Genetic linkage between WntRE-GFP transgene and albinism in *Xenopus laevis* frogs

Collin Taylor, Gregory F. Weber
Department of Biology

ABSTRACT

African clawed frogs (*Xenopus laevis*) are an important model organism, used globally to study cell and developmental biology. A WntRE-GFP gene has been inserted into the genome of *X. laevis* to observe Wnt cell signaling during development. However, albino offspring were unexpectedly generated from the mating of two pigmented WntRE-GFP *X. laevis*. This occurrence suggests the presence of a recessive gene allele leading to albinism. We hypothesized that the WntRE-GFP transgene was inserted within a gene encoding enzymes required for pigmentation. To test this, multiple backcrosses were conducted to determine whether the alleles for albinism and the WntRE-GFP are linked. All backcrosses resulted in zero recombinant offspring indicating that the alleles for albinism and the reporter transgene are completely linked. We began isolating genomic DNA from transgenic frogs by Targeted Locus Amplification (TLA), a technique that will provide specific DNA sequences surrounding the transgene. This sequence can be compared to known *X. laevis* genomic sequence to determine the loci of the transgene within the genome.

INTRODUCTION

Albinism is characterized by reduced pigment in the retinal pigment epithelium (RPE) of the eye and in the melanophores and melanocytes of the skin during the late embryonic stage in a variety of animals, from frogs to humans (Fukuzawa, 2020; U.S. National Library of Medicine, 2020; Videira, 2013). The process of pigment, or melanin, is produced through melanogenesis and can be disrupted through alterations of any of the proteins or enzymes necessary for proper function.

A synthetic reporter gene, WntRE-GFP, was previously randomly inserted into the *X. laevis* genome. The Green Fluorescence Protein, or GFP, gene can be expressed within tissues of the frog where Wnt signaling is active, illuminating green. This provides a useful means of examining Wnt signaling in various biological processes. GFP can be expressed and observed in an organism by shining blue light upon the species. Random insertion of a gene can disrupt a variety of processes. Due to the generated albino offspring, melanogenesis enzymes/proteins are the key suspect.

METHODS

Perform backcrosses of the following crosses:

- WntRE-GFP/albino x wildtype → albinism
- WntRE-GFP/albino x WntRE-GFP/albino → true-breeding
- WntRE-GFP/albino x heterozygous parent → GFP test
- heterozygous parent x wildtype → heterozygosity test

1. Inject the frogs with chorionic gonadotropin
2. Place into mating tanks overnight
3. Collect embryos from tanks
4. Let grow into tadpole development
5. Observe pigmentation and GFP expression

Targeted Locus Amplification (TLA) protocol consisting of:

a. Selecting region of interest
b. Crosslinking
c. Digestion
d. Ligation
e. Reverse crosslink of DNA
f. Digestion #2
g. Amplification with PCR
h. Sonication and adaptor ligated
i. PCR purification
j. DNA sequencing

Figure 1. Albino WntRE-GFP *X. laevis* offspring (left) were generated by mating two heterozygous WntRE-GFP frogs that have normal skin and eye pigmentation (right). The albino frogs were hypothesized to be homozygous for alleles of both albinism and the WntRE-GFP transgene.

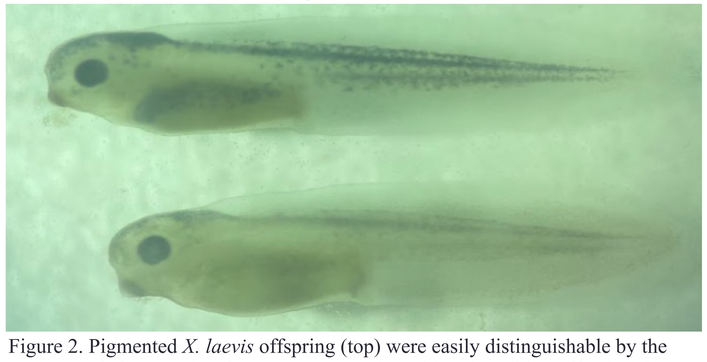

Figure 2. Pigmented *X. laevis* offspring (top) were easily distinguishable by the presence of pigmented melanocytes among the epidermis. Albino offspring (bottom) did not have apparent melanocytes and any pigmentation granules that were maternally contributed from the egg cell were lost by tadpole stages.

RESULTS

The first test cross provided 50% albino and 50% wildtype offspring all containing the GFP gene. The chi-squared value is 8.28, allowing for the null hypothesis to be accepted. The second cross concluded with 100% albino offspring all with the GFP gene, leading to a 0 chi-squared value. The sum of the third test cross led to approximately 50% pigmented offspring without the GFP gene and 50% pigmented offspring with the GFP gene, obtaining a 0.02 chi-squared value. The final testcross brought forth questioning to experimentation and the research. The euploidy of *X. laevis* as a species were reinvestigated and distinguished to be allotetraploid, and not diploidy, which was the original hypothesis. The fourth testcross ultimately allowed for us to construct a variety of Punnett squares dealing with the possibilities of heterozygous parentals. The final observations of raw data concluded with roughly 75% pigmented GFP positive offspring with the other 25% being albino GFP positive.

CONCLUSION

- *X. laevis* are allotetraploid organisms (4n).
- Albino WntRE-GFP *X. laevis* are 100% true-breeding.
- Albino offspring cannot be seen without the GFP gene.
- GFP can be expressed with only a single allele; however, intensity of GFP increases as the number of alleles for the gene increase.
- Albinism is a recessive trait, requiring homozygosity across likely two pairs of homologous chromosomes to be phenotypically observed.
- The inserted WntRE-GFP gene is inseparable genetically to one or more genes important to melanin production in melanogenesis.
- As a result of the disruption of melanogenesis gene(s) or regulatory components, such as promoters, albinism occurs in homozygous WntRE-GFP frogs.
- TLA protocol fully amplified the GFP genetic material.

Figure 3. Albino WntRE-GFP *X. laevis* offspring illuminated by blue light to reveal areas of GFP, depicted by arrows.

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