PASPCR Newsletter

Volume 5 Number 3 **September,1997**

Introduction . . . **by the Publications Committee**

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 the **PASPCR** *Newsletter*; help us to update the Job Listings, Calendar of Events, Meeting Reports, and other items of general membership interest. 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 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 any of the members of the Publications Committee.

The PASPCR now has its own **WWW** home page. We plan this to be a major source of current information for the PASPCR membership. The address for the page is: **http://lenti.med.umn.edu/paspcr**. This site contains information on the goals of the society, future meetings, council information, past issues of the **PASPCR** *Newsletter* as well as links to other sites including the InterPig DataBase, the International Pigment Cell Conference in Nagoya and the International Federation of Pigment Cell Societies (IFPCS).

We have now included the membership directory on that page; please notify us if you wish any or all of your information to be deleted or modified on that site.

Please check out the PASPCR web site and send any comments and/or suggestions to either the PASPCR WebMaster Bill Oetting at bill@lenti.med.umn.edu or to Vince Hearing at hearingv@nih.gov.

IN THIS ISSUE . . . . .

PanAmerican Society for Pigment Cell Research

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Calendar of Events :

Oct 9- 11, 1997 7th ESPCR Annual Meeting, to be held in Bordeaux, France (contact: 7th ESPCR Meeting Bordeaux, c/o Congres Seminaires Organisation, 81, Boulevard, Pierre 1er, 33110 Le Bouscat, Bordeaux, France)

Dec 13 - 17, 1997 American Society for Cell Biology Annual Meeting, to be held in Washington DC (contact: FASEB, 9650 Rockville Pike, Bethesda, MD 20814, USA; FAX: +1 301/530-7014)

Aug 15 - 18, 1998 VIIIth PASPCR Annual Meeting, to be held in Snowmass, CO (contact: Dr. David Norris, Dermatology Dept, Univ of Colorado Medical Center, Denver, CO 80262 USA; FAX: +1 303/372-1159)

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

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

Nobuhiko Kobayashi **Peter K. Lee**

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

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.

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Call for Nominations

by James J Nordlund

Our Nominations Committee will soon be in action to select nominees for the PASPCR Council Members to be included on the 1997 ballot. We invite you to submit names for consideration and please send those to Dr. Richard King, chair, Nominations Committee.

In addition to selecting 3 Council Members to begin terms in 1998, we will also be considering candidates for the PASPCR Career Achievement Award and the Gelb Lecturer, both of which will be presented at our next meeting in Snowmass, CO (cf below). In addition to those Awards, we will also consider nominees as Honorary Members to the PASPCR. Please forward any of your suggestions for any of these Awards to any of the PASPCR Officers and we will make sure that they are duly considered.

Finally, the PASPCR Council decided that the PASPCR meeting in the year 2000 will be in Texas and will be organized by Dr. Lynn Lamoreux with assistance from Dr. Estela Medrano. The dates for this meeting will be announced in the future. Anyone who would like to propose meeting sites for the year 2001 and beyond should contact the office of the Secretary/Treasurer.

Meeting Report - VIIth Annual Meeting of the PASPCR

June 15th - 18th, 1997 The Westin Hotel Providence, RI

Overview / Summary of Meeting -

Walter Quevedo (co-organizer)

On behalf of Hal Swartz and myself, I would like to congratulate the 129 scientists that participated in the 7th Meeting of the PanAmerican Society for Pigment Cell Research and the fine Providence weather for making it a good one. The clambake organized by Tom Holstein and the Reception and Banquet arranged by Fae Best Carletti were standout features of the social program.

The scientific sessions were well attended throughout and discussions following presentations were of high quality. One had the feeling that pigment cell research is at the cutting edge of science not only in pursuing leads provided by workers in other fields but also in compensating such workers with new investigative directions that they might follow. Hal's concept of the sunrise sessions was vindicated by the consistently high attendance and unabating enthusiastic exchanges between "teachers" and "students" during classes that ended all too soon. There were moments of pride and sentimentality such as those accompanying Vince Hearing's Gelb Award, Joe Bagnara's Career Investigator Award, and Aaron Lerner's and Tom Fitzpatrick's acceptance of the Meeting Chairmen's Award for Outstanding Contributions to Pigment Cell Research and its Pursuit in New England. It was an honor for Providence to host the 7th Meeting.

Symposium I – Molecular Aspects of Malignant Melanoma **Frank Meyskens**

This was an exciting symposium that elicited many questions and comments. Two major types of papers were presented: strategies related to the underlying basis for immunotherapy in human melanoma and characterization of molecular changes in certain genes related to pigmentation.

The symposium opened with a useful historical overview of the field of biologic and immunologic approaches to the management of melanoma (H Wanebo). To date, almost all approaches to melanoma management have produced no or modest benefit in the clinical situation, although the studies presented by D Shrayer and colleagues suggested that in an animal model of human disease that both primary melanoma tumor growth and lung metastases could be prevented. The emphasis of subsequent speakers was on the necessity of characterizing the specific features of immunologic alterations. Precise identification of human melanoma antigens by tumor infiltrating T lymphocytes (Y Kawakami), the immune response to melanosomal differentiation antigen induced by altered antigen (S Bartido). The new human melanocyte specific gene factor MSG1 was presented in two papers; T Shioda showed that the factor was a nuclear protein, and was induced by phorbol ester and was transcriptionally regulated. M Fenner provided further information that MSG1 promoter activity was correlated with pigmentation.

In two studies from Yale, it was shown that the regulation of FGF-2, a requirement for melanocyte proliferation, was regulated by c-Myb proto-oncogene (M Miglasese). In a second paper, R Halaban and her colleagues showed that when tyrosinase was inadvertently retained in the endoplasmic reticulum, its degradation was accelerated, contributing to the amelanotic phenotype, and suggesting perhaps that this property is phenotypical rather than from direct genetic alteration. Finally, a paper fitting neither of the two major areas, but dealing with an important, relatively neglected, aspect was presented (F Meyskens). NKFB and AP-1 were shown to be regulated differently in melanocytes and melanoma cells and the response in terms of redox suppression between the two cell types was markedly different. Overall, this symposium offered considerable anticipation that new approaches to the management of melanoma may evolved from these and other basic advances in understanding described throughout the conference.

Symposium II – Role of Melanocyte Death during Development and Adaptive Responses of Skin to Damaging Agents **Raymond Boissy**

This symposium began with the keynote address presented by James H Wyche entitled "A model system for studying cell death regulation". Dr Wyche presented a review of molecular events occurring in the initial stages of apoptosis prior to DNA damage by describing the molecules modulating susceptibility to apoptosis (i.e., bax, bcl2, etc.) and the various caspases involved in proteolysis during initiation of apoptosis. He highlighted this review by describing his research on staurosporine treated promyelocytic leukemia cells which exhibited activation of caspase-3 causing the proteolysis of the DNA-dependent protein kinase which then correlated with the initiation of apoptotic chromosomal DNA degradation. Zalfa Abdel-Malek then presented "Elucidation o f the signaling pathway which mediates the responses of human melanocytes to UVB light" in which she demonstrated that cultured human melanocytes exposed to UVB undergo proliferation arrest and can progress to apoptosis after overexpression of p53. In addition, cAMP inducers like MSH can

accentuate the increased melanization response of cultured melanocytes to UVB and promote movement of cells to the S phase of proliferation. Fan Yang next presented "The effects of tyrosinase activity and commonly used mitogens on the cytotoxicity of 4-tertiary butyl phenol (TBP) to human melanocytes" in which she demonstrated that the development of apoptosis in cultured human melanocytes after exposure to 4TBP was not influenced by the level of tyrosinase activity or melanin content. In contrast, the presence of either MSH or bFGF could accentuate cytotoxicity of 4TBP exposed melanocytes. William Pavan then presented "Met-HGF signaling is critical for melanocyte development: Implications for Waardenburg Syndrome type II" in which he demonstrated, using genetically engineered mice as either knockouts or overexpressers for the Met tyrosine kinase receptor or its ligand HGF/SF respectively, that this signaling pathway is crucial for continued development of ckit/steel positive neural crest cells towards melanocytes. E. Michelle Southard-Smith next presented "Physical mapping and embryological analysis of Dominant Megacolon, a mouse model of Hirschsprung's disease" in which she described the mapping of the Dom locus to a 0.01cM region proximal to D15Mit2 by linkage disequilibrium and physical mapping. In addition, expression analysis in embryos demonstrated that homozygous and heterozygous mutant animals exhibited an absent or reduced expression of TRP-2/EDNRB positive neural crest cells respectively. Nels Granholm then presented "Effects of agouti alleles, hair plucking, and aging on thiol concentrations in mouse livers" in which he demonstrated using HPLC analyses of GSH and cys that there was a casual relationship between hair plucking, aging, and liver thiol mobilization. J. Robert Smyth next presented "Do viruses and/or growing environment affect the expression of autoimmune vitiligo in the Smyth line chicken?" in which he demonstrated that the development of feather amelanosis could be increased by prior vaccination for the turkey herpes virus suggesting that priming of the immune system could influence melanocyte destruction in this model. P. Bernhardt Koch then presented "Pigment synthesis and molecular mechanisms of color pattern formation in butterfly wings" in which he first review the biology of wing development and pigmentation. The expression pattern of dopa decarboxylase (DDC), which generated dopamine melanin, was correlated with the development o f the dark eye spot on the wings of normal and mutant animals was then presented. Finally, Roger Bowers presented "Further analysis of genetic sensitivity causing premature cell death in vitiliginous avian melanocytes: low antioxidant protection" in which he demonstrated that exposure of cultured avian melanocytes to oxygen radicals generated by the addition of xanthine:xanthine oxide to the medium induced more cell death in mutant cells as opposed to control cells. In addition, the former group demonstrated greater radical oxygen species as evaluated by direct fluorometer measurements.

Symposium III – Melanosomes: Biogenesis and Structure **Seth J Orlow**

V Hearing and J Hammer cochaired a session entitled "Melanosomes: Biogenesis and Structure". S Orlow (NY) began the session with a discussion of the *pink-eyed dilution* gene product and its role in melanogenesis and melanosome formation. Melanocytes cultured from p-null mice possess smaller, more numerous melanosomes, and this is corrected in part by culture in high concentrations of exogenous tyrosine, as are alterations in levels of tyrosinase and some other melanosomal proteins. V Setaluri (Winston-Salem) discussed the sequences responsible for exit from the ER and trafficking to melanosomes of gp75 (aka TRP-1). By the use of site-directed mutagenesis, the information necessary has been shown to be present in the short cytosolic tail of gp75, and the presence of a dileucine motif is an important criterion for proper trafficking, though it may not be the only one. VJ Hearing (Bethesda) gave the Gelb Keynote Address in the context of this symposium. He summarized a large body of work from his own lab and others, focusing especially on the differences between pheomelanosomes and eumelanosomes. Levels of tyrosinase are decreased and levels of TRP-1, TRP-2, p and silver proteins are almost undetected in pheomelanic hair follicle extracts. Cultured melanocytes can now be treated with recombinant agouti signal protein, enabling the process of pheomelanosome formation in response to ASP to begin to be dissected *in vitro*. J Hammer (Bethesda) presented work from his laboratory on myosin V^a, the *dilute* locus gene product. This unconventional myosin can be shown to be present at the melanosomal surface by immunofluorescence, immuno-EM and by biochemical

techniques. In cells from mice with dilute mutations, melanocytes extend dendrites, but melanosomes fail to traffic down these dendrites and remain in a perinuclear distribution, thus implicating myosin V^a in the transport of melanosomes as a prelude to their transfer. J Bhawan (Boston) discussed various theories regarding the transfer of melanosomes from melanocyte to keratinocyte. Careful EM studies provide evidence in support of not one, but rather two or three modes of transfer that may be operative in sun-exposed skin.

P Samaraweera (Orlow lab) described a 65 kDa protein which localizes to the cytosolic face of the melanosome, and unlike previously described melanosomal proteins is peripheral rather than integral to the membrane. It shows altered detergent solubility in melanocytes cultured from buff mice, which may have a melanosomal transport defect. K Sato (Orlow lab) used site directed mutagenesis and the construction of chimeric molecules to show that both TRP-1 and TRP-2 are able to stabilize tyrosinase protein in cotransfection experiments. The amino terminal portion of TRP-1 is critical to its ability to effect this stabilization. B Potterf (Hearing/Gahl labs) examined the transport of sulfhydryl compounds by melanosomes. Although glutathione transport seems not to be an issue, melanosomes show robust transport of cysteine. The role of such transport in the control of melanogenesis was discussed. Finally M K Cullen (St. Louis) examined tyrosine uptake by isolated melanosomes. Cytosolic factors appear to regulate this accumulation, and interestingly, extracts from melanocytes cultured from mice with "melanolysosomal" defects such as ruby exhibit an altered ability to enhance this tyrosine transport.

Symposium IV – Ocular Melanocytes and RPE – Role of Transcription Factors in Pigment Cell Biology

Seth J Orlow

A symposium on Ocular Melanocytes and RPE/ Transcription Factors in Pigment Cell Biology was chaired by Seth Orlow. Richard Sidman (Southborough) delivered the keynote address. He and his colleagues have examined the effects of two white-spotting mutations, namely mi^{vit} and W^{sh}, on RPE and choroidal melanocytes. Careful analysis of the RPE of mi^{vit} mutant mice reveals that this gene controls not only pigmentation but proliferation as well, with more numerous taller RPE cells, many of them hypopigmented, noted early on in the mutants. An unusual tigroid pattern can be discerned in ocular tissue from W^{sh} mice, with the additional unexpected observation that giant clumped melanosomes are present in pigment cells from this mutant, reminiscent of those in beige mice. Since W^{sh} mi^{vit} double mutant mice are indistinguishable from the mi^{vit} single mutants, Dr Sidman deduced that Kit may act downstream of MITF. Ongoing studies are geared towards determining how nuclear localization may play a role in controlling MITF function.

Sylvia Smith (Augusta) presented work on the expression of the TRP family in RPE/choroid of mi*vit mice. Levels of TRP-1 are dramatically reduced even at the earliest time points examined, and levels of tyrosinase diminished to a lesser extent, whereas levels of TRP-2 were unaffected, suggesting that MITF *in vivo* plays its most critical role in TRP-1 gene expression regulation. A Zervos (Charlestown) and his group have identified an MITF-interacting protein using the yeast 2-hybrid system. This protein is the human homolog of RKR2, which contains both multiple zinc finger repeats and basic regions. The protein interacts with and inhibits the transcriptional regulatory function of MITF.

Rivka Rachel (NY) discussed her efforts to determine how the ability of RPE cells to make melanin affects the retinofugal projections during embryonic development. She has looked at both albino mice which lack all tyrosinase activity as well as those from mice homozygous and heterozygous for dark-eyed albinism (c^{44H}) which have low but demonstrable levels of tyrosinase activity to ask whether the pigment-producing effects are graded or there is a "gate" level needed below which normal development will not occur. Her results suggest that the reality may be a mixed-situation. Dan-Ning Hu (NY) and Ray Boissy (Cincinnati) each discussed their laboratories efforts to study melanogenesis and growth of cultured human ocular melanocytes. These cells can now be cultured from the uveal tract and will synthesize melanin and melanosomes in culture. Agents which upregulate cyclic AMP stimulate growth and melanogenesis by these cells, including via the bet adrenergic receptor. By

contrast muscarinic agonists inhibit their growth. MSH receptors appear not to be expressed by ocular melanocytes *in vitro*. Finally Brian Potterf (Hearing/Gahl labs) expanded on his previous work to show that melanosomal tyrosine transport is upregulated by pretreatment of cells with MSH or dbcAMP. Whether this regulation is at the translational or transcriptional levels is under investigation. Of a wide range of small molecules, those that compete with tyrosine best are L-phenylalanine, L-DOPA and L-leucine. The potential role of tyrosine transport in the regulation of melanogenesis and in the switch from eumelanin to pheomelanin was discussed.

MiniSymposium I – Pigment Cell Genetics and Molecular Biology **Gregory Barsh**

The large number of previously existing mouse coat color mutations, combined with ease of experimental accessibility and availability of inbred strains, make the mouse much more then a "model" for human disease, a point exemplified by presentations in the section on Pigment cell genetics and molecular biology.

Several years ago, Heinz Arnheiter and colleagues recognized that mutations in the *Microphthalmia* (*Mi*) gene were caused by altered structure or expression of a novel transcription factor, Mitf, whose human homolog was later found to be mutated in individuals affected with Type II Waardenburg syndrome. *Mitf* is one of the earliest genes expressed in pigment cell precursors, yet also may activate expression of melanogenic genes in differentiated melanocytes. In Providence, Arnheiter described recent work by his group that reveals multiple roles for Mitf in the hierarchy of genes that control pigment cell development. At or shortly after neural tube closure, expression of Mitf is detected in only a handful of cells that contribute to the dorsolateral pathway of neural crest, and therefore are likely to correspond to melanoblast precursors. These cells also express the tyrosine kinase receptor Kit, but surprisingly, Mitf is not required for Kit expression since the latter is still present in embryos or cells that lack a functional Kit protein. By contrast, expression of *Dopachrome tautomerase* (*Dct*) is extinguished in *Mitf* mutant cells (at least those derived from neural crest); therefore, *Mitf* is required for early expression of *Dct*. A second role for *Mitf* becomes apparent 1-2 days later in explant culture, when cells that initially express low levels of *Kit* fail to up-regulate *Kit* expression in the absence of functional *Mitf* protein. A priori, failure to up-regulate *Kit* might be caused by defects in cell migration such that mutant cells lack accessibility to one or more paracrine regulators such as Steel factor (also known as Mast cell growth factor, Mgf) or endothelin 3, but this appears not to be the case, since neither factor can rescue *Mitf* mutant cells *in vitro*. Arnheiter's results indicate that pigment cell development is more complicated then previously apparent, in which signaling pathways that are initially parallel (Kit and Mitf) later become dependent on one another. To add to this complexity, what initially seemed like a common theme- a requirement for Mitf in both retinal- and neural crest-derived pigment-clearly proceeds by different mechanisms, since expression of *Dct* depends on *Mitf* in the latter but not in the former.

By contrast to *Mitf*, whose biochemical mechanism of action was suggested by analysis of its structure, identification of the gene responsible for Hermansky-Pudlak syndrome has raised a number of questions for cell biologists that have yet to be answered. As reported by Richard Spritz at last year's IFPCS meeting in Anaheim, the gene mutated in HPS encodes a ubiquitously expressed intracellular protein of unknown function whose absence leads to structural abnormalities in melanosomes, platelet storage pool granules, and probably lysosomes. These findings were confirmed by Richard King and Scott Wildenberg, who have also characterized a splice variant of the same gene they described in Providence. Naturally there is a mouse model for HPS (in fact there are more than a dozen), but in a rare victory for the human geneticists, the HPS gene was identified from human pedigrees before defects in the homologous gene were recognized as the cause of the mouse *pale ear* (*ep*) mutation, as described in Providence by Murray Brilliant. Several mutant alleles have been described for HPS in humans, but the sole mouse mutation is caused by insertion of a retrotransposon that presumably disrupts transcription and/or splicing. No doubt we will learn in the near future why the number of loci mutated in mouse "HPS", such as *ruby*, *light ear*, and *pallid*, to name a few, seems to exceed those recognized in humans by nearly an order of magnitude; an answer to this question may

also help in understanding the relatively mild pigmentation defects in *ep*/*ep* mice compared to humans affected with HPS.

Some of the most interesting questions about pigmentation genetics lie not in the realm of identifying new genes, but understanding how ones recognized for some time interact with one another. In this regard, Bill Pavan gave an update on work of his laboratory in characterizing genetic modifiers of *piebald* (*s*), a hypomorphic allele of the *Ednrb* gene. As reported two years ago by Pavan in Kansas City, differing sensitivity to *Ednrbs*-induced spotting in the Mayer and C3H strains of mice can be ascribed, in part, to different alleles these strains carry for a gene on chromosome 10 that lies in the vicinity of the *Mgf* locus. But, the simple hypothesis that *Mgf* alleles modify the effects of *Ednrb* is, in fact, not so simple. Using the resource of previously existing *Mgf* alleles, Pavan found that a point mutation would, indeed, modify modify *Ednrbs*-induced spotting, but that additional closely linked genes (defined by a 1.5 cM deletion that includes *Mgf*) were responsible for the difference in spotting phenotypes displayed by Mayer and C3H.

Additional work presented in this session included that of Tom Hornyak, who has been characterizing regulatory elements required for *Dct* expression, and from Teichmann *et al.*, who described how gene expression profiles determined using cDNA microarrays can be used to study mouse and human pigmentation. All in all, the session represented a nice mixture of biology and technology, old questions and new, and provided much grist for the mill in Snowmass next year !

MiniSymposium II – Signaling Pathways in Pigment Regulation **Zalfa Abdel-Malek**

Identification of the signaling pathways that mediate the regulation of cellular proliferation, survival, and differentiation is one of the hottest topics of science. In this Symposium, the signaling pathways that control the proliferation and/or melanizaton of non-mammalian as well as mammalian pigment cells were discussed.

Ana Maria Castrucci compared the regulation of physiologic color change among different vertebrate species. In the teleost fish, pigment dispersion is triggered by activation of the cAMP/PKA pathway and pigment aggregation results from inhibition of adenylate cyclase as well as activation of inositol triphosphate/DAG pathway. The tetrapods differ from teleost fish in that pigment dispersion is induced by activation of the cAMP/PKA as well as the PKC pathways, while pigment aggregation results from inhibition of the cAMP pathway. In this, the tetrapods are similar to mammals in that they share the same regulatory pathways for melanin synthesis.

Ruth Halaban explored the role of ectopic expression of the dominant negative E2F1 in conferring TPA-independent proliferation of melanocytes. Normally, E2F acts as a transcription repressor when bound to the retinoblastoma protein family. Expression of the mutant non-DNA binding E2F1-E132 in immortalized B10.BR mouse melanocytes resulted in TPA-independent proliferation, a faster growth rate, morphologic changes, but not tumorigenicity. These changes seem to be due to overexpression of the CDK inhibitor p21, cyclin A, and PCNA, and increased CDK2 activity. Since E2F1-E132 could bind pRb, it was concluded that this binding would free the host E2F from the repressive effect of pRb and thus lead to increased melanocyte proliferation.

Hee-Young Park discussed the roles of PKCβ and PKA on melanogenesis. She has found that activation of PKC by TPA or overexpression of PKCβ resulted in increased tyrosinase activity. This effect is thought to be due to the phosphorylation of tyrosinase on serine residues 505 and 509. Transfection of S91 melanoma cells with the PKA inhibitor PKI resulted in inhibition of tyrosinase activity as well as mRNA and protein levels in α-MSH treated cells.

Glynis Scott described the complimentary signaling of growth factors and the extracellular matrix (ECM) in melanocytes, and its effects on apoptosis, migration, dendrite formation, and melanogenesis. She found that fibronection protects melanocytes from programmed cell death, and that melanocyte survival depends upon an intact cytoskeleton. By comparing the effects of endothelin-1 (ET-1) and/or α-MSH, she found that ET-1 increases migration, while α-MSH increases the adhesion of melanocytes plated on fibronectin. Endothelin-1 antagonized the effect of α-MSH and resulted in the phosphorylation of p125FAK, a tyrosine kinase associated with focal contact formation and cell

migration. Since both growth factors and ECM regulate the organization of the melanocyte cytoskeleton, it was suggested that their combined action might regulate pigmentation through regulation of actin assembly which in turn is involved in dendrite formation and melanosome movement.

Estela Medrano discussed the cell cycle regulatory events that are involved in the process of terminal differentiation of human melanocytes. Previously, she had shown that cAMP inducers stimulate melanogenesis and ultimately result in cell cycle withdrawal of human melanocytes. She found that terminally differentiated, hyperpigmented melanocytes accumulate the CDK inhibitor p27, have reduced cyclin D1 expression, CDK2 activity, and poor pRb phosphorylation, as well as loss of E2F1 protein expression. Induction of hyperpigmentation by cAMP inducers seems to be prerequisite for terminal differentiation, since albino melanocytes did not become post mitotic or lose E2F1 expression upon treatment with these agents.

Zalfa Abdel-Malek presented data on the signaling pathways of ET-1 and α-MSH. ET-1, but not α-MSH, caused intracellular Ca+ mobilization, and had a dose-dependent stimulatory effect on proliferation. ET-1, at concentrations which mobilized Ca+ from intracellular stores, inhibited tyrosinase activity and protein level, but at concentrations which did not affect Ca+ mobilization, it increased tyrosinase activity and protein level. α-MSH had a dose-dependent stimulatory effect on tyrosinase activity. ET-1, but not α-MSH or other cAMP inducers, phosphorylated the cAMP response element binding protein (CREB) and its kinase pp90 RSK . Both effects were dependent on mobilization of Ca^{+2} but not on PKA activity . Increasing extracellular Ca^{+2} concentration to 1 mM enhanced melanocyte proliferation and inhibited tyrosinase activity. This suggests a role for Ca^{+2} in human melanocyte regulation.

Minao Furumura reported the results of screening differentially expressed genes in melanocytes following treatment with recombinant agouti signaling protein (ASP). Differential display revealed three up-regulated genes and 6 down-regulated genes. The former included a potential DNA replication control protein, a putative basic helix-loop-helix transcription factor, and a novel gene. The latter included tyrosinase and TRP-2. Subtractive hybridization of C57BL6 lethal yellow (A^y/a) and black (a/a) mouse skin cDNA revealed additional genes upregulated in yellow skin. These genes might be involved in pheomelanogenesis and possibly other pleiotropic changes in yellow mice.

MiniSymposium III – Comparative Aspects of Vertebrate Pigmentation **Joseph Bagnara**

In his introduction to the session, Joe Bagnara pointed out that in most life science disciplines, the word "comparative" is inferred immediately to mean cold-blooded vertebrates and, as such, are looked at as model systems by those who investigate mammals. He emphasized that this is a misconception and that lower and higher vertebrate pigmentation systems are related primarily by homology rather than by analogy. He enumerated some of these homologies and several of these facets were discussed in more detail by subsequent speakers. Thus, Ken Mason next discussed molecular, genetic, and evolutionary aspects of the pigmentation of the Mexican axolotl, a salamander species for which many mutants are available. Comparative analysis of these mutants was discussed in relation to relevant mammalian mutants. Mason indicated that "the cladistic analysis of the TRP-1 cDNA from the axolotl along with members of the tyrosinase gene family from a number of species, clarified the evolutionary relationships of these molecules." The molecular biology of other axolotl pigmentary enzymes was discussed. Several shorter presentations related to the development of pigmentation were presented later. Thus, Mark Reedy disclosed that, in the chick embryo, later chromatoblasts emigrating from the neural crest follow a dorsolateral route and differentiate into melanocytes. He suggested that specification of these neural crest cells as melanoblasts occurred *in situ* and dictated their later migration and their route. Mark Moody then discussed his work on developmentally regulated expression of TRP-1 in the axolotl. The axolotl was also used by Susan Holder who discussed developmental regulation of xanthophore differentiation by region-specific influences. Perhaps, one such region-specific factor is melanization inhibiting factor (MIF) which appears to function much as does the product of the *agouti* gene implicated in several aspects of

mammalian pigmentation. Thus, J Newton presented his work on the cloning and molecular characterization of two genes, the *agouti* and *extension*, that are involved in the regulation of pheomelanogenesis in breeds of domestic dogs. The Smyth line chicken (SL), long considered a model for autoimmune human vitiligo, was discussed by G.P. Sreekumur who presented a penetrating analysis of the genetic and molecular linkage of the Smyth line chicken system. For his presentation, he was later honored with the award for the most outstanding presentation by a graduate student. The functional aspects of chromatophores were reviewed by Mac Hadley who emphasized the role of MSH and its receptor MC1R. The physiology of chromatophores was further discussed by Ana Maria Castrucci who considered the roles of a variety of neural and hormonal agents in the normal regulation of chromatophore function in fishes. A remarkable means of chromatophore control was considered by Mark Rollag who discussed photo-transduction in *Xenopus* melanophores. He reported that such light-sensitive melanophores contain a unique opsin that represents a class of opsins that became distinct at about the time that vertebrate and invertebrate opsins diverged during evolution.

MiniSymposium IV – Photobiology and Biophysics of Melanin and Melanocytes **Helene Z Hill**

This symposium was held on Tuesday, June 17th and was cochaired by Drs. Harold M Swartz and Helene Z Hill. Three of the papers dealt with effects of iron in one form or another on melanin. Jeffrey Tosk noted that the brain stem has one of the highest concentrations of ferritin in the body and this iron-rich substance may interact with catecholaminergic precursors to form melanin. This could account for the presence of melanin in this area of the brain. Dr. Eisner is also interested in neuromelanins and the structure around the Fe-site. Natural and synthetic neuromelanins were compared and showed small but significant differences. Dr. Jacobson's talk focused on the role of extracellular melanin of pathogenic yeast as a redox buffer the activity of which is enhanced by Fe^{++}. Iron may provide the reducing equivalents needed in order for melanin to neutralize pro-oxidants generated by the immune system, thereby enhancing the pathogenesis of the yeast.

Determining the routes taken by various precursors to synthesize the various melanins is key to our understanding of the balance of pigments in the final products and ultimately to the understanding of pigment function. Dr. Ito showed in a most elegant fashion that phaeomelanin synthesis is favored in the presence of high cysteine and low melanogenic activity. Eumelanin will dominate when the opposite conditions exist. In the presence of TRP1, DHICA is oxidized to its corresponding quinone and this in turn will oxidize DHI resulting in a mixed polymer.

Understanding the nature of the radical spectra generated by melanins of various types and sources is key to understanding the myriad effects attributed to melanins. Mark Nilges, in his studies of the free radicals generated by melanins, cast doubt on the hypothesis that dehydrohydroxybenzothiozine is the source of the radical spectrum of phaeomelanin.

'Is melanin photoprotective, photosensitizing or neutral?' remains a question that has yet to be answered. The literature is rife with conflicting results which have only served to muddy the waters. Endpoints studied are survival, pyrimidine dimers, growth arrest, to mention a few. Yet it is to skin cancer and especially melanoma that we should direct our attention. In tissue culture studies, this boils down to studying cellular transformation and mutation. These endpoints are technically difficult to investigate. Ms. Kaur, a graduate student in Dr. Hill's lab, has developed an artificial melanocyte so-to-speak by transfecting a plasmid containing the tyrosinase gene into Chinese hamster ovary cells which are exquisitely sensitive to mutation. Her preliminary studies show that cells which express pigment are less sensitive to killing by ionizing radiation and UVA and more sensitive to UVC than plasmid controls lacking the gene. The responses of the pigmented cells were no different from the controls after exposure to UVB and FS20 radiation. The pigment in the cells was eumelanin by ESR analysis. These cells will be useful in future mutational analyses.

Meeting Report – Uveal Melanocytes

by Dan-Ning Hu

May 14th, 1997 Fort Lauderdale, FL

The Symposium "Uveal Melanocytes: What We Know and What We Need to Know" was held in the Fort Lauderdale Convention Center (Florida) on May 14th, 1997 during the Annual Meeting of The Association for Research in Vision and Ophthalmology. This meeting was organized and sponsored by the Ocular/Extracutaneous Pigmentation Expert Group of the International Federation of Pigment Cell Societies. Drs Dan-Ning Hu, Laszlo Bito and Steven A McCormick were the organizers of this meeting. This Symposium was composed of 2 sessions, which included 9 formal presentations and 8 organized discussions. More than 100 ophthalmologists and basic scientists from all over the world joined this meeting.

The first session "Physiology of Uveal Melanocytes (UM)", was chaired by Dr Laszlo Z Bito of Columbia University. Dr Dan-Ning Hu of the New York Eye & Ear Infirmary, presented a lecture on the "Regulation of growth and melanogenesis of UM". He showed that cultured adult human UM respond to various growth factors, hormones, neurotransmitters and inflammatory mediators. Stability of UM *in vivo* is controlled by a balance of these factors. Stimulators include bFGF, insulin, hydrocortisone, adrenergic agonists, and prostaglandins, while inhibitors included TGFβ, muscarine agonists and others, to maintain homeostasis. Dr Linda Smith-Thomas of the University of Sheffield reported on "Extracellular matrix, MSH and UM", worked performed by her and her colleague, Dr Sheila MacNeil. They found that many extracellular matrix proteins are capable of affecting morphology, proliferation and tyrosinase activity of UM *in vitro*. αMSH stimulated melanogenesis of cultured UM but not uveal melanoma. Dr John Stjernschantz of Pharmacia/Upjohn, Inc and Dr David F Woodward of Allergan, Inc presented "Prostaglandins and iris pigmentation" and "Pharmacology of prostanoids induced melanogenesis", respectively. A new glaucoma medication, latanoprost (a PGFα analog), causes iris pigmentation in approximately 15% of treated glaucoma patients (mainly in those with mixed-color irides). It does not stimulate the growth of cultured UM, nor does it increase the melanin content of UM from pure-color irides. However, tyrosinase RNA expression in UM from mixed-color irides is increased. Prostaglandins E1, E2, A2 and E2 receptor agonists stimulated growth and melanogenesis of UM *in vitro*.

The second session "Pathology of UM" was chaired by Dr Steven A McCormick of the New York Eye & Ear Infirmary. He reviewed various pathologic conditions associated with UM, especially uveal melanoma, the most common malignant tumor of the eye. Dr Anselm Kampik, Muenchen University, presented "Non-neoplastic proliferation of UM". He reported the retrocorneal pigmentation and pigmented pupillary membranes caused by proliferation of UM occurred mainly after injury. Dr Joan Roberts of Fordham University presented "Effect of melatonin and free radicals on uveal melanoma cells", demonstrating that melatonin at physiologic concentrations inhibited growth of cultured uveal melanoma cells but not normal UM. Dr Gonzalo Blanco, University of Valladolid (Spain), presented "Rabbit model of uveal melanoma". He developed an experimental model of uveal melanoma from human uveal melanoma cells in immunosuppressed rabbits.

In discussions of physiology of UM, Dr Ulrich Schraermeyer (Germany) discussed the effect of strong light on the melanin of UM. Dr Rudolph D Glickman (USA) discussed melanin as a photosensitizer. In discussion of pathology of UM, Dr Arun D Singh (USA) discussed the risk of developing uveal melanoma from oculardermal melanocytosis. Drs Ian Cree (UK), Martin J Jager (Netherlands), Peter W Chen (USA), Theresa R Kramer (USA) and Mouriaux Frederic (France) discussed the proliferation of UM in Tpras transgenic mice, expression of various oncogenes and modulation of HLA expression of uveal melanoma.

IPCC Proceedings

A limited number of bound volumes featuring the published Proceedings of the ***1996 International Pigment Cell Conference*** held in Anaheim last October will be available for an outstanding price (~$45-50 each). Anyone who is interested in obtaining one of these volumes should immediately contact Dr. Frank Meyskens to place an order, at: Dr. Frank L Meyskens, Director, UCI Clinical Cancer Center, Division of Hematology/Oncology, University of California Irvine Medical Center, 101 The City Drive, Orange, CA 92668 USA; Tel: +1 (714) 456-5039; Email: flmeyske@uci.edu

Members in the News

Joseph Bagnara – received the 1997 PASPCR Career Achievement Award at the PASPCR Meeting

Vincent Hearing – presented the Gelb Lecture at the PASPCR Meeting

Aaron Lerner – received special recognition at the Providence PASPCR Meeting and was presented with the Chairman'es Award.

Young Investigator Awards presented at the PASPCR Meeting in Providence –

G.P. Sreekumar: (Graduate Student): "Genetic and molecular linkage analysis of the Smyth line chicken model for autoimmune human vitiligo."

Mark R. Miglarese (Postdoctoral): "Regulation of FGF-2 expression in SK-Mel-2 melanoma cells by the c-MYB proto-oncoprotein."

William Pavan (Young Faculty): "Genetic Regulation of melanocyte patterning."

Pigment Cell Research Wants YOU!

by James J Nordlund

THE PRICE OF THE JOURNAL *PIGMENT CELL RESEARCH* COMES TUMBLING DOWN

Hope that the stock markets don't crash like the cost of our pigment journal. Your subscription to ***Pigment Cell Research*** will be about 45% less than in previous years. The real price was over $200 but most were getting it at discount for about $160. At the recent meeting in Anaheim, Munskgaard agreed to revise its pricing schedule for the journal ***Pigment Cell Research*** for Society members only. For an annual fee of $95 total you can have your own copy of ***Pigment Cell Research***, the official journal of the International Federation of Pigment Cell Societies.

You get a lot for this small price. For example, you will get the bimonthly issues of the latest and best of pigment biology. In addition you will get the proceedings of the meeting of the European Society for Pigment Cell Research to be held in France later this year. You will get the proceedings of the upcoming PASPCR meeting in Providence, RI under the chairmanship of Walt Quevedo. You will get the proceedings of the XVIth meeting including the abstracts and the published manuscripts.

We need everyone's support. We need to get most of the members subscribing to keep this journal flourishing. Joe Bagnara did a yeoman's job getting it up and started and Dr. Jiro Matsumoto is doing a great job making the issues bigger and better.

An application for the journal will be included with your dues statement for 1997.

SUPPORT YOUR JOURNAL AND SOCIETY. SUBSCRIBE TO THE JOURNAL WHEN YOU RENEW YOUR MEMBERSHIP TO PASPCR.

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 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

Predoctoral and Postdoctoral Positions - available for molecular biologists in the areas of drug discovery and metabolism research. Requires experience in gene cloning, DNA sequencing, recombinant protein expression and cell culture methods. Prior experience in dermatology research is desirable. Southern Research Institute is a diversified research and development organization. Our Life Sciences Division provides comprehensive preclinical drug development and testing capabilities as well as basic research in drug design and synthesis, pharmaceutical formulations, toxicology, virology, microbiology, and pharmacology. To apply, send resume or curriculum vitae to: Southern Research Institute, Attention: Suzann Allen, Human Resources, Department 118, P.O. Box 55305, Birmingham, AL, 35255-5305.

Faculty Position - Massachusetts General Hospital, Harvard Medical School, Cutaneous Biology Research Center. The Cutaneous Biology Research Center (CBRC) seeks a molecular, cellular or developmental biologist to establish a program in fundamental research relevant to skin pigmentation. Areas of research can include but are not limited to pigment synthesis and transfer in melanocytes, genetics of mouse coat color and development/migration of neural crest cells. Applicants must have a Ph.D. and/or M.D. degree and relevant postdoctoral experience. Only applicants with a strong research record and the potential to develop extramurally supported research programs will be considered. Individuals with a demonstrated ability to develop imaginative approaches to important biological questions are particularly encouraged to apply. Rank/salary/start-up funds and space are negotiable depending on experience and qualifications. The CBRC occupies 45,000 square feet of fully equipped laboratory space in a new multidisciplinary research facility. Interested individuals should send curriculum vitae, reprints, a statement of research and future directions, along with the names, addresses and telephone numbers of three references to: Dr. Paul F. Goetinck, Chair, Faculty Search Committee, Cutaneous Biology Research Center, Massachusetts General Hospital - East, Building 149, 13th Street, Charlestown, MA 02129

INTERPIG DataBase

by Vincent Hearing

The INTERPIG database is on the InterNet! You can now access the InterPig DataBase at the following address: **http://lenti.med.umn.edu/paspcr/interpig.html**. Please note that as of this time, I estimate that less than 5% of the various IFPCS members have contributed entries. Think of how useful and complete this list would be if everyone took the time to supply their own information. Please take a moment to fill out the database data entry form (either online through the Web page or via Email) and send it back to Dr. Hearing. Please contact Vince Hearing or Bill Oetting if you need more information about these mechanisms of submission.

Check out the New Table on Pigment Genes & Associated Diseases ! ! !

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The Bibliography published in this issue covers the period May, 1997 through July, 1997. 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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