PANAMERICAN SOCIETY for PIGMENT CELL RESEARCH

PASPCR Newsletter

Volume 6 Number 1 **March,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; 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 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 URL address for the home page is: **http://lenti.med.umn.edu/paspcr**. The PASPCR site 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 Pigment Cell Conference in Nagoya, 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 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 page*** and send any comments and/or suggestions to 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 :

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)

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)

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)

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** . . .

Vaishali A. Chaubal

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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Ortho Pharmaceutical Corp	Shiseido Co, Ltd	

Call for Applications – 1998 IFPCS Travel Awards

by James J Nordlund / Raymond Boissy

The IFPCS has again offered each of the regional Pigment Cell Societies an IFPCS Travel Award to promote international collaborations. Details of this program can be found on the IFPCS Web Site, which can be readily accessed from the PASPCR home page. The deadline for 1998 applications will be May 1st, 1998 and decisions will be announced by the end of that month. Again, applications will be competitive and can be up to 1 page in length and should note the laboratory to be visited, the project or collaboration to be performed, estimate the expenses involved (Travel Stipends can be for up to $3,000) and the time frame of the proposed travel. Applications should be submitted to the office of Dr. James J. Nordlund, Secretary-Treasurer of the PASPCR.

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).

Members in the News

Murray Brilliant, moved from the Fox Chase Cancer Center to the University of Arizona.

Jim Nordlund, is currently on sabbatical leave in Tanzania for 1998. Ray Boissy is taking over the office of PASPCR Secretary/Treasurer for the year while Jim is away. You can write to Jim at: RDTC at KCMC, P.O. Box 8332, Moshi, Tanzania; FAX: +255 55 50330.

Susan Kidson, received a promotion to the rank of Associate Professor in the Faculty of Medicine at the University of Cape Town.

Pigment Cell Research Wants YOU! by James J Nordlund

Hope that the stock markets don't crash like the cost of our pigment journal; your subscription to ***Pigment Cell Research*** continues to be about 45% less than in previous years. The list subscription price is over $200 but Munskgaard has agreed to continue its discounted pricing schedule for ***Pigment Cell Research*** for Society members only. For an annual fee of $95 you can now have your own copy of ***Pigment Cell Research***, our official journal. We need to get most of the members subscribing to keep this journal flourishing. An application for the journal will be included with your dues statement for 1998.

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 :

Research Associate Position. Successful candidate should have experience with and interest in mouse genetics. Position includes organizational responsibilities with opportunity also to undertake own research projects so long as they relate to the mouse genetics. For further information please contact Lynn Lamoreux at: 409-845-6084 (leave message) or LLamoreux@cvm.tamu.edu

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 - position with the pigmentation team at the J&J Skin Research Center. The Skin Research Center is located in Skillman, NJ, and is responsible for research leading to new prescription drugs and consumer skin products. Current research involves the understanding of keratinocyte-melanocyte interactions and their regulatory role in melanogenesis. A novel, receptor-mediated pathway that affects melanogenesis has been identified, which is dependent on keratinocyte-melanocyte contact. We are looking for a molecular and cellular biologist to investigate this pathway. The candidate will be challenged to develop model systems, to study molecules involved in the regulation of melanogenesis, and to investigate keratinocyte-melanocyte interactions and their role in pigmentation. The position requires a PhD in biological science, preferably in molecular and cellular biology. Skills in the area of skin biology and pigmentation are preferred, but not required. If you, or someone you know, might be interested this position, please discuss this opportunity with them. Please contact Dr. Miri Seiberg for additional details of the position at: Johnson & Johnson, 199 Grandview Road, Skillman, NJ 08558-9418; Phone: 908-874-2325; Fax 908-874-2323; Email: MSEIBER@CPIUS.JNJ.COM. J&J provides a competitive salary and comprehensive benefits. We are an equal opportunity employer and support diversity in the workplace. This program provides basic and industrial research experience to the individual, whether they plan to pursue academic or industrial careers.

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

Meeting Report - 7th ESPCR Meeting, Bordeaux, France Oct 9 - 11, 1997 by Sheila Mac Neil

This report has been compiled with input from several colleagues who kindly shared their highlights from this meeting - Dorothy Bennett, David Gawkrodger, Marco d'Ischia, Mauro Picardo and Tony Thody.

For those of you who may not have attended a European Pigment Cell Research meeting before, there are perhaps a few facts I should tell you - you will have a **good time socially** (and the food and drink this year were chosen with particular care - Tony Thody wishes congratulations to be passed on to Jean-Etienne Surleve-Bazeille and Alain Taieb on the excellence of both) - however, you will also need **stamina** and to keep your wits about you if you are to do justice to a 2½ day meeting which typically includes presentations from 6 guest lecturers, 55 oral communications and 72 posters on a range of topics which span the biochemistry of the melanins, developmental and cellular biology of the melanocytes, their genetics through to their behaviour in vitiligo and melanoma. There are no simultaneous sessions at these meetings and it is tough being a member of the audience at a European Society for Pigment Cell Research meeting.

So, is it worth listening to talks in areas outside of your own field or speciality? The answer is a clear "yes". If you are willing to listen and learn, the chances of **setting up collaborations** to extend and broaden your research are excellent. Many scientific friendships have been forged at these meetings and continue to flourish. A lot of the presentations at the meeting were visible fruit of such collaborations. Not that I am biased, of course, but as the new Secretary for the ESPCR, I would say that this meeting (more than any other that I know of) has just the right chemistry (Professor Prota) to set up enjoyable collaborations - the meetings are the right size - they contain a number of disciplines all focussed on the melanocyte and you can be pretty sure of meeting up with your colleagues on an annual basis. If you have never attended one of these meetings before, don't be put off - we are a very easy society to join, very friendly, newcomers are made very welcome and guest lecturers are chosen with infinite care to provide topical overviews of particular areas of melanocyte biology. That was certainly the case at this meeting where the quality of the guest lectures was excellent throughout. So, enough advertising - here are some of the highlights from this meeting.

GUEST LECTURES

Particularly commended by many colleagues was the guest lecture by **Jonathan Rees** on genetic approaches to melanoma susceptibility in which he reviewed recent work from Newcastle and other Centres on mutations affecting the melanocortin-1 receptor gene. "There are now known to be a considerable number of mutations of this receptor and Professor Rees' group is leading the research into looking at the possible relationships between gene variants and inheritance of red hair and between gene variants and the likelihood of developing melanoma. This work is likely to open new vistas not only on the origin of skin tumours but especially on the complex mechanisms affecting skin type and hair colour - these studies favour a gradual shift of interest from the eumelanin to the phaeomelanin pathway as the key to the understanding of melanoma susceptibility - it will be interesting to see how these results will be integrated into the current knowledge of the biochemistry of phaeomelanin pigmentation" - Marco d'Ischia.

Other excellent guest lectures were from **Seth Orlow** on the comparative genetics of oculo-cutaneous pigmentation (detailing the different stages at which differences in genetics could have an

effect on pigmentation encompassing melanocyte migration, melanosome biogenesis and melanosome trafficking and transfer) and from **Barbara Gilchrest** who addressed the issue of how melanocytes *in vivo* are undoubtedly under the control of factors produced by adjacent cells which will themselves change in response to UV. **Professor Gualde** also reviewed the very wide array of approaches to inducing immunogenicity in melanoma that are currently under investigation in a very clear presentation.

Another excellent guest lecture was from **Nicole Le Douarin** on factors controlling the development of the melanocytic lineage from the neural crest. This was closely followed by a presentation from Laure Lecoins from Professor Le Douarin's laboratory on a novel sequence for an avian endothelin receptor which may help in understanding the evolution of function of these receptors (Dorothy Bennett).

AND NOW TO SOME OF OUR FAVOURITE THINGS ...

With respect to **interactions between α-MSH and cytokines**, things are looking interesting - on one hand, we learned that inhibition of melanogenesis by TNF-α is mediated through a downregulation of the tyrosinase promoter activity (Englaro et al.) - on the other hand, we learned that α-MSH itself can oppose the action of TNF-α in melanocytes (Hedley & Mac Neil) and melanoma cells (Morandini & Ghanem). The implications of α-MSH opposing the response of melanocytes and melanoma cells to cytokines are potentially quite exciting - time and further work will tell.

α-MSH was also found to increase production of **nitric oxide** in melanocytes and to potentiate UV-induced nitric oxide production (Alison Graham and Tony Thody) - whether this will relate to any melanogenic actions of α-MSH or other actions of this busy little hormone, time will tell.

How the **agouti protein** fits into things is slowly emerging. Vincent Hearing showed that agouti protein is capable of upregulating some and downregulating other genes. "These genes are likely to be important in control of eumelanin and phaeomelanin synthesis and determining hair colour and may provide further key information on the biochemical pathways responsible - possibly some important missing pieces of our jigsaw" - Dorothy Bennett. Dorothy also singled out a talk from **Fritz Anders** on a new class of **oncodeterminants** as the most important talk of the meeting - Dorothy explains - "the Xiphorus fish work produced the first oncogene and the first tumour suppressor gene and mammals followed fish on both these occasions. In his talk, Dr. Anders presented evidence of retrotransposons as heritable and amplifiable tumour-suppressor-suppressors. He also pointed out that all of these sequences are found in telomeres and one repeat sequence that he gave in detail was crammed full with methylation sites (of CG). Methylated DNA goes heterochromatic and can silence neighbouring genes by spread of this state - apparently a well known phenomenon". Once again, these little fish in Dr. Anders' capable hands may be pointing the way.

I am indebted to Marco d'Ischia for highlights of the session on the **biophysics and biochemistry of melanins**. "The effect of thiol compounds on melanogenesis was the central theme of two contributions by Smit, Pavel et al. and Potterf, Benathan, Hearing and co-workers. While the first paper focussed on the effects of varying concentrations of tyrosine and cysteine on melanogenesis highlighting a role of melanin production on glutathione depletion in melanocytes cultured with high tyrosine and low cysteine concentrations, the second paper addressed the relationship between agouti signal protein, cysteine transport and uptake in melanosomes and cysteinyl dopa formation to reinforce the notion that it is cysteine and not glutathione that is the actual ultimate precursor of cysteinyldopas. In another partially related paper, Dr. Benathan went on to show that tyrosinase could play a protective role against UV irradiation by catalysing the formation of cysteinyldopa conjugates".

Dr. Mars and Professor Larsson used a microautoradiographic technique to conclude that persons with a high content of phaeomelanin in their skin have toxicological risks of melanin-related adverse effects compared to those of dark skinned people.

Similarities and differences in the process of pigment formation between cutaneous melanocytes and melanogenesis in the ink gland of Sepia Officinalis were presented by Dr. Palumbo. Analysis of the nature of melanins is improving - Wakamatsu Ito and co-workers have used an improved version of a spectrophotometric method for melanin analysis combined with gel filtration HPLC to evaluate the DHICA content of eumelanins and their molecular size.

Also, in what must be the biggest ever group of related posters - 4 related poster presentations by Riley, Land, Pavel, Smit and co-workers on the mechanism of tyrosinase activation and the synthesis and properties of novel indoliumolate derivatives - these workers concluded that the actual product of the

action of tyrosinase on tyrosine is dopaquinone and not dopa which is formed only by a reduction of dopaquinone by leucodopachrome. Other significant contributions dealt with the structural modifications of synthetic eumelanins under aerobic conditions by Prota, d'Ischia and co-workers, the characterisation of melanins from tetrahydroisoquinolines by Rosei, Mosca and colleagues and a MALDI/MS study of oligomers of 5-hydroxytryptimine by Allegri, Traldi et al.

Putting the melanocyte back where it belongs ...

A number of groups are now looking at the behaviour of the melanocyte in *in vitro* models of reconstructed skin. Clearly leading this field is work from Professor Taieb's laboratory in which a number of basic and fundamental parameters about the behaviour of melanocytes from different skin types and from vitiligo skin have been established. These models are obviously going to prove very useful for studies of pigmentation research and may yield a few surprises - a poster by Hedley et al. showed that fibroblasts added to such an epidermal/dermal reconstituted skin tended to reduce spontaneous pigmentation of these composites. To date, the majority of composites have examined melanocyte/keratinocyte interaction in either an artificial collagen matrix or on acellular human dermis. Such composites are now also being used to investigate invasion of melanoma cells and should be a particularly useful tool for studying melanoma cells/ECM interactions (Bizik et al.).

Do melanocytes differ from melanoma cells in their ability to cope with oxidative stress?

Further evidence supporting this hypothesis was presented in a poster by Chau, Meyskens and Buckmeier showing essentially that melanoma cells cope less well than melanocytes with reactive oxygen species suggesting impairment of redox regulation in melanoma. Following on from this, this same group (Meyskens, Buckmeier and Tohidian) showed that the transcription factor NFκB which is activated by a variety of stimuli including inflammatory cytokines and reactive oxygen species appear to be differently regulated in melanoma cells compared to melanocytes. Differences in expression of rel family members between normal human melanocytes and metastatic melanoma were noted which may go some way to explain the differing abilities of the cells to cope with reactive oxygen species.

POSTER AWARDS

A first prize of 2,000FF was awarded to N.P.M. Smit, S. Pavel, C.J. Cooksey, C.A. Ramsden, P.A. Riley for a poster entitled, "The mechanism of tyrosinase auto-activation. Part IV: Methylation of indoluminolates by COMT". (This was the fourth part of a blockbuster poster presentation which represented combined work from scientists in Leiden, London, Manchester and Keel. The breadth of the work accomplished in these four posters was very evident).

Two joint second prizes of 1500FF were awarded to Y. Gauthier, F. Nagy, M.F. Harmand, A. Taieb for a poster entitled, "Transplantation of cultured melanocytes in piebaldism" and to S.A. Gordan, M.A.F. MacKenzie, P.S. Budd and I.J. Jackson for a poster entitled, "Melanoblast proliferation in W,PhRw mutants".

HONORARY ESPCR MEMBERSHIP

The Society took great pleasure in making two Honorary Lifetime (ESPCR) Membership Awards to Professor Hans Rorsman and to Professor Fritz Anders. The awards were made by Professor Patrick Riley with the unanimous approval of the ESPCR Council members - judging by the applause on the evening, it was clear that Professors Rorsman and Anders have earned the respect and affection of many many people.

In conclusion, apologies to everyone's work that I haven't mentioned - I hope I have given you a flavour of the meeting and to persuade you that you really cannot afford to miss the next one in Prague in September 1998.

Meeting Report - 12th JSPCR Meeting, Chiba, Japan Dec 6 - 7, 1997 by Jiro Matsumoto

This meeting was organized by Toho University with Professor Noriko Oshima (Department of Biomolecular Science, Faculty of Science) as chairperson. The science programs consisted of a presidential address, an invited lecture, 2 special lectures, a Symposium, 4 research seminars and 28 oral presentations, with a special emphasis on the symposium.

The symposium entitled with "Phylogenic aspects of tyrosinase genes" was organized with an expectation to provide up-to-date, unified concepts on the evolution of genes playing a central role in pigmentation. Four speakers reported their own recent works on tyrosinase or its associated genes deriving from different phyletic levels of organisms and their biological significance.

Melanogenesis and prokaryotes: Dr. Y. Nishimura reviewed recent findings on the wide distribution of homologues of eukaryotic tyrosinase in prokaryotes and pointed out that melanin-like pigments in bacteria are formed mostly by the tyrosinase catabolic pathway forming homogentisic acid as observed in human alkaptonuria. It was emphasized that bacteria with melanins or alike indicate a high survival rate against UV-irradiation, and that melanogenic Escherichia coli carrying plasmid tyrosinase genes from actinomycete (S. antibioticus) is available for screening of inhibitors for melanogenesis.

Insect pro-phenol oxidase and its activation mechanism: Dr. M. Ashida reported the homology of pro-phenol oxidase and hemocyanin present in the hemolymph of arthropods based on the similarity in the base sequence of their cloned cDNA, and presumed possible evolution of these two molecules from the common ancestral binuclear copper protein. He mentioned that dimeric phenol oxidase in insect hemolymph is activated by breakage of the peptide-bond at Arg (50)-Phe (51) under the presence of zymogen proteinase and then is released into tissues, and that quinones thus produced by activated phenol oxidase cause hardening of exoskeleton, attributing the protection against bacterial infections.

Expression of the ascidian tyrosinase gene in the developing brain: Dr. H. Yamamoto and his associate cloned tyrosinase gene from ascidian species, Halocynthia roretzi, and found that its open reading frame has 36 - 39% homology with its counterpart of vertebrate origin in terms of amino acid sequence. He also reported that this gene is expressed in the neural plate of early neurulae, even though the neural crest of this species shows no further differentiation, yielding only two sensory cells having different functions in the brain. Based on the presence of melanin granules, these two sensory cells were considered to be an ancestry type of pigment cells.

Albinism in fish: insertion of an Ac-like transportable element in the tyrosinase gene: Dr. H. Hori reported that albinism in the i4-mutant of Medaka fish is caused by insertion of a 4.6 kb sequence into the exon 5 of the tyrosinase gene, and that the inserts, Tol-2 in their designation, are homologous in its basic structure and mobility inside the genome to a maize transposable element, Ac. It was also shown that the gene for tyrosinase, TRP1 and TRP2 in this fish are similar in their basic structure to those of mammalian origin, but are different with regard to the size of their introns which are about 10 kb smaller than those of mammals.

MITF isoform multiplicity and a new aspect in melanin research: Dr. S. Shibahara reported that microphthalmia-associated transcription factor (MITF) plays an important role in cell type-specific transcription of the tyrosinase and the TRP-1 gene, and that MITF fails to transactivate the TRP-2 promoter, despite the presence of an MITF-binding site in the promoter of this gene. He pointed out that transgenic insertional mutation and/or mutation at the mi locus are closely associated with various dysfunctional phenotypes such as small non-pigmented eyes, a lack of melanocytes in the inner ear associated with deafness, a deficiency in mast cell and osteopetrosis, suggesting an intimate correlation of such disorders with the multiplicity of MITF isoforms.

Melanin monomer genesis investigations leading to therapeutic control of malignant melanoma and melanin pigmentation - molecular to clinical level : Dr. Y. Mishima reviewed a recent successful development of the selective boron neutron capture therapy by application of ^{10}B-p-boronphenylalanine (^{10}B-BPA) which had accentuated polymer forming ability within melanoma cells. He emphasized that 1) the accumulation of ^{10}B-BPA becomes possible by formation of complexes with DHICA and DHI which are abundant in these cells, 2) this therapy becomes applicable even to amelanotic melanoma upon transfection of the genes for tyrosinase or its related proteins, and 3) complex forming ability of BPA with DHICA and DHI *in vitro* led to the idea of using this compound as an inhibitor for melanogenesis in hyperpigmented human skin. At the end of this presentation, he emphasized the necessity of conceptual designs in the research.

Structural and enzymatic components of mammalian melanosomes: Dr. V. J. Hearing, an invited speaker, addressed current understanding of mammalian melanosomes with a particular emphasis on the role of two intercellular signaling molecules, melanocyte stimulating hormone (MSH) and agouti signal protein (ASP), which switch between eumelanin and pheomelanin synthesis. He indicated based on his recent findings that ASP elicits down-regulation in transcription of several pigment specific loci such as

albino, brown, slaty, silver and pink eyed-dilution, all of which encode proteins associated with catalytic and structural components of melanosomes. He set forth a view that melanosomes emerge as organelles common to lysosomes, which then differentiate into two distinguishable forms with spherical or elongated fibrillar phenotypes known as pheomelanosomes and eumelanosomes, and that selection of such differential courses of maturation can be defined by combination of specific proteins that are synthesized and deposited under the guidance of signaling molecules.

Signals and molecules regulating melanosome biosynthesis and transport of tyrosine-related protein: Dr. K. Jimbow reported his group's recent findings regarding the fate of tyrosinase-related protein (TRP-1) in the course of melanosome formation, with particular interest in biosynthesis and structural integration of this protein in the ER and Golgi and its transport via the trans Golgi network to the melanosomes under guidance of signal molecules. He pointed out that (1) calnexin plays an important role in folding of tyrosinase and TRP-1 in the ER, suggesting its correlation with melanosomal proteins, (2) newly synthesized TRP-1 is fully glycosylated in the trans Golgi network and then transported to late endosomes installed with the mannose-6-phosphate receptors, possibly by binding with its tyrosine-dileucine residues, (3) several small GTP-binding proteins present in melanosomes, particularly rab 7, are implicated in trapping of TRP-1, together with PI-3-kinase.

Pigment cells and gene transfer in Medaka: Dr. K. Ozato reviewed recent progress in gene-transfer studies using Medaka fish and reported his own success in production of transgenic Medaka carrying the gene for salmon melanin concentrating hormone (MCH) with CMV promoter. He addressed that the transgenes of salmon origin are expressed in a variety of tissues of homozygous progenies, indicating the presence of a detectable level of MCH in their serum and thereupon causing a distinguishable paler body coloration over 10 generations. He also reported his current success in establishing embryonic stem cell lines from Medaka embryos, suggesting the usefulness of the nuclear transplantation technique in production of chimeric animals.

At the research seminars, four topics were dealt with: the chemistry of melanins (Dr. K. Wakamatsu), the effects of ACF (stem cell factor) on the development of cultured neural crest cells into melanocytes (Dr. H. Ono), the role of c-KIT expression in melanoma (Dr. Y. Funasaka), and the expression of SCF and c-KIT in cutaneous mastocytosis (mastocytoma) (Dr. Y. Kubota).

In this seminar, Ono reported clear evidence indicating the necessity of stem cell factor for melanocyte differentiation which is considered to be inducible solely by endothelin-3, based on *in vitro* neural crest culture using homozygous sl mutant mice. Dr. Funasaka reported a marked decrease of c-kit expression in terms of mRNA levels in dysplastic nevus and malignant melanoma cells. She predicted, based on the inhibitory effects of SCF on the growth of melanoma cells, that apoptosis would be inducible by application of phosphorylation to c-kit proteins.

In the regular presentation, Dr. T. Hirobe reported that the differentiation-inducing potential of ACTH for murine melanocytes exists in the site corresponding to 1 to 13 amino acid residues, showing a higher activity with shortening of accessory residues and reaching to its maximal with the sequence similar to MSH.

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