

Tyrosinase Activity in the Skin of Three Strains of Albino Gecko (*Eublepharis macularius*)

Fig. 1. Adult wild-type leopard gecko.

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Introduction

The color and pattern of reptile skin is produced by a combination of pigments and structural compounds. Three types of specialized skin cells, or chromatophores, namely melanophores, xanthophores (including erythrophores) and iridophores contain pigments. The first cell type, melanophores, is found in the dermis and epidermis. Melanophores produce the pigment melanin that is stored in organelles called melanosomes (Bechtel, 1995).

Melanin is responsible for black and brown colors and occasionally some yellow and red coloration. The second cell type, xanthophores (and erythrophores) are cells found predominantly in the dermis that produce a class of pigments called pteridines which are stored in pterinosomes, organelles similar to melanosomes. Pteridines are predominately red while carotenoids are predominantly yellow to orange. Xanthophores also store fat-soluble carotenoids which are obtained from an animals' food. Xanthophores and erythrophores are distinguished primarily by color, yellow and red respectively, which is ultimately determined by the proportion of carotenoids to pteridines in each cell (Cooper and Greenberg, 1992; Bechtel, 1995). The third variety of chromatophore, the iridophores, contains crystalized purines stacked on top of one another in organelles called reflecting platelets. Platelets are colorless but highly reflective and create different colors depending on how the purine crystals are stacked. Iridophores are found in the dermis and are responsible for iridescence and blue coloration in reptile skin (Bechtel, 1995).

Defects in the normal production of skin pigments, while rare, do occur. Perhaps the



Fig. 2. Adult Tremper albino leopard gecko.



Fig. 3. Adult Rainwater albino leopard gecko.



Fig. 4. Adult Bell albino leopard gecko.

most striking of these defects is albinism, the congenital failure to produce normal amounts of pigment. The inability to create normal amounts of pigment by melanophores is termed amelanism, in xanthophores it is called axanthism, and in erythrophores it is called anerythrim. Amelanism has been reported in many species of snake but relatively few lizards (Bechtel, 1995).

Because there are multiple varieties of chromatophore in reptile skin the failure to produce pigment in one cell type will typically not alter the ability of other chromatophores to function normally. The contrast of functioning pigment cells to unpigmented patches makes

many albino reptiles quite colorful, particularly when compared to albino mammals. Mammals possess only melanophores and familiar strains of albino mammals (e.g. lab mice and white rabbits) are white as albinos because there are no other pigment cells to produce color.

The leopard gecko (*Eublepharis macularius*) is a medium-sized lizard commercially bred in large numbers for the pet trade. In the last ten years, several color and pattern mutations have arisen that have been shown to be heritable. Three of these mutations, known as the Tremper albino, Rainwater albino, and Bell albino (named for the commercial breeders that popularized them) produce amelanistic phenotypes (Figures 2–4). Each of the mutations are descendants of normal colored, wild-caught leopard geckos imported from Pakistan in the early 1990's (R. Tremper, personal communication) and are not thought to be related to each other. Breeding experiments have shown that each of the three strains of albino leopard gecko is the result of a single, recessive gene (J. Aherns, unpublished data). Test-crosses have also shown that all three varieties are incompatible with each other. Crossing a Rainwater albino leopard gecko with a Tremper albino, for example, will produce all normal offspring. Available evidence indicates that each variety results from either mutations on different genes or different mutations, or alleles, of the same gene. Phenotypes of all three strains are characterized by a reduction or elimination of black pigment. Areas of skin that would be brown or black in wild-type leopard geckos are typically white or lavender and occasionally light brown in albinos.

The production of melanin is a process that takes several steps and is controlled by multiple genes (Figure 5). The first step in the melanin pathway is the transformation of the amino acid tyrosine to L-3,4-dihydroxyphenylalanine (dopa). Dopa is oxidized to dopaquinone, which is eventually converted to melanin. The first two steps in this process are catalyzed by the enzyme tyrosinase. All of these reactions

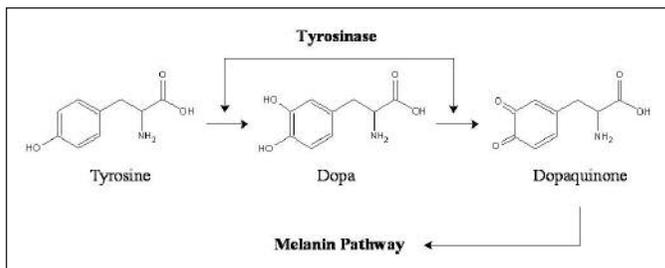


Fig. 5. Tyrosinase catalyzes the first two steps in the melanin pathway. The hydroxylation of the amino acid tyrosine to dopa (dihydroxyphenylalanine) and the subsequent oxidation of dopa to dopaquinone goes through a series of non-tyrosinase-mediated reactions to become the polymer, melanin.

take place in the melanosomes within melanophores (Bechtel, 1995).

The lack of the enzyme tyrosinase is a common cause of albinism in people and animals (Bechtel, 1995). However, not all albinos lack tyrosinase. Some albinos still possess tyrosinase, a mutation known as tyrosinase positive albinism. The inability to produce normal amounts of melanin in these individuals is the result, therefore, of some non-tyrosinase-dependent defect in the melanin pathway or a defect involving the transport of melanic precursors or products (Bechtel, 1995). Because tyrosinase positive albinos can still potentially produce some melanin and/or melanic precursors they are often darker in color than albinos lacking tyrosinase. Two species of reptile have been shown to possess both tyrosinase positive and tyrosinase negative forms of albinism: the black ratsnake (*Elaphe obsoleta*) and the San Diego gopher snake (*Pituophis catenifer*) (Bechtel et al., 1980; Bechtel and Bechtel, 1981; Bechtel and Bechtel, 1985). In both species, the tyrosinase positive albinos are more colorful and have a more defined pattern than the pale, tyrosinase negative albinos.

Tyrosinase activity can be detected in the skin using the dopa test, which involves incubating a piece of skin in a solution of dopa (Bechtel et al., 1980). A tyrosinase negative albino will show no reaction and melanocytes will remain unpigmented. A tyrosinase positive albino, on the other hand, will develop heavily pig-

mented melanocytes due to the creation of melanin produced from the tyrosinase-catalyzed reaction. The dopa test has been used to assay tyrosinase activity in the skin of albino snakes (Bechtel et al.,

1980; Bechtel and Bechtel, 1981; Bechtel and Bechtel, 1985). This paper describes the results of the dopa test for tyrosinase activity in three strains of albino leopard gecko and offers some comments on the mutations.

Materials and Methods

Skin samples were taken from four individual geckos: a wild-type leopard gecko, a Tremper albino, a Rainwater albino, and a Bell albino. The distal portion of each gecko's tail was removed and from this clipping, two 3-4 mm biopsies were cut out using a scalpel. The leopard geckos regenerated their lost tails and this procedure did not seem to cause undue stress to the animals. The tail samples were divided into two treatments: a reaction treatment and a control treatment. Reaction samples were subject to the dopa test as described below. The control samples followed the same protocol with the exception of the incubation in dopa solution.

All biopsies were fixed in 10% formalin for two hours at room temperature. The reaction biopsies were removed and incubated in a 0.01% buffered dopa solution. The dopa solution was made by dissolving 0.01 g of L-3,4-dihydroxyphenylalanine (dopa) into 100 ml of phosphate buffer at pH 7.4 (Shimizu et al., 1994). Biopsies were allowed to react, at room temperature, for 24 hours. This reaction time is three times longer than recommended by Bechtel et al. (1980) but normal (wild-type) gecko skin samples incubated for 8 hours failed to show any change after 8 hours. The control biopsies were incubated in phosphate buffer without dopa. All biopsies were returned to formalin after incubation pending histological examination.

Samples were embedded in paraffin, mounted, and cut with a microtome. Sections were mounted on glass slides and examined

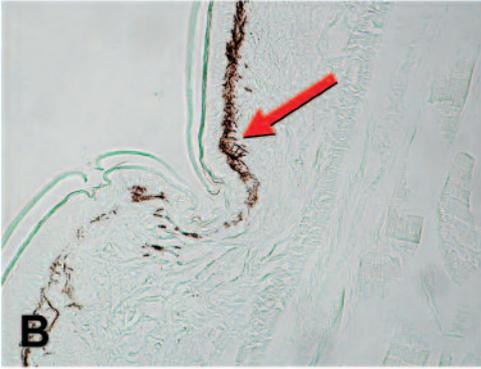
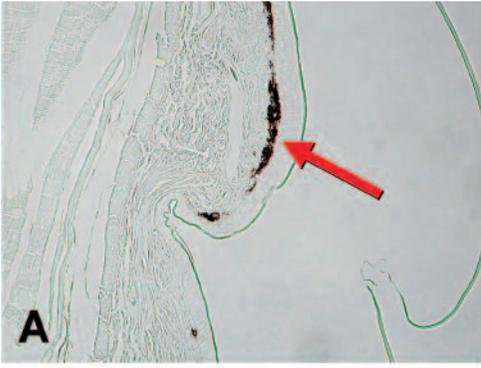


Fig. 6A - Section of tail from wild-type leopard gecko, control treatment. Arrow indicates area with large concentration of melanophores.

Fig. 6B - Section of tail from wild-type leopard gecko incubated in dopa. Arrow indicates area with large concentration of melanophores. Notice the increased visibility of the dendritic processes extending out from the melanophores.

under a light microscope. At least six sections were cut of each sample. Every section was examined and digital photos were taken of representative sections.

Results

Dopa treated melanophores in the wild-type gecko were unchanged in terms of color compared to the control treatment but the dendritic processes that extended out from the melanophores were much more visible in the dopa treatment (Figures 6A & 6B).

Melanophores were visible in control treatments from all three strains of albino gecko although the melanophores were appreciably lighter in color in the albino geckos than the

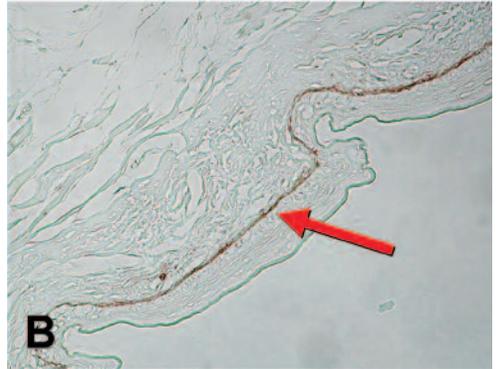
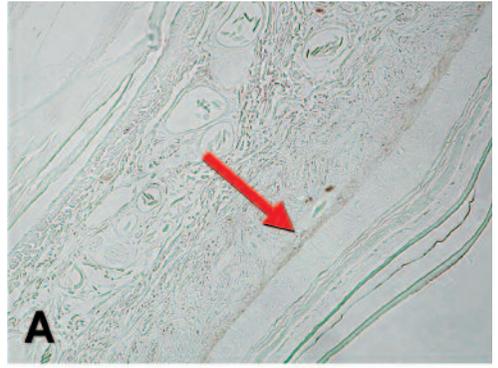


Fig. 7A - Section of tail from Tremper albino leopard gecko, control treatment. Arrow indicates area with large concentration of melanophores.

Fig. 7B - Section of tail from Tremper albino leopard gecko incubated in dopa. Arrow indicates area with large concentration of melanophores which were substantially darker than the control sample.

wild-type. Tremper albino melanophores darkened significantly after incubation in dopa because of the production of melanin which confirmed the presence of tyrosinase (Figures 7A & 7B). Rainwater albino melanophores, after incubation in dopa, were significantly darker than Rainwater control treatments confirming the presence of tyrosinase (Figures 8A & 8B). Bell albino melanophores, after incubation in dopa, were minimally darker when compared to the control treatment although melanophores in the Bell control treatment were much darker than either the Tremper or Rainwater control treatments to begin with, again indicating the presence of tyrosinase (Figures 9A & 9B).

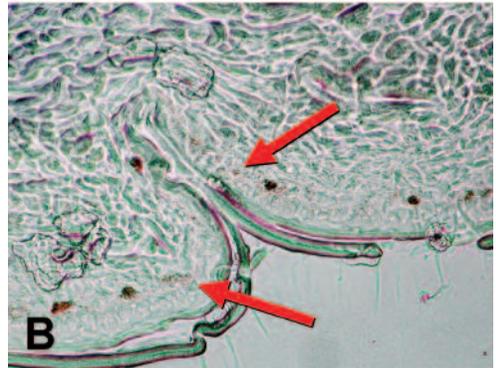
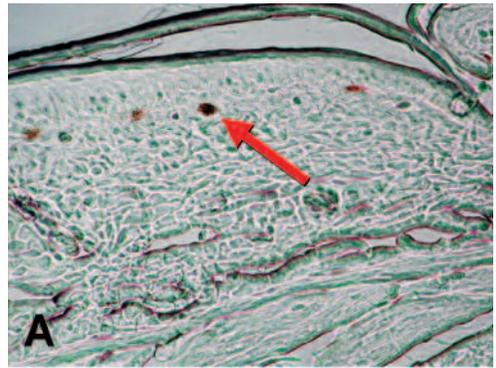
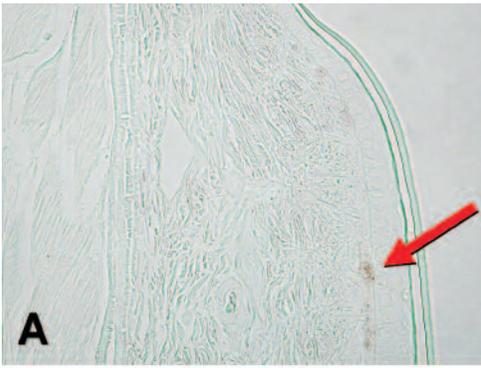


Fig. 8A - Section of tail from Rainwater albino leopard gecko, control treatment. Arrow indicates area with a concentration of melanophores.

Fig. 9A - Section of tail from Bell albino leopard gecko, control treatment. Arrow indicates a single melanophore.

Fig. 8B - Section of tail from Rainwater albino leopard gecko incubated in dopa. Arrow indicates area with large concentration of melanophores which were substantially darker than the control sample.

Fig. 9B - Section of tail from Bell albino leopard gecko incubated in dopa. Arrows indicate areas with a concentration of darkened melanophores in the control sample.

Discussion

All three forms of albino leopard gecko tested here were tyrosinase positive. Increases in the amount of melanin, which resulted in darker melanophores in the dopa treatments confirmed the presence of tyrosinase. The clear visibility of lightly pigmented melanophores in the albino control treatments also indicated the existence of at least some melanin prior to incubation in dopa and provided additional evidence that tyrosinase was present. Reduced melanin production was perhaps the result of tyrosinase that did not work very efficiently or cells that were unable to move melanic precursors or products efficiently into or within the cell.

Assessing the presence of tyrosinase is an important step in describing and differentiating the three strains of albino leopard gecko. While all three forms were tyrosinase positive that does not mean the three forms are identical. Non-allelic forms of albinism are relatively common. There are, for example, over 800 alleles at 127 loci (genes) that affect pigmentation in mice alone (Bennett and Lamoreux, 2003)! There are also numerous examples in reptiles including black ratsnakes (*Elaphe obsoleta*), western diamondback rattlesnakes (*Crotalus atrox*), and San Diego gophersnakes (*Pituophis catenifer*) (Bechtel, 1995). Non-allelic forms of albinism could result from mutations of different genes involved in melanin production or different mutations on the same gene.

Albinism has been well studied in humans and mice and existing research can provide information about the mechanism of albinism in other species. There may be some similarity between the tyrosinase positive albinism in leopard geckos, for example, and several human and murine forms of oculocutaneous albinism, an assortment of disorders that result in reduced or no melanin production. Oculocutaneous albinism 1 (OCA1) is caused by mutations of the tyrosinase gene. One variation of this mutation (OCA1B) is a “leaky” mutation, which produces tyrosinase enzyme with reduced efficiency, typically 1 – 10 % of normal tyrosinase (Oetting et al., 1996). Individuals with the OCA1B mutation can vary from being completely unpigmented to having some freckles and even the ability to get a light tan (Carden et al., 1998). Oculocutaneous albinism 2 (OCA2) has historically been referred to as “tyrosinase positive” albinism in humans because this mutation does not affect the tyrosinase gene but instead affects the gene that codes for P protein (Carden et al., 1998). The function of the P protein is unknown but it may be involved in the transport of tyrosine into the melanosomes (Rinchik et al., 1993). Oculocutaneous albinism 3 (OCA3) affects the gene for tyrosinase-related-protein 1 (TRP-1). TRP-1 stabilizes tyrosinase and other enzymes in the melanosome (Carden et al., 1998). Melanin is still produced by individuals with OCA3 but the melanin is always brown rather than black and the skin and hair are therefore much lighter in color.

Further work is needed to determine the exact mechanism of the three albino leopard gecko mutations discussed here. The next logical step would be to sequence likely candidate genes such as genes that code for tyrosinase, P protein, and tyrosinase-related-protein 1. The ability to find the affected locus and characterize the mutations at a molecular level would provide insight into the production of reptile melanin and, as a consequence, the behavioral and thermoregulatory behaviors associated with reptilian coloration.

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