Commentary: Unraveling the Melanosome

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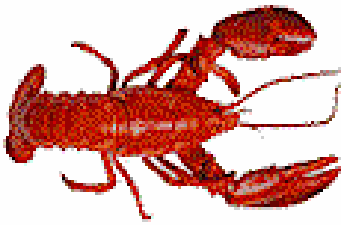
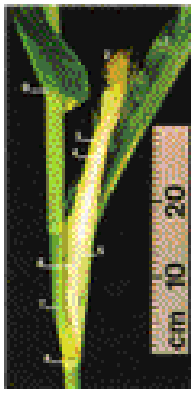
Introduction

The story underlying how I became involved in studying pigmentation, and then developed that into my scientific career is a convoluted one built on a large number of coincidences. As background, I was born into and grew up in a military family and with my 4 younger siblings, moved on the average of every 1.5 years until I was 18. We lived in France, Germany, Hawaii, Kansas, Alabama, Virginia (3 times) and Washington DC (twice). I suppose that explains why I've taken roots in the Washington DC area and haven't moved my home base since then. It also probably explains why I love to travel and visit other cultures/countries at every opportunity. To me, the chance to travel to scientific meetings around the world and collaborate with other scientists on a global basis is perhaps the most rewarding and surprising aspect of a scientific career.

Materials and Methods

To receive a B.S. degree in Biology at Georgetown University (at least back then) you needed to do a 1 year senior science research project. That was my introduction to the scientific method. At the time, among the faculty there, Dr. Sanford Vernick was an energetic young Professor who had a great sense of humor and who was willing to mentor me; he had only 1 drawback, i.e. he was an ichthyologist, and to work with him I had to take on a project in that field. He interested me in learning and then using electron microscopy (then a relatively new technique) to study the blood cells of lobsters; it turns out their blood is green because of the pigment hemocyanin and I also found that they were quite delicious once the blood had been collected each Friday at lunch-time. We managed to publish a scientific paper on that study which introduced me to the publication process and to the rewards and challenges there (1). Following graduation, I enrolled at The Catholic University of America in a Ph.D. program majoring in Cell & Molecular Biology (a field also growing rapidly back then). My mentor was Prof. John O'Brien, a celebrated botanist, but he also required me to perform my dissertation research in his field, which was an abrupt switch in direction. He interested me in studying the mechanism by which plants grown in the dark are unpigmented but then rapidly begin to produce the pigment chlorophyll and turn green when exposed to sunlight. Again, it was a fascinating study that involved pigment, albeit pigment in organelles (chloroplasts) but not yet of the mammalian variety (2). That final step arrived the following year when I met a gorgeous young Chemistry student and it became quite clear that I would need to improve every aspect of my dubious character to attract her and also that I needed a source of additional income to support us if I was successful in doing that. Back in those days, the stipend for teaching graduate students was about $3,000 (a year, not a month), but it was tax-free and one person, but not two, could survive on that. The National Institutes of Health was close by and the Dermatology Branch there was recruiting a part-time technician to run their EM facility. I applied for that position and was successful. The pet project of Dr. Marvin Lutzner, the Chief of Dermatology, was the Chediak-Higashi syndrome and he wanted me to do the ultrastructural analysis of the mouse model for that disease - the beige mouse. While we were at it, we decided that we might as well look at the other pigment mutants available from the NIH mouse colony, which included albino, pink, brown, dilute and a few other types of mice that have since become famous.



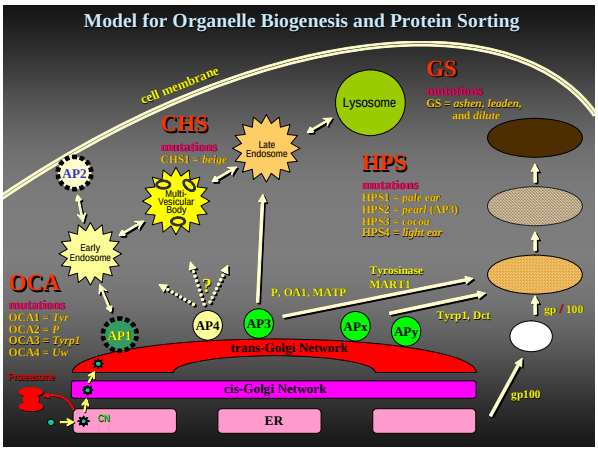


We even cross-bred many of those mutants to look at the effects of various combinations. So my days were spent during daylight hours studying plant pigmentation at Catholic University and much of the night studying mammalian pigmentation at NIH. Not only did both of those projects turn out successfully (3), but my pursuit of Betsy Brown was equally successful and we were married during my sophomore year. Although I had a few offers for post-doctoral studies, I decided to accept the one offered by the National Cancer Institute because I was now hopelessly entranced with the process of melanogenesis, and, given the tremendous resources at NIH, I felt that was THE place to be, and a foot in the door as a post-doc would be a good start. Of course, it worked out that way and within a few years I converted to a Staff Fellow here, and a few years after that was given tenure and became a Principal Investigator. Back in those days, we were each allowed to have one post-doctoral fellow and one clinical associate. Prof. Makoto Seiji contacted me to ask if I would accept Dr. Yasushi Tomita from his group as my first foreign post-doc; given Seiji's reputation in the field I was quick to agree and Tomita (who has now progressed to Prof. and Chair of Dermatology at Nagoya University) became my first post-doc. Seiji hosted the IPCC at that time and died soon thereafter, but fortunately, Prof. Yoshiaki Hori took over the role of sending me outstanding post-docs from his University, which included Koichiro Kameyama, Katsuhiko Tsukamoto, Kazunori Urabe and Minao Furumura. The restrictions of space in a small laboratory and having only one post-doc at a time was impeding progress in a time filled with explosive advances in techniques and understanding of regulatory processes at the subcellular level.

Just about that time, Marvin Lutzner decided to move on and the new Chief was Steve Katz, whose specialty was the immune functions of the skin, and melanocyte biology was no longer an emphasis of that group. Fortunately, Dr. Lloyd Law, who had discovered the combined chemotherapy approach to treating leukemia and was thus a celebrity at NCI, was very interested in studying the immune responses to melanoma (and why they aren't very successful). He recruited me to join the Laboratory of Cell Biology with the promise of more space (always a major problem at NIH) and better resource support. He did that (and more) to advance my career, really pushing me to design fail-safe experiments with all appropriate controls that ask the most critical questions. He piqued my interest in melanoma biology and I piqued his in the regulation of pigmentation, so we had an excellent collaborative spirit in our laboratories. With more space and resources, I was able to gradually expand my research group from 2 to its current level of 9; this allowed me to take outstanding post-docs not only from Japan, but also from Europe and other Asian countries as well as from the Americas. That list is very long now, but includes >60 names, and I am very proud that most of them are still actively involved in clinical or basic research.

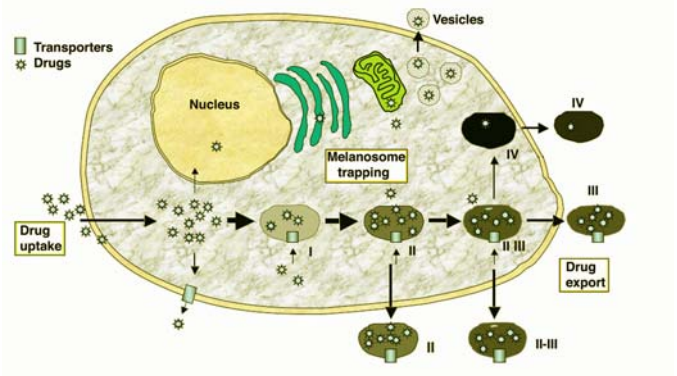
Results

The evolution of my research interests thus went from invertebrate pigmentation (hemocyanins) to plant pigmentation (chlorophyll) to mammalian pigmentation (melanins). As noted above, our studies on melanogenesis began with ultrastructural and enzymatic analysis of pigment cells in mammals and amphibians and, aside from the fascinating process of their maturation from premelanosomes to melanosomes (as first described by Seiji), the fact that active tyrosinase could be detected in the Golgi apparatus and in coated vesicles en route to melanosomes, but was not normally active in those organelles was just too much of a challenge to resist. Back then, only tyrosinase was known as an important melanosomal protein, but later the issue became even more complex as other enzymatic and structural components of melanosomes were discovered (Tyrp1, Dct, Pmel17, etc). One then had to consider whether they were trafficked together to melanosomes or were segregated in different vesicles and were only brought together at melanosomes (which might explain the delay in melanin synthesis). As we are all aware, tremendous strides have been made in defining all of this (and pigmentary diseases that result when the process goes awry) but not all questions have been resolved about the trafficking of



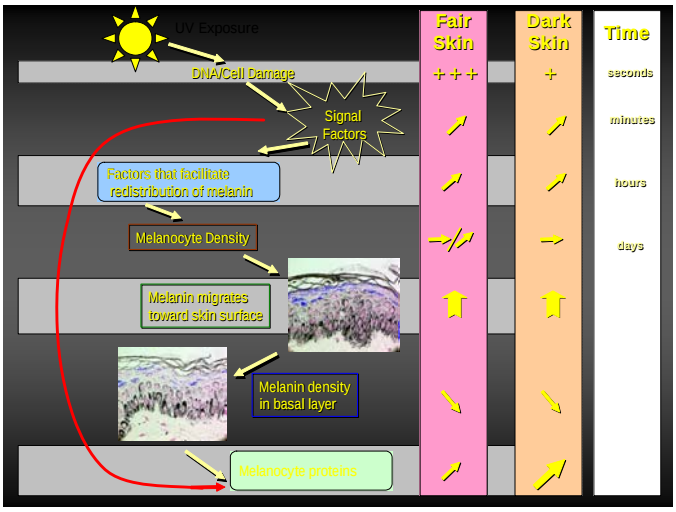
melanosomal proteins, or even about the functions of some melanocyte-specific proteins (e.g. P, MATP and OA1) and how they regulate that process. Proteomic analysis of different stages of melanosomes has revealed the incredible number of components required to make melanosomes and to push them through their maturation process (4;5). In sum, much remains to be done to fully characterize the melanosome and I don't foresee a time in my career when all interesting questions on this topic have been resolved and I'll need to look around for something else to work on.

When I transferred my research group to the Laboratory of Cell Biology, Dr. Law followed through on his promise to expand my space and resources, so in turn I expanded my research effort into studying melanoma immunology with him. We soon found that one could relatively easily immunize mice against subsequent challenge by tumor cells (either subcutaneous or intravenous to look at effects on primary tumor growth or 'metastasis', respectively). In fact, melanoma vaccines prepared from any one type of mouse melanoma (except S91) was able to protect against the others (6). The problem came when trying to treat existing tumor burden and, although we were able to get some prolonged survival of tumor-bearing mice using a number of approaches, none could be said to be effective in eradicating the tumors, something that is unfortunately also true in the clinic with human melanomas. Dr. Law retired about a decade later and his successor was Dr. Michael Gottesman, who had made his fortune by cloning and characterizing the first multi-drug transporter (MDR1), a pump that can effectively keep most chemotherapeutics out of tumor cells, thus allowing their survival. Even worse, expression of MDR1 is often stimulated by drug treatment so the most drug-resistant cells persist and form an even more intractable tumor. Interestingly, a large number of MDR family genes have now been cloned (>40) and one of them, ABCB5, is expressed only in melanocytic cells. Of particular interest, while most MDR proteins are on the cell surface, ABCB5 is localized intracellularly on the melanosome membrane (7). MDR proteins seem to have important functions in normal cells where expressed, which work to their disadvantage when they become transformed to malignant cells. We think that the normal function of ABCB5 may be to pump toxic intermediates (produced during melanin synthesis) to keep them within melanosomes and thus avoid toxicity to the cells (8). However, in melanoma cells, this pump would efficiently transport any drugs taken up into melanosomes, thus sequestering them and increasing the resistance of melanoma cells to chemotherapy (also a well known phenomenon in the clinic).



As the regulation of melanin biosynthesis was gradually being clarified at various levels (by ours and many other laboratories), we began to address a critical basic question about the role(s) of melanins/melanosomes in photoprotection of the skin. This had been assumed for many years based on a large number of in vitro studies, but the actual mechanisms involved and whether eu- or pheo-melanin was to be preferred was not known about human skin in situ. This is of course of critical interest to the NCI with respect to photocarcinogenesis. We had been working on this question using mouse models, but the architecture of mouse skin which is so distinct from human skin with respect to melanocyte (and melanin) distribution had made such studies far from appropriate for physiological understanding. When

we were unexpectedly approached by the FDA to see if we were interested in analyzing human skin specimens of varying racial/ethnic origin that were UV-irradiated in situ, it was the ultimate gift. We were able not only to look at DNA damage in human skin of varying phenotypes, but also to use those specimens to examine the basic physiology of melanocyte function in human skin. In recent years, we have published a number of studies that have provided important insights into the DNA damage that occurs in various types of cells in the skin, and which (in my view at least) provide interesting clues to the dramatic differences in incidence of all types of skin cancers in light as opposed to dark skin (9;10).



Discussion

Working at NIH has freed me from many time-consuming constraints, such as grant writing (yes, at NIH we have to write annual reports and we have Site Visits every 4 years, but I realize this is minimal effort compared to academia) and teaching (yes, we have some students and of course lab workers, but teaching lab techniques is a breeze compared with teaching courses). So while space has always been a critical issue here, budget and other resources have not been, although those have gradually eroded in recent years to become challenging here as well. Since I didn't have to spend excess time in those duties, I decided that I should make every effort to use the time saved towards promoting research in the field. This has taken the form of holding a large number of political offices, developing and distributing critical reagents, serving as editor on a number of books and our society's journal, and organizing meetings to facilitate interactions in the field. Beginning near the end of the -80's, this has turned into an almost comical sequence of events that has just now concluded:

- 1989 - Organizer, 2nd PASPCR Meeting - the formation of the PASPCR (led by Dick King and Jim Nordlund) in 1987 led to its first meeting in Minneapolis in 1988, but where would the 2nd more formal meeting occur? Thanks to my supportive boss, who helped ensure that NCI would underwrite most of the costs of that meeting, we could rapidly organize that meeting in Bethesda.
- 1991 - 1993 - PASPCR Council and President - probably due to name recognition as Organizer of the PASPCR Meeting, I was elected to the Council and then as President.
- 1993 - 1996 - IFPCS Council and then President - by virtue of being the PASPCR President, I was automatically on the IFPCS Council, by virtue of being on the IFPCS Council when the rotation dictated that the IFPCS President come from the PASPCR - voila!
- 2000 - Editor, Pigment Cell Research - by virtue of retiring as IFPCS President and thus being available, at the time when the next Editor of PCR was to be selected from the PASPCR or ESPCR - voila!
- 2005 - Organizer, 19th IPCC - by virtue of retiring as Editor of PCR at the time when the IPCC was due to be hosted by the PASPCR - voila!

Not that there weren't duties and responsibilities, and financial requirements and support personnel required by all of those functions above, but there is no doubt that the resources of the NIH made that possible whereas had I not been at NIH, I would not have considered doing much, maybe all, of that.

It has been extremely rewarding to see that over the years, interest in pigment research at NIH has grown exponentially. When I first began working here, mine was the only basic research lab in this field

at NIH, but over the past 15-20 years, many Institutes and other local government agencies have become involved in pigment research, many of them studying development, differentiation, dermatology, photobiology, etc. The support of those other groups and our underlying focus group (the NIH Pigment Cell Interest group) made the recent IPCC a rousing success at every level and a relative breeze to organize and fund. It is my hope that interest in pigment research will continue to grow and that NIH will recruit and expand laboratories studying these various important topics. Given past success by pigment groups here and the important problems that remain, I think it is a safe bet to turn out that way.

My political and other outside duties now behind me, I am refocusing my efforts for the balance of my career to run my laboratory, to catch up on my writing and to help others get started. It seems a bit strange to have this extra time now but also shocking at how far behind in my scientific work I had gotten. I'm looking forward to at least trying to reach an equilibrium point before retiring from NIH.

Acknowledgements

Finally, there is no way to begin to adequately thank everyone for their support, but let me briefly try. First, my wife Betsy (who also grew up in a military family and thus shares my love of travel), who has a fantastic career of her own, for always putting that secondary to supporting and pushing me forward at all times. My children (Brian, Laura and David), who tolerated my odd sense of humor and dedication to work and somehow prospered despite that. My mentors, all through my schooling and career at NIH who gave me almost anything I asked for (or at least what I semi-deserved). Finally, my post-docs and collaborators over the years, who are now legion and who have enabled my scientific achievement every step of the way.

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