# P A S P C R

## *N e w s l e t t e r*

*Volume 1 Number 4* *December, 1993*

## Introduction . . .

The **PASPCR** ***Newsletter*** is published quarterly and 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, Calendar of Events, Meeting Reports, Abstracts in press and other newsworthy items. 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.

## Welcome to New Members

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

Roger D. Cone
Seung-Kyung Hann
Soverin Karmiol
William J. Pavan

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

**IN THIS ISSUE . . . .**

**PanAmerican Society for Pigment Cell Research**

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The PASPCR Newsletter is published quarterly; for further information and/or to submit contributions, please contact:

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## Calendar of Events :

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

***April 10 - 13, 1994*** Annual Meeting of the American Association for Cancer Research, to be held in San Francisco, California (contact: AACR Office, Public Ledger Building, 620 Chestnut Street, Suite 816, Philadelphia, PA 19106-3483 FAX: 215/440-9313)

***April 27 - 29, 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*** V$^{th}$ PASPCR Annual Meeting, to be held in Philadelpia, Pennsylvania, (contact: Dr. Gert Jacobsohn, Department of Biological Chemistry, Hahnemann University, Broad and Vine, Philadelphia, PA 19102-1192, FAX: 215/762-3722)

***Oct 19 - 22, 1994*** 5$^{th}$ ESPCR Annual Meeting, to be held in Vienna, Austria, (contact: ESPCR '94, Vienna Academy of Postgraduate Medical Education and Research, Alser Straße 4, A-1090 Vienna, Austria, FAX: 43-1-42-138323)

***June , 1995*** VI$^{th}$ PASPCR Annual Meeting, to be held in Kansas City, Kansas, (contact: Dr. Sally Frost-Mason, Department of Physiology, University of Kansas, 3038 Hayworth Hall, Lawrence, KS 66046-2106, FAX: 913/864-5321)

***Oct 29 - Nov 3, 1996*** XVI$^{th}$ International Pigment Cell Conference, to be held in Anaheim, California, (contact: MMC/UCI Center for Health Education, P.O. Box 1428, Long Beach, California 90801-1428, FAX: 310/933-2012)

## Corporate Sponsors

The **PASPCR** would like to acknowledge and thank our Corporate Sponsors. Financial gifts from these sponsors have allowed our Society to increase benefits to the membership far out of proportion to the actual dues collected from members. Monies contributed by these sponsors have been used over the years to support various **PASPCR** functions including our Young Investigator Award program, meeting travel stipends, annual meeting expenses and this ***Newsletter***.

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## PASPCR Election Results

We congratulate the winners of the most recent election and thank all who participated in the process. New Council Members who will serve from January, 1994 through December, 1996 are:

Dr. Raymond Boissy, University of Cincinnati
Dr. DeWayne Townsend, University of Minnesota
Dr. Gert Jacobsohn, Hahnemann University

Our next election will be held in the fall of 1994; the Nominations Committee will again be chaired by Dr. Sally Frost-Mason, as required by PASPCR By-Laws. Anyone who is interested in being considered for nomination in the next election could express that interest to Dr. Frost-Mason and/or employ the mechanism outlined in the By-Laws for petitioning to be on the ballot.

## PASPCR Young Investigator Awards

**by Vincent Hearing**

Two PASPCR Young Investigator Awards were presented at the XIVth IPCC held recently in London. They were given to Dr. William Pavan (Princeton University) for his platform paper entitled "Piebald lethal acts early to disrupt the development of neural crest derived melanocytes" and to Dr. Setaluri Vijayasaradhi (Memorial Sloan Kettering Cancer Center) for his platform paper entitled "Identification of signals for sorting and targeting of melanosomal membrane proteins". Congratulations to both of these young investigators for their outstanding presentations and we will look forward to hearing of your future progress at upcoming meetings. We would also like to thank the Anonymous Committee that selected these awards; they were looking for novel strategies and findings in pigment cell biology in their deliberations. This is an especially difficult task to cover all platform and poster sessions and we thank them for their conscientious work in this regard.

## PASPCR Career Achievement Award

**by James Nordlund**

The PASPCR council formally established the PASPCR Career Achievement Award. It shall be the most prestigious award given by the Society to an individual for a career distinguished by outstanding contributions to pigment cell research. The receipt of the Award and the unique medal, without a monetary honorarium, are to be considered to be a recognition by the membership and the officers of the distinction and accomplishment achieved by the recipient. The Award and the method of selection of the awardee is as follows:

(a) The recipient will be a senior investigator generally with an academic rank of professor or its equivalent for those in industry and government service.

(b) The recipient shall be a member of the Society except in those very rare times in which an individual in another discipline makes such discoveries and contributions that the field of pigment cell research clearly advances to the benefit of all investigators.

(c) The Society shall have a special plaque designed unique for this Award upon which are inscribed the full name of the Society, the logo of the Society, the name of the Award, the name of the Awardee, the year in which it was presented, and other appropriate information.

(d) the Award will be presented at the annual meeting of the Society.

(e) the nominating committe appointed by the President shall be charged to initiate immediately following each annual meeting to prepare a slate of three candidates along with supporting documentation which will be presented to the Council no later than January of the following calendar year. This slate is confidential for the Committee and the Council only.

(f) The Society will request additional nominations from the membership at large. Such nominations will require the same dossier and documentation as required by the nominating committee. At least five members at large must write letters of support for the nominee, such letters shall be included in the dossier.

(g) the Council shall vote by secret ballot for one or several candidates. A majority of votes shall be required for election. If necessary at least the two candidates receiving the highest number of votes shall be voted on by a second secret ballot.

(h) Usually only one Award shall be given each year except in rare instances where two or more individuals have worked as a team or made such outstanding accomplishments to be deserving equally of the award. It is not required that an Award be given annually.

## 1994 PASPCR V$^{th}$ Annual Meeting

The V$^{th}$ 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*** and in the direct mailings of the Announcements of the meetings. By now everyone should have received the first announcement. The Call for Abstracts will be sent out early in 1994 and the Abstract deadline will be March 15, 1994. We hope that all members of the **PASPCR** will plan to attend this meeting - be sure to mark it on your calendar.

## 1995 PASPCR VI$^{th}$ Annual Meeting Site :

The **PASPCR** Council unanimously and enthusiastically voted to accept the bid by Dr. Sally Frost-Mason to host the VI$^{th}$ Annual Meeting of the **PASPCR** in Kansas City in June, 1995. We are very excited about this site for the meeting and feel that Sally will do an outstanding job in maintaining the high standards set for the **PASPCR** meetings to date. She already has drawn some tentative plans for the meeting that promise to make it a memorable one and further details about the 1995 Annual Meeting will be forthcoming in future Newsletters and mailings.

## PASPCR Secretary / Treasurer's Report : by Richard King

Following is a synopsis of the **PASPCR** Council Meeting held by telephone conference call on July 22, 1993.

Hearing opened the meeting and the minutes of the telephone council meeting of April 22, 1993, were reviewed and accepted.

Nordlund gave the report for the *ad hoc* Committee on a Senior Investigator award. The committee proposed the following: 1) an award will be given to an individual with a life-long career devoted to pigment cell research and distinguished by outstanding accomplishments and distinguished scholarship; 2) the award will be a medal; 3) the award will be given to PASPCR members only; 4) the Nominating Committee of the society will nominate three candidates, and the council will select the recipient by secret ballot; and 5) the award will be the most pretigious given by the Society. Nordlund was instructed to prepare a final Career Achievement Award proposal for adoption as a rule and regulation during the next council meeting.

Abdel-Malek gave the Publication Committee report. Possible review of the London IPCC meeting in the Newsletter was discussed since all PASPCR members will not be attending. The Publication Committee had felt that it was not necessary to review PASPCR or IPCC meetings but may consider some IPCC material in an upcoming issue. There was general congratulations to Abdel-Malek and her committee for the newsletter.

Financial support for the XVI IPCC Anaheim meeting in 1996 was reviewed. F. Meyskens, chairman, has requested $10,000 of support for this meeting The council agreed (given in increments of $2,500 per year) for the 1996 Anaheim meeting. The Council also agreed as follows: (1) the organizing committee of the XVI IPCC will be informed that they will receive $10,000 from the Society (given in increments of $2,500 per year); (2) any excess funds after the meeting should be refunded to the society; and (3) if there were insufficient funds to pay for the meeting, then the society could help the organizing committee with this debt within the budgetary limits of the society.

Financial support for the PASPCR meeting to be held in Philadelphia in 1994 was discussed. No outside support has been identified for this meeting and it was suggested that the PASPCR pledge financial support in the range of $5,000. It was felt that there was a need to keep the registration fee at a moderate level so that young investigators and students would attend the meeting. This item was tabled until the next Council meeting.

Frost-Mason has proposed having the 1995 meeting in Kansas City, Missouri, and the initial plans were reviewed. This meeting would be held in June, 1995. Further discussion will be held at the next Council meeting.

King gave the election update. The biographical sketches have been received from the 5 nominees for council positions. No outside nominations have been proposed.

There being no further old business or new business, the meeting was adjourned. Minutes have been prepared by R. King, Sec.-Treas.

## 1993 PASPCR General Membership Business Meeting

**by Richard King**

Minutes of the General Membership meeting held in London, Kensington Town Hall on September 29, 1993

Opening remarks were delivered by President Vincent J. Hearing. He welcomed the 28 new members who have joined the Society in 1993. He asked for a moment of silence for departed member, Carl J. Witkop, Jr. President Hearing distributed Certificates of Service to James J. Nordlund for service as founding president, 1989-1992; to Raymond E. Boissy, Nels H. Granholm and Herbert F. Haberman, for founding Council membership, 1989-1992; and to Joseph T. Bagnara, John A. Brumbaugh and Kowichi K. Jimbow for Council membership from 1991-1994. R. King, secretary-treasurer, gave the treasurer's report. The PASPCR account balance was $23,255.04 at January 1, 1992, and $23,958.37 on December 31, 1992. The account balance as of August 31, 1993, is $33,420,39. The minutes of the June 10, 1992, general membership meeting were accepted. President Hearing gave the results of the Committee reports. The *ad hoc* Committee on a Career Achievement Award, chaired by J. Nordlund, has proposed a PANAMERICAN Society for Pigment Cell Research Career Achievement Award to be given with a medal on an annual basis to a senior investigator with a prestigious and meritorious career in pigment cell research. The proposal has been accepted by the Council and the first Award will be given in 1994. The Newsletter Committee, chaired by Z. Abdel-Malek, has worked hard to produce a viable Newsletter of the society. Several new items of the Newsletter have been suggested and a quarterly Newsletter will be continued. Future annual meeting locations were discussed. The 1994 meeting will be held in Philadelphia, and the host will be Gert M. Jacobsohn. The 1995 meeting will be held in Kansas City, Missouri, and the host will be Sally Frost-Mason.

The 1993 Young Investigator Awards were announced. These awards were presented to William J. Pavan, Ph.D., for his work entitled "Piebald Lethal acts early to disrupt the development of neural crest derived melanocytes" and to Setaluri Vijaysaradhi, Ph.D., for his work entitled "Identification of signals for sorting and targeting of melanosomal membrane proteins".

## Announcement of Dues Increase for 1994

The Council of the PASPCR has voted to raise the annual dues from $63 to $100 for regular members and from $28 to $35 for student members. The PASPCR dues will include annual dues of $25 for the International Federation of Pigment Cell Societies (IFPCS). This dues increase will take effect for 1994.

The PASPCR was founded in 1987, and has grown into an active, vital society for pigment cell biology. From the beginning, the Council has been devoted to the promotion of pigment cell research and the development of young scientists who can carry on and expand the many wonderful areas of research. To achieve these goals the Council has established the following over the past seven years:

1. A PASPCR Newsletter that is published quarterly.
2. Travel Awards for students and junior faculty. A total of $4,200 (up to $300 per individual) is provided annually for each PASPCR meeting.
3. Young Investigator Awards. Up to three awards for $250 each are given annually at each PASPCR meeting.
4. Honorary Membership in the PASPCR. Honorary members (usually no more than one per year) are honored at the annual meeting, and travel expenses are provided.

5. PanAmerican Society for Pigment Cell Research Career Achievement Award. One award each year in the form of a medal.
6. Travel stipend for PASPCR officers to attend the annual council meeting of the International Federation of Pigment Cell Societies when the meeting is held outside of the U.S.
7. Support for the XVI International Pigment Cell Conference. to be held in Anaheim in 1996. The support will be $2500 per year for four years (1993-1996).
8. Support for the annual PASPCR meeting.

The current estimated annual costs for the PASPCR, including the items above and other expenses (e.g., office expenses, quarterly telephone council meetings) is approximately $14,000. The increase in dues is necessary to continue with these activities and to keep the Society solvent.

## Meeting Report : by PASPCR Council Members

***XIVth International Pigment Cell Conference, September 26-30, 1993, London, England***

There were many different Symposia, Workshops and Plenary Sessions that covered virtually all aspects of pigment cell biology. The Seiji Memorial Lecture was delivered by Prof. T Takeuchi and the IFPCS Presidential Address was given by Prof Y Mishima. The Myron Gordon Award was given to three scientists, Prof P Riley, Prof H Rorsman and Prof T Takeuchi. A new award (the Raper Prize) was also established for an outstanding scientist in the field of melanin chemistry and/or biochemistry; it was given to Dr. J Pawelek The following summaries of the various sessions were compiled by members of the PASPCR Council who attended those sessions. If any members who attended sessions at the London IPCC not reported on below would like to write short synopses of those sessions for publication in the next **Newsletter**, it would be greatly appreciated.

Prof. Riley was kind enough to send a limited number of program booklets for distribution to those interested but unable to attend the meeting. These will be distributed on a first-come first-served basis. Please request from Dr. Vincent Hearing, Bldg 37 Room 1B22, National Institutes of Health, Bethesda, MD 20892 USA

### - - - - - *Monday, Sept 27* - - - - -

**Plenary Session I - Normal Melanocyte Biology and Development - by Joe Bagnara**

The session began with a presentation by DC Bennett who showed how the cultured melanocyte carrying mutations at color loci such as the albino tyrosinase locus, can be used to show complementation by a cloned candidate gene, characterization of the mutation, and biochemical consequences that ensue. Genes controlling the development, growth, and function of normal melanocytes were considered. These included the receptor and proto-oncogene W/kit as well as the recessive spotting (rs), and piebald spotting (s) which affect melanocyte/melanoblast proliferation. The extension of knowledge about melanocytes to malignant melanoma cells was considered in a comparison of gene expression in cultured melanomas and melanocytes. Various non-random changes were revealed in several tyrosine kinase receptors.

Quite appropriately, this was followed by a penetrating presentation on cell signalling and protein kinase c (PKC) by PJ Parker. The PKC family consists of some nine genes. The differential expressions of PKC genes, activated on binding diacylglycerol (DG), suggest specific roles for PKC isotypes. Individual PKC isotypes present in particular cell types, display characteristic locations and activation patterns in response to stimuli.

JR Woodgett then spoke about Nuclear Onco-Protein targets of signal transduction pathways. He pointed out that since oncoproteins are components of signal transducing pathways, they are extremely important in the control of cell growth and differentiation. He focused his talk on the acute regulation of two nuclear

oncoproteins, cJun/API and cMyc. He considered the various protein kinases and phosphatases that regulate nuclear oncoproteins.

Signal transduction was again the focus of the last two presentations of the session as A Bernstein spoke about "Genetic approaches to the elucidation of signal transduction pathways that control normal melanocyte development" and R Halaban considered "Growth factor signal transduction in normal and malignant melanocytes".

**Cellular Effects of Light - by Kowichi Jimbow**

"Photooxidation of retinal pigment epithelium - A model for melanin aging" by M Rozanowska et al. This study was aimed to characterize the anti-oxidant ability of retinal pigment epithelium (RPE) melanin changes with aging and what factors might be involved in melanin aging. Isolated melanosomes from RPE were exposed to intense light, resulting in bleaching of these melanosomes under electron microscopy. Similar changes were observed in human RPE melanin with age, corresponding to ESR signals of RPE cells from middle aged and older donors, indicating that aging is also associated with partial bleaching of melanin within RPE. Discussion followed regarding the actual images of melanin bleaching from the melanosomes under electron microscopy. It appears that bleached melanosomes may show some unique, fine structural changes.

"Ultraviolet light, the cell cycle, and expression of MSH receptors in the epidermal-melanin unit" by J Pawelek et al. This paper discussed the stimulation of MSH receptors by UV light. They found that the MSH receptor from mouse melanoma cells and immortalized human keratinocytes are similar in affinity receptors, affinity classes, and binding competition. They concluded that coordinated regulation of the epidermal melanin unit by UV light is central to the MSH receptor system.

"The superoxide anion may have a role in mediating UV-induced melanogenesis" by D Tobin et al. In this study, B16 F1 murine melanoma cells were exposed to UV light (UVB plus UVA). The combined UVA and UVB exposure directly acted on the melanoma cells, having both short and long-term effects on melanogenesis. Importantly, the long-term effects were not dependent on the generation of reactive oxygen species, but the short-term action could be mediated in part, by this oxygen species.

"Abnormal stress gene induction in human malignant melanoma cell lines" by LA Applegate et al. This was another paper which discussed oxidative stress after UV exposure. All five melanoma cell lines revealed abnormal expression of human hemoxygenase (HO-1) gene. Two of them revealed the induction of HO-1 mRNA similar to those of other human cell types with maximum induction at 2 to 4 post-irradiation. In contrast, the remaining 3 revealed extremely low expression of HO-1 mRNA and no sign of induction, indicating there is heterogeneity in the expression of HO-1 mRNA as well as its induction.

"Role of gene expression and protein synthesis of tyrosinase, TRP-1, LAMP-1 and CD63 in UVB induced melanogenesis" by H Hara et al. This paper dealt with the expression of melanogenic genes as well as melanogenesis-associated genes after exposure to UVB. They found that tyrosinase was a key enzyme responsible for UV-induced melanogenesis and the TRP-1 and LAMP-1 coordinates this melanogenesis in UV-induced melanogenesis, TRP-1 being more important than LAMP-1. In contrast, there is no CD63 gene involvement in melanogenesis.

"A possible unique mechanism for UVB-induced hyperpigmentation" by Z Abdel-Malek et al. This paper discussed the activation of cultured normal human melanocytes after exposure to UVB, focusing primarily on melanogenesis. They found that stimulation of melanogenesis by UVB resulted primarily from increased tyrosinase activity without any significant increase in the amount of enzymes. There was a decreased expression of TRP2 which might result in preferential synthesis of insoluble dark brown melanin pigment enriched with 5,6-dihydroxyindole. This paper had some conflicting findings with a previous paper by Hara et al, regarding the activation of gene expression encoded tyrosinase.

**Melanogenesis Symposium - by John Pawelek**

Several papers were presented on the potential catalytic functions(s) of TRP-1.

Kobayashi, Winder, Urabe, Brewington, Imokawa and Hearing transfected a TRP-1 gene into fibroblasts and demonstrated that the corresponding cell extracts were able to recognize DHICA as a substrate. Extracts of B/B mouse melanocytes acted similarly to the transfected fibroblasts, but extracts of b/b mouse melanocytes showed significant reductions in the process. Conclusion: TRP-1 can catalytically convert DHICA to an (as yet) unknown product.

Jimenez-Cervantes, Solano, Lozano and Garcia-Borron identified two forms of melanosomal "tyrosinase", designated LMT and HMT. Unlike HMT, LMT was a poor tyrosinase or dopa oxidase, but it was more efficient than HMT as a DHICA conversion factor. The product of the conversion reaction was not characterized. A side-by-side comparison of LMT and immunopurified TRP-1 showed similar DHICA conversion characteristics, as described by Kobayashi et al. (see above). Conclusion: LMT may be identical to TRP-1.

Zhao, Nordlund and Boissy used immunopurified TRP-1 and showed that it had tyrosine hydroxylase activity, but no dopa oxidase activity. The ability to hydroxylate tyrosine was retained in B/B melanocytes but deficient in b/b melanocytes. Conclusion: TRP-1 may initiate melanogenesis through the production of L-dopa from L-tyrosine.

Tsukamoto, Kobayashi, Winder, Urabe, Potterf and Hearing using immuno-purified tyrosinase, TRP-1 and TRP-2 as well as extracts of fibroblasts transfected with the corresponding genes to demonstrate that tyrosinase exhibits increased thermostability when incubated in the presence of TRP-1. Conclusion: TRP-1 may bind to tyrosinase, thus regulating melanogenesis through protein/protein interactions.

Winder, Wittbjer, Odh, Rosengren and Rorsman used mouse fibroblasts stably expressing TRP-1 to demonstrate that TRP-1 exhibits dopachrome tautomerase activity. Assays were carried out with cell extracts, where DHICA could be isolated as a product, as well as with living cells where the culture media contained methylated carboxylated indoles (DHICA metabolites). They could not demonstrate tyrosinase or catalase activities in parallel experiments. Conclusion: TRP-1 acts as a dopachrome tautomerase *in vivo* as well as *in vitro*.

[Note: In addition to these multiple functions reported above for TRP-1, Halaban and Moellmann (Proc Natl Acad Sci USA 87:4809-4813, 1990) have reported an associated catalase activity. Thus, a perplexing array of activities and functions have all been assigned to this enigmatic protein. The one biological function that is certain is that in the wild-type state, TRP-1 causes mice to be black, whereas in the mutated state, mice are brown. (Perhaps TRP-1 should be referred to as the "black" protein)].

Chakraborty, Osber and Pawelek demonstrated that a glycoprotein partially purified from a variety of melanomas and normal melanocytes can convert DHICA into a water-soluble melanin (the solubility in aqueous buffers presumably attributable to the retention of DHICA carboxylic acid groups by the resulting melanin product). Evidence that this factor is not TRP-1 came from the fact that it is abundant in Cloudman S91 cells that originated in b/b mice. However, there was evidence that the activity is associated with the pmel-17 gene which maps to the *silver* locus in mice. The DHICA conversion reaction was inhibited by the addition of superoxide dismutase. Conclusion: There is evidence for a DHICA oxidative polymerase.

Benathan, Scaletta and Frenk compared the levels of glutathione (GSH) levels and various GSH regulatory enzymes to the levels of dopa oxidase and melanin content of 4 human melanoma cell lines. Conclusion: GSH levels can balance the oxidative stress associated with melanogenesis.

Palumbo, d'Ischia, Misuraca and Prota described a new enzymatic activity in the ink of *Sepia officinalis* that catalyzes the conversion of dopachrome to 5,6-dihydroxyindole (DHI). They propose that this activity is related to the mammalian TRP-2 (dopachrome tautomerase) in that it catalyzed the rearrangement of dopachrome, however, a decarboxylation step was also present that resulted in the formation of DHI. It was not determined whether the activity was due to single or multiple enzymes. Conclusion: Invertebrate melanogenesis may be different from mammalian melanogenesis.

Peter presented a summary of melanogenesis in insects where dopamine and DHI are principle precursors for melanin. Melanins in insects are important not only for cuticle (exoskeleton) pigmentation, but also for wound healing and immune responses. The phenoloxidases in the cuticle include tyrosinase, o-diphenol oxidase, laccase, peroxidase and a tautomerase (to name a few). Conclusion: Insect

melanogenesis clearly differs from mammalian melanogenesis in at least some aspects, however, understanding the pathways of melanogenesis throughout the phyla should be of great help when focussing on any particular pigmentary system.

## - - - - - *Tuesday, Sept 28* - - - - -

### Genetics and Disorders of Pigmentation - by Roger Bowers

IJ Jackson ("Genetics and Molecular Biology of Mouse Pigmentation") reviewed the current information on selected mouse pigmentation genes that control transcription (mi), morphology (d, ash, ln, dsu), transport (c, b, slt) and MSH response (a, e). The characterization of the TRP proteins and pmel 17 (si locus) was discussed. TRP-1 (b locus) works downstream from TRP-2 (dopachrome tautomerase, slt locus) and works only on eumelanin. Two mutations of b (TRP-1) cause melanocyte death and these are $B^{lt}$ (arg to cys substitution) and $B^{W}$ (white based brown). $B^{W}$ may act by ecotropic activation of a lethal gene. Since $B^{lt}$ melanocytes live in albino mice, the melanocyte death due to the $B^{lt}$ mutation is not due to a protein product, but due to toxicity produced during melanogenesis.

RA King ("The Genetic Complexities of Human Oculocutaneous Albinism") reviewed the mutations associated with OCA 1 (tyr neg) and OCA2 (tyr pos) and stated that the real key to diagnosing OCA is ocular problems (reduced pigment in retina and iris and misrouting of optic fibers) as well as reduced skin pigmentation. In OCA1 the majority of the mutations are missense (30-40), frameshift (9) and nonsense (5). The correlation between animal models and human OCA was discussed and in particular OCA1ts, temperature sensitive OCA. OCA2 may be genetically heterogeneous. The P gene mutations are correlates of mouse pink-eye dilution genes and are associated with tyr-pos OCA and Prader-Willi and Angelman Syndromes.

In his Presidential address, Y Mishima ("Molecular and Biological Controls of Melanogenesis - Through Tyrosinase Genes and Intrinsic and Extrinsic Inhibitory Factors") reviewed the current status of knowledge of the intracellular pathways of melanogenesis including the GERL-CV membrane system and the enzyme tyrosinase and TRP proteins. The major portion of his presentation focused on the question "Are melanosomes specialized lysosomes?". Four lines of evidence were presented to support the concept that melanosomes are specialized lysosomes: 1) melanosomes will phagocytosize latex particles like lysosomes; 2) both receive tyrosinase and hydrolases from coated vesicles; 3) both possess LAMP1; and 4) amelanotic melanosomes form lysosomal-like myelin figures. The remaining portion of his presentation discussed his neutron bombardment treatment of melanoma.

DA Norris ("Melanocyte Destruction and Repopulation in Vitiligo") reviewed the previously suggested causes of vitiligo (immune, cytotoxic and neural secretory) and discussed possible new causes of vitiligo based on current research. These include defective anti-oxidant defense mechanisms against reactive oxygen species, defective growth factors, defects in endoplasmic reticulum processing of melanin, abnormal responses to cytokines, and genetic determinants of vitiligo. He discussed repigmentation of vitiliginous areas by melanocytes from hair follicles, skin grafts and melanocyte autographs, and the effects of PUVA-induced mediators, cytokines and growth factors to stimulate melanocyte proliferation and migration during repigmentation. Leukotrienes C4 and D4 stimulated melanocyte proliferation and collagen type IV enhances melanocyte migration through integrin receptors.

JP Ortonne ("Clinical Aspects of Human Disorders Related to Altered Embryogenesis of the Melanin Pigmentary System") reviewed the clinical aspects of vitiligo and other hypopigmentation diseases of the skin. He gave case studies and treatment responses for vitiligo and those hypopigmentation diseases that affect the embryological proliferation, migration or survival of melanocytes in humans such as piebaldism. He presented an excellent review of the current literature on these diseases of the skin.

### Molecular Biology and Genetics of Pigmentation - by Murray Brilliant

Papers presented in this Session covered some of the major genes involved in pigmentation in mice and man and included the genes tyrosinase, pink-eyed dilution, silver, piebald, brown and a new protein

encoded by the p90 cDNA. In addition, a new amino acid motif that may function to target proteins to the melanosome was reported. A synopsis of each talk, in the order of presentation follows:

MH Brilliant reported that mutations in the mouse pink-eyed dilution gene (encoding a protein with twelve membrane spanning domains) are associated with hypopigmentation and only a small amount of pheomelanin is produced in mice with null alleles. The human gene maps to an area commonly deleted in patients with Prader-Willi (PWS) and Angelman Syndrome (AS) who are often hypopigmented. A subset of PWS and AS patients also have OCA type II. In these patients, mutations in the remaining chromosomal homologue of the human P gene were detected, rendering them null for expression of the P gene. Mutations in both homologues of the P gene of OCA type II patients who do not have PWS or AS were also detected. Thus, the human P gene is associated with OCA type II.

BS Kwon reported that the gene encoding Pmel 17, a 95 kD membrane glycoprotein with three internal 26 amino acid repeats, was mapped to mouse chromosome 10, near the silver (*si*) locus. To determine whether Pmel 17 was the product of the silver gene, the sequence of Pmel 17 cDNA from C57BL/6 melanocytes was compared with that from silver melanocytes. Two single base insertions (one in the transmembrane domain, the other in the cytoplasmic domain) distinguish the silver Pmel 17 sequence from wild-type Pmel 17. These insertions cause a frameshift in the predicted protein sequence leading to a truncated product. Thus, Pmel 17 and the silver gene product are the same. The phenotype of the silver mutation suggests that Pmel 17 is an important gene for normal melanin biosynthesis.

WJ Pavan presented results wherein the developmental timing of expression of the piebald letha ($s^l$) mutation was examined. Mice homozygous for $s^l$ lack hair follicle melanocytes and have a completely white coat. An antibody directed against TRP-2 (tyrosinase related protein 2) was used as an early differentiation marker to identify melanocyte precursors in wild-type and $s^l$ homozygous mice. In wild-type mice, TRP-2 positive cells were first detected in embryos starting at day 10; TRP-2 positive cells were found in the pigmented retinal epithelium and choroid layers of the eye, in the skin and hair follicles, inner ear, endolymphatic duct, and telancephalon. However, in $s^l$ embryos TRP-2 staining cells were observed only in regions where non-neural crest melanocytes are derived. Thus the action of the $s^l$ mutation is very early in development and its effects are limited to neural crest derived cells.

WS Oetting reported on the cumulative results of more than 40 mutations in the human tyrosinase gene that lead to OCA1. Analysis of these mutations reveal the relationship of tyrosinase structure to its function. Among the essential functional regions of the protein is the signal peptide sequence. Without this sequence the tyrosinase message is translated on free ribosomes instead of membrane bound ribosomes. The resulting protein is cytosolic and non-functional. Other essential regions are the membrane spanning region and copper binding sites.

L. Breimer reported on mutations in the tyrosinase gene in British albinos. In addition to several different point mutations resulting in amino acid changes, the common exon III 373 C to A transversion was detected in 3 of 8 patients, close to the published 25% frequency. Other mutations and their significance to tyrosinase function were presented.

RE Boissy reported on studies of a patient with Brown OCA and his unaffected fraternal twin brother used as a control. Melanocytes from these siblings were cultured and characterized. The Brown OCA melanocytes contained predominantly amelanotic premelanosomes, a few early stage melanosomes, and were positive for tyrosinase as determined by classic DOPA histochemistry. No TRP-1 protein was identified in the Brown OCA melanocytes by a variety of anti-TRP-1 antibodies, nor were transcripts of TRP-1 detected by Northern analysis. Differences in insoluble melanin as well as the kinetics and stability of tyrosine hydroxylase activity were noted between the Brown OCA melanocytes and controls. None of the differences noted were common with other forms of tyrosinase positive OCA.

K Jimbow reported on the c90 cDNA clone, encoding a novel melanosomal membrane protein. This clone was initially obtained from a human melanoma cDNA expression library using a rat polyclonal antibody against human melanosomal proteins. The predicted c90 protein shares 93% homology with dog calnexin, a chaperone-like ER protein, and anti-dog calnexin recognizes the human protein. Immuno-EM gold labeling localized the c90 protein to the melanosome membrane and to the ER. Sequence analysis predicts that the c90 protein will have a Ca-binding loop, PKC and PKA phosphorylation sites.

S Vijayasaradhi reported that melanosomal membrane proteins may share a common sequence that targets these proteins to the melanosomal membrane. In melanocytes, gp75 (TRP-1) is targeted to melanosomes. However, when gp75 is transfected into fibroblasts, the protein is targeted to lysosomes. In contrast, in fibroblasts CD8 is targeted to the cell surface. Various chimeric gp75/CD8 cDNA constructs were transfected into fibroblasts and gp75 negative melanoma cells. The subcellular location of the chimeric proteins were determined to help identify the sorting signals. A short amino acid sequence in the cytoplasmic tail of gp75 was found to contain a signal that targets the protein to the melanosome in melanocytes (or the lysosome in non-melanocytes). In addition to being found in the TRP-1 sequence, this same signal was detected in other melanosomal proteins including tyrosinase, pmel 17, and P. However, this signal is absent in TRP-2.

**Evolution and Expression of the Tyrosinase Protein Family - by Sally Frost-Mason**

The papers that were selected to be part of this workshop all had as their common theme the molecular genetics of the tyrosinase gene family. The organizer and moderator for the discussion was IJ Jackson.

The first presentation, by R Morrison, was a cladistic analysis of the evolutionary relationships of the tyrosinase and related gene family based on sequence data. His parsimony-based analyses demonstrated that sequence similarities, among tyrosinase family genes and related genes for molecules such as hemocyanin, can be used to construct phylogenies. Such genes are highly conserved especially within their copper-binding domains. Cladistic analyses suggest that tyrosinase is, evolutionarily, a very 'old' gene, at least as old as eukaryotes. The TRP's are also 'old' genes, at least as old as vertebrates.

The second presentation in this session, by B Bouchard, involved the isolation and characterization of a cDNA for human TRP-2. Using primers from Jackson's group, a sequence from human cells that is 80% similar (at the amino acid sequence level) to mouse TRP-2 was identified (70% sequence identity at the nucleotide level). This same sequence is only 40-46% homologous to either mouse or human TRP-1 and tyrosinase. Notable characteristics of the human TRP-2 include: (1) the human cDNA for this sequence can detect the mouse sequence, but mouse TRP-2 does not hybridize with human melanoma RNA; and (2) human TRP-2 probably has dopachrome tautomerase activity.

The third paper in this workshop, presented by R Boissy, represented work done with L Austin on avian TRPs. Using cDNAs and antibodies for the TRPs, Smyth line chickens have been shown to have TRP-1 activity that is localized to melanocytes. Similarly, TRP-2 activity has also been shown to be present in chickens. Thus, not only are both of these gene products present in avian systems, but they must also possess significant similarity to the mammalian genes because the antibodies and cDNAs used to identify avian TRP-1 and -2 activity were all from mammalian sources.

Then followed a report by K Mason on the cloning and characterization of TRP-1 from a lower vertebrate, the Mexican axolotl. Using mouse primers obtained from I Jackson, Ken was able to clone and sequence a 1-kb fragment of a tyrosinase-like gene from the axolotl. This gene shares about 77% similarity with the mouse gene at the amino acid level. Preliminary data show that TRP-1 is expressed in varying amounts in albino, white and melanoid mutant axolotls as well as in the wild type.

H Yamamoto compared the 5' flanking sequences from a variety of vertebrates, including mouse, human, turtle, quail and chicken. Using cDNAs to the 5' flanking sequences, minigene constructs were made and inserted into mouse cells. Despite the fact that not all of the sequences were similarly homologous, each nevertheless could direct the expression of mouse tyrosinase cDNA when both were introduced into cultured albino mouse melanocytes. These results thus signify the functional relatedness of these genes and may be useful when extended to transgenic experiments.

F Beerman next reported on the many potential regulatory sites in the 270-bp mouse tyrosinase gene promoter region. Deletion analysis showed at least three different regulatory domains. Mutational analysis identified two positive regulatory cis elements. Combined mutations revealed a negative-acting region. Analysis of the positive-acting cis elements by *in vitro* protein binding revealed no cell specificity. The individual upstream and downstream elements do not appear to confer cell specificity. Thus, if minigenes containing the 270-bp promoter regions are introduced into transgenic mice the two-cis-acting

elements can be shown not to be exclusively necessary for promoter activity and do not abrogate expression of tyrosinase when mutated.

In another study on the expression of POU-domain genes in human melanoma cells, R Sturm spoke of characterizing regulatory elements in human melanoma cells that are involved in pigment differentiation. Using a DNA motif that binds a family of proteins known as Oct factors, which appear to be stage and/or lineage specific in mammalian embryos, human melanoma cells were screened. Melanoma cells express at least two Oct factors, and Oct factor expression can be seen to vary depending on the source of *in vitro* cells and their time in culture. The mouse *brn-2* gene was shown to encode both of the Oct factor activities, and this gene is being studied with regard to its involvement in melanocyte specification and differentiation.

Finally, S Porter reported on more long-range controls that might be exerted on the mouse tyrosinase gene. For example, the $c^{m}$ (*chinchilla*$^{mottled}$) gene in mice results in aberrant chromatin in melanocytes. The defect is such that a large chunk of an upstream site on the tyrosinase gene is missing. Several constructs were made yielding the following results: 3.5 kb of tyrosinase message does not induce much pigmentation. A larger segment (UPT series), however, leads to a strongly pigmented phenotype. In this latter case, all neural crest derivatives exhibit a 20-fold increase in expression, while the optic cup does not. Thus, the larger construct may be useful as a potential neural crest cell lineage marker, with melanocytes/Schwann cells clustering apart from neuronal derivatives.

## - - - - - *Wednesday, Sept 29* - - - - -

### Molecular Genetics of Cancer, Melanoma Genetics - by Richard King

Sir Walter Bodmer introduced this session with an outstanding review of the current concepts of cancer genetics. He outlined how cytogenetic changes had provided much of the initial insight into the genes involved in cancer formation, and how currently identified oncogenes and tumor suppresser genes function to promote the formation and progression of cancer. Colon cancer as discussed as model system of cancer, and Sir Bodmer hypothesized that melanoma would involve a similar series of genetic changes in initiation and progression. This lecture served as a perfect introduction to the following two lectures on the Xiphophorus melanoma model and the ongoing attempt to identify tumor suppresser genes in human melanoma. Manfred Schartl of the University of Wurzburg reviewed the Xmrk oncogene of the dominant genetic Tu locus in the teleost fish Xiphophorus. The Xmrk encodes a novel receptor tyrosine kinase and the protein is overexpressed in melanoma. Furthermore, using gene transfer experiments, overexpression alone is sufficient to induce tumorigenesis in transgenic fish. The Tu locus arose from nonhomologous recombination of Xmrk with a previously uncharacterized D sequence, producing an additional copy of Xmrk with a new promoter, and suppression of the new Xmrk by the R locus inhibits tumor formation in the parental fish, and its deregulation explains tumor formation in hybrid fish. The last lecture of this session was breathtaking as an example of using new techniques in the identification of chromosomal regions and specific genes in a human disease such as melanoma. Jeffery Trent, a member of the newly formed Human Genome laboratory at NIH, described how his laboratory is using chromosome microdissection and fluorescent in situ hybridization to identify chromosomal regions that may contain tumor suppresser genes involved in human melanoma. These three lectures were an outstanding demonstration of the intense and diversified approach to understanding the genetic basis of melanoma.

### Enzymatic Aspects of Melanogenesis - by Vincent Hearing

Kameyama and Sakai reported on "Expression of tyrosinase, tyrosinase related proteins 1 and 2, c-kit and c-kit ligand and regulation of melanin production by them". In this study, the expression of the above proteins were studied using murine and human melanoma cell lines with varying degrees of pigmentation, and in normal skin and hyperpigmentation disorders. There was a positive correlation between the expression of those enzymes and the amount of melanin produced, and all those factors were specifically

expressed by human melanocytes as has been shown for murine melanocytes. The results suggest that tyrosinase, TRP1 and TRP2, as well as c-kit play important roles in the regulation of melanogenesis; they further suggest that melanoblasts exist in the dermis of normal skin.

del Marmol *et al.* reported on "TRP-1, cysteine and eumelanogenesis". They observed that under standard culture conditions, TRP-1 is only detectable in eumelanin containing cells. They have studied the effect of cysteine concentration on pigment synthesis by pheomelanotic cells, and find that under culture conditions where cysteine is reduced 10 or 100-fold, the phenotype of the cells became eumelanotic, as judged by EM and chemical analysis. Northern blot anlaysis showed that TRP-1 synthesis was not induced, even though eumelanogenesis was induced. The data suggest that cysteine concentration is a critical regulator in controlling the switch between eu- and pheomelanogenesis.

Smit *et al.* presented work entitled "O-Methylation in the conversion of tyrosine to melanin". In this study they examined the methylation of DHICA by catechol O-methyl transferase (COMT) which is thought to be important in the protection of the cell from cytotoxic intermediates. They have shown now that DOPA is also a substrate of the enzyme, evolving 3-O-methyl-DOPA which is apparently detectable within melanocytes. Two forms of COMT could be detected in melanocytes - a soluble and a membrane-bound form, and the latter form is present primarily in the endoplasmic reticulum. A minor portion of the enzyme is found in the melanosome fraction.

Shibata et al. reported on "Tyrosinase-related protein 2: the role in melanin monomer synthesis and subcellular localization in pigment cells". They have examined the subcellular localization of TRP2 in melanocytes, using biochemical and immunological approaches following fractionation of subcellular particles of melanoma cells, and using electron microscopy. They found that TRP2 is present in a much higher concentration in coated vesicles than in the premelanosome fraction, and they postulate that this is not only consistent with high DHICA content in those fractions, but may be instrumental in the delay of melanin production in those vesicles prior to delivery to the melanosome.

Rieber and Rieber presented a paper on "Specific tyrosinases associated with melanoma replicative senescence and melanogenesis". They demonstrate that induction of differentiation in B16 cells deprived of growth factors leads to an irreversible arrest of growth, although the cells remain viable. Concurrent with this, novel forms of glycosylated tyrosinase (polymers?) are forms which are specific indicators of the irreversible growth arrest. Similar changes were not found with TRP1 or TRP2.

**Melanoma - by Vincent Hearing**

MacNeil *et al.* reported on their study of "Tamoxifen inhibition of ocular melanoma cell attachment to matrix proteins". They have previously reported that B16 melanoma attachment to extracellular matrix components is inhibited by calmodulin antagonists - one of the functional mechanisms of tamoxifen action. Human ocular melanoma lines were established from choroidal melanomas and used in this study; they attached readily to plastic, collagens, fibronectin, laminin, etc. Tamoxifen showed a sginficant ability to inhibit that attachment, and they conclude that tamoxifen inhibits melanoma cell attachment to matrix proteins in vitro, and may be of potential use against metastasis in vivo.

Easty and Bennett presented "Protein tyrosine kinase expression during melanoma progression and metastasis". They used Northern blotting to examine expression of protein tyrosine kinases in a number of melanocyte and melanoma cell lines. Based on the pattern of expression, they propose that one type (JTK-14) may be involved in the adhesion to endothelium while another (ECK) is a receptor for a growth factor (neither of these was present in normal melanocytes). They found that expression of KDR was markedly reduced in metastatic melanomas compared to normal melanocytes.

Dore *et al.* discussed "Selective expression of PNA-binding glycoconjugates by invasive human melanomas: a new marker of metastatic potential". They have shown that highly metastatic human melanoma cells have an increased ability to bind to peanut agglutinin (PNA) and that this ligand can be used to detect highly invasive melanoma cells in tissue samples. The reactive material was found in the cytoplasm and on the plasma membrane of reactive cells, and this property may provide an important clue to changes whereby tumor cells become metastatic.

Burton and Armstrong presented "An analysis of a melanoma epidemic". In the past several years, the incidence of melanomas detected has almost doubled; this has in part been due to earlier diagnosis due to public awareness programs. However, they feel that this is only part of the explanation and that a truly significant increase in the incidence of malignant melanoms is also occurring.

Hersey *et al.* reported that "IL-10 is produced by melanoma cells and may have a role in immunosuppression mediated by melanoma". IL-10 functions by inhibiting production of cytokines involved in cell mediated responses. This has now been implicated in the pathogenesis of some infections, and it was found by PCR analysis that mRNA for IL-10 could be found in 66%of the melanoma cell lines. IL-10 produced by melanoma cells could inhibit TNF-$\alpha$ and IFN-$\gamma$ production by lymphocytes. They therefore feel that IL-10 production by these melanoma cells may be important in decreasing host immune responses to the tumor.

Wakeling *et al.* showed that "Murine melanoma cells show suppressed tumorigenicity following transfection with a human genomic library and selection for properties of normal melanocytes". They have transfected a human genomic library into B16 cells and selected colonies for their growth inhibition and reduced ability to grow in semisolid agar (i.e. normal phenotypic properties). This apparently worked since most of the clones showed growth stimulation by TPA and a reduced ability to grow as primary tumors in mice.

Garbe *et al.* reported on the "Prolongation of life in stage IV melanoma by combined treatment with rIFN-a-2$\alpha$ and vindesine". Patients treated with this combined therapy tolerated the treatment well; 4 patients had a significant improvement in their disease and 8 other patients remained stable in their disease; survival of the treated group was markedly increased over the controls, suggesting that this combination of therapy might be useful for melanoma treatment.

Klein *et al.* reported on the "Expression of the human $\alpha 2\beta 1$ collagen receptor of integrins (VLA-2) in mouse melanoma cells confers the ability to recognize collagen 1". Using transfection of human VLA-2 to BULT murine melanoma cells, they proved that the title of their abstract was true. Expression of the human $\alpha 2\beta 1$ collagen receptor seemed to be correctly on the cell surface.

Hill *et al.* reported on the "Properties of an autocrine multitherapy resistance factor from melanoma cells". This study characterized a multitherapy resistance factor (MTRF) which increases survival of S91 cells following exposure to a number of toxic agents. The MTRF was characterized from S91 conditioned medium and some of its physical properties suggest is is a high molecular weight, relatively stable protein, perhaps a growth factor. Further characterization of MTRF is underway and it seems to be a novel autocrine growth factor.

Hearing *et al.* reported on the "Resection of local melanoma tumors followed by postoperative treatment of mice with melaoma vaccines in combination with interleukin-2 (IL2) leads to prolonged survival". A novel model for spontaneous melanoma metastasis was shown and significant protective effects were demonstrated for several different vaccine preparations. The efficacy of those preparations could be increased by formalin treatment, and concomitant treatment with IL2 further increased survival of tumor bearing mice.

Kroumpouzos *et al.* discussed "Other malignancies in 1371 patients with malignant melanoma". A number of other types of tumors were diagnosed on melanoma patients, including basal cell carcinomas, breast, colon, lund, lymphomas and other types of tumors. a 15-fold increased Relative Risk for melanoma patients to develop other skin cancers and a 5-fold increased Relative Risk to develop other melanomas was demonstrated, compared to the normal population.

Cochran *et al.* reported "Xenografting of human melanoma to immune suppressed neonatal rats: a model for the study of early metastases". Discussed a new experimental system to study metastasis in neonatal T-cell depleted rats were explanted with human tumor xenografts; these animals develop successful metastases. Organ specificities and kinetics of implantation were discussed, and further study will be directed to examining the effects of cytokines and other therapies using this new model system.

**Vitiligo Symposium -** **by James Nordlund**

P Grimes reviewed the classical data on aberrant immune functions found in patients with vitiligo. She also reviewed the circumstantial clinical data on immune related disordrs and vitiligo. Antibodies of several types have been found in the sera of patients with vitiligo. Some are antimelanocytic, others are against other organs. Cytotoxic lymphocytes have been identified. She is conducting a clinical trial with isoprinosine, an immunomodulator, as a treatment for vitiligo and observing some responses.

W Westerhof reviewed data on the number of OKT-6 cells in vitiligo skin. He found the number increased although the controls were not clear. He found normal numbers of CD3, CD68 (macrophages) in perlesional skin but increased CD4 cells in the same area.

RB Goudie has noted the remarkable symmetry of vitiligo of the generalized type. He notes that lymphocytes home to the skin by markers such as the HECA-452 ligand. He proposes vitiligo is a clonal lymphoma that destroys melanocytes. Using PCR techniques he looked for evidence of clonality in the lymphocytes of vitiligo skin. The results were negative in a large series of patients.

B Salzer studied the personality profiles of 117 patients with vitiligo. Vitiligo patients do not differ from normals. However vitiligo patients were found to be less content, oversensitive, over stressed, inhibited and quick tempered compared to normals. Possibly this is the result and not the cause of vitiligo.

R Bowers studies oxidative stress enzymes in chickens exhibiting acquired depigmentation. He found catalase levels to be normal but SOD levels were reduced to 1/3 normal. He proposed that oxidative stress might be responsible for melanocyte destruction.

S Passi has reported elevated levels of catecholamines and metabolites in the urine of patients with vitiligo. Elevated catechols in the skin might cause hypoxia and release of oxygen radicals. Levels of antioxidants in the blood of vitiligo patients are normal. He proposed that lack of tissue antioxidant factors rather than generalized deficiencies might be injurious to melanocytes.

A Ramaiah noted that vitiligo melanocytes in some culture conditions do not grow normally or as well as normal melanocytes. This suggests that they have some inherent defect that is made manifest by culture conditions. Determination of the culture factors might provide clues to the cause of melanocytotoxicity in humans.

K Schallreuter noted that vitiliginous keratinocytes have higher than normal number of ß-adrenergic receptors on their surface, an observations which correlates with abnormal calcium uptake. Biopterin dependent tyrosine hydroxylase activity was higher in epidermal extracts from vitiligo patients. She suggested there is an excessive concentration of catecholamines in the epidermis related to biopterin. She suggests that vitiligo is a biopterin defect.

MJ Olsson has treated a large number of patients with cultured melanocytes applied to dermabraided skin. The original studies used only pure melanocytes. He now is using mixed cultures containing keratinocytes and melanocytes. They have very successful results although the technique is expensive.

## - - - - - *Thursday, Sept 30* - - - - -

**MSH Symposium -** **by Zalfa Abdel-Malek**

The presentations in this symposium focused on the following issues: the biochemical and molecular mechanisms underlying the MSH induced effects on melanocytes, the responses of normal human melanocytes to MSH and related melanotropins, the diversity of the MSH receptor alleles and the modulation of the synthesis and immunoregulatory functions of POMC derived peptides.

P Aroca presented "Patterns of Melanin Biosynthesis in Murine Melanoma Cells Following Hormonal Stimulation". Treatment of JB/MS and B16 murine melanoma cells with MSH for 4 days resulted in ~ 30 fold increase in melanin production and a significant decrease in the incorporation of carboxylated precursors into the melanin polymer. The increase in melanin synthesis seems to be mediated primarily by increased tyrosine hydroxylase and DOPA oxidase activities, and increased transcription and translation of the tyrosinase gene. No change in mRNA or protein synthesis, or in the catalytic activities of TRP-1 and TRP-2 was observed following MSH treatment.

R Cone reported on "Variant MSH Receptor Alleles and the Pigmentation Phenotypes of the Extension Locus". Cone's group recently established that the murine extension locus encodes the MSH receptor. Mutations at this locus may result in a non-functional receptor, as in the recessive yellow allele, or a hyperactive receptor, as in the sombre or tobacco darkening alleles. While investigating the possible existence of multiple MSH receptor alleles in man, it was found that there are 3 MSH receptor types: MC-1, which contains specific phosphorylation sites and is expressed on melanocytes, MC-3, localized in the hypothalamus and limbic system, and MC-4 present in the brain stem. MC-2 was found to be the ACTH receptor.

G Hunt presented a paper entitled "Do Cultured Human Melanocytes Respond to MSH Peptides?" Normal human melanocytes maintained in culture in the absence of artificial mitogens respond to α-MSH and [$Nle^4$, D-$Phe^7$]-α-MSH with increased dendricity, tyrosinase activity, mRNA and protein, and melanogenesis. About 15% of treated cultures had no response to α-MSH, possibly due to lack of or insufficient expression of the receptor. In some cultures, the increase in dendricity and melanogenesis were independent of one another. It was found that α-MSH increases the attachment of melanocytes to laminin and fibronectin. In addition, distinct morphologic changes were observed when MSH treated melanocytes were plated on these matrix proteins, or on collagen type IV. Therefore, α-MSH is melanogenic for human melanocytes *in vitro*.

Z Abdel-Malek presented work on "Mitogenic and Melanogenic Effects of α-MSH and ACTH on Cultured Normal Human Melanocytes". Cultured human melanocytes respond to α-MSH and ACTH with increased proliferation, dendricity and melanogenesis. These effects are significant only when melanocytes are deprived of any cAMP inducer (eg bovine pituitary extract) from the culture medium. The increase in melanogenesis is preceded by stimulation of tyrosinase activity and increased expression of tyrosinase, TRP-1 and TRP-2, as determined by western blotting. Stimulation of proliferation appears to be due to the synergistic interaction between α-MSH and the two mitogens TPA and basic bFGF. Therefore, in addition to their role as melanogenic factors, both α-MSH and ACTH are mitogenic for normal human melanocytes in culture.

P Donatien presented a study entitled "α-MSH Increases Tyrosinase Messenger RNA in Normal Human Melanocytes". Human melanocytes maintained in culture in the absence of artificial mitogens and treated with $10^{-8}$M [$Nle^4$, D-$Phe^7$]-α-MSH for 48 hours demonstrate a 50% increase in melanin content. When compared to amelanotic DX3 melanoma cells, and melanized B16F1 melanoma cells, which show a 162% increase in melanin content after 48 hours of treatment with [$Nle^4$, D-$Phe^7$]-α-MSH, normal human melanocytes and B16 melanoma cells but not DX3 cells, show a 92% increase in tyrosinase mRNA. Since the increase in melanin content in human melanocytes did not parallel the change in the transcription of the tyrosinase gene, additional posttranscriptional mechanisms may exist to regulate the response to MSH.

T Luger spoke on "Immuno-Modulating Capacities of Pro-Opiomelanocortin Peptides". Pro-opiomelanocortin derived peptides are synthesized by many different cell types, including lymphocytes and keratinocytes. Both α-MSH and ACTH elicited a biphasic effect on IgE synthesis by peripheral blood mononuclear cells, in combination with IL-4. At physiologic concentrations, α-MSH and ACTH increased IgE synthesis, and at higher concentrations they inhibited it. Interferon γ production is known to downregulate IgE production. The effect of physiologic concentrations of ACTH and α-MSH on IgE synthesis was consistent with their inhibitory effect on interferon γ production and mRNA synthesis by con A stimulated peripheral blood mononuclear cells. In addition, the synthesis of α-MSH by keratinocytes and A431 cells was enhanced by the immune modulators IL-1 and UV, as well as TPA. This provides further evidence that POMC derived peptides participate in immunoregulatory functions, and that the synthesis of these peptides is in turn affected by immune modulators.

## IFPCS General Assembly

**by James Nordlund**

Y Mishima (IFPCS President) opened the meeting and welcomed the membership to the XVth IPCC. He thanked Patrick Riley and the members of the scientific and organizing committee for their outstanding working in making this meeting a scientific success.

J Nordlund (IFPCS Secretary/Treasurer) reported that the IFPCS had $13,700 in reserve. He noted that the Council had voted a $5 increase in dues to cover the costs of the various activities of the IFPCS such as the IPCC, the Journal, and the administrative costs. He also noted that the current membership for the JSPCR was 236, for the ESPCR 204 and for the PASPCR 130. The total for the IFPCS is 570. Nordlund also noted that the Publications Committee was negotiating with Munksgaard regarding the next editor of the Journal following the conclusion of the term of the current editor, subscribing memberships with a reduced cost for the Journal, and other issues. Subscribing members would receive the Journal of Pigment Cell Research; nonsubscribing members would not receive the journal. Each regional Society would establish these categories of membership.

The next IPCC will be held in Los Angeles and will be chaired by F Meyskens with cochairmen of A Cochran and R Bowers. Dr Meyskens gave a short presentation on the Los Angeles site which will be held at the Disneyland hotel.

The recipients of the Myron Gordon Awards were Drs Patrick Riley, Hans Rorsman and Takuji Takeuchi.

The new officers of the IFPCS are President G Prota (ESPCR); Vice-President R King (PASPCR); and Secretary/Treasurer S Ito (JSPCR).

Professor Mishima passed the gavel to President Prota at the reception. President Prota thanked the membership for the trust and honor and pledged to continue the progress of the IFPCS as the world's leading Society for research on pigment cell biology.

## Abstracts in press

**"Contribution of Melanogenic Proteins to the Heterogeneous Pigmentation of Human Melanocytes" Z Abdel-Malek, V Swope, C Collins, R Boissy, H Zhao and J Nordlund (in press: *J. Cell Science)***

**Abstract:** Human melanocytes from individuals with different skin types, as well as from the skin of the same individual, are heterogeneous in their melanin content. This heterogeneity may be attributed to differences in the activity and expression of the three melanogenic proteins: tyrosinase, tyrosinase related proteins 1 and 2 (gp75 or DOPAchrome tautomerase, respectively), which in turn are affected by certain regulatory factors. Established melanocyte strains that exhibited intrinsic melanogenic heterogeneity could be separated into subpopulations according to density and melanin content by Percoll density gradient centrifugation. The least melanotic subpopulation consisted of melanocytes that contained an active tyrosinase enzyme and a low amount of melanin. Tyrosinase activity and the quantities of tyrosinase enzyme, tyrosinase related protein-1 and DOPAchrome tautomerase gradually increased with increased melanin content and percoll density of the isolated melanocyte subpopulations. We have found a direct correlation between melanin content, tyrosinase activity and the expression of the three melanogenic proteins in melanocyte strains established from different skin types. Addition of the two epidermal cytokines, tumor necrosis factor-$\alpha$ or interleukin-1$\alpha$, to cultures of human melanocytes from different skin types caused decreased proliferation, tyrosinase activity and expression of tyrosinase, tyrosinase related protein-1 and DOPAchrome tautomerase. Similar results were obtained when Percoll derived melanocyte subpopulations were treated with tumor necrosis factor-$\alpha$ and interleukin-1$\alpha$. These results indicate that the variation in melanin content in human melanocytes is due to differences in the activity and expression of the melanogenic proteins, which are influenced by autocrine and paracrine factors.

## 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: Postdoctoral Fellowship. Applications are invited for a postdoctoral fellowship to join a group working on the molecular mechanisms of host defence in invertebrates primarily arthropods. The project is to isolate and characterize pattern recognition receptors and to study the regulation of immune responses in arthropods. The candidate must have a PhD and a strong background in molecular biology techniques. Experience with invertebrates is not required. The fellowship is for two years and applications including a CV and the names of two referees should be sent to: Prof. Kenneth Söderhäll, Department of Physiological Botany, University of Uppsala, Villavägen 6, 752 36 Uppsala, Sweden. phone: 46-18-182818; FAX: 46-18-559885

## Bibliography :

The Bibliography published in this issue covers the period mid-August through mid-November, 1993. 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.

### MELANINS & MELANOGENS


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