

PASPCR

Newsletter

Volume 1 Number 2

June, 1993

Introduction . . .

The quarterly **PASPCR Newsletter** is intended to serve as an informal means for the members of our Society to communicate with one another. As such, we invite our membership to actively contribute to the *Newsletter*. Help us to update the Job Listings and Calendar of Events. If you attend a scientific meeting at which you heard about work which you think will be of interest to the membership of the **PASPCR**, please write a few paragraphs summarizing what was presented and share it with us. If you should have a change of affiliation and address, we'd like to know that, too. This is **your Newsletter**, and we depend upon you to help us make sure it best serves the Society's needs. Contributions and comments can be sent to any of the members of the Publications Committee listed in this issue.

Welcome to New Members :

We welcome the following new members to the **PASPCR**

Jean-Claude Bystryn	Bryan P Murphy
Donna Durham-Pierre	Yoshimichi Nakatsu
Jamal Z Farooqui	J Michael Newton
Bryan B Fuller	Richard M Niles
John M Gardner	Regina Pietruszko
Alan N Houghton	Brian Potterf
W Gerald Klingler	Michael Rachkovsky
Gary D Kruh	Ian R Scott
Kenneth A Mason	Richard T Swank
Katsuo Matsumoto	Ram K Tripathi
Kenji Mori	

If anyone is interested in joining our Society or wishes to sponsor a member, application forms can be obtained from Dr. Richard King.

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Pigment Cell Research**

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Roger Bowers
Murray Brilliant
John Brumbaugh
Alistair Cochran
Kowichi Jimbow
Seth Orlow
John Pawelek

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Past-President

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Dr. Seth J. Orlow
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New York Univ Med Center
550 First Avenue, RM H100
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Calendar of Events :

May 30 - June 1, 1993

American Society for Biochemistry & Molecular Biology, to be held in San Diego, California (contact: FASEB, 9650 Rockville Pike, Bethesda, MD 20814; FAX: 301/530-7014)

September 21 - 23, 1993

Fifth World Congress on Cancers of the Skin, to be held Berlin, Germany (contact: Dr. Claus Garbe, Department of Dermatology, University Medical Center Steglitz, Hindenburgdamm, 30, 1000 Berlin 45, Germany; FAX: 49/30/798-4141)

September 26 - 30, 1993

XVth International Pigment Cell Conference, to be held in Kensington, UK (contact: Mrs. Rosemary Barton, IPCC Conference Office, P.O. Box 1773, London E17 9LW, United Kingdom, FAX: 44/81/503-6463)

October 28 - 31, 1993

Tricontinental Dermatology Conference, to be held in Kyoto, Japan (contact: Dr. Kouichi Ikai, Dept of Dermatology, Kyoto University Faculty of

Medicine, Kyoto 606, Japan)

December 11 - 15, 1993 American Society of Cell Biology Meeting, to be held New Orleans, Louisiana, (contact: ASCB National Office, 9650 Rockville Pike, Bethesda, MD FAX: 301/530-7139)

March 11 - 12, 1994 Melanin Symposium, Melanin: Its Role in Human Photoprotection, to be held in Alexandria, Virginia, (contact: Dr. Ago Ahene, 3696 Haven Avenue, Redwood City, California, 94063, FAX: 415/368-4470)

April 27 - 30, 1994 Annual Meeting of the Society for Investigative Dermatology, to be held in Baltimore, Maryland, (contact: SID Office, Department of Dermatology, University Hospitals of Cleveland, 2074 Abington Road, Cleveland, OH 44106, FAX: 216/844-8993)

June 26 - 29, 1994 5th PASPCR Annual Meeting, to be held in Philadelphia, Pennsylvania, (contact: Dr. Gert Jacobsohn, Department of Biological Chemistry, Hahnemann University, Broad and Vine, Philadelphia, PA 19102-1192, FAX: 215/762-3722)

1994 PASPCR Vth Annual Meeting

The Vth Meeting of the **PASPCR** will be held in Philadelphia, Pennsylvania from June 26-29, 1994. Dr. Gert Jacobsohn is the Organizer of this meeting, and he and his Program Committee have already assembled a number of exciting keynote speakers and the outline of a most interesting scientific program. More information about this meeting and the tentative program will follow in future *Newsletters*. We hope that all members of the **PASPCR** will plan to attend this meeting - be sure to mark it on your calendar.

1995 PASPCR VIth Annual Meeting Site :

We are currently soliciting applications from anyone interested in hosting the VIth Annual Meeting of the **PASPCR**, preferably in the middle of 1995. If you are interesting in bidding for this Conference, please contact one of the officers of the **PASPCR**. Previous meeting sites to date have been: I - Minneapolis, Minnesota; II - Bethesda, Maryland; III - Edmonton, Alberta; IV - Cincinnati, Ohio. The next meeting will be held in Philadelphia, Pennsylvania (cf above).

XVth International Pigment Cell Conference

By now, everyone should have received all of the information regarding the meeting site and date of the next IPCC to be held in London from September 26-30, 1993. However, information on hotels and housing available was not sent in the original Announcement, and following is some preliminary data on the hotels and their prices that will be offered to attendees. In addition, we include some information on other types of housing that is available and might be cheaper on a group basis. If anyone has any additional knowledge of relatively cheap housing in the London area, we invite them to send it to us for inclusion in our next Newsletter, which will be sent to members of our Society prior to the IPCC meeting in London.

<u>Hotel Name</u>	<u>Location</u>	<u>Single</u>	<u>Double</u>
Copthorne Tara	W8	88	100
Kensington Close	W8	77	86
Kensington Palace Thistle	W8	80	90
Hospitality Inn	W2	75	86
Park Court	W2	75	85
Charles Dickens	W2	65	80
Phoenix	W2	55	72
Julius Caesar	W2	28	38
The Gloucester	SW7	65	70
The Bailey's	SW7	65	75

* prices are in British pounds and include breakfast and VAT

1993 PASPCR Young Investigator Awards The

We have information on apartments that can house up to 8 people for one week; these apartments are located in South Kensington and in Blumbury. The price including all taxes are \$1,925 and \$1,665 per week respectively (this would work out to \$200/\$250 per person per week). Contact us if you wish further information about these rentals.

PASPCR will present three Young Investigator Awards to its members attending the XVth IPCC; these awards are sponsored by our Society and consist of a plaque and a \$250 each. Winners of these awards will be chosen by an anonymous Committee during the actual meeting and will be announced at the **PASPCR** Business Meeting to be held during the IPCC in London.

PASPCR Secretary / Treasurer's Report :

Synopsis of the **PASPCR** Council Meeting held by telephone conference call on January 28, 1993.

Hearing opened the meeting and the minutes of the previous meeting held on October 15, 1992 were accepted as amended. Nordlund reviewed the Council Meeting of the IFPCS held in Tokyo in November, 1992 (issues discussed at that meeting were published in Issue #1 of the PASPCR Newsletter). The report on the annual meeting to be held in Philadelphia, 1994 was given; a Program Committee meeting had already been held and planning for that meeting has been initiated. The date for the meeting has been set at June 26-29, 1994. Hearing reviewed the Nomination Committee's recommendation that Elizabeth Russell be made the first honorary member of the PASPCR and this was unanimously accepted by the Council. Hearing discussed the need for a Newsletter that would improve communication within the membership; following discussion it was decided that Hearing and King would serve as the coordinators of publication and mailing and that the first year Council Members (Abdel-Malek, Brilliant, Orlow) serve as an *ad hoc* Publication Committee to generate future issues. The need for a Membership Committee was reviewed; it was noted that there were 17 new members in 1992 and that many individuals working in pigment research are not members. An *ad hoc* Membership Committee consisting of the third year Council Members (Bagnara, Brumbaugh and Jimbow) was asked to prepare a report on mechanisms for having an ongoing active membership campaign. The Nominating Committee, automatically chaired by the President-Elect (Frost-Mason) will develop a slate of candidates to fill the three Council positions; R Bowers will act as the second member of the Committee and they will select three additional members at large. The Nominations Committee was directed to select candidates from the broad range of pigment cell research; nominations are to be completed by the July, 1993 Council meeting. The issue of travel stipends for the London IPCC was discussed; it was moved and approved that travel stipends be provided for the London meeting, and it was decided to approve them as early as possible so that attendees would have such support committed when planning their trip. The question of the PASPCR Young Investigator awards was also discussed and the Council unanimously approved the motion to give those awards during the London IPCC, limiting eligibility to members of the PASPCR. It was decided to present those awards during the PASPCR business meeting in London, and recipients will be selected by an anonymous Committee. Nordlund reviewed the proposal that the PASPCR agree to cosponsor a Melanin Symposium to be held in March, 1994 in Crystal City, Virginia. There was general discussion as to the appropriateness of the PASPCR sponsoring this meeting. It was generally felt that the idea of this meeting was good, but that the PASPCR should have some role in organizing the meeting if they were to act as sponsor. It was suggested that perhaps this meeting could be held in conjunction with the Philadelphia PASPCR meeting, but it was noted that the organizers wanted to hold it close to Washington DC so that members of the regulatory agencies dealing with new drugs could attend. It was moved and seconded to table this discussion to a later meeting following receipt of additional information. There being no further new business, the meeting was adjourned. Minutes prepared by King, Secretary/Treasurer, PASPCR.

Positions - Wanted and Available :

Available: Postdoctoral Fellowship: NIH-funded position (for up to 3 years) to study the biogenesis of the melanosome and genetic disorders which affect it. Prior training in either immunomicroscopy or molecular biology desirable. US citizen or permanent resident only. Contact: Dr. Seth Orlow, Dermatology Room H-100, NYU Medical Center, 550 First Avenue, New York, NY 10016. phone: 212/263-5070, FAX: 212/263-8752.

Available: Two postdoctoral positions are available to study the recently described receptors for the proopiomelanocortin peptides (Science, 257:1248, 1992). Ongoing projects include structure/function analysis of the MSH receptor, isolation and characterization of neural-specific proopiomelanocortin receptors, and study of the functions of melanocortins in the CNS. One position requires a background in peptide and protein chemistry for receptor structure/function analysis. The second position would ideally be filled by an individual with training in neurobiology and a strong background in molecular biology. Applicants should send a Curriculum Vitae, a statement of research interests, and three letters of recommendation to: Dr. Roger D Cone, Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97201-3098. phone: 503/494-4732; FAX: 503/494-4534.

Available: One or two (NIH grant-funded) postdoctoral positions in the laboratory of Dr. Murray Brilliant at the Fox Chase starting the summer of 1993. The research involves Mammalian Genetics, Pigmentation Biology and Mouse Models of Human Genetic Disease. Recent efforts have been aimed at understanding the mouse pink-eyed dilution locus (Science 252:566, 1991; PNAS 89:2969, 1992; PNAS 90:297, 1993) and its role in human albinism, Prader-Willi Syndrome and Angelman Syndrome (Mammalian Gen 3:187, 1992; Science 257:1121, 1992). Ongoing projects include characterization of the pigment gene and adjacent genes that mediate other intriguing developmental functions, in both mouse and man. The laboratory research group is small, active, productive and amiable. The laboratory is part of the basic sciences division (The Institute for Cancer Research), located in a new research building on a large wooded campus in a suburban-like setting, just inside the city limits of Philadelphia. The Fox Chase Cancer Center has a large postdoctoral community that benefits from active inter-lab collaborations and weekly seminary/pizza/beer gatherings. In addition to a generous benefits package, other attractive amenities include subsidized housing and excellent day-care facilities, all within walking distance. Contact Dr. Murray Brilliant, Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Ave, Philadelphia, PA 19111; phone: 215/728-2864, FAX: 215/728-3616.

Meeting Report - Society for Investigative Dermatology - 1993

The 1993 Annual Meeting of the Society for Investigative Dermatology was held in Washington, DC from April 28-May 1, 1993. The following summary of abstracts presented was compiled by Dr. Zalfa Abdel-Malek (who is to be congratulated for her tremendous effort - Ed. note)

I. CYTOKINES, NEUROPEPTIDES AND OTHER HORMONES

Expression of Insulin-like growth factor (IGF-1) Message in Pigmented Lesions. *Fleming MG, Graf LH.* IGF-1 message was detected by *in situ* hybridization in common nevi, dysplastic nevi, early primary melanomas, advanced primary melanomas and metastatic melanomas.

Melanocyte Function in Human Skin is Modulated by Neurotrophic Factors Through TRK Receptors. *Yaar M, Eller MS, Di Benedetto P, Reenstra WR, Zhai S, Gilchrist BA.* Human melanocytes stimulated with TPA, UV, treated with keratinocyte conditioned media, or co-cultured with KC, express high affinity receptors of the TRK family that bind NT-3, a neurotrophin related to nerve growth factor

- (NGF). These receptors are functional, as evidenced by their rapid tyrosine phosphorylation upon the addition of NGF to melanocytes.
- The Behavior of Cytokines, Melanotropic Peptides and Other Proteins in Hyperpigmentation of Human Skin Grafts on Nude Mice. *Matsumoto K, Robb E, Warden G, Nordlund J.* Human skin xenografts (on athymic nude mice) spontaneously hyperpigment. Hyperpigmentation is obvious one week after grafting and increases with time. The tyrosinase related protein (TRP)-1 is activated one week for a subsequent four weeks after grafting. The cytokines IL-1 and TNF- α are transiently increased from 24-48 hours after the graft. The expression of IL-6 is not detected. Some cells in the dermis express α -MSH and ACTH 2-4 weeks after grafting.
- Inhibition of B16 Melanoma Growth *In Vivo* After Treatment with Interleukin-1 Receptor Antagonist. *McKenzie RC, Sauder DN, Dinarello C.* Interleukin (IL)-1 and IL-6 enhanced the growth of B16-F10 melanoma cells only when co-injected with the tumor cells. Interleukin-1 receptor antagonist (IL-1RA), which blocks the binding of IL-1 to its receptors, and lacks agonist activity, reduced tumor growth in a dose-dependent manner. The possible role of IL-1 in melanoma tumor development and the therapeutic value of IL-1RA are suggested.
- Involvement of Interleukin-6 in the Biology of Human Malignant Melanoma. *Kocoschka EM, Mittermayer F, Schauer E, Micksche M.* Seventeen out of twenty human malignant melanoma cell lines secreted IL-6. The production of IL-6 was stimulated by PMA (TPA), IL-1 β and TNF- α . IL-6 was shown to bind IL-6 receptors, and binding was increased by preincubation with interferon- γ and TNF- α .
- Effect of 9-cis Retinoic Acid on Human Primary Melanocytes and Metastatic Melanoma Cells. *Yamanishi DT, Voboril JE, Wagner EA, Meyskens FL, Jr.* 9-Cis retinoic acid resulted in a slight increase in the proliferation of human melanocytes, but inhibited the proliferation of three out of four melanoma cell strains. Melanocytes expressed retinoic acid receptor α , β and γ RNA transcripts, while melanoma cells expressed retinoic acid receptor α and γ , and only two out of the four lines expressed β -transcripts. The relationship of the retinoic acid receptor isotypes to cellular response is still to be determined.
- MSH Receptors in Immortalized Human Epidermal Keratinocytes: A Potential Mechanism for Coordinate Regulation of the Epidermal Melanin Unit by UV Light. *Pawelek JM, Chakraborty AK.* Primary human epidermal keratinocytes immortalized in culture expressed MSH receptors. Like S91 melanoma cells, keratinocytes expressed high and low affinity receptors, the former showing positive cooperativity. The MSH receptors in both cell types had similar K_i values, identical gel migration patterns, were associated with plasma membrane and coated vesicles, and were upregulated by UV light and MSH. It is suggested that the presence of MSH receptors on keratinocytes translates UV light into a single hormonal initiation signal in the skin.
- Purification and Characterization of Allergy-Induced Melanogenic Factor in Brownish Guinea Pig Skin. *Imokawa G, Yada Y, Higuchi T.* Phenylazo-naphthol (PAN) allergy induces late-appearing hyperpigmentation of brownish guinea pig skin. This hyperpigmentation accompanies the appearance of an epidermal melanogenic soluble factor with a molecular mass of 7.9 kDa, capable of stimulating Ins (1,4,5) P₃ formation and increasing intracellular Ca⁺² in guinea pig melanocytes. The growth and melanogenic activities of this factor seems to be mediated by the PKC signal transduction pathway.
- Identification of Functional Endothelin-1 Receptors in Cultured Human Malignant Melanoma Cells. *Yohn J, Smith S, Stevens T, Morelli J, Dormish J, Kane M, Zamora M.* Endothelin-1 is a peptide growth factor for human melanocytes which is produced in large amounts by vascular endothelium (it is also produced by human keratinocytes). Three human malignant melanoma cell lines were evaluated for specific ET-1 binding, intracellular calcium mobilization and growth response. Human melanoma cell lines SK Mel 28, SK Mel 30 and CU 800 had specific, saturable, high affinity endothelin-1 binding, and responded to endothelin-1 treatment with increased intracellular Ca⁺² and enhanced proliferation. The expression of functional endothelin-1 receptors by human melanomas may be associated with enhanced perivascular proliferation of malignant melanoma.

II. EXTRACELLULAR MATRIX, INTEGRINS

Extracellular Matrix of Keratinocyte Mediated Cell to Cell Interactions can Control Normal Human Melanocyte Differentiation and Proliferation. *Nakazawa K, Damour O, Collombel C.*

Extracellular matrix produced by proliferating or differentiating keratinocytes regulate dendrite formation, growth and melanization of melanocytes, possibly by enhancing attachment of melanocytes to extracellular matrix components or induction of cell adhesion molecules.

Spatial Organization of Integrin Subunits on Cultured Normal Human Epidermal Melanocytes Examined by Confocal Microscopy. *Searles GE, Munoz V, Jimbow K.*

Normal human melanocytes were stained by immunofluorescence methods using specific antibodies against integrins. No expression of α_4 or β_2 was found. α_2 had a random distribution along the perikaryon and dendrites, α_3 stained the cell body and dendrites, α_5 and β_1 had a random wide range distribution, α_v and β_3 were localized at the tip of dendrites, and β_4 at hillock and proximal dendrites.

TNF- α and IL-1 α Modulate Melanoma Integrin Expression and Cell Migration. *Dekker SK, Vink J, Vermeer BJ, Brijn JA, Mihm MC, Byers HR.*

The two cytokines IL-1 α and TNF- α upregulate the expression of α_4 and α_5 integrin subunits on metastatic melanoma MM-RU cells plated on plastic, and increase cellular migration rate on fibronectin matrix. Migration is inhibited by monoclonal antibodies against the α_4 , α_5 and β_1 subunits, and against TNF- α and IL-1 α receptors.

Human Melanocyte Movement on Type IV Collagen is Inhibited by Antibodies to α_2 and α_3 , but not α_5 Integrins. *Morelli JG, Yohn JJ, Zekman T, Norris DA.*

Human neonatal melanocyte movement *in vitro* is enhanced on a matrix of type IV collagen. This movement was found to be regulated by α_2 and α_3 , but not α_5 integrin.

Stimulation of Skin Melanocyte Tyrosinase Activity by Hair Bulb Dermal Papilla Cells. *Buffey JA, Messenger A, Taylor M, Ashcroft ATT, Westgate GF, MacNeil S.*

Dopa oxidase activity of tyrosinase in neonatal human melanocytes was increased when melanocytes were (1) co-cultured with hair bulb dermal papilla cells, (2) treated with conditioned medium derived from dermal papilla cell cultures, or (3) cultured on dermal papilla extracellular matrix components.

III. SIGNAL TRANSDUCTION

Protein Kinase C- β Activates Tyrosinase. *Park H-Y, Fernandez E, Russakovsky V, Gilchrest BA.*

A human non-pigmented melanoma line NP-MM4 lacks PKC- β and tyrosinase activity despite normal expression of tyrosinase protein. Tyrosinase activity was stimulated by mixing NP-MM₄ lysates with normal melanocyte lysates, and by transfecting NP-MM₄ with PKC- β cDNA, but not by transfecting with a mutated inactive PKC- β cDNA.

Protein Kinase C Stimulates Melanogenesis Through Phosphorylation of Tyrosinase. *Park H-Y, Russakovsky V, Fernandez E, Gilchrest BA.*

Neonatal human melanocytes treated with TPA undergo acute activation followed by reduction of PKC and tyrosinase within 24 hours. Incubation of melanocytes with TPA (to increase PKC activity) and [ortho ³²P]-phosphate for 15 minutes, followed by immunoprecipitation of tyrosinase resulted in a single ³²P band of 70 kD molecular weight. Irradiation with UV increased PKC α and β mRNA 2-3 fold within 1 hour, and the levels returned back to baseline by 2 hours.

Ionizing Radiation Directly Stimulates Pigment Production in Human Melanocytes. *Rubeiz NG, Park H-Y, Rogers GS, Gilchrest BA.*

Neonatal human melanocytes responded to 46 γ X-irradiation with decreased proliferation and increased melanin content which was noted 5 days post-irradiation. PKC- β and α were induced within 2 hours, and PKC- β returned to baseline within 24 hours post-irradiation. These effects on PKC were specific to X-irradiation and were not induced by heating melanocytes to 40° C.

IV. PHOTOBIOLOGY

Biochemical Analysis of UVB Induced Hyperpigmentation of Human Melanocytes In Vitro. *Abdel-Malek Z, Swope V, Babcock G, Dawes S, Nordlund J.* Human melanocytes in culture respond to UVBR with decreased proliferation, arrest in S/G₂-M phase and increased melanin synthesis. Irradiated melanocytes exhibit an increase in tyrosinase activity, no change in the expression of tyrosinase and TRP-1, and a decrease in the expression of TRP-2. This UVBR induced mechanism might be unique, since cholera toxin and IBMX increase melanogenesis by a mechanism that involves increased expression of tyrosinase, TRP-1 and TRP-2.

Effects of Ultraviolet Light on the Cell Cycle. *Bologna JL, Sodi SA, Chakraborty AS, Pawelek JM.* UVB irradiation increased MSH binding and responsiveness of mouse Cloudman melanoma cells to MSH. Irradiation of Cloudman melanoma cells, mouse carcinoma cells and 3T3 mouse fibroblasts resulted in a significant increase in the percentage of all three cell types in the G₂ phase of the cell cycle, and increased ¹²⁵I-βMSH binding to Cloudman melanoma cells. The effects of UVB on MSH binding and melanogenesis seem to be mediated by cell cycle regulation.

Pre- and Post-Translational Expression of UV-Induced Melanogenesis in Melanoma Cells. *Hara H, Chen H, Luo D, Jimbow K.* Irradiation of melanotic and amelanotic human melanoma cells with a fractionated low dose of UVB resulted in increased melanin synthesis, tyrosinase activity and gp75 in the melanotic melanoma cells. The mRNA levels of tyrosinase and gp75 were also increased in these cells. Therefore, in melanoma cells, low doses of UVB increase melanogenesis by pre- and post-translational means.

Ultraviolet A Induces Incorporation of ³H-Dihydroxyphenylalanine in Normal Human Melanocytes. *MacFarlane D, De Leo V.* Human melanocytes demonstrated a dose-dependent increase in dendricity in response to irradiation with UVA. Doses between 10-20 J/cm² UVB resulted in increased ³H-DOPA incorporation after 72-96 hours, and significant cytotoxicity with 24 hours.

Endonuclease Treatment Enhances UV-Induced Melanogenesis in Human Melanocytes and S91 Melanoma Cells. *Zhai S, Yaar M, Eller MS, Yarosh DB, Gilchrist BA.* T₄ endonuclease V (T₄N₅) is a DNA repair enzyme involved in the removal of thymidine dimers following UV irradiation. Incubation of UV-irradiated human melanocytes and S91 melanoma cells in a medium containing T₄N₅ overnight, resulted in increased tyrosinase activity and melanin content to levels higher than those in irradiated melanocytes and melanoma cells that were not treated with T₄N₅ or treated with heat inactivated T₄N₅. It was concluded that UV induced melanogenesis is stimulated by DNA repair.

V. MELANOGENIC PROTEINS

A Factor in Mouse and Human Melanoma Cells that Catalyzes the Conversion of 5,6-Dihydroxyindole-2-carboxylic Acid to Melanin. *Chakraborty AK, Pawelek JM.* DHICA conversion factor is expressed in human and murine melanoma cell lines and in a human keratinocyte cell line. It is suggested that this factor is involved in the incorporation of DHICA into melanin and to have the following properties: it is a membrane associated glycoprotein, whose activity is reduced by metal chelators but stimulated by ATP and GTP, Mg⁺² or Mn⁺², and is elevated in COS fibroblasts transfected with Pmel-17 gene.

Co-Transfection and Expression of Both Human Tyrosinase and Tyrosinase-Related Protein Genes in a Single COS Cell. *Luo D, Chen H, Hara H, Jimbow K.* Human tyrosinase and TRP-1 genes were constructed into two separate expression vectors so that cloned genes were under the control of human cytomegalovirus promoters. Monkey COS-7 cells were transfected with either or both genes. The expression of tyrosinase and TRP-1 in transfected cells was confirmed by: RNA-PCR amplification, immunocytochemistry, electron microscopy and DOPA histochemistry. Expression of tyrosinase and TRP-1, however, did not produce mature eumelanin or pheomelanin.

VI. MELANOSOMAL PROTEINS

Identification of a Mammalian Melanosomal Matrix Glycoprotein. *Orlow SJ, Zhou B-K, Boissy RE.* Antisera raised in rabbits against the Triton X-100 insoluble fraction of melanosomes from mouse

melanoma cells specifically decorate the internal matrix of melanosomes by immunoelectron microscopy. This was confirmed by subcellular fractionation followed by immunoblotting. The antisera recognized a protein of Mr 90,000 which is processed to a broadly migrating band of Mr 53-55,000. The processed protein required SDS for solubilization. Tunicamycin, but not deoxymannojirimycin and swainsonine, reduces the Mr of the nascent protein to 75,000. This protein is regulated differently and is not immunologically related to tyrosinase or tyrosinase related proteins, suggesting a bipartite pathway for melanosomal biogenesis.

Identification of cDNAs for Calnexin, β -Galactosidase and Melanotransferrin-like Proteins Associated with Melanosomes in Human Melanoma. *Dakour J, Vinayagamoorthy T, Jimbow K.* Human melanoma cDNA library was developed and immunoscreened with polyclonal antibodies raised against the melanosomal fraction isolated from human melanoma cells. Four different cDNAs were found, two of which had homology with the calnexin, the major Ca^{+2} binding transmembrane ER phosphoprotein. This seems to be a new melanosomal protein, possibly associated with melanogenesis. A third clone showed homology with β -galactosidase which might participate in post-tyrosinase melanogenesis. A fourth clone was homologous to human melanotransferrin which binds and translocates iron within melanoma cells.

Possible Functional Domains of a New Melanosomal Calnexin-like Protein Originating from Endoplasmic Reticulum. *Vinayagamoorthy T, Dakour J, Jimbow K.* Five functional motifs were predicted by analyzing a 3.8 Kb cDNA clone isolated from human cDNA library: (a) Ca^{+2} binding loop, (b) two sites of protein kinase C phosphorylation, (c) cAMP dependent protein A site (both b and c could be involved in signal transduction, docking of coated vesicles from Golgi complex, and translocation of melanosomes), (d) cell cycle kinase phosphorylation site, and (e) membrane spanning region. This clone also had homology with dog calnexin, whose antigenic epitope was present in the endoplasmic reticulum and melanosomal surface membrane.

VII. PIGMENTARY ABNORMALITIES AND MELANOCYTE TRANSFORMATION

Quantitative Investigations of Nucleolar Organizer Regions Associated Protein in Melanocytic Skin Lesions.

Oh CH, Lee SY. Several morphologic criteria have been used to compare the frequency and size of nucleolar organizer regions in malignant melanoma, congenital nevi, and acquired nevi. A significant difference was found between malignant melanoma and the two types of nevi, but not between congenital and acquired nevi.

Normal Human Melanocytes Expressing Viral Oncogene Products Display Altered Growth Characteristics.

Zepter K, Haffner AC, Elmetts CA. Incubation of neonatal human melanocytes in supernatants of fibroblast cultures shedding retroviral vectors encoding the SV40 large T-antigen (Tag) resulted in populations of Tag positive melanocytes. These melanocytes displayed accelerated population doubling times, grew to highly confluent monolayers, and could be passaged more than twice the passage number of normal melanocytes before reaching senescence. The Tag positive melanocytes, however, did not become malignant, remained anchorage dependent, and failed to form colonies in soft agar.

Melanin Content and Its Morphology in Cutaneous Corneocytes. *Ando N, Edwards C, Pearse AD, Marks R.*

Observation of surface corneocytes revealed a higher melanin content in darkly pigmented than in lightly pigmented skin, and in sun-exposed sites than in non-exposed Caucasian skin. Following UV irradiation, melanin could be detected in corneocytes 17 days after irradiation. Cap-like melanin structures could mostly be detected in surface corneocytes of lentigo, and might be a marker for pigmentary anomalies.

Neurocutaneous Melanosis: Immunohistologic and Ultrastructural Tissue Culture Study. *Reyes-Mugica M,*

Chou P, Alvarez-Franco M, Strauss L, Gonzalez-Crussi F. Nevomelanocytes cultured from neurocutaneous melanosis, a very rare and highly lethal childhood disorder, were compared to nevus cells cultured from congenital pigmented nevi. Ultrastructurally, no significant differences

were found in the two types of nevomelanocytes to account for the difference in their clinical behavior.

Evidence for Genetic Instability in Melanocytes Isolated from Atypical Moles. *Medrano EE, Yang F, Nordlund JJ.* Melanocytes isolated from normal or atypical moles exhibited loss of territoriality *in vitro*, as they did *in vivo*. Unlike normal melanocytes which do not spread and die within a few days after plating in plastic dishes with poor anchorage capacity, nevocytes spread and proliferate. Some nevocyte cultures grow as a monolayer, while others from tumor-like spheroids. Most nevocyte cultures maintain the requirement for melanocyte growth factors and the ability to terminally differentiate. Some, however, become responsive to, and express receptors for epidermal growth factor, and have increased levels of the mutant form of the tumor suppressor gene p-53. This *in vitro* system may help identify the molecular events in melanoma tumor progression.

Assay to Distinguish Between the Catalytic Activity of Tyrosine Hydroxylase with and without Regulatory Components in Cultured Human Melanocytes. *Zhao H, Boissy R.* Comparison of tyrosine hydroxylase (TH) activity using cell lysates (*in vitro*) or intact cells (*in vivo*) of normal human melanocytes and melanocytes cultured from Chediak-Higashi syndrome (CHS), tyrosinase-positive and tyrosinase-negative albino patients revealed the following. TH activities using intact cells correlated with melanin content of all the above types of melanocytes. The value of TH activity of CHS melanocytes was lower in the *in vitro* than the *in vivo* assay. The increase in activity in these cells following IBMX/cholera toxin stimulation was measurable in the *in vivo*, but not the *in vitro* assay. Tyrosinase-negative albino melanocytes showed no *in vitro* but minimal *in vivo* values. TH activity of two tyrosinase-positive albino melanocyte strains had normal *in vitro* but decreased *in vivo* values. In normal melanocytes, the values for TH activity were comparable in both the *in vivo* and *in vitro* assays. The *in vitro* assay may evaluate the catalytic function of tyrosinase while the *in vivo* assay may reflect additional regulatory components affecting TH activity.

Cutaneous Hyperpigmentary Lesions in Neurofibromatosis-1 are not Due to a Functional or Structural Defect in the Melanocyte. *Abdel-Malek Z, Swope V, Boissy Y, Boissy R, Severs S, Nordlund J.* Cultured melanocytes derived from hyperpigmented (café-au-lait) skin and adjacent non-lesional skin of neurofibromatosis-1 patients had similar (1) tyrosinase activity, (2) melanin content, (3) amounts of tyrosinase, TRP-1 and TRP-2, (4) proliferative rates, and (5) responses to melanogenic stimulators and inhibitors. Neurofibromatosis-1 melanocytes lacked ultrastructural abnormalities and were comparable to normal melanocytes derived from skin type matched donors in all the above criteria.

A highlight of the meeting were two elegant presentations at General Plenary Sessions entitled "*Lessons From the Mouse Regarding Human Pigment Disorders: Steel, Dominant Spotting and Microphthalmia*", by Neal Copeland, and "*Cutaneous Pigmentation: Lessons from the Bedside to the Gene*", by our own Richard King.

Current Active Members :

The following list of active members is included to make us all aware of those who belong to the Society, but also to point out the number of active investigators in the field who are not members. Each of us should make an effort to recruit potential members to our Society - both the Society and the member should benefit from this. If each member recruited only a single new member, our Society would obviously double in size !

Zalfa Abdel-Malek, University of Cincinnati
Ago Ahene, Advanced Polymer Systems

Robert Angus, University of Alabama-Birmingham
Pilar Aroca, National Institutes of Health

Lisa Austin, University of Cincinnati
Joseph Bagnara, University of Arizona
Jag Bhawan, Boston University
Karen Bijwaard, Georgetown University
Kenneth Bloom, University of Minnesota
Raymond Boissy, University of Cincinnati
Jean Bologna, Yale University
Frédéric Bonté, LVMH Recherche - Cosmetics
Roger Bowers, California State University
Murray Brilliant, Fox Chase Cancer Center
Stephanie Brown, University of Minnesota
John Brumbaugh, University of Nebraska
Jean Burnett, Michigan State University
Jean-Claude Bystryn, New York Univ Med Center
Ana Castrucci, University of São Paulo
Catherine Causse, L'Oreal
Miles Chedekel, MEL-CO
Alistair Cochran, UCLA Medical Center
John Conlee, University of Utah
Roy Cosan, Raritan, New York
Mark Costlow, Schering-Plough Corporation
Alan Dean, University of Kansas
John O Dejordy, Weizmann Institute of Science
Vincent DeLeo, Columbia-Presbyterian Med Center
Donna Durham-Pierre, Fox Chase Cancer Center
Magdalena Eisinger, Pearl River, New York
Jamal Farooqui, University of Cincinnati
Philip Fernandez, Department of Natural Sciences
William Fletcher, Oregon Health Sciences Univ
Paul Forlot, Laboratoires Pharmaceutiques
Timothy Frew, University of Nebraska
Thomas Froiland, Northern Michigan University
Sally Frost-Mason, University of Kansas
Bryan Fuller, University of Oklahoma
John Gardner, Fox Chase Cancer Center
Nels Granholm, South Dakota State University
Herbert Haberman, Toronto Western Hospital
Mac Hadley, University of Arizona
Ruth Halaban, Yale University
Rebat Halder, Howard University
Herlina Handoko, University of Minnesota
Elizabeth Hearing, Data Abstract Search Service
Vincent Hearing, National Cancer Institute
Meenhard Herlyn, The Wistar Institute
George Hill, New Jersey Medical School
Helene Hill, New Jersey Medical School
Thomas Holstein, Roger Williams College
Alan Houghton, Sloan Kettering Cancer Center
Rae Janet Jacobs-Cohen, Columbia University
Gert Jacobsohn, Hahnemann University

Myra Jacobsohn, Hahnemann University
Kowichi Jimbow, University of Alberta
Warren Johnson, University of Arizona
William Johnson, New York, New York
Koichiro Kameyama, Kitasato Institute Med Cen
John Kenney, Howard University
Sang Tae Kim, Kosin Medical College
Richard King, University of Minnesota
W Gerald Klingler, Springfield, Illinois
Joan Krakowsky, University of Cincinnati
Gary Kruh, Fox Chase Cancer Center
Byoung Se Kwon, Indiana University
M Lynn Lamoreux, Texas Veterinary Medical Cen
Maria Lamy-Freund, University of Sao Paulo
Jeffrey Laskin, Robert Wood Johnson Med School
Peter Lea, University of Toronto
Aaron Lerner, Yale University
Norman Levine, University of Arizona
Kenneth Mason, University of Kansas
Katsuo Matsumoto, University of Cincinnati
John McLane, Roche Dermatologics
David McLean, University of British Columbia
Estela Medrano, University of Cincinnati
Aravind Menon, University of Toronto
J Julian Menter, Morehouse School of Medicine
Frank Meyskens, University of California
Nadine Milos, University of Alberta
Beatrice Mintz, Fox Chase Cancer Center
Yutaka Mishima, Kobe University
Gisela Moellmann, Yale University
Kenji Mori, Kanebo, Ltd
Randall Morrison, University of Kansas
Bryan Murphy, Clairol R & D Laboratory
Yoshimichi Nakatsu, Fox Chase Cancer Center
John Naughten, Northern State University
J Michael Newton, University of Arizona
Richard Niles, Marshall University
James Nordlund, University of Cincinnati
William Oetting, University of Minnesota
Alice Oliveira, Fund Oncocentro de São Paulo
Seth Orlow, New York University

Jean Paul Ortonne, Hopital Pasteur
Madhu Pathak, Harvard Medical School
John Pawelek, Yale University
Andrew Pawlowski, University of Toronto
Regina Pietruszko, Rutgers University
Seymour Pomerantz, University of Maryland
Brian Potterf, National Cancer Institute
Neelu Puri, Academic Medical Center
Walter Quevedo, Brown University
Michael Rachkovsky, Yale University
Arthur Rhodes, University of Pittsburgh
Manuel Rieber, Centre de Microbiologica
Mark Rollag, Uniformed Services University
Robert Sayre, Rapid Precision Testing Laboratory
Uwe Schlehaider, New Jersey Medical School
Stefan Schmitz, University of Alberta
Thomas Schultz, Zotos International
Ian Scott, Unilever Research
Wade Sherbrooke, Amer Museum Natural History
Ie-Ming Shih, The Wistar Institute
Willys Silvers, University of Pennsylvania
Andrzej Slominski, Albany Medical College
J Robert Smyth, University of Massachusetts
Arthur Sober, Massachusetts General Hospital
Richard Spritz, University of Wisconsin
Karla Stoner, Gillette Research Institute
Manickam Sugumaran, Univ of Massachusetts
Richard Swank, Roswell Park Cancer Institute
Harold Swartz, Dartmouth-Hitchcock Med Center
Viki Swope, University of Cincinnati
John Taylor, Wayne State University
Kenneth Tomecki, Cleveland Clinic Foundation
DeWayne Townsend, University of Minnesota
Ram Tripathi, Fox Chase Cancer Center
Maria Carolina Tuma, University of São Paulo
Kazunori Urabe, National Cancer Institute
Setaluri Vijayasaradhi, Sloan-Kettering Cancr Cen
Maria Visconti, University of Sao Paulo
Michael Wick, Dana Farber Cancer Institute
Leon Wilkins, Organogenesis, Inc
Joseph Yohn, University of Colorado
Shimatani Youichi, Shiseido Co., Ltd
Lisa Zeise, MEL-CO
Huiquan Zhao, University of Cincinnati

Book Review :

Two reviews of a recently published book are reprinted here in their entirety

Melanins and Melanogenesis. Giuseppe Prota. Academic Press, San Diego, CA. ISBN: 0-12-565970-9; 290 pages; 1992. \$ 55.00

from *Pigment Cell Research*, **6:(in press), 1993**. "This long overdue book on melanin pigmentation should prove to be a landmark publication on the biosynthesis, structure, physicochemistry, and function of melanins. Dr. Giuseppe Prota has been a leading authority on melanin structure and synthesis for over 30 years and he continues as an active researcher on the subject. Although the author has purposely avoided being encyclopedic his selection of topics summarizes in adequate detail the basic concepts and controversies that relate to the biochemical processes related to melanogenesis within melanocytes. The major chapter captions of the monograph reveal the broad coverage provided and indicate that the book should be of interest not only to pigment cell biologists/chemists but also to: physiologists, geneticists and clinicians of several disciplines such as dermatology and oncology. Following an introductory chapter on the major issues of melanin research and the biological significance of melanin pigmentation in animals including man, the author provides a very well organized series of chapters that include: Melanin-Producing Cells, Tyrosinase, Natural and Synthetic Melanins, Eumelanins, Neuromelanin, Pheomelanins and Trichochromes, Pigment Cell Metabolism, Enzymatic and Chemical Control, Genetic and Hormonal Regulation of Melanogenesis, Photobiology and Photochemistry of Melanogenesis. Over 1100 pertinent references are provided in this book of about 300 pages in length.

Of the 224 pages or so of text (about 62 pages of references) only about one-fourth is related to the biology of pigment. Nevertheless, the book is about "Melanins and Melanogenesis" and as a world authority on the topic, the author has provided an in-depth coverage of what is known about melanin pigments which play such an important role in the cellular physiology of animals including man. Although much of the book concerns itself with the chemistry and biosynthesis of melanin, as a biologist/endocrinologist I was struck by how well the author integrated the chemistry with biology to provide a book of real relevance to the biologist and clinician.

This very readable monograph certainly belongs on the bookshelf of anyone interested in pigment cell biology. I suspect that "Melanins and Melanogenesis" will also find its way into the hands of a much larger audience.

Mac E Hadley, Dept of Anatomy, University of Arizona

from *Melanoma Research* **3:81, 1993** A book summarizing the state of the art in melanin research has finally made an appearance; in spite of the recent surge in interest in melanin research internationally, it had been almost 25 years since Nicolaus wrote his book entitled "Melanins". Dr. Prota has undertaken the formidable task of summarizing research on pigmentation from the biological, medical and especially chemical perspectives. This is quite an ambitious undertaking since advances in numerous relevant areas that have taken place even in the last decade alone are staggering. What has evolved in this book is an extremely interesting synopsis of current thinking of melanogenesis, and the reader should find it a useful research summary as well as an excellent reference work. The citations are up-to-date (through early 1992) and I think that most scientists, even those highly familiar with the topic, would appreciate this addition to their library.

The book recounts current understanding on a variety of topics, some of which might be considered somewhat peripheral to the field, but the sum of which constitute the encompassing field of melanin research. Included as topical headings of chapters are: • An Introduction to Melanin Research, • Melanin-Producing Cells (which discusses not only melanocytes but melanophores and other pigment producing cells), • Tyrosinase (activation and inactivation), • Natural and Synthetic Melanins, • Eumelanins, • Neuromelanins, • Pheomelanins and Trichochromes, • Enzymatic and Chemical Control of Pigment Cell Metabolism, • Genetic and Hormonal Regulation of Melanogenesis, and • Photobiology / Photochemistry of Melanogenesis. As you can see this is a very ambitious undertaking to summarize research in such disparate areas, and yet Professor Prota has done an outstanding job in this regard. He has provided an overview of

each topic that is up-to-date, yet includes enough details of each section to make the reading interesting and pertinent even to those active in each area of expertise. An added bonus is the rich bibliography of approximately 1,000 references - many of which I must admit I was unaware of (even those in my own field).

Professor Prota considers each topic as a part of the whole concept of the field of melanin research and ties each aspect of study nicely into the overall scheme presented. He does an admirable job of presenting the data as an objective scientist; it is obvious that this book has been a long time in the making and is truly a labor of love. In sum, I would suggest that this book would be a valued member of any research library, particularly so for those of us actively engaged in research in the field.

Vincent J Hearing, Laboratory of Cell Biology, National Institutes of Health

Bibliography :

Some questions have arisen about the criteria being used to generate the Bibliography published in the **PASPCR Newsletter**, both with respect to its focus and its contents. The Bibliography is being generated from the Life Sciences edition of Current Contents (600 journal edition), and will include listings from the three month period immediately prior to publication of each **Newsletter**. Thus the Bibliography published in the February issue covered the three month period from mid-November, 1992 through mid-January, 1993. The Bibliography published in this issue covers the period mid-February, 1993 through mid-May, 1993. We realize that this approach does not cover all disciplines and time frames and hope that everyone will bear these constraints in mind when they scan these Bibliographies. Nevertheless, the great majority of papers published in the field should be covered in this search, and we hope that the members of our Society will find this a useful supplement to their own literature searching strategies. If you notice a paper that was not detected by this search that should be included, please send it to us and we will include it in the next issue. We have highlighted publications which include a member of the **PASPCR** with an asterisk.

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