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The *golden* Mutation in Zebrafish
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1. How did you come to work with the zebrafish** golden **mutation?

I came to use the zebrafish *golden* mutation in my work in cancer genetics, specifically, as a tool for studying genomic instability. During medical and graduate school, I came to understand the importance of the mutator phenotype (elevated rate of spontaneous mutation) in cancer (Nowell 1976), which led me to postdoc in the lab of Larry Loeb. The mutator hypothesis of cancer states that a mutator phenotype is required to account for all the mutations needed to cause human cancer. Towards the end of my postdoc with Larry in 1991, I committed to testing this hypothesis in a vertebrate model system in which one could pursue a screen for somatic mutators. I decided that a pigment mutation would be a good genetic "light bulb" for mutation, and began searching for a vertebrate model system in which mutation would be represented by pale cells on a black background (for easy detection). In 1991, Gary Ostrander, a fish glycolipid biochemist visiting the lab, showed me George Streisinger's NCI monograph article demonstrating detection of somatic mutation in zebrafish in the mosaic eye assay (Streisinger, 1984). This assay uses the recessive pigment mutant, *golden*, which causes lighter pigmentation in the body melanophores and retinal pigmented epithelial (RPE) cells of 2- to 3-day old embryos. Streisinger showed that treatment of *golden* heterozygotes with chemical or physical mutagens cause a dose-responsive appearance of pale cells in the RPE, yielding mosaic eyes. This is the assay I was looking for – detection of somatic mutation in the context of an otherwise normal organism. Subsequent visits to the strikingly generous zebrafish biologists at the University of Oregon, including Charlene Walker, Steve Johnson, Monte Westerfield and others, confirmed the feasibility of using this phenotype for a genetic screen for somatic mutators. The mutant screen would hopefully identify new genes that play a role in determining mutation rates, thereby affecting the rate of evolution, whether associated with the generation of biological diversity or the evolution of cancer cells from normal cells in each cancer patient.

Interestingly, George Streisinger found the *golden* mutation in a pet store in Oregon in the early '70's. This is the first mutation studied in zebrafish, serving as one of the most important tools Streisinger used to develop methods of generating haploid and half-tetrad zebrafish (see the historic cover article in *Nature*- Streisinger 1981), mapping of distances of mutations from the

centromere using gynogenetic half-tetrads (Streisinger *et al*. 1986), and detecting mutation (Streisinger 1984).

I began my new career at Penn State in 1992 with the intent to set up this mutant screen. The screen, funded locally and then by the American Cancer Society and NSF, yielded its first mutants in 1996. As will soon be reported, one of those mutants causes a 10x increase in susceptibility to cancer in heterozygotes. In order to determine the mechanism for genomic instability in these genomic instability "*gin*" mutants, we needed to clone the *golden* gene. It seemed most likely that the gene would have been already discovered among the over 100 genes identified already in human and mouse mutants. Even though pigmentation was not my specialty, I was curious about the cellular basis of the decreased pigmentation in the *golden* mutants. As shown in our paper in *Science*, dissecting microscope examination showed lighter cells of the same pattern and number as in wild-type, eliminating developmental cell fate as the mechanism of change. Since I knew that pigment was contained in melanosomes, I wanted to know whether the mutation would affect the number, size or density of pigment of the melanosomes. To my surprise, the mutation affected all three of these features of melanosome morphogenesis - precisely the same features affected in light as compared with dark-skinned humans. This suggested to me that the mechanism affected by *golden*, if not the same gene, would be relevant to pigmentation in humans.

2. What do you know about the gene and its function so far?

As presented in our work, the *b1* allele of zebrafish *golden* is a likely null mutation (a nonsense mutation that truncates the protein to 40% of its normal length and causes mRNA degradation) and yet still allows melanosome formation. This suggests that the gene is likely to play a modulatory rather than essential role in melanosome morphogenesis. We know from the bioinformatics that the *golden* gene, also called *SLC24A5* or *NCKX5*, is part of a large superfamily of transmembrane proteins that includes sodium, calcium exchangers (NCX), and potassium-dependent sodium, calcium exchangers (NCKX), whose studied members reside in the plasma membrane. The amino sequence of this gene is sufficiently conserved through evolution that the human gene can restore pigmentation in melanophores (the fish counterpart of the melanocyte) of homozygous *golden* zebrafish. It should be noted that rescue is mosaic in zebrafish, caused by unequal distribution of injected RNA (we have not seen, as reported in newspapers, rescue of stripes in adults, though it is theoretically possible in a transgenic). The mutation that represents the predominant allele in Europeans changes one amino acid from the ancestral (the one preserved in all vertebrates sequenced, including Africans and Asians). This change presumably diminishes its function, since the zebrafish nonsense mutation also causes lighter skin. We know from analysis of GFP and HA-tagged protein that slc24a5 is associated with an intracellular membrane compartment, presumably the melanosome and/or a precursor.

3. What are the implications of the discovery for future research?

There presently exists no direct evidence of SLC24A5's cation exchanger activity. We anticipate study of its activity to be challenging due to its organellar localization. A simplified model of SLC24A5 function presented in the paper (developed largely by Nancy Mangini and Victor Canfield) includes three proteins, but we recognize that a full understanding of the effect of these

ion gradients on melanosome morphogenesis will require identification of the relative roles of other melanosome proteins. Furthermore, the expression of this protein in multiple tissues, perhaps in lysosomal structures, might be expected to affect any physiological process involving cation exchange and/or lysosomes. The extreme difference in population distribution of this gene suggests that we should be on the lookout for potential impacts on physiology, disease susceptibility and drug efficacy.

The analysis of eye color in humans would seem likely to benefit from knowledge of *SLC24A5* genotype. While the *Thr111* allele is predominant in Europeans, the rare presence of the ancestral *Ala111* allele can be expected to affect eye and hair color in Europeans. It will be interesting to determine whether heterozygosity at this locus will be associated with brown hair and eye color, and whether homozygosity for the derived allele is required for lighter eye and hair colors.

Our genomic analysis shows that *SLC24A5* is uninvolved in the overall lighter skin color in Northeastern Asian populations such as Han Chinese or Japanese. The intermediate melanosomal phenotype of East Asians suggests the possibility that whatever gene/s is/are affected in East Asians serves in the same or parallel genetic/signaling pathway affected by *SLC24A5*. It will be incredibly interesting to determine how many genes contribute to skin pigmentation through this same pathway. I expect that future work focusing on this new genetic pathway affecting melanosome morphogenesis will lead to a much greater understanding of pigmentation in vertebrates.

The most obvious medical implication of this work is that inheritance of the *Thr111* allele confers susceptibility to sun-induced skin cancers. While this makes the *SLC24A5* perhaps the most prevalent cancer-susceptibility gene in humans, the most important actions to take - encouraging the use of sunscreen, and discouraging the use of tanning salons in an attempt to increase pigmentation in light-skinned individuals - require no direct knowledge of the gene. It has yet to be determined whether or not knowledge of the role of this gene in pigmentation will facilitate more effective interventions. This gene does add to the toolbox of researchers wishing to use *SLC24A5* as a target for regulating skin color - hopefully safer than the UV light used in tanning salons or currently-used skin lightening creams. It also serves as one more potential target for immunotherapy against malignant melanoma. It is not known whether or not *SLC24A5* is any better or worse than any other of the many proteins associated with melanosomes, such as those being identified using proteomic approaches (Basrur *et al*. 2003).

As pointed out to me by Nancy Mangini, progression of the most common form of acquired blindness in the elderly, age-related macular degeneration (ARMD) is known to be associated with lighter skin and hair color (Frank *et al*. 2000). It will be of interest to determine not only the strength of the impact of SLC24A5 on ARMD but also the extent to which ARMD may be prevented or slowed by interventions targeting this pathway.

There may conceivably be affects of SLC24A5 on other organs. For example, dark-skinned soldiers appear to be more susceptible to frostbite than their lighter-skinned compatriots (Overfield 1995), which can be imagined to be the result of a direct physical effect via the cellular density of melanosomes or some unknown effect on the dermis or vasculature. The

incidence of Parkinson's disease is also higher in black than white populations, with Asians in between (Van Den Eeden *et al.* 2003). It remains to be determined what effect SLC24A5 allelism may play in the survival of neurons in the neuromelanin-containing substantia nigra.

It is worth noting the importance of interdisciplinary approaches. Tying the discovery of *SLC24A5* to its role in human pigmentation required a broad array of disciplines, including model system genetics, reverse genetics, bioinformatics, use of the HapMap database, and human population genetics. Interdisciplinary investigation is recognized and encouraged by NIH leadership in the form of the "roadmap," especially for research questions that require connection of mechanism to relevance to humans. Of particular importance is that the increasing use of SNP chip databases will commonly yield multiple candidate genes. Model systems will be an important tool for distinguishing key genes from tag-alongs. Furthermore, as a relatively approachable problem, the scientific process used to deepen our understanding of human pigmentation can serve as a model for the study of other human diseases.

4. What are the implications of the discovery for society?

Many groups, internationally, have paused to note that a simple one-base pair change out of three billion in our genome has contributed to some of the most terrible actions in the history of man. A reflection of interest in this realization is the peaking of the number of Google hits for *SLC24A5* at more than 28,000 from more than 60 countries in January 2006.

It has been interesting and challenging to tackle the impact of the discovery of the *Thr111* variant on the issue of race. Our traditional tact in the scientific community regarding race has been one of denial. I had been suggested to me by many of my well-meaning colleagues to instruct the public to deny the existence of race. I found myself uncomfortable with this denial. Everyone uses the word, and it is admittedly often used in ugly ways. Stephen Oppenheimer, who is known for his mtDNA-based out-of-Africa work, helped me to validate this discomfort, saying that self-censure can be worse than talking about difficult issues. More recently, I note the words of Unitarian Universalist minister Joshua Pawelek, who said that "…the denial of race will not work, for it leads to a denial of racism—and you can't address a problem if you don't think it exists."

While addressing the problem of racism was not the planned activity of my scientific pursuit, we agreed in a December 16, 2005 meeting of NHGRI's Race, Ethnicity and Genetics working group that society may best benefit from this discovery by using it to demystify the concept of race in two ways. The first is to challenge us to question the purpose of such discussion of race – it is aimed at a deeper understanding of race, or to assign value to differences? The former deepens understanding, while the latter antisocial purpose serves no greater purpose than an expression of primitive group instincts, well known in all primate species, to dominate others. As individual members of society, we can decide whether or not to consciously engage in conversation that is antisocial. The second demystification contributes to our understanding of genetics and environment, referred to in the lay press as nature and nurture. Race is a complex concept that, as commonly used, includes both biological and sociological components. The biological components, such as pigmentation, are genetic. Other components, such as language and nationality, are non-genetic. The most contentious issues, however, have to do with

intelligence, which have both genetic and non-genetic components. Scientifically unjustifiable use of *SLC24A5* by white supremacist groups within a week of publication of the paper establishes the importance of confronting this difficult issue, rather than avoiding it.

It is interesting to note that the discovery of the role of the *Thr111* polymorphism in the evolution of people of European ancestry from their African ancestors has already been used in school discussions of evolution (http://evolution.berkeley.edu/). We, as scientists, have an opportunity to facilitate active discussion of this issue, aimed at the acceleration of our society's evolution away from divisive debate, so that we can focus on scientific approaches to solving some of the truly important issues of the day – such as how to live with each other without destroying the very biosphere upon which our existence depends, and how to engage in international cooperation to solve global problems.

Finally, it is worthwhile noting that the spirit of cooperation that permeated much of the work contributed to its success. We are especially grateful to the embodiment of that cooperation in George Streisinger, his colleagues in the zebrafish community, and to the many unnamed individuals involved in a key component of this work–the HapMap project. The HapMap is a wonderful multinational model for how humanity can and should solve problems - a cause for celebration.

Keith

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