Why is Science Making Albino Opossums?

By: Stephen Gergeni

Genetically modified or transgenic animals, are those whose DNA has been manipulated through genetic engineering, often involving the insertion of a gene from another species, for purposes like research, disease modeling, or enhancing desirable traits.[1,3] Many common animal species have been genetically engineered, including livestock animals and laboratory staples like mice and rabbits and even fish.[3] A famous example of genetic engineering is the creation of glow-in-the-dark mice. Researchers inserted a gene from jellyfish that produces green fluorescent protein, or a gene from fireflies that creates the luciferase enzyme, into the mouse's DNA. This causes some of the mice's cells to glow under UV light.[2] The goal is to turn the glowing genes on in specific types of cells so that researchers are able to follow any changes by easily differentiating the cells visually.[2]

When it comes to lab animals, opossums are a developmental biologist's dream. They have many unique characteristics that can be exploited by researchers, especially for those studying early mammalian development.[5,7]

Among marsupials—pouched mammals like kangaroos, koalas and Virginia opossums—the gray short-tailed opossum (*Monodelphis domestica*) stands out as one of very few established laboratory models.[7] When marsupials are born, they are strikingly underdeveloped, looking more like a fetus more than a fully formed newborn. With closed eyes and only their front limbs partially developed, they must make their way blindly up their mother's belly to reach a pouch filled with nipples, where they remain until they complete their final development.

Gray Short-tailed Opossum Adult. Photo Credit: Dawson

However, the gray short-tailed opossum deviates from the typical marsupial anatomy by lacking a true pouch ![9,10]

Gray short-tailed opossum females give birth to joeys after just 15 days of gestation. After climbing out of the womb, the joeys latch on to a nipple and finish developing externally. This unique form of external development offers scientists a rare glimpse of a process that, in all other mammals, is hidden from view.[7,9]

Despite this potential boon to developmental biology, marsupials lag far behind more common laboratory mammals in gene function research. The main challenge lies in the lack of reliable techniques for manipulating their genomes.[5,7] In typical non-marsupial laboratory mammals, like mice, scientists can easily access and modify their genomes just days after fertilization using tried and true methods.[7] In contrast, almost immediately following fertilization, marsupial embryos quickly form a thick protective covering, making manipulation of their genomic DNA far more difficult.

But scientists have finally figured out a way to overcome this obstacle. Thanks to the opossum's similarities in size and reproductive traits to common rodent models like mice and rats, scientists leveraged that knowledge to revise methods for tracking when opossum eggs are fertilized, which

allowed them to safely pierce the thicker embryonic membranes. This makes it possible to deliver or remove DNA to early opossum embryos for genetic studies.[6, 7]

One powerful tool scientists use to manipulate an organism's genome is CRISPR/Cas9. This method works by "snipping" apart a section of DNA, allowing researchers to add, remove, or replace genetic material at that spot. For example, CRISPR/Cas9 can be used to correct mutations at specific locations in the genome, creating a permanent change in the organism's DNA.[12] CRISPR/Cas9 editing techniques can also be used to manipulate gene expression, allowing scientists to precisely turn genes on or off. Using these genetic editing techniques in opossums could potentially yield tantalizing new insights about the complexities of development.

Scientists attempted to apply CRISPR/Cas9 editing in the gray short-tailed opossum by "knocking out" (removing) the *TYR* gene, which is required for melanin production.[7] One of the easiest ways to confirm successful genetic modifications using CRISPR/Cas9 is to target a gene that influences an easily observable trait, such as fur color. Mutations in *TYR* are a primary cause of oculocutaneous albinism (OCA), a genetic disorder characterized by a lack of pigment in the skin, hair, and eyes.[8] Thus, if the genetic modification was successful, it would lead to albino joeys.

The *TYR* knockout experiment yielded a range of results: some joeys were fully albino, others showed partial pigment loss, and some appeared unaffected.

Crucial to the generation of genetically engineered animals is establishing a reliable breeding colony. This ensures that genetic modifications can be reliably passed on to future generations.[12]

Excitingly, researchers successfully bred the albino offspring, establishing a stable breeding line of opossums that all carry the knockout *TYR* gene.[7] This achievement not only confirmed the marsupial genome can be precisely modified using CRISPR/Cas9, but also that these genetic modifications are heritable—thus, paving the way for opossums to become powerful new models in developmental biology.

Opossum babies with genetically modified pigmentation. Photo Credit: RIKEN

F1 (first generation of offspring produced by a cross between two parental types) all offspring showing albino phenotype. Photo Credit RIKEN

Beyond developmental biology, the ability to genetically modify marsupials opens up new avenues for research in mammalian embryology, genomic imprinting, reproduction, neurobiology, immunogenetics, cancer biology, and comparative evolution. Researchers are also exploring the potential of genetic modification to help conserve endangered marsupial species, such as the northern quoll, by developing toxin resistance against cane toads.[4]

The findings could even open new doors for neuroscientists studying nerve cell regeneration. For example, unlike adults, young opossums can regenerate a severed spinal cord. Understanding the genes at play may help scientists unravel the molecular alchemy underlying this regenerative potential.

Whether you've seen opossums as charming nighttime visitors or peculiar pests, it may be time to see them in a new light—unlikely marsupial heroes with the potential to help unlock cures for human diseases.

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