants. Antisense morpholino oligonucleotides (AMOs) designed to knockdown tyrosinase gene expression in wild-type embryos led to reduced pigmentation in the retina and skin of embryos at 25 and 35 dpf, respectively. Furthermore, the tyrosinase activities of AMO-treated embryos were significantly reduced. We conclude that both Tyr-1 and Tyr-2 are crucial for melanin synthesis in rainbow trout embryos. Furthermore, we describe a potential application of AMOs in the treatment of hyperpigmentation.

- Bubacco L, van Gastel M, Benfatto M, Tepper AW, Canters GW.
  What are the structural features of the active site that define binuclear copper proteins function? Micron. 35(1-2):143-5, 2004.
The structural basis that define the physiological functions of binuclear copper enzymes is discussed in the frame of the data generated by a broad spectroscopic approach, spanning from paramagnetic NMR and pulsed EPR to x-ray absorption spectroscopies. The structural features discussed for the different oxidation and ligation states accessible to a binuclear copper sites are the coordination geometry for the first and second shell, the metal-metal distance and the role of the bridging exogenous ligand(s). A structural model will be presented to rationalize both the differentiation in function within the protein families and the reaction mechanism of those proteins that are enzymatically active.

- Chen P, Solomon EI. 

Reaction thermodynamics and potential energy surfaces are calculated using density functional methods to investigate possible reactive Cu(O2) species for H-atom abstraction in peptidylglycine alpha-hydroxylating monoxygenase (PHM), which has a noncoupled binuclear Cu active site. Two possible mononuclear Cu(O2) species have been evaluated, the 2-electron reduced Cu(II)(M)-OOH intermediate and the 1-electron reduced side-on Cu(II)(M)-superoxo intermediate, which could form with comparable thermodynamics at the catalytic Cu(M) site. The substrate H-atom abstraction reaction by the Cu(II)(M)-OOH intermediate is found to be thermodynamically accessible due to the contribution of the methionine ligand, but with a high activation barrier (approximately 37 kcal/mol, at a 3.0-A active site/substrate distance), arguing against the Cu(II)(M)-OOH species as the reactive Cu/O(2) intermediate in PHM. In contrast, H-atom abstraction from substrate by the side-on Cu(II)(M)-superoxo intermediate is a nearly isoenergetic process with a low reaction barrier at a comparable active site/substrate distance (approximately 14 kcal/mol), suggesting that side-on Cu(II)(M)-superoxo is the reactive species in PHM. The differential reactivities of the Cu(II)(M)-OOH and Cu(II)(M)-superoxo species correlate to their different frontier molecular orbitals involved in the H-atom abstraction reaction. After the H-atom abstraction, a reasonable pathway for substrate hydroxylation involves a "water-assisted" direct OH transfer to the substrate radical, which generates a high-energy Cu(II)(M)-oxyl species. This provides the necessary driving force for intramolecular electron transfer from the Cu(H) site to complete the reaction in PHM. The differential reactivity pattern between the Cu(II)(M)-OOH and Cu(II)(M)-superoxo intermediates provides insight into the role of the noncoupled nature of PHM and dopamine beta-monoxygenase active sites, as compared to the coupled binuclear Cu active sites in hemocyanin, tyrosinase, and catechol oxidase, in O(2) activation.


Melanins are UV shielding pigments found in skin and other light exposed tissues. However, a kind of melanin, named neuromelanin (NM), is found in those deep brain loci that degenerate in Parkinson's disease (PD), where no such a function may be imagined. The NM synthetic pathway, different from the one of eumelanin based on tyrosinase, is still obscure as well as its physiological function. Here we show that under conditions of excess of toxic quinine concentration, nonmelanocytic cell strains (i.e., primary keratinocytes) may accumulate a dark cytoplasmatic pigment that proved to be a melanin. The ability of pigment deposition, possibly driven by peroxidases, is restricted to diploid cells and increases cell survival acting as a sink for potentially hazardous quinones. We suggest that in the basal nuclei, exposed to high level of catecholaminergic neurotransmitters, NM deposition is a relevant antioxidant mechanism by trapping quinones and semiquinones, thus protecting neurons from accumulating damage over many years. In this perspective, just as a hypothesis, we may imagine that PD neuron degeneration is the consequence of a reduced/abrogated ability to produce neuromelanin.

- Elliott RJ, Szabo M, Wagner MJ, Kemp EH, MacNeil S, Haycock JW. 

alpha-MSH signals by binding to the melanocortin-1 receptor (MC-1R) and elevating cyclic AMP in several different cells. The anti-inflammatory properties of this peptide are also believed to be cyclic AMP dependent. The carboxyl terminal tripeptides of alpha-MSH (KPV / KP-D-V) are the smallest minimal sequences reported to prevent inflammation but it is not known if they operate via MC-1R or cyclic AMP. The aim of this study was to examine the intracellular signalling of key MSH and ACTH peptides in human keratinocytes. No elevation in cyclic AMP was detected in either HaCaT or normal human keratinocytes in response to alpha-MSH, KPV or ACTH peptides. Rapid and acute intracellular calcium, however, were observed in HaCaT keratinocytes in response to alpha-MSH (10(-15)-10(-7) M), KPV (10(-15)-10(-7) M), KPD-V (10(-15)-10(-7) M) and ACTH (10(-15)-10(-7) M), but only in the presence of PIA, an adenosine agonist that inhibits the cyclic AMP pathway. Normal keratinocytes responded to all the above peptides but in addition responded to ACTH 1-17 (10(-13)-10(-7) M) in contrast to the HaCaT keratinocytes. Stable transfection of Chinese hamster ovary cells with the MC-1 receptor showed that alpha-MSH and the KPV peptides elevated intracellular calcium.


Congenital defects in retinal pigmentation, as in oculocutaneous albinism Type I (OCA1), where tyrosinase is defective, result in visual abnormalities affecting the retina and pathways into the brain. Transgenic animals expressing a functional tyrosinase gene on an albino genetic background display a correction of all these abnormalities, implicating a functional role for tyrosinase in normal retinal development. To address the function of tyrosinase in the development of the mammalian visual system, we have generated a transgenic mouse model with inducible expression of the tyrosinase gene using the tetracycline (TET-ON) system. We have produced two types of transgenic mice: first, mice expressing the transactivator rtTA chimeric protein under the control of mouse tyrosinase promoter and its locus control region (LCR), and; second, transgenic mice expressing a mouse tyrosinase cDNA construct driven by a minimal promoter inducible by rtTA in the presence of doxycycline. Inducible experiments have been carried out with selected double transgenic mouse lines. Tyrosinase expression has been induced from early embryo development and its impact assessed with histological and biochemical methods in heterozygous and homozygous double transgenic individuals. We have found an increase of tyrosinase activity in the eyes of induced animals, compared with littermate controls. However, there was significant variability in the activation of this gene, as reported in analogous experiments. In spite of this, we could observe corrected uncrossed chiasmatic pathways, decreased in albinism, in animals induced from their first gestational week. These mice could be instrumental in revealing the role of tyrosinase in mammalian visual development.

- Guyonneau L, Murisier F, Rossier A, Moulin A, Beermann F. Melanocytes and pigmentation are affected in dopachrome tautomerase knockout mice. Mol Cell Biol. 2004 Apr;24(8):3396-403. The tyrosinase family comprises three members, tyrosinase (Tyr), tyrosinase-related protein 1 (Tyrp1), and dopachrome tautomerase (Dct). Null mutations and deletions at the Tyr and Tyrp1 loci are known and phenotypically affect coat color due to the absence of enzyme or intracellular mislocalization. At the Dct locus, three mutations are known that lead to pigmentation phenotype. However, these mutations are not null mutations, and we therefore set out to generate a null allele at the Dct gene locus by removing exon 1 of the mouse Dct gene. Mice deficient in Dct [Dct(tm1(Cre)Bee)] lack Dct mRNA and dopachrome tautomerase protein. They are viable and do not show any abnormalities in Dct-expressing sites such as skin, retinal pigment epithelium, or brain. However, the mice show a diluted coat color phenotype, which is due to reduced melanin content in hair. Primary melanocytes from Dct knockout mice are viable in culture and show a normal distribution of tyrosinase and tyrosinase-related protein 1. In comparison to the knockout, the slaty mutation (Dct(slt)/Dct(slt)) has less melanin and affects growth of primary melanocytes severely. In summary, we have generated a knockout of the Dct gene in mice with effects restricted to pigment production and coat color.

- Hall AM, Krishnamoorthy L, Orlow SJ. 25-hydroxycholesterol acts in the Golgi compartment to induce degradation of tyrosinase. Pigment Cell Res. 2004 Aug;17(4):396-406. Oxyysterols play a significant role in cholesterol homeostasis. 25-Hydroxycholesterol (25HC) in particular has been demonstrated to regulate cholesterol homeostasis via oxysterol-binding protein and oxysterol-related proteins, the sterol regulatory element binding protein, and the rate-limiting enzyme of cholesterol biosynthesis, hydroxymethylglutaryl coenzyme A reductase. We have examined the effect of 25HC on pigmentation of cultured murine melanocytes and demonstrated a decrease in pigmentation with an IC(50) of 0.34 microM and a significant diminution in levels of melanogenic protein tyrosinase. Pulse-chase studies of 25HC-treated cells demonstrated enhanced degradation of tyrosinase, the rate-limiting enzyme of melanin synthesis, following endoplasmic reticulum (ER) and Golgi maturation. Protein levels of GS28, a member of an ER/cis-Golgi SNARE protein complex, were also diminished in 25HC-treated melanocytes, however levels of the ER chaperone calnexin and the cis-Golgi matrix protein GM130 were unaffected. Effects of 25HC on tyrosinase were completely reversed by 4alpha-allylcholestan-3alpha-ol, a sterol identified by its ability to reverse effects of 25HC on cholesterol homeostasis. Finally, the addition of 25HC to lipid deficient serum inhibited correct processing of tyrosinase. We conclude that 25HC acts in the Golgi compartment to regulate pigmentation by a mechanism shared with cholesterol homeostasis.

- Hosaka E, Soma Y, Kawa Y, Kaminaga H, Osumi K, Ooka S, Watabe H, Ito M, Murakami F, Mizoguchi M. Effects of ultraviolet light on melanocyte differentiation: studies with mouse neural crest cells and neural crest-derived cell lines. Pigment Cell Res. 17(2):150-7, 2004. To evaluate the etiologic role of ultraviolet (UV) radiation in acquired dermal melanocytosis (ADM), we investigated the effects of UVA and UVB irradiation on the development and differentiation of melanocytes in primary cultures of mouse neural crest cells (NCC) by counting the numbers of cells positive for KIT (the receptor for stem cell factor) and for the L-3,4-dihydroxyphenylalanine (DOPA) oxidase reaction. No significant differences were found in the number of KIT- or DOPA-positive cells between the UV-irradiated cultures and the non-irradiated cultures. We then examined the effects of UV light on KIT-positive cell lines derived from mouse NCC cultures. Irradiation with UVA but not with UVB inhibited the tyrosinase activity in a tyrosinase-positive cell line.
have used deletion mutants of the Dct promoter, transfections with developmentally relevant transcription factors, embryogenesis, prior to other members of the tyrosinase gene family important for regulating pigmentation. We The murine dopachrome tautomerase (Dct) gene is expressed early in melanocyte development during activation of dct expression with mitf.


The murine dopachrome tautomerase (Dct) gene is expressed early in melanocyte development during embryogenesis, prior to other members of the tyrosinase gene family important for regulating pigmentation. We have used deletion mutants of the Dct promoter, transfections with developmentally relevant transcription factors, and/or endothelin-1.


The endothelin receptor B gene (Ednrb) encodes a G-protein-coupled receptor that is expressed in a variety of cell types and is specifically required for the development of neural crest-derived melanocytes and enteric ganglia. In humans, mutations in this gene are associated with Waardenburg-Shah syndrome, a disorder characterized by pigmentaion defects, deafness and megacoloni. To address the question of whether melanocyte development depends entirely on a cell-autonomous action of Ednrb, we performed a series of tissue recombination experiments in vitro, using neural crest cell cultures from mouse embryos carrying a novel Ednrb-null allele characterized by the insertion of a lacZ marker gene. The results show that Ednrb is not required for the generation of early neural crest-derived melanoblasts but is required for the expression of the differentiation marker tyrosinase. Tyrosinase expression can be rescued, however, by the addition of Ednrb wild-type neural tubes. These Ednrb wild-type neural tubes need not be capable of generating melanocytes themselves, but must be capable of providing KIT ligand, the cognate ligand for the tyrosine kinase receptor KIT. In fact, soluble KIT ligand is sufficient to induce tyrosinase expression in Ednrb-deficient cultures. Nevertheless, these tyrosinase-expressing, Ednrb-deficient cells do not develop to terminally differentiated, pigmented melanocytes. Pigmentation can be induced, however, by treatment with tetradeconyl phorbol acetate, which mimics EDNRB signaling, but not by treatment with endothelin 1, which stimulates the paralogous receptor EDNRA. The results suggest that Ednrb plays a significant role during melanocyte differentiation and effects melanocyte development by both cell non-autonomous and cell-autonomous signaling mechanisms.


Seven kinds of hydroxycinnamic acid derivatives identified as 3-cafeoylquinic acid (3-CQA), 4-cafeoylquinic acid (4-CQA), 5-cafeoylquinic acid (5-CQA), 5-feruloylquinic acid (5-FQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA), and 4,5-dicaffeoylquinic acid (4,5-diCQA) by MS, (1)H NMR, and HPLC analyses were isolated from low-quality (immature) and commercial quality green coffee beans. The quantity of chlorogenic acid isomers (10.4 g/100 g), especially 5-CQA, in commercial green coffee beans [West Indische Bereiding (West India processing beans from Sumatra Island, Indonesia, WIB)] was higher than that in low-quality beans [9.1 g/100 g, Eerste Kwaliteit (Export low-quality beans from Java Island, Indonesia, EK-1, grade 4)], whereas little difference in diCQAs was detected between the two kinds of beans. The free radical scavenging activity of these isolates was evaluated in assay systems using DPPH free radicals and superoxide anion radicals generated by xanthine-XOD. The diCQAs showed strong (1.0-1.8-fold) free radical scavenging activity compared to commonly used antioxidants such as alpha-tocopherol and ascorbic acid. The potency order of superoxide anion radical scavenging activity was diCQAs > caffeic acid, CQAs > 5-FQA. The activities of the diCQAs were twice as effective as those of CQAs and 4 times as effective as that of 5-FQA. The diCQAs also exhibited more potent (2.0-2.2-fold) tyrosinase inhibitory activities compared to CQAs, arbutin, and ascorbic acid. The isolates exhibited antiproliferation activities in four cancer cell lines, U937, KB, MCF7, and WI38-VA. Among these, KB cells were most sensitive (IC(50) = 0.10-0.56 mM).


Crustacean hemocyanin as oxygen carrier and catecholoxidase as enzymes belong to the same protein family (type 3 copper proteins) sharing very similar active sites. Treatment with SDS of these hemocyanins results in an opening of the entrance to the active site for bulky phenolic compounds. This demonstrates, that almost all copper proteins (sharing very similar active sites) are capable of catalyzing the oxidation of phenolic compounds. This is further supported by results from experiments with recombinant enzymes.

and gel shift assays to define transcriptional determinants of Dct expression. Deletion mutagenesis studies show that sequences within the proximal 459 nucleotides are critical for high level expression in melanocytic cells. This region of the promoter contains candidate binding sites for the transcription factors Sox10 and Mitf. Transfections into 293T and NIH3T3 cells show that Sox10 and Mitf independently activate Dct expression, and, when co-transfected, synergistically activate Dct expression. To support the notion that Sox10 acts directly upon the Dct promoter to activate gene expression, direct interaction of Sox10 was demonstrated using gel shifts of oligonucleotide probes derived from promoter sequences within the region required for Sox10-dependent induction. These results suggest that a combinatorial transcription factor interaction is important for expression of Dct in neural crest-derived melanocytes, and support a model for sequential gene activation in melanocyte development whereby Mitf, a Sox10-dependent transcription factor, is expressed initially before an early melanocyte differentiation gene, Dct, is expressed.


Pterin-dependent tyrosine hydroxylase has been described to occur occasionally in melanocytes. It is therefore important to quantify the mRNA of this enzyme in pigment cells to understand whether this enzyme can take an active part in pigment formation. A real-time reverse transcription-polymerase chain reaction method was used to quantify tyrosine hydroxylase mRNA in melanocytes and melanoma cells. The calibrator was obtained by amplification of a segment of cDNA from tyrosine hydroxylase mRNA, which included the target thus allowing enumeration of the number of transcripts per cell. In melanocytes (n = 3), tyrosine hydroxylase mRNA ranged from non-detectable to 0.000492 transcripts/cell and in melanoma cells from non-detectable to 0.005340 transcripts/cell. In neuroblastoma cells, the median tyrosine hydroxylase mRNA number was 0.4 transcripts/cell (range 0.02-25 transcripts/cell). The amount of tyrosine hydroxylase mRNA in the pigment cells was far less than the mRNA concentrations of four melanocyte-specific proteins measured in the same melanocytes and melanoma cells. We conclude that on the average less than 1 of 1000 melanocytes and melanoma cells contains at least one tyrosine hydroxylase mRNA molecule. Consequently, in 999 of 1000 cells translation into the corresponding enzyme protein cannot occur because of the lack of an mRNA template. Thus, in these cells there is no pterin-dependent tyrosine hydroxylase that can contribute to pigment formation by producing priming amounts of l-dopa for proper function of tyrosinase.


The involvement of phospholipase D (PLD) in the regulation of melanogenesis was examined. Treatment of B16 mouse melanoma cells with 12-O-tetradecanoylphorbol-13-acetate (TPA) resulted in the activation of PLD and a decrease in melanin content. 1-Butanol, but not 2-butanol, completely blocked the TPA-induced inhibition of melanogenesis, suggesting the involvement of PLD in this event. Reverse transcription-PCR and immunoblot analyses revealed the existence of both PLD isozymes, PLD1 and PLD2, in B16 cells. When PLD1 or PLD2 was introduced into those cells by an adenoviral gene-transfer technique, both PLD1 and PLD2 were activated by TPA. When PLD1 and PLD2 were overexpressed, PLD2 potently caused a decrease in melanin content, whereas the effect of PLD1 expression on melanin content was minimal. Over-expression of PLD2 itself did not affect protein kinase C activity, as assessed by the intracellular distribution and levels of expression of each isoform expressed in B16 cells. The effects of TPA on the down-regulation of basal or alpha-melanocyte-stimulating hormone-enhanced melanogenesis were almost completely blocked by expressing a lipase activity-negative mutant, LN-PLD2, but not by LN-PLD1. Further, the PLD2-induced decrease in melanin content was accompanied by a decrease in the amount and activity of tyrosinase, a key enzyme in melanogenesis, whereas the mRNA level of tyrosinase was unchanged by the over-expression of PLD2. Moreover, treatment with proteasome inhibitors completely blocked the PLD2-induced down-regulation of melanogenesis. Taken together, the present results indicate that the TPA-induced down-regulation of melanogenesis is mediated by PLD2 but not by PLD1 through the ubiquitin proteasome-mediated degradation of tyrosinase. This suggests that PLD2 may play an important role in regulating pigmentation in vivo.


The quality of nascent protein folding in vivo is influenced by the microdynamics of the proteins. Excessive collisions between proteins may lead to terminal misfolding, and the frequency of protein interactions with molecular chaperones determines their folding rates. However, it is unclear how immature protein dynamics are regulated. In this study, we analyzed the diffusion of immature tyrosinase in the endoplasmic reticulum (ER) of non-pigmented cells by taking advantage of the thermal sensitivity of the tyrosinase. The diffusion of tyrosinase tagged with yellow fluorescence protein (YFP) in living cells was directly measured using fluorescent correlation spectroscopy. The diffusion of folded tyrosinase in the ER of cells treated with brefeldin A, as measured by
fluorescent correlation spectroscopy, was critically affected by the expression level of tyrosinase-YFP. Under defined conditions in which random diffusional motion of folded protein was allowed, we found that the millisecond-order diffusion rate observed for folded tyrosinase almost disappeared for the misfolded molecules synthesized at a nonpermissive high temperature. This was not because of enhanced aggregation at the high temperature, as terminally misfolded tyrosinase synthesized in the absence of calnexin interactions showed comparable, albeit slightly slower, diffusion. Yet, the thermally misfolded tyrosinase was not immobilized when measured by fluorescence recovery after photobleaching. In contrast, terminally misfolded tyrosinase synthesized in cells in which alpha-glucosidases were inhibited showed extensive immobilization. Hence, we suggest that the ER represses random fluctuations of immature tyrosinase molecules while preventing their immobilization.

- **Kim DS, Park SH, Park KC.**

- **Kim H, Choi J, Cho JK, Kim SY, Lee YS.**

- **Land EJ, Ramsden CA, Riley PA.**

- **Li CY, Gao TW, Wang G, Han ZY, Shen Z, Li TH, Liu YF.**

- **Mamnone T, Marenus K, Muizzuddin N, Maes D.**

  The biochemical synthesis of melanin in humans is understood in some detail. However, little is known about melanin degradation and catabolism of melanin. We hypothesize that human skin contains enzymes that degrade melanin.
and these enzymes can be used to reduce skin color. To test this hypothesis, HaCaT keratinocytes and normal human keratinocytes in culture were pulse labeled for one hour with radiolabeled synthetic melanin. This melanin was synthesized in vitro using tyrosinase enzyme from mushrooms and using radiolabeled [14C]3,4-dihydroxyphenylalanine (DOPA) as a substrate. After the initial pulse labeling, samples of both the cells and media were taken at 2, 4, 6 and 18 hours. Over these time periods the counts remaining in the media and cell fraction were significantly decreased. This data suggests the need for new protein synthesis and the lysosome organelle function for the degradation. Melanin degrading extracts isolated from Aspergillus fumigatus and Saccharomyces cerevisiae were applied to human skin. These extracts cause significant reduction in UVB induced pigmentation. These extracts may be useful in developing new whitening products to even skin color and tone.

Nerya O, Musa R, Khatib S, Tamir S, Vaya J.

The inhibition of tyrosinase is one of the major strategies to treat hyperpigmentation. Various limitations are associated with many of these inhibitors, such as high cytotoxicity, poor skin penetration and low stability in formulations. In continuation of our previous study [J. Agric. Food Chem. 51 (2003) 1201], showing that isoliquiritigenin chalcone (ILC) is a potent tyrosinase inhibitor, the present study aims to characterize the chalcone family as new tyrosinase inhibitors, and demonstrate their potential whitening potency. Nine mono-, di-, tri- and tetrahydroxychalcones were tested as inhibitors of tyrosinase mono- and diphenolase activities, showing that the most important factor in their efficacy is the location of the hydroxyl groups on both aromatic rings, with a significant preference to a 4-substituted B ring, rather than a substituted A ring. Neither the number of hydroxyls nor the presence of a catechol moiety on ring B correlated with increasing tyrosinase inhibition potency. 4-Hydroxychalcone (4-HC), ILC and Butein inhibited tyrosinase and shortened the lag period of enzyme monophenolase activity from about 490 min (control) to 30 min (ILC). As pigmentation also results from auto-oxidation, the antioxidant activity of 4-HC, ILC and Butein, were tested. Results showed that chalcones are also potent antioxidants, with Butein the most potent. We may conclude that chalcones are potentially potent new depigmentation agents, with their double effect of reduction and antioxidant activity.


Protein kinase C (PKC), a family of at least eleven isoforms, mediates numerous cell functions. In human melanocytes, alpha, beta, delta, epsilon and zeta isoforms of PKC are expressed, but uniquely PKC-beta activates tyrosinase, the key and the rate-limiting enzyme in melanogenesis, by phosphorylating specific serine residues on its cytoplasmic domain. To investigate the mechanism by which only PKC-beta phosphorylates tyrosinase, we examined the expression of receptor for activated C-kinase-I (RACK-I), a receptor specific for activated PKC-beta, on the surface of melanosomes, the specialized organelle in which melanogenesis occurs. Immunoblot analysis of purified melanosomes revealed that RACK-I is readily detectable. Immunoprecipitation of RACK-I from purified melanosomes, followed by immunoblot analysis using antibody against PKC-beta, revealed abundant PKC-beta, whereas PKC-alpha was not detected when immunoblot analysis was performed using antibody against PKC-alpha. Activation of PKC in melanocytes increased the level of PKC-beta co-immunoprecipitated with RACK-I, while the level of melanosome-associated RACK-I decreased when melanocytes were treated chronically with the 12-0-tetradecanoyl-phorbol 13-Acetate (TPA), a condition known to deplete PKC and reduce tyrosinase activity. Immunoprecipitation with RACK-I antibody co-precipitated fewer PKC-beta in the presence of UV-activated 1, 1'-decamethylenebis-4-aminoquinaldinium di-iodide (DECA), known to disrupt the interaction between activated PKC-beta and RACK-I. Treatment of intact melanocytes with DECA also decreased tyrosinase activity. Moreover, suppression of RACK-I expression by transfecting melanocytes with siRNA against RACK-I reduced the basal tyrosinase activity and blocked TPA-induced increases in tyrosinase activity. Taken together, these results demonstrate that RACK-I anchors activated PKC-beta on the melanosome membrane, allowing PKC-beta to phosphorylate tyrosinase.

Partridge JM, Weatherby SJ, Woolmore JA, Highland DJ, Fryer AA, Mann CL, Boggild MD, Ollier WE, Strange RC, Hawkins CP.

Multiple sclerosis (MS) risk is determined by environment and genes. The authors investigated in 419 cases and 422 controls if polymorphism in the vitamin D receptor (VDR), melanocortin-1 receptor (MC1R), and tyrosinase (TYR) genes is linked with MS risk and outcome. VDR ff was associated with reduced (odds ratio [OR] = 0.59) and MC1R His294-encoding alleles with increased (OR = 2.21) risk. MC1R Glu84/Glu84 was linked with disability (OR = 5.65). These preliminary data suggest a role for these genes in MS pathogenesis.

Prohaska JR, Gybina AA.
Copper is an essential cofactor for approximately a dozen cuproenzymes in which copper is bound to specific amino acid residues in an active site. However, free cuprous ions react readily with hydrogen peroxide to yield the deleterious hydroxyl radical. Therefore, copper homeostasis is regulated very tightly, and unbound copper is extremely low in concentration. Copper imported by the plasma membrane transport protein Ctr1 rapidly binds to intracellular copper chaperone proteins. Atox1 delivers copper to the secretory pathway and docks with either copper-transporting ATPase ATP7B in the liver or ATP7A in other cells. ATP7B directs copper to plasma ceruloplasmin or to biliary excretion in concert with a newly discovered chaperone, Murr1, the protein missing in canine copper toxicity. ATP7A directs copper within the transgolgi network to the proteins dopamine betamonooxygenase, peptidylglycine alpha-amidating monoxygenase, lysi1 oxidase, and tyrosinase, depending on the cell type. CCS is the copper chaperone for Cu,Zn-superoxide dismutase; it delivers copper in the cytoplasm and intermitochondrial space. Cox17 delivers copper to mitochondria to cytochrome c oxidase via the chaperones Cox11, Sco1, and Sco2. Other copper chaperones may exist and might include metallothionein and amyloid precursor protein (APP). Genetic and nutritional studies have illustrated the essential nature of these copper-binding proteins; alterations in their levels are associated with severe pathology.


The synthesis of melanin intermediates through tyrosinase (TYR) involves the production of cytotoxic free radicals. By using recombinant adenoviruses that express TYR, tyrosinase-related protein 1 (TYRP1) or DOPAchrome tautomerase (DCT), we analyzed the biological function of these proteins with regard to melanin production and the growth of melanocytes, fibroblasts, melanoma cells and nonmelanoma cancer cells. High-level expression of TYR produced newly synthesized melanin and induced cell death in all of these cells. However, when TYRP1 or DCT was coexpressed with TYR in melanocytes and melanoma cells, TYR-mediated cell death was clearly decreased. This decrease was not observed in nonmelanocytic cells. Western blot analysis and measurement of enzyme activity revealed that the expression of TYRP1 or DCT had little effect on the amount or activity of coinroduced TYR in either the melanocytic or nonmelanocytic cells. In cells expressing both TYR and TYRP1 or TYR and DCT, the total amount of melanin and/or eumelanin increased substantially more than that in cells expressing TYR alone. On the other hand, the level of pheomelanin was similar in these three cell types. These findings suggest that TYRP1 and DCT play an important role in suppressing TYR-mediated cytotoxicity in melanocytic cells without decreasing TYR expression and/or activity. These biological activities of TYRP1 and DCT may work through the interaction with TYR in melanosomal compartment.

- Rundshagen U, Zuhilke C, Opitz S, Schwinger E, Kasmann-Kellner B.


Oculocutaneous albinism (OCA) is caused by a deficiency of melanin synthesis and characterized by generalized hypopigmentation of skin, hair, and eyes. Due to the hypopigmentation of the retinal pigment epithelium, OCA is usually associated with congenital visual impairment, in addition to an increased risk of skin cancer. OCA is a genetically heterogeneous disease with distinct types resulting from mutations in different genes involved in the pathway which results in pigmentation. OCA1 is associated with mutations in the TYR gene encoding tyrosinase. OCA2 results from mutations in the P gene encoding the P protein and is the most common form of OCA. OCA3, also known as rufous/red albinism, is caused by mutations in the TYRP1 gene, which encodes the tyrosinase-related protein 1. Recently, OCA4 was described as a new form of OCA in a single patient with a splice site mutation in the MATP gene (or AIM1), the human ortholog of the murine underwhite gene. The similarity of MATP to transporter proteins suggests its involvement in transport functions, although its actual substrate is still unclear. We screened 176 German patients with albinism for mutations within the MATP gene and identified five individuals with OCA4. In this first report on West European patients, we describe 10 so far unpublished mutations, as well as two intronic variations, in addition to two known polymorphisms.

- Sasaki M, Kizawa K, Igarashi S, Horikoshi T, Uchiwa H, Miyachi Y.


Nitric oxide (NO) is a potent intercellular mediator of melanogenesis, whereas metallothionein (MT) is an inducible intracellular antioxidant that has been reported to scavenge NO. We investigated the existence and induction of MT in melanocytes, and its inhibitory effect on NO-induced melanogenesis. The expression of MT was detected in melanocytes, however, at a lower level than in keratinocytes, and its induction was possible by the addition of zinc chloride. Further, an NO-stimulated increase of tyrosinase activity in melanocytes was remarkably suppressed, when MT was induced prior to NO stimulation. Melanogenesis was also suppressed, when dexamethasone was used to induce MT. However, an NO-stimulated increase of tyrosinase expression was not suppressed at the gene and protein level, when MT was induced in melanocytes. The same suppressive effect of melanogenesis was also observed, when alpha-melanocyte-stimulating hormone or endothelin-1 was used as a stimulator. Because these results implied a mechanism other than NO scavenging to explain the suppressive effect of MT induction on melanogenesis, the direct inhibition of tyrosinase by MT was examined. Melanosome
fractions were prepared from melanocytes, whose melanogenesis was suppressed by the induction of MT. Tyrosinase suppression was observed in the melanosome fractions, which was neutralized by the addition of anti-MT antibody. These results suggest that MT induction may be effective to suppress melanogenesis stimulated by NO as well as other melanogens, and these suppressive effects might be due to a direct inhibition of tyrosinase activity in melanosome and not a scavenging effect of NO.

- Schmutz SM, Berryere TG, Ciobanu DC, Mileham AJ, Schmidt BH, Fredholm M.
  **A form of albinism in cattle is caused by a tyrosinase frameshift mutation.** Mamm Genome. 15(1):62-7, 2004. We used PCR amplification of cDNA prepared from skin biopsies to determine the full-length protein-coding sequence of tyrosinase (TYR) in cattle of several coat colors. An insertion of a cytosine was detected in an albino Braunvieh calf, which resulted in a frameshift which caused a premature stop codon at residue 316. This insertion was found in the homozygous state in this calf and the genomic DNA of two related albino calves. All six parents of these calves were heterozygous for this insertion. However, an albino Holstein calf did not have this insertion, nor was any other mutation detected in the partial TYR sequence obtained from the genomic DNA available. Diagnostic genotyping tests were developed to detect this mutation in Braunvieh cattle.

- Schweikardt T, Jaenicke E, Decker H.

- Siddiqui NI, Preaux G, Gielens C.

- Siegbahn PE
  **The catalytic cycle of catechol oxidase.** Biol Inorg Chem, 2004 Jun 5 [Epub ahead of print]

- Smith DR, Spaulding DT, Glenn HM, Fuller BB.

- Svedine S, Wang T, Halaban R, Hebert DN.

  The endoplasmic reticulum (ER) quality-control machinery maintains the fidelity of the maturation process by sorting aberrant proteins for ER-associated protein degradation (ERAD), a process requiring retrotranslocation from the ER lumen to the cytosol and degradation by the proteasome. Here, we assessed the role of N-linked glycans in ERAD by monitoring the degradation of wild-type (Tyr) and albino mutant (Tyr(C85S)) tyrosinase.
Initially, mutant tyrosinase was established as a genuine ERAD substrate using intact melanocyte and semi-permeabilized cell systems. Inhibiting mannose trimming or accumulating Tyr(C85S) in a monoglucosylated form led to its stabilization, supporting a role for lectin chaperones in ER retention and proteasomal degradation. In contrast, ablating the lectin chaperone interactions by preventing glucose trimming caused a rapid disappearance of tyrosinase, initially due to the formation of protein aggregates, which were subsequently degraded by the proteasome. The co-localization of aggregated tyrosinase with protein disulfide isomerase and BiP, but not calnexin, supports an ER organization, which aids in protein maturation and degradation. Based on these studies, we propose a model of tyrosinase degradation in which interactions between N-linked glycans and lectin chaperones help to minimize tyrosinase aggregation and also target non-native substrates for retro-translocation and subsequent degradation.


In order to investigate the role of tryptophan and its metabolites in biogenesis of melanins, a study on the enzymatic reaction of 3-hydroxykynurenine with tyrosinase and peroxidase was performed. The reaction at different pH values was monitored by sampling at different times, with ultrafiltration used before analysis by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The data obtained in this way showed that oligomerization processes take place with both enzymes, but with different behaviour, also depending on pH. 3-Hydroxykynurenine in the presence of tyrosinase at pH 6.0 leads to formation of xanthommatin, and at pH 8.0 hydroxanthommatin is formed in the first step of the reaction followed by formation of black-brown pigments. In contrast, the formation of oligomerization products by peroxidase action is observed in high yields under both acidic and basic conditions; however, at pH 6.0, a more extensive oligomerization process is observed. Thus peroxidase is able to activate oligomerization analogous to that observed in the case of tyrosinase without depending on the variation of pH. Due to the early formation of decarboxylated hydroxykynurenine, hydroxanthommatin and decarboxylated hydroxanthommatin, the enzymatic reaction leads to mixed oligomers, which can be considered as precursors of new pathways in pigment production.


Melanosomes, specific organelles produced only by melanocytes, undergo a unique maturation process that involves their transition form amorphous rounded vesicles to fibrillar ellipsoid organelles, during which they move from the perinuclear to the distal areas of the cells. This depends upon the trafficking and processing of gp100 (also known as Pmel17 and the silver protein), a protein of great interest, because it elicits immune responses in melanoma patients but in which specific function(s) remains elusive. In this study, we have used biochemical and immunological approaches to more critically assess the synthesis, processing, glycosylation, and trafficking of gp100. We now report that gp100 is processed and sorted in a manner distinct from other melanosomal proteins (such as tyrosinase, Tyrp1 and Dct) and is predominantly delivered directly to immature melanosomes following its rapid processing in the endoplasmic reticulum and cis-Golgi. Following its arrival, gp100 is cleaved at the amino and at the carboxyl terminus in a series of specific steps that result in the reorganization of immature melanosomes to the fibrillar mature melanosomes. Once this structural reorganization occurs, melanogenic enzymes begin to be targeted to the melanosomes, which are then competent to synthesize melanin pigment.


There is no effective treatment for melanoma, a fatal skin cancer occurring with increasing frequency. Dietary tyrosine restriction lowers systemic tyrosine and suppresses the growth of melanoma in mice, but this is not tolerated by human resulting in nausea, vomiting, and weight loss. We report here the successful use of oral polymeric microcapsules containing tyrosinase to lower the systemic tyrosine level in the rats. We found that microencapsulated tyrosinase incubated with intestinal content of rats selectively lowered the tyrosine level. We then studied the daily oral administration of microencapsulated tyrosinase in rats of one dose a day, two doses a day, and three doses a day over a period of up to 22 days. With three doses a day, the tyrosine levels in the test group decreased to 68.8% of the control group by day 4 and then decreased to 52.6% after this and remained at this level throughout the 22 days test period. This is the level shown earlier by other workers using dietary restriction of tyrosine to result in suppression of growth of melanoma. However, unlike dietary tyrosine restriction, oral tyrosinase microcapsules did not result in adverse effects nor significant differences in growth (weight gain) when compared to the control group. This approach can also be used for the lowering of systemic tyrosine in hypertyrosinemia, an inborn error of metabolism.
8. Melanosomes
(Dr. J. Borovansky)

Literature on melanosomes has been very rich and diverse recently. As for the transport, molecular mechanisms underlying the disrupted melanosome transport in pigmented basal epithelioma (Sakurada et al) and a role for spectrin in dynactin-dependent melanosome transport (Aspengren&Wallin) were described. Molecular mechanisms of Rab-dependent myosin-driven organelle motility on actin tracks were reviewed (Seabra&Coudrier). Ultrastructural studies revealed melanosome irregularities in the skin of DBA/2J mice (Nguyen&Wei) and membrane defects and irregular shape of melanosomes in hair follicles of llamas (Cecchi et al). These results are surprising because melanosome abnormalities are typical of tumour melanosomes (or melanosomes in cultured cells). First ultrastructural characterization of „animal” type of melanoma in man mentioned a high number of aberrant melanosomes (Kazakov et al). Defects of melanosome morphogenesis were observed in four of five studied murine models of Hermansky&Pudlak syndrome (Nguyen&Wei). Increased concentration of tyrosine in cultivation media induced characteristic changes in melanosome shape in relation to the skin type origin of cultured melanocytes (Van Nieuwpoort et al). Melanosomes can act as energy-converting device. This unique feature can be exploited in selective killing pigment cells in the treatment of RPE disorders (Roegener et al). X-ray microanalysis detected a high amount of calcium and iron in melanosomes of dysplastic naevi which can be interpreted as an evidence for high phaeomelanin content in and a predisposition to oxidative stress of dysplastic naevi cells (Pavel et al). More details concerning mechanisms of tyrosinase activation by protein kinase C-β in melanosomes (Park et al) and possible participation of sodium/hydrogen exchanger in the regulation of intramelanosomal pH (Smith et al) were reported. The study of Vetrini et al of the dependence of Oa1 expression on the microphthalmia transcription factor established a link between genes involved in melanin synthesis and melanosome biogenesis. Pseudopapillary tumour of pancreas extended the group of non-melanoma neoplasms containing melanosomes (Chen et al). Gliomas can be targetted by immune-based strategies that prime T cells to epitopes from melanoma (and melanosome) antigens (Prins et al). Two excellent reviews dealing with the secretory function of lysosomes (Stinchcombe et al) and with genes mutated in murine Hermansky-Pudlak syndromes (Wei et al) make the list of contemporary melanosome literature complete.

- Cecchi T, Cozzali C, Passamonti P, Cecarelli P, Pucciarelli F, Gargiulo AM, Frank EN, Renieri C. Melanins and melanosomes from llama (lama glama L.). Pigment Cell Res 17(3): 307-311, 2004. Comments: Ultrastructural study of melanosomes in hair follicles of 8 coloured (black, dark brown and light brown with different shades) Argentinian llamas. Immature melanosomes had often an irregular shape and even fully melanized melanosomes displayed an irregular outline, deposition of melanin was mostly granular, limiting membranes sometimes missing. Total melanin, eu- and phaeomelanin concentrations in hair were studied by means of spectrophotometric methods.
- Chen C, Jing W, Gulati P, Vargas H, French SW. Melanocytic differentiation in a solid pseudopapillary tumor of the pancreas. J Gastroenerol 39(6): 579-583, 2004. Comments: Case report of a patient with solid pseudopapillary tumour of the pancreas (SPT) demonstrating for the first time melanocytic differentiation of SPT cells. The tumour cells were positive for S100 and HMB45 and gave positive Fontana reaction. Electron microscopy revealed the presence of premelanosomes and melanosomes, mostly of ovoid lamellar type but round granular melanosomes were also present. Many melanosomes exhibited uneven melanin deposition and/or signs of disintegration. The authors conclude that SPT cells are derived from neural crest.
- Kazakov D, Rutten A, Werner K, Michal M. Melanoma with prominent pigment synthesis (animal-type melanoma): A case report with ultrastructural studies. Am J Dermatopathol 26(4): 290-297, 2004. Comments: Histopathological and ultrastructural study of a very rare melanoma type. Tumour cells stained positively inNSE, NKI/C3, tyrosinase, p53, CD68 and MiTF (nuclei). The cytoplasm contained numerous single stage II-IV melanosomes, compound melanosomes were rare. There was a high number of aberrant melanosomes. Melanophages were a minor component of the lesion.
trafficking proteins, nine novel genes found only in higher eukaryotes encode members of 3 protein complexes termed BLOCs (Biogenesis of Lysosome-related Organelle Complexes). The article contains tables summarizing positional cloning of murine/human HPS genes with their functional links, physiological and cellular effects of mutations of murine HPS genes, colour figure with coat colour of all known HPS mutant mice and a colour scheme of the structure of BLOC complexes.

- Nguyen T, Wei ML.
*Comments:* A quantitative analysis of five murine models of the HP syndrome. (For another ten models see Nguyen et al/ J Invest Dermatol 119, 2002, 1156). Four of the studied strains (sandy, muted, buff and subtle gray) had defects of melanosome morphogenesis unlike the ashen strain which had problem in melanosome secretion. Three cellular mechanisms contributing to hypopigmentation seen in the HP syndrome were documented: 1) exocytosis of immature melanosomes from melanocytes with subsequent keratinocyte uptake; 2) decreased intramelanocyte steady state numbers of melanosomes available for transfer to keratinocytes; 3) accumulation of melanosomes within melanocytes due to defective exocytosis (e.g. in ashen strain). In addition, melanosomes in normal DBA/2J strain were reported to be abnormal.

- Park HY, Wu H, Killoran CE, Gilchrest BA.
*Comments:* Immunoblot analysis of purified melanosomes showed that RACK-I was easily detectable. Immunoprecipitation of RACK-I from purified melanosomes followed by immunoblot analysis using antibody against protein kinase C-β revealed abundant PKC-β; similar experiment with antibody against PKC-α did not detect any PKC-α. Immunoprecipitation with RACK-I antibody coprecipitated fewer PKC-β in the presence of DECA known to disrupt the interaction between activated PKC-β and RACK-I. These findings suggested the conclusion mentioned in the title.

- Pavel S, van Nieuwpoort F, van der Meulen H, Out C, Pizinger K, Cetkovská P, Smit NPM, Koerten HK.
*Comments:* X-ray microanalysis of melanosomes in situ revealed a significantly higher content of sulphur (considered as an indicator of melanogenesis) and iron in melanosomes in dysplastic naevi and melanoma cells in comparison with those in normal melanocytes and pigment cells of banal naevi. Calcium level in melanosomes in dysplastic naevi and in melanoma cells was also higher than in melanocytes and banal naevi cells. These data bring direct evidence for higher phaeomelanogenesis in melanosomes of dysplastic naevi predicted in studies analyzing phaeomelanin in whole tissue (Salopek et al / Pigment Cell Res 4, 1991, 172; Jimbow et al / J Invest Dermatol 100, 1993, 259s) and explain oxidative stress in dysplastic naevi and melanoma cells demonstrated in this study.

- Prins RM, Odesa SA, Liau LM.
*Comments:* Melanocytes and astrocytes are both derived embryologically from neural ectoderm and their malignant counterparts - melanoma and glioma have been shown to share common antigens. In this study two murine glioma cell lines –GL26 and GL261 – expressed melanoma antigens gp100 and TRP-2. These melanoma-associated antigens (MAA) on GL26 cells could be targetted by lymphocytes primed with gp100 and TRP-2 peptide-pulsed dendritic cells vaccination. MAA peptide-pulsed dendritic cells induced a protective immunity against GL26 cells implanted into mouse brains.

- Roegener J, Brinkmann R, Lin CP.
*Comments:* Upon laser irradiation the retinal pigment epithelium melanosomes form hot spots and initiate microscopic cavitation bubbles that expand and collapse on the time scale of 0.1-1 µs. The mechanical action associated with bubble expansion and implosion imparts damage to cells. (See also Lin&Kelly/App Phys Let 72, 1998,2800). This time authors used a new method to measure bubble formation within the RPE based on the transient increase in probe backscattering signal during the life time of the bubble. For single 12nm pulses the threshold for bubble formation was the same as the ED50 for cell death. Cells die when a single bubble was detected in a multiple pulse sequencer.

- Sakuraba K, Hayashi N, Kawashima M, Imokawa G.
Comments: Tumour cells of pigmented basal cell epithelioma (BCE) contained significantly lower population of melanosomes than normal keratinocytes in the perilesional normal epidermis. Expression of protease-activated receptor 2 (PAR-2) mRNA transcripts was lower and PAR-2 protein expression suppressed compared to normal perilesional keratinocytes.

  
  Comments: Review discussing molecular mechanisms of Rab-dependent, myosin-driven organelle motility on actin tracks. A special attention has been paid to melanosome-Rab27A – melanophilin - myosin Va system operating in skin melanocytes, to melanosome -Rab27A - MyRIP (myosin and Rab interacting protein)=Slac-2c – myosin VIIa system regulating melanosome distribution in the RPE and to two further systems not engaged in melanosome motility.

  
  Comments: Black and white melanocytes expressed both mRNA and protein for sodium/hydrogen exchangers (NHEs), namely NHE-1, NHE-3, NHE-5, NHE-6 and NHE-7. NHE-7 and NHE-3 colocalized with melanosomal protein TRP-1. NHE inhibitor – ethyliso-propyl amiloride – caused a rapid dose-dependent inhibition of tyrosinase activity in black melanocytes and only little inhibition in white melanocytes. Vesicular proton pump – vesicular ATPase – was found to be present both in white and black melanosomes indicating that organelles from both racial skin types are capable of being acidified. One or more NHEs may help to regulate melanosomal pH and tyrosinase activity.

  
  Comments: An elegant review focused on the secretory function of lysosomes. Secretory lysosomes are also found in many different cell types of the immune system. Melanosomes belong among lysosome-related organelles. Links between albinism and defects of immunity in patients have uncovered a number of key proteins required for lysosomal secretion. Human diseases and their mouse models giving rise to defects in secretory lysosomes are summarized; special attention is paid to Chediak-Higashi, Griscelli and Hermansky-Pudlak syndromes and to immunodeficiency without albinism. A colour figure summarizing the protein machinery identified in lysosomal secretion in spermatid, platelets, fibroblasts, cytotoxic lymphocytes, melanocytes and mast cells.

  
  Comments: Under in vitro conditions increased tyrosine concentration in media induced different changes in melanosomes in melanocytes from light and dark skin type. Increased pigmentation in the light skin melanosomes was accompanied by their elongation and a reduction in width unlike dark skin melanosomes which enlarged their size in all the dimensions. Ratio between phaeomelanin and eumelanin concentration in light skin melanosomes as well as their sulphur content increase more than in melanosomes in dark skin types.

Calendar of events

12th ESPCR Paris September 2004

New Members

Pigment Cell Research: Editor’s notice

The Vitiligo Society Call for Research Projects

Calendar of events

2004  Annual Meeting of the New Zealand Dermatological Society
August 18-21, Queenstown, Australia.
Contact: Ken Macdonald
New Zealand Dermatological Society
202 Bealey Avenue,
AUS - Christchurch
Phone: +03 379 9467    Fax: +03 366 8607
E-mail: macdonald@derm.co.nz
Web: www.dermnetnz.org

2004  34th European Society for Dermatological Research
September 9-11, Vienna, Austria
Contact: AIMS International Congress Services
“34th Annual ESDR Meeting – Vienna”
Mariannengasse 32
1090 Vienna, Austria
Tel: +41 402 77 55 – 97/ -38    Fax: +43 1 402 77 31
E-mail: esdr2004@ahr-aims.com
Web: www.esdr.org

2004  25th Symposium of the International Society of Dermatopathology (ISDP)
September 16-18, Lisbon, Portugal
Contact: Saudade Leitao
International Society of Dermatopathology (ISDP)
Mundiconvenius,
Rua do Embaiwador, 13-2°
1300-215 Lisbon, Portugal
Phone: +351 21 364 94 98    Fax: +351 21 364 95 23
E-mail: saudade_leitao@mundiconvenius.pt
Web: www.intsocdermpath.org

2004 XIIth Meeting of the ESPCR
September 22-25, Paris, France
Contact: Dr. Lionel LARUE
E-mail: Lionel.Larue@curie.fr
Congress Secretariat:
2004  The International Society of Dermatology’s 7th International Congress of Dermatology
September 29-2 October, Tehran, Iran
Contact: Yahya Dowlati
Iranian Society of Dermatology
PO Box 14155-6383, Tehran, Iran
Phone: +98 218 97 8190  Fax: +98 218 96 3804
E-mail: info@iranderm.org
Web: www.iranderm.org

2004  International Congress on Epidemiology Causes and prevention of Skin diseases
October 10-12, Venice, Italy
Contact: Luigi Naldi
International Dermatoepidemiology Association (IDEA) & European Dermatoepidemiology Network (EDEN)
U.O. Dermatologia
Ospedali Ruiniti
Largo Barozzi
I - 24128 Bergamo
Phone: +39 035 400 625  Fax: +39 035 253 070
E-mail: luiginal@tin.it
Web: www.esdr.ch/announcements.htm

2004 Perspectives in Melanoma VI
November 13-14, Miami, Florida, USA
Contact: IMEDEX
70 Technology Drive
Alpharetta, GA 30005-3969 USA
Tel +1 (770) 751 7332  Fax: +1 (770) 751 7334
E-mail: meetings@imedex.com
Web: www.imedex.com

2004 13th Congress of the European Academy of Dermatology & Venereology (EADV)
November 17-21, Florence, Italy
Contact: President Office
Viale Matteotti, 7
50121 Florence, Italy
Phone: +39 055 50 35 342  Fax: +39 055 50 35 341
E-mail: president@eadv2004.org
Web: www.EADV2004.org

2004 18th Annual Meeting of the Japanese Society for Pigment Cell Research
November 27-28, Kumamoto City, Japan
Chair: Prof. Tomomichi Ono of Kumamoto University
Contact: Dr Toshiro Kageshita

2005 8th International Conference on Solar Energy and Applied Photochemistry
February 20-26, Photoenergy Center, Upper Egypt [Luxor/Aswan]
Contact: Prof. Sabry Abdel-Mottaleb
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Abbassia, 11566 Cairo, Egypt
Cellular: + 2012 216 9584
Fax: + 202 484 5941 OR + 202 634 7683
E-mail: solar05@photoenergy.org
Web: www.photoenergy.org

2004 The 10th World Congress on Cancers of the Skin
May 13-17, Vienna, Austria
Contact: Elfriede Pomp
Dept of Dermatology, Vienna General Hospital
University of Vienna
Währinger Gürtel 18-20
A - 1090 Vienna
Tel: +00431 40400 7707 Fax: +00431 40400 7699
E-mail: info@wccs.at
Web: www.wccs.at

2005 XIVth International Pigment Cell Conference (IPCC)
September 18-23, Reston, Virginia, USA
Contact: Dr. V. HEARING
E-mail: hearingv@nih.gov
Web: www.ipcc.info

2006 XIIIth Meeting of the ESPCR
Barcelona, Spain
Contact: Dr. L. Montoliu
E-mail: montoliu@cnb.uam.es
Dear Fellow Members,

The organizers of the upcoming 12th European Society for Pigment Cell Research have decided to give an award to the best poster presented over the Meeting.

While reading the presentations the jury will take several criteria into consideration including scientific quality, iconography and answers to questions formulated by the members of a nominated jury to be able and select the winning communication.

The award will consist in a French surprize and a free registration for the 19th International Pigment Cell Conference to be held in Reston, Va. From September 18th through 22nd, 2005. (www.palladianpartners.com/IPCC05)

We wish you luck and look forward to meeting you in Paris.

Best regards,
Dr. Lionel LARUE
Institut Curie
NEW MEMBERS

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

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Pigment Cell Research is pleased to introduce Prof. Colin R. Goding as its Editor as of Jan 1, 2005. The transition to the new Editorial Office in the UK will occur over the remainder of this year. As of June 1, 2004, all new manuscripts should be submitted directly to Prof. Goding at the email address noted below. All manuscripts currently in review or in revision will continue to be handled in my office.

Submit articles to:

Prof. Colin R. Goding  
Pigment Cell Research Editorial Office  
Marie Curie Research Institute  
The Chart, Oxted  
Surrey, RH8 0TL, UK  
email: pcreditor@mcri.ac.uk

I look forward to the continued growth of the journal under Prof. Goding's direction,

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THE VITILIGO SOCIETY

CALL FOR RESEARCH PROJECTS

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

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