PASPCR Newsletter

Volume 6 Number 3 **September,1998**

Introduction . . .

The **PASPCR** ***Newsletter*** is published quarterly and is intended to serve as a means of communication for the members of our Society. As such, we invite our membership to actively contribute to it; if you attend a scientific meeting at which you heard 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 or 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 Vince Hearing, preferably by Email to **hearingv@nih.gov**.

The **PASPCR** ***Web*** page is the major, up-to-date source of current information for the **PASPCR** membership. The home page page has a new URL address which will result in faster transfer of information to your computer than before. The new address is http://www.cbc.umn.edu/paspcr. Please update your existing **PASPCR** link to this new address (the old one will disappear in a few months). We have now included a page that has positions available and positions wanted. Postings for Positions Available will be open to all individuals so long as the position is related to pigment cell research. Postings for Positions Wanted will be open only to members of the **PanAmerican Society for Pigment Cell Research** or its sister societies (**JSPCR** and **ESPCR**). Send postings to Bill Oetting at **bill@lenti.med.umn.edu**. Please provide an expiration data for any submitted postings. The **PASPCR Web** page also contains information on the goals, ByLaws and Rules of the Society, future meetings, past issues of the **PASPCR** Newsletter as well as links to other related sites including the InterPig DataBase, the International Federation of Pigment Cell Societies (**IFPCS**) and the regional Pigment Cell Societies from Europe and Japan. In addition, the **PASPCR** membership directory is available on the **PASPCR Web** page; please notify us if you wish any or all of your information to be deleted or modified on that site. If there is additional information that you wish to have added to this web page, please let us know. Send any comments and/or suggestions to the **PASPCR** WebMaster, Bill Oetting at **bill@lenti.med.umn.edu** or to Vince Hearing at **hearing@nih.gov**.

IN THIS ISSUE . . . . .

PanAmerican Society for Pigment Cell Research

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Calendar of Events :

Sept 11 - 13, 1998 Cutaneous Neuroimmunomodulation: The Proopiomelanocortin System, to be held in Munster, Germany (contact: Science & Technology Meetings Dept, New York Academy of Sciences, 2 East 63rd St, New York, NY 10021 USA; FAX: +1 212 838-5640)

Sept 23 - 26, 1998 8th ESPCR Annual Meeting, to be held in Prague, Czech Republic (contact: Dr. Jan Borovansky, Department of Biochemistry, Charles University, 1st Faculty of Medicine, U nemocnice 5, 128 53 Prague 2 Czech Republic; FAX: + 42 2-2491-5449)

Oct 1 - 4, 1998 Frontiers in Melanoma, to be held in Vienna, Austria (contact: Scientific and Administrative Secretariat, Vienna Academy of Postgraduate Medical Education and Research, Alserstrasse 4, A-1090 Vienna, Austria; FAX: +43 1 405-138323)

Dec 5 - 6, 1998 13th JSPCR Annual Meeting, to be held in Kobe, Japan (contact: Dr. Masamitsu Ichihashi, Department of Dermatology, Kobe University School of Medicine, 5-1 Kusunoki-cho, 7-chome, Chuo-ku, Kobe 650 Japan; FAX: +81 78 382-2497)

Dec 12 - 16, 1998 American Society for Cell Biology, Annual Meeting to be held in San Francisco, CA (contact: http://www/faseb.org)

Oct 30 - Nov 3, 1999 XVIIth International Pigment Cell Conference, to be held in Nagoya, Japan (contact: Dr. Shosuke Ito, Fujita Health University School of Health Sciences, Toyoake, Aichi 470-11, Japan; Phone: +81-562-93-2595; Fax: +81-562-93-4595; Email: sito@fujita-hu.ac.jp)

Welcome to New Members

by James J Nordlund / Raymond Boissy

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

Sumayah Jamal	**Hoon Kang**	**Regina M. Kuliawat**
Luiz Eduardo M. Nery	**Nuning Nurcahyani**	**Carolyn D. Roberson**
Arturo Solis-Herrera	**Itaru Suzuki**	**Takako Takakuwa**
Siak-Kim Tan	**M. Carolina Tuma**	

If anyone is interested in joining our Society or wishes to sponsor a member, application forms can be obtained from Dr. James J. Nordlund at the PASPCR Secretary/Treasurer's office.

Corporate Sponsors

by James J Nordlund / Raymond Boissy

The PASPCR would like to acknowledge and thank our Corporate Sponsors; the list below reflects contributions over the past 2 years. 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.

GOLD Corporate Patrons	*SILVER Corporate Patrons*	*Corporate Patrons*
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PASPCR Elections

by Richard A King

The Nominations Committee has nominated the following candidates to be placed on the ballot for 1998; their terms will run for 3 years beginning January 1, 1999. Members are reminded that additional names can be placed on the ballot by provided a petition to the office of the Secretary/Treasurer signed by 5 active members. Such petition ballots should include a statement from the individual being nominated that he/she is willing to run for that office. Petition ballots are due in the office of the Secretary/ Treasurer by November 1, 1998. Ballots will be mailed in mid-November. Nominees are (in alphabetical order only):

For President-Elect :	Zalfa Abdel-Malek	John M. Pawelek	
For Secretary / Treasurer :	James J. Nordlund		
For Council (3 to be elected) :	Ashok Chakraborty	Mark K. Cullen	Bryan Fuller
	Meenhard Herlyn	Helene Z. Hill	Giselle Thibadeau

Invitation to the 8th ESPCR Meeting in Prague

by Jan Borovansky

The 8th Meeting of the European Society for Pigment Cell Research will be organized by 1st Faculty of Medicine, Charles University in Prague, September 23-26, 1998. In addition to usual topics (melanin, melanogenesis, melanosome, melanocyte, melanoma, disorders of pigmentation) two special sessions

devoted to Photoprotection and Phototherapy will be scheduled in cooperation with the European Society for Photobiology. Details on the scientific program can be obtained from dr J. Borovansky (fax: +42 2 2491-5449; Email: jborov@lf1.cuni.cz); registration forms can be obtained from the Congress Office, KAHLEN spol, Vlkova 24, 130 00 Prague 3, Czech Republic (fax +42 2 6719-5304; Email: kahlen@kahlen.cz).

Invitation to the XVIIth IPCC (International Pigment Cell Conference) by Shosuke Ito

Invitation to the XVIIth International Pigment Cell Conference Nagoya Congress Center
Nagoya, Japan October 30 - November 3, 1999

Dear Colleague:

After the inauguration of the International Federation of Pigment Cell Societies (IFPCS) in Kobe in 1990, the International Pigment Cell Conferences (IPCC) rotate among the European, American, and Asian continents, hosted by one of the three regional societies: the ESPCR, the JSPCR, and the PASPCR. The 15th IPCC was thus held in London in 1993, chaired by Professor Patrick A. Riley, and the 16th IPCC was recently held in Anaheim, California, chaired by Professor Frank L. Meyskens, Jr.

It is our great honor and real pleasure to inform you that the next 17th IPCC will be held in Nagoya, Japan in 1999, co-organized by the IFPCS and the JSPCR. We heartily hope that pigment cell biologists and clinicians will join together in Nagoya in October 1999 to present their latest achievements in the exciting world of pigment cell research. Your participation will be most important for the scientific success of this meeting.

The city of Nagoya, the 4th largest in Japan, enjoys a rich history of traditional culture and a reputation for world-renowned high-tech industries. Nagoya is located at the center of Japan and is easy to access: the Nagoya International Airport is directly connected with 30 cities around the world. The conference site, the Nagoya Congress Center, is newly built and has ample spaces for the participants to discuss and exchange ideas, which we believe will certainly bring about fruitful collaborations.

We will follow the good tradition of the IFPCS leadership in directing scientific programs to unify the three regional societies. Within such a framework, we wish to place special emphasis on poster presentations. We hope to provide a certain number of travel grants for young investigators to attend this meeting. In order to be eligible for such a grant, an applicant has to be a member of one of the three regional societies for at least one year prior to the meeting. We are also planning banquet and social activities in such a way to make your visit to Nagoya most enjoyable and memorable. It will be our great privilege to welcome you and your colleagues to Nagoya in 1999.

Shosuke Ito, Ph.D. — *Chair,* IPCC Nogoya
Kazumasa Wakamatsu, Ph.D. — *Secretary-General,* IPCC Nagoya

For further information please contact us at: Fujita Health University School of Health Sciences, Toyoake, Aichi 470-11, Japan; *Phone*: +81-562-93-2595; *Fax:* +81-562-93-4595; *Email*: sito@fujita-hu.ac.jp

Positions - Wanted and Available :

Postdoctoral Positions. in the Department of Cell Biology at the NYU School of Medicine are available to study the biogenesis of melanosomes using a combined cellular, molecular and genetic approach. Prior experience in molecular or cell biology required. Applications from those with prior experience with yeast, Drosophila etc. interested in applying their skills to a mammalian system with strong genetics are especially welcome. A track record of productivity is essential. Send CV, brief description of experience and names of 3 references to: Seth J. Orlow, MD, PhD, NYU Medical Center, 560 First Avenue Room H-100, New York, NY 10016. Fax 212-263-5819, email: orlows01@mcrcr.med.nyu.edu

Postdoctoral Research Associate - Position available to study the biology of human inherited disorders of pigmentation using mouse knockout technology. The successful applicant will have a Ph.D. and/or M.D. with experience in cell biology and molecular biology. Experience with production of knockout

mice using ES cell technology preferred. Please send curriculum vitae along with the names of three references to Dr. Richard King, Division of Genetics, Department of Medicine, Box 485 Mayo, 420 Delaware St. S.E., University of Minnesota, Minneapolis, MN 55455. Equal Opportunity Employer.

Postdoctoral Position - Ph.D. in molecular biology, biophysics, genetics or biochemistry. Position available to conduct research on molecular mechanisms of cellular response to oxidative stress in human melanocytes and melanoma cells and its regulation for preventive and therapeutic indications. Contact Dr. Frank L. Meyskens Jr., Director, University of California-Irvine, Chao Family Clinical Cancer Research Center, 101 The City Drive, Orange, CA 92668, USA. Fax (714) 456-5039 Email flmeyske@uci.edu

Meeting Report - by Dan-Ning Hu

Iris Pigment Epithelium (IPE) Transplantation May 12, 1998 Fort Lauderdale, FL

The symposium " IPE Transplantation: Theoretical and Practical Considerations" was held in the Fort Lauderdale Convention Center, (Florida, USA) on May 12, 1998 during the 1998 Annual Meeting of The Association for Research in Vision and Ophthalmology. This meeting was organized by the Ocular/Extracutaneous Pigmentation Expert Group of the International Federation of Pigment Cell Societies. This symposium was composed of 2 sessions, which included 7 presentations. More than 150 ophthalmologists and basic scientists from all over the world joined this meeting.

Dr. Uri Shabto of The New York Eye & Ear Infirmary (USA) gave the introduction, "Why IPE transplantation?". He mentioned that subretinal neovascular membranes associated with age-related macular degeneration are a major cause of legal blindness. Surgical excision of these membranes always leaves a retinal pigment epithelium (RPE) defect, which may lead to further damage to the neural retina and the visual function. RPE transplantation usually fails because of rejection of the RPE allograft. It is easy to obtain autologous IPE from iridectomy specimens. Therefore, it is worthy to study subretinal IPE transplantation as a substitute for RPE in various retinal degeneration diseases related to RPE defects.

The first session, "Comparison of physiology and cell biology of IPE and RPE" was chaired by Dr. Dean Bok of University of California Los Angeles (USA). Dr. Ulrich Schraermeyer of the University of Cologne (Germany) presented on "Phagocytosis of photoreceptor outer segments by IPE". They found that the IPE possess phagocytic capacity *in vivo* and *in vitro*, which is one of the important function of the RPE. Dr. Dan-Ning Hu of The New York Eye & Ear Infirmary (USA), presented "Comparison of IPE and RPE *in vitro*". He showed that adult human IPE and RPE contain melanin that is similar in amount and nature. Both do not demonstrate any melanogenesis *in vitro*. Both cells reduced exogenous NO in the culture medium, and each responded similarly to various growth factors and cytokines and produced similar growth factors and neurotrophic factors. Drs. Dean Bok of UCLA and Ron P. Gallemore of Duke University (USA) presented "Retinoid metabolism of RPE" and "Water and ion transport by RPE", respectively. They discussed these two important functions of RPE, which have not yet been studied thoroughly on the IPE.

The second session "Animal models and clinical experience" was chaired by Dr. Jason S. Slakter of Columbia University (USA). Dr. Kouros A. Rezai of Chicago University (USA) presented "IPE transplantation". He reported the studies on IPE transplantation *in vitro* and *in vivo* and documented that IPE have phagocytic activity and can form a blood-retinal barrier. Dr. Schraermeyer presented "IPE transplantation in rabbits and RCS rats". He reported that transplanted IPE could survive in subretinal space in both rats and rabbits. They took up photoreceptor outer segments and had a beneficial influence on photoreceptors of RCS rats. Dr. Amparo Navea of the University of LA FE (Spain) presented "Autologous transplantation of IPE into the subretinal space in humans". She reported 6 cases of IPE transplantation in age-related macular degeneration patients. IPE transplantation seems to be well tolerated. Three cases showed improvement of vision.

Based on this meeting, it is clear that much work has been done in the study on IPE transplantation, both *in vitro* and in experimental animals; preliminary clinical experiences have obtained encouraging results. However, many problems still exist and require further investigation in this exciting, nascent field.

Meeting Report -

VIII[th] Annual Meeting of the PASPCR Aug 15-18, 1998 Snowmass, CO

The PASPCR meeting this year was held in Snowmass, CO under the chairmanship of Dr. David Norris. The Editor would like to thank the following authors (as noted) for contributing commentaries on each of the sessions held.

Sunday morning Sessions **by Zalfa Abdel-Malek**

The Gelb lecture for the eighth meeting for the PASPCR was given by John Pawelek, Ph.D., and was entitled "Melanoma/macrophage hybrids and the development of metastases in melanoma". In his presentation, Dr. Pawelek provided a historical overview of reports by various investigators stating that such hybrids indeed exist and are associated with metastatic disease. Dr. Pawelek presented results from his laboratory, using mouse Cloudman melanoma cells that are known to have a poor metastatic potential. He reported that Cloudman melanoma X macrophage hybrids greatly enhanced the metastatic ability of tumor cells. It was observed that these hybrids became significantly more melanotic than the original melanoma cells. The proposed mechanism for the enhancement of metastasis and melanization is increased N-glycosylation of proteins, that included several melanogenic proteins. The melanoma/macrophage hybrids acquired a macrophage-like glycosylation system that resulted in enhancement of N-glycosylation. This mechanism offers one explanation for increased aggressiveness of melanoma tumors during disease progression.

The first plenary session was entitled "Control of Melanocyte Development and Differentiation". The abstract by Southard-Smith et al. was presented by Bill Pavan. He stated that premature termination of SOX 10, one of the SRY-like HMG box transcription factors resulted in the absence of neural crest derivatives in Dom mice, a model of Waardenburg-Hirschprung disease. SOX 10 was found to function intrinsic to melanocytes and to be expressed in early melanoblasts. The potential target genes for SOX 10 are to be identified using cDNA expression microarrays. The abstract by Donatien and Bennett was presented by Dorothy Bennett who described the procedure for establishing long-term culture of human fetal melanoblasts. For this, murine keratinocyte feeder cells were used, and the growth medium consisted of RPMI 1640 supplemented with 10% fetal calf serum, 1.5 μM hydrocortisone, 20 pM thyroxine, 40 pM basic fibroblast growth factor, 20 ng/ml stem cell factor, 20 pM cholera toxin, 100nM endothelin-3, and 20 nM TPA. In this medium, the cells doubled every 3-5 days, and expressed TRP-2 and Pmel-17, but not P protein, and were DOPA-negative. Growth could be further enhanced by the addition of NDP-MSH, and melanin content could be increased by the addition of oleoyl acetyl glycerol or cAMP inducers. The abstract by Xu et al., was presented by Estela Medrano. The authors used the yeast two-hybrid system to identify human proteins that interact with the transcription factor MITF. By screening the melanoma cell line IIB-Mel-J cDNA library, they found that most clones with a positive interaction with MITF encoded the human ubiquitin-conjugation enzyme hUBC9 (UBE21). This was confirmed by in vitro GST "pull down" assay, using 6 x His-tagged MITF, by co-localization of MITF and hUBC9 in the nucleus, and by increased degradation of MITF upon co-transfection of MITF and hUBC9 into Cos 7 cells. Dong and Vijayasaradhi investigated the role of the transcription factor MITF in the coordinated regulation of tyrosinase and TRP-1 in human melanocytes and melanoma cells. Upon treatment of these cells with the differentiation inducer hexamethylene bis-acetamide (HMBA), the level of TRP-1 mRNA was drastically reduced, an effect that was not due to increased rate of mRNA degradation, and could be abolished by inhibition of de novo protein synthesis. The protein level of TRP-1 correlated with the level of its mRNA. On the other hand, tyrosinase and MITF mRNA levels were up regulated, with no change in the protein level of MITF. It was concluded that while expression of tyrosinase and other pigmentary genes correlated with MITF expression, TRP-1 expression was regulated independently of MITF and required other transcriptional factors.

The second Plenary Session was entitled "Control of Pigmentation by MSH, The Melanocortin Receptor and The Agouti Signaling Protein". Suzuki et al. reported on the regulation of the human MC1R expressed on human epidermal melanocytes. Brief treatment of melanocytes with α-MSH, ACTH or endothelin-1 resulted in increased MC1R mRNA level. This might account for lack of desensitization of the receptor following prolonged treatment with its ligands. Treatment of melanocytes with agouti signaling protein (ASP) or with UV radiation down regulated MC1R mRNA level. Unlike melanocytes, human keratinocytes did not express functional MC1R, as determined by Northern blot analysis, receptor binding assay, and cAMP radioimmunoassay. Matsunaga et al.used αPEP 16, a rabbit polyclonal antiserum generated against a synthetic peptide that corresponds to the carboxy teminus of mouse agouti protein, to characterize the expression of this protein in 3, 6, and 9 day old non-agouti

black, agouti, and lethal yellow mouse skin specimens. Expression of agouti protein was very low in black mice, was noticeable and increased with age in hair matrix cells adjacent to melanocytes in lethal yellow mice, and was observed during the pheomelanogenic phase in agouti mice. Abdel-Malek et al. addressed the question whether ASP antagonizes the effects of α-MSH by exclusively binding to the MC1R, or by additionally binding to another unknown receptor. Melanocytes cultured from C57 BL6J E+ /E+ mice, congenic e/e or Eso /Eso mice were compared for their responsiveness to ASP. Only E+ /E+ responded to ASP with inhibition of basal and α-MSH stimulated tyrosinase activity, significant reduction of tyrosinase, TRP-1 and TRP-2 protein levels, and inhibition of α-MSH induced cAMP level. This demonstrates that expression of normal MC1R is pivotal for the responsiveness of mammalian melanocytes to ASP. Miltenberg et al. examined the biological significance of the highly conserved basic domain, adjacent to the cysteine-rich agouti C-terminal. Deletion of this entire region and expression of the mutant gene in transgenic mice resulted in mice with yellow coat color, but without hypoglycemia, insulinemia, or obesity. This mutation rendered the protein inactive in neural tissues, but functional in regulating pigmentation, with a reduced capacity to inhibit MC1R-induced stimulation of cAMP level. This suggests that the basic domain in the agouti gene is involved in the proteolytic processing of agouti signaling protein, affects the secretory pathway, and/or facilitates melanocortin receptor binding. Virador et al. attempted to identify bioactive domains of ASP by synthesizing overlapping 15 mer peptides that encompass its entire sequence. The biological effects of these peptides on melan-a cells were investigated by determining total melanin synthesis, and the expression of tyrosinase and TRP-1 mRNA. Peptides in the regions 30-52 and 57-91 generated about 10% reduction in total melanin production, compared to 30% reduction generated by recombinant ASP. The functional agouti region was narrowed down to 5 amino acids, Lys82Pro86, which resulted in significant reduction in melanin formation, tyrosinase expression and function. Johansen et al. described the isolation and sequencing of the bovine and porcine agouti genes, and the expression of this gene in bovine tissues. Using murine agouti cDNA as a probe, they found that cattle and pigs possess an agouti gene with 161 bp products that hybridize to murine agouti exon 2. This bovine and porcine agouti region was 75 and 76% similar to murine, 82 and 79% similar to human, respectively, and 88% similar to each other. The predicted amino acid sequences showed about the same percent homology. Furumura et al. reported the results of differential display study in which they identified three genes that were up regulated during the switch from eumelanin to pheomelanin synthesis. One of these genes is ITF2, an E type basic helix loop helix transcription factor. Upon transfection of Melan-a cells with the murine ITF2 gene, ITF2 trans-activated the TRP-1 promoter to the same extent as MITF, was less efficient than MITF in trans-activating the tyrosinase promoter, and had no trans-activating effect on the TRP-2 promoter. ITF2 over expression up regulated TRP-1 level, but down regulated the levels of tyrosinase and TRP-2, suggesting that ITF2 regulates melanogenesis by acting as an E-box binding protein. Abdel-Malek et al reported on the differential responsiveness of cultured melanocytes established from different skin types to α-MSH. Five out of five cultures with high constitutive melanin contents, and four out of five cultures with very low melanin contents exhibited the typical dose-dependent responses to α-MSH, evidenced by stimulation of cAMP formation, proliferation, and tyrosinase activity, beginning at a dose of 0.1 nM. One of the five cultures with a very low melanin content demonstrated a significantly reduced response to α-MSH, evident as a shift to the right in the dose-response curves for cAMP formation and tyrosinase activity, with a minimal effect dose of α-MSH equal to 10 nM. RT-PCR and sequencing of the entire coding region of the MC1R gene revealed the presence of these point mutations in this culture: Phe147Leu, Ile155Leu, Arg160Trp,Thr177Arg, and Ile264Met. The significance of these mutations on the loss of function of the MC1R is being investigated.

Sunday afternoon Sessions **by John Pawelek**

The afternoon Plenary Session was on the Biochemical Control of Pigmentation.

1) Keynote Lecture: Perspectives on biochemical control of pigmentation and pigment cell survival, K. Schallreuter. Dr. Schallreuter discussed the hypothesis, proposed by Dr. John Wood and herself, that (6R)-L-erythro 5,6,7,8 tetrahydrobiopterin (6BH4) through regulation of phenylalanine hydroxylase (PAH) and tyrosinase activities is a key, rate-limiting factor in the control of melanogenesis. The proposal is based in part on their observations that L-Phe is actively transported by human melanocytes, whereas L-tyr enters cells much more slowly, through passive diffusion. 6BH4 regulates melanogenesis through specific binding sites on both tyrosinase and MSH. MSH activates tyrosinase by removing 6BH4 from a tyrosinase:6BH4 inhibitory complex. UVB, through photooxidation of 6BH4, activates both PAH and tyrosinase.

2) Macrophage migration inhibitory factor (MIF) has enzyme activity towards oxidized catecholamines and recues cells from dopaminechrome induced death. J. Matsunaga et al. Evidence was presented that MIF is able to catalyze the conversion of DOPaminechrome and norepinephrinecrome, toxic qinone neurotransmitter by-products, to indole-quinone derivatives that may serve as precursors of neuromelanin. Cytotoxicity experiments showed that MIF can rescue cells from DOPaminechrome induced death in culture. Since MIF is highly expressed

in brain, the possibility is raised that MIF detoxifies catecholamine products and therefore could have a protective role for neural tissues.

3) The function of the pink-eyed dilution protein. N. Puri and M.H. Brilliant. Using immunohistochemistry and confocal microscopy, the authors showed that at least one function of the pink-eyed dilution protein (p protein) is to
regulate melanosomal pH. Acidic compartments of melanocytes were detected by DAMP incorporation and indirectly visualized using fluorescein-conjugated antibodies. In wild type melanocytes, virtually all melanosomes were acidic and virtually all acidic compartments were melanosomes. In contrast, melanosomes of p-deficient cell lines were almost never acidic. As tyrosinase activity in melanosomes is dependent on a low pH, the authors postulated that the minimal melanin synthesis observed in p-deficient mutants is due to insufficiently low pH.

4) Cell density-mediated induction of tyrosinase-related protein gene expression and differential regulation of TRP-2 glycoforms. T.J. Hornyak et al. The effects of cell density on the expression of TRP-1 and TRP-2 in cultured melan-a mouse melanocytes were investigated. The relative levels of mRNA for both proteins increased with increasing cell density and decreased when confluent cells were replated at low density. Compared to TRP-2, TRP-1 both decreased more rapidly with low density and increased more rapidly as density increased. Western blotting showed that TRP-2 existed in two distinct glycoforms under separate regulation with cell density changes. The results showed the potential importance of cell density or cell contact in determining the extent melanogenesis.

5) Tyrosinase-related proteins (TRPs) modulate tyrosine hybroxylase activity of tyrosinase in genetically defined mouse melanocytes. R. Sarangarajan et al. Tyrosine hydroxylase (TH) activity of cultured mouse melanocytes from wild type B/B mice was compared to mice with mutations in TRP-1 and TRP-2. Using ^{3}H-L-tyrosine in the Pomerantz assay, it was determined that TH activity was higher in wild type over mutant cells, even though tyrosinase itself was wild type in all cases except for albino (c/c) negative controls. Conclusion: TRPs are positive regulators of TH activity.

6) The regulation of pigmentation by serine proteases and their inhibitors. M. Seiberg and S.S. Shapiro. Multilayered epidermal equivalents expressing UVB-inducible melanogenesis were used to study the effect of protease inhibitors on melanogenesis. Several serine protease inhibitors were also effective melanogenesis inhibitors. The pigmented Yucatan swine treated with one of the protease inhibitors showed a visible lightening effect. Data suggested that the protease inhibitors interfered with melanosomal transfer from melanocytes to keratinocytes.

7) Supermelanotic and metastatic melanoma x macrophage fusion hybrids: Altered N-glycosylation as an underlying mechanism. S. Sodi et al. A number of fusion hybrids between weakly metastatic Cloudman S91 cells and peritoneal macrophages were shown to have increased metastatic potential and this correlated with a dramatic increase in both basal and MSH-inducible pigmentation. Using Western Blotting of LAMP-1 and TRPs, incorporation of ^{3}H-glucoseamine, and glycosylation inhibitors, it was determined that the increased pigmentation was likely to be a result of a macrophage-like N-glycosylation system expressed in the metastatic hybrids, and that this glycosylation system might be an underlying cause for increased metastasis as well. Further, the data revealed for the first time that N-glycosylation may be an important pathway for MSH-induced melanogenesis.

8) Chemical characterization of dopamine-melanin: Application to identification of melanins in *Cryptococcus neoformans*. S. Ito, et al. Melanin has long been associated with virulence in *C. neoformans* and is believed to be produced by laccase. Several isolates of C. neoformans were incubated with dopamine or DOPA and subjected to improved melanin analyses using alkaline H_2O_2 oxidation and HI hybrolysis. The results indicated that laccase from *C. neoformans* indeed oxidizes catecholamines to produce eumelanic pigments.

9) Molecular characterization of c-kit from the Mexican axolotl. K.A. Mason et al. Using alignments of known c-kit sequences, degenerate PCR primers were designed and used to amplify a small fragment of c-kit from axolotl RNA by RT-PCR. With this fragment, 3 positive clones were identified in an axolotl cDNA library. Two clones were identical and appeared to encode a complete cDNA sequence for axolotl c-kit. Genetic mapping excluded c-kit as a candidate for the axolotl white mutant, but showed that c-kit is tightly linked to PDGFα, seen in other vertebrates, and additionally confirming that this represented at least one form of axolotl c-kit.

10) An analysis of pigment patterns in leopard and golden mutant zebrafish and related taxa of danios. R. Morrison and K. Nagashima. The zebrafish, *Danio rerio*, was studied as a model organism for pigment pattern formation. There are at least four types of chromatophores in zebrafish: xanthophores, iridophores, leucophores, and melanophores. There is a distinct embryonic pigment pattern composed of four melanophore stripes that transform into the adult pattern of alternating blue-black and silver yellow stripes. The golden mutant, which lacks melanophores and iridophores as adults, and the leopard mutant, which shows a spotted phenotype were studied, along with the pearl danios, which lacks alternating striping elements as an adult but contains a fifth type of chromatophore, the erythophore, and the giant danios that has a different arrangement of chromatophores than that

seen in wild-type zebrafish. It was proposed that analyses of these fish should further clarify the critical events and time-points in zebrafish pigment pattern formation.

11) LiCl is involved in the pigmentation of the embryonic zebrafish (*Brachydanio rerio*). E-J Jin and G. Thibaudeau. Zebrafish embryos were treated with various signalling-related molecules. LiCl and LiCl/forskolin treatments each increased pigmentation. The LiCl/forskolin-induced pigmentation was not accompanied by an increase in melanophore number, but did result in increased tyrosinase activity and increased expression of MSH-1, a pigment specific protein, and TRP-2 as assessed by immunoblotting.

Monday morning Sessions **by Raymond Boissy**

The morning began with a sunrise session entitled "New Perspectives on the Treatment of Human Vitiligo". David Norris presented a review of the processes of programmed cell death (i.e., apoptosis) and necrosis and discussed the balance between these two mechanisms as they pertain to cell survival. Molecular regulators of apoptosis (i.e., the bcl family of proteins, FAS and the caspases) were reviewed. It was proposed that melanocyte destruction in vitiligo occurs via apoptosis and intervention of this may provide potential therapeutic opportunities. Raymond Boissy discussed occupational/contact vitiligo resulting from exposure to phenolic/catecholic agents. The phenolic agent, 4-tertiary butyl phenol, is cytotoxic to melanocytes via an apoptotic process. The antioxidant catalase could provide some protection against this form of melanocyte destruction. Pranab Das reviewed the immunological components of vitiligo. The role of T-cell autoreactive clones, conducting a hit and run affect on melanocytes in vitiligo was discussed. Immunomodulary therapy was proposed as a perspective for treatment for vitiligo. Finally, Karin Schallreuter discussed the biochemical aberration in skin of patients with vitiligo. The role of biopterin in the regulation of tyrosinase activity was reviewed. Successful results of the daily topical application of pseudocatalase on repigmentation in vitiligo was presented and discussed. It was concluded in this sunrise session that the etiology of vitiligo is both complex and diverse and that multiple theraputic regimes will have to be developed to successfully treat this disease.

A keynote lecture was then presented by Richard Spritz entitled "New Approaches in Genetics and Their Application to Pigment Cell Research" and subtitled "How to find a gene". Functional versus Positional Cloning methods were presented and the difference between discussed. Mapping a gene directly to a chromosome was first discussed and techniques using autoradiography, FISH, and chromosome abnormality was presented and the c-kit associated Waardenberg Syndrome and OCA2 were provided as examples. Genomic analysis (i.e., linkage) was discussed next. Examples of using RFLP assessment, Simple Tandem Repeats, and the utilization of chromosome site markers were presented. Finally, current methods for gene mapping were described. This included the need for large family trees, Yeast Artificial Chromosomes, Sequence-tagged-site, homozygosity mapping, linkage disequilibrium mapping, and the generation of a physical map.

A session focussing on the Hermansky-Pudlak Syndrome followed. Richard Spritz presented genetic and functional studies of Hermansky-Pudlak syndrome. The characteristics of this syndrome consist of oculocutaneous albinism, a platelet aggregation dysfunction, and the development of a ceroid like material in the lungs resulting in pulmonary fibrosis. The cloning of the gene (and the murine counterpart) and the identification of various protein-null mutations was presented. The HPS gene product appears to be soluble and unglycosylated. It is predominantly unassociated with organelles or granules in fibroblasts and lymphoblastoid cells and loosely associated with large granules in melanoma cells. The HPS gene product demonstrated some co-localization with tyrosinase, LAMP-1, and Myo5A (the product of the *dilute* locus). Richard King next presented the identification of an alternative 1.5 kb transcript (in addition to the 3.6 kb full length transcript) also present in bone marrow and a melanoma cell line. Raymond Boissy next discussed cytological aberrations in melanocytes cultured from patients with mutations in the HPS gene resulting in the lack of transcript expression. These hypopigmented melanocytes demonstrated muted tyrosinase activity, large membranous complexes, and DOPA positive 50 nm vesicles distributed throughout the cell. Tyrosinase, TRP-1 and ME491 were not efficiently trafficked to melanosomes in the mutant cells. Localization studies suggested that the HPS gene product was associated with the endoplasmic reticulum and melanosomes in the normal melanocytes.

The final session of the morning was entitled "Vitiligo: mechanisms of depigmentation and repigmentation". Pranab Das presented data demonstrating the appearance of immunocytes at the border of a vitiligo lesion and the associated development of apoptosis in some melanocytes. In addition, studies demonstrating that immune cells can lead to *de novo* generation of nitric oxide in melanocytes resulting in apoptosis. Caroline LePoole presented the immortalization of a line of vitiligo derived melanocytes using the E6 and E7 gene of HPV. These cells were passaged over 60 generations, demonstrated dilated RER, and exhibited alterations in proteins identified in fractionated RER. Marna Ericson presented data in which biopsies both pre and post treatment with topical steroids were immunocytochemically processed for the identification of melanocytes, Langerhans cells and nerve cells, and viewed with confocal microscopy and computer reconstruction. Demonstration of a decrease in the appearance of nerve fibers in the epidermis after successful treatment was provided. Fan Yang presented data

demonstrating the 4-tertiary butylphenol (4-TBP) acts as a specific competitive inhibitor of tyrosinase at concentrations well below the threshold that generated a cytotoxic response in normal melanocytes. Finally, Caroline LePoole demonstrated that shortly after exposure of melanocytes to 4-TBP several transcripts are differentially expressed. One of these upregulated proteins was an adenosine receptor that has been implicated in the activation of apoptosis.

Monday afternoon Sessions **by Frank Meyskens**

The session on malignant melanoma covered a diversity of topics. Meenhard Herlyn started the session with an eloquent keynote presentation of their skin reconstruction model, which is used to study progression. Using the model, Hsu has shown that adherence of keratinocytes by E-cadherin to melanocytes controls their growth and phenotype and when this adhesion molecule is shut off a cell cluster, a nevus, forms. The overall important point was made that the model more closely resembles the biology in intact skin than that exhibited by cells under typical culture conditions. There were several papers on the role of various proteins in controlling melanocyte growth including its positive regulation by retino-blastoma tumor suppressor protein (R. Halaban, Yale), and negative regulation by p15(INK4B) (T. Pacheco, University of Colorado). In a similar vein Rearden at the University of Colorado used T-cell receptor knock-out mice to show that transduction of B16 F10 melanoma cells with M-CSF increased survival of the inoculated animals suggesting that cells other than T lymphocytes (eg NK or macrophages) are involved in anti-melanoma immunity, at least in this model.

In a fasciniating presentation, Bill Robinson of Australia presented the zebrafish as a powerful developmental model for understading the role of p16 and other regulators in melanocyte proliferation. This model impresses this reviewer as possibly one of the most important new systems to come along in quite some time.

The other Keynote lecture was entertainingly provided by John Cohen of the University of Colorado and apoptosis was reviewed in detail. Two papers were presented dealing with apoptosis in melanoma. Shellman from Colorado convincingly showed that apoptosis was controlled by the dimension status of tumor growth; i.e.under monolayer growth conditions, ras-alterated cells underwent apoptosis in response to various stress conditions while under 3-dimensional spheroid growth no or little apoptosis occurred. Since most culture studies are done in monolayer these studies have obvious and important consequences for the interpretation of studies of melanoma growth and drug resistance in the *in vivo* setting. From my own laboratory, Spillane has found that human melanoma cells have high levels of endogenous reactive oxygen species that may induce high levels of constitutive NFKB and a protective stress response. Interestingly, the complex antioxidant PDTC induced apoptosis while classical antioxidants(e.g. α-tocopherol) did not; suprisingly heavy metal chelators could largely mimic the effect PDTC. The most intriguing and interesting study of the session (and perhaps of the conference) was presented by Stan Pavel of the Netherlands. They measured the sulfer and phaeomelanin content of atypical nevi; and found their concentrations to be elevated, as was the concentration of calcium. Normal melanocytes from these same subjects showed no such changes suggesting that an abnormality in melanosomal regulation exists. Since phaemelanin metabolites are considerably more toxic that those of eumelanin. metabolites. One wonders if the generation of atypical nevi results from a simple error of metabolism, e.g. in the Agouti signaling protein. All in all this session was quite stimulating and a lot of new ideas were heard and vigorous discussion ensued.

Tuesday morning Sessions **by Joe Bagnara & Vince Hearing**

The keynote lecture by Karl H. Pfenninger presented basic insights into cell motility through the use of nerve growth cones as an example. Thus, he set the stage for the subsequent three presentations which were specifically directed toward an understanding of the migration of melanocytes and melanoma cells. Dr. Pfenninger illustrated the importance of pseudopod attachment, release, and reattachment as the basis for cell migration and he considered the means by which these steps are accomplished. The subsequent presentation by Hiroaki Yagi demonstrated through the use of Boyden chamber assays that insulin-like growth factor-1 (IGF-1) is a potent chemoattractant for both human melanocyes and melanoma cells. Endothelin-1 (ET-1) and basic fibroblast growth factor (bFGF) enhanced migration of normal human melanocytes and enhanced the invasion activities of WM35 cells (a human melanoma with low invasiveness). IGF-1 induced maximal movement in both these cell types and this action could be blocked by CDC, a selective 12-lipoxygenase inhibitor. cPLA2, a critical enzyme in pseudopod activation in both cell types is stimulated by ET-1, bFGF and IGF-1. The implications of these findings toward the progression of melanoma were discussed. This presentation was followed by that of Tatsuya Horikawa, et al. who considered the motility and proliferative responses of an H-ras-transfected murine melanocyte cell line, melan-A. In a Boyden chamber assay it was found that H-ras-transfected melanocytes show a higher incidence of migration than do wild type melanocytes. Cell motility was induced by ET-1 and bFGF in wild type melan-A, but not in H-ras-transfectants. TPA was required to grow parental melan-A cells, but the ras-transfectant was TPA-independent and in fact was inhibited by TPA. It was suggested that H-ras plays a key role in the induction of melanocyte locomotion and proliferation. The last talk on acquired MSH-sensitive chemotaxis by highly

metastatic melanoma/macrophage fusion hybrids by Rachkovsky et al. was presented by John Pawelek. It was a most fitting follow up to the very first presentation of the conference, John Pawelek's Gelb Lectureship on melanoma/macrophage hybrids and melanoma metastases. In this symposium talk, it was pointed out that of the various fusion hybrids between normal macrophages and Cloudman S91 melanoma cells, the most metastatic also showed dramatically increased motility. Metastatic hybrids demonstrated increased migration in response to 3T3-conditioned medium, lung fibroblast-conditioned media, and lung explants. Treatment of the hybrid cells with MSH/BMX markedly increased migration through enhanced chemotaxis rather than chemokinesis. Evidence was presented suggesting that different glycosylation pathways were expressed in hybrid and parental cells and that motility was probably regulated by means of N-glycosylation. It was suggested "that the enhanced metastatic potential of macrophage x melanoma hybrids may have its basis in a new, MSH-inducible chemotactic phenotype, with altered N-glycosylation as one of the underlying regulatory mechanisms."

After a short break, Barbara Gilchrest presented a Keynote Lecture on the "Effects of UV on melanocytes: speculative relationship to the epidemiology of melanoma." Melanocyte responses to UV were reviewed with special emphasis on their DNA repair mechanisms. The role these responses play in melanocytes and keratinocytes might explain the differences in malignant transformation in those cell types to produce melanomas and carcinomas, respectively. The importance of sunscreen use to protect from UV damage throughout life was emphasized.

N. Kobayashi then reported on studies examining the photoprotective effects of melanins. Immuno-histochemistry with antibodies specific for different types of DNA photoproducts allows assessment of DNA damage in UV irradiated skin at the cellular level. A direct correlation was found between melanin content in a cell and protection from UV light. Current studies are aimed at examining the efficiency of different types of melanins using mouse tail skins of different genotypes as models. Z. Abdel-Malek then reported on responses of melanocytes from different human skin phototypes to UV light. The sensitivities of melanocytes from different donors to UV light was measured for DNA damage, bcl2 expression and other parameters of cellular injury. There were significant differences in the responses measured depending on skin phototype in melanocytes, keratinocytes and fibroblasts, and the increased sensitivity of keratinocytes and fibroblasts to UV damage was proposed as the reason for the higher incidence of carcinomas in those cell types. Finally, F. Meyskens reported on the response of metastatic melanoma cells to UVB stimulation, particularly with respect to activation of the NFkB transcription factor. There were dramatic differences in activation of 2 NFkB family members (p50 and p75) in malignant melanoma cells and in normal human melanocytes, often by an order of magnitude, suggesting basic differences in response mechanisms of normal and transformed melanocytes. It was suggested that such changes are associated with the metastatic potential of those malignant cells, and that understanding the reason behind such differences may offer novel approaches to the prevention, diagnosis and therapy of melanoma.

Poster Session **by Roger Bowers**

The poster sessions at this PASPCR meeting were well attended. There were only 12 posters due to the numerous oral presentations but they were of the highest quality. Holder and Thibaudeau showed that increased melanization in the melanoid defect in axolotls may involve aspects in addition to cellular plasticity whereas cellular plasticity is implicated to be involved in enhanced xanthophores from albino axolotl embryos. Parker and Mason demonstrated that extracts from the white axolotl mutant caused a decrease of pigment cells and inhibited their movement in cultured melanoma cells. They are currently working to characterize this extract. Roberson and Thibaudeau showed a two stage time-dependent differentiation of *in vivo* melanophores in zebrafish. Similar results were found in *in vitro* zebrafish melanophores and LiCl increased pigmentation in these same cells. Nurcahyani and Thibaudeau demonstrated cellular parameters influencing ant./post. responses of neural crest-derived pigment cell linages in axolotls. For example, ant. neural crest cells gave rise to more pigment cells whereas post. neural crest cells yielded more melanophores. Gonzalez, Buckner, Ruiz and Bowers, with the use of the glutathione inhibitor BSO, other treatments and parameters, showed the importance of antioxidants in the viability of avian melanocytes. Maxwell, Walsh and Maxwell demonstrated the infection of human melanoma cells by parvoviruses and suggested therapeutic uses of these viruses and their vectors for melanoma. Murakami, Baba, Kawa and Mizoguchi showed that inflammation in atopic dermatitis plays a role in developing acquired dermal melanocytosis along with the hereditary disposition of having immature dermal melanocytes. Sarangarajan, LePoole and Boissy demonstrated that NHE-1 isoform of sodium hydrogen exchanger is expressed in M14 melanoma cells and in keratinocytes but not in human melanocytes. Ahn, Jang, Cho, Lee, Hong and Lee showed that a kojic acid derivative, kojyl caffeic acid, has a greater depigmenting effect than kojic acid. Forest, Nofsinger, Drake and Simon demonstrated that the photochemistry of melanin in the UV is wavelength dependent. Shi, Krauss and Woodward showed evidence for the presence of EP2- and IP- receptors coupled to melanin production via stimulation of tyrosinase activity in S91 Cloudman cells. Butts and Naughten demonstrated that melanocytes decreased with time in new wound closure epidermis in the corneal region of the frog.

Members in the News -

Eun-Jung Jin - received a pre-doctoral **Young Investigator Award** at the PASPCR meeting for her presentation entitled "LiCl is involved in the pigmentation of the embryonic zebrafish (*Brachydanio rerio*)"

I. Caroline LePoole - received a Junior Faculty **Young Investigator Award** at the PASPCR meeting for her presentation entitled "4-TMP treatment induces A2BR adenosine receptor expression in human melanocytes"

Rosalynn Miltenberger - received a post-doctoral **Young Investigator Award** at the PASPCR meeting for her presentation entitled "An agouti mutation lacking the central basic domain promotes yellow pigmentation but not obesity in transgenic mice"

Randall Morrison - received a Junior Faculty **Young Investigator Award** at the PASPCR meeting for his presentation entitled "An analysis of pigment patterns in leopard and golden mutant zebrafish and related taxa of Danios"

James J. Nordlund - was presented the PASPCR Career Achievement Award at the Snowmass Meeting; this Award is the most prestigious honor bestowed by the PASPCR "to an individual(s) for outstanding and distinguished contributions to pigment cell research and/or the Society"; congratulations to Jim, who certainly is most deserving of this distinction.

John M. Pawelek - presented the Lawrence Gelb Lecture at the Snowmass meeting; this lecture is presented by an active and outstanding researcher who is currently making a significant impact on the field of pigment cell research; the title of John's lecture was "Melanoma/Macrophage Hybrids and the Development of Metastases in Melanoma"

George Szabo - was selected as an Honorary Member of the PASPCR; he was presented this award at the Banquet in Snowmass; it was a pleasure to see George again and he still retains his unique sense of memory and humor, as was evident by his remarks that evening.

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The Bibliography published in this issue covers the period May, 1998 through July, 1998. 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 attempted to highlight any publications which include a member of the PASPCR with a star (*sorry if we missed you but let us know and you'll get a free marked repeat in the next issue*).

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