QnAs with Carlos Taboada

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A molecule that would be toxic to most animals provides the characteristic blue-green color of hundreds of frog species. In a recent PNAS article (1), biologist Carlos Taboada reports how the typically toxic pigment biliverdin binds to members of a family of serpin proteins to produce a pathway for green coloration in frogs. Taboada is a postdoctoral fellow in biology at Duke University and has been working with frogs since his undergraduate days in Argentina, shifting focus from studies of anatomy and histology to biochemistry and engineering. Taboada discusses the findings from his PNAS article.

PNAS: To the human eye, the frogs you studied appear green. How are they different?

Taboada: Most green animals—chameleons, lizards, geckoes, [and] many frogs and salamanders, too—have chromatophore cells. In those animals, all of the color that we see comes from that really thin layer of cells.

In the species that we describe [in the article] (1), there is a huge concentration of this previously unidentified blue protein that binds biliverdin. In the frog, it was a mystery. How can the animals have such huge concentrations of biliverdin? The physiology of that was weird because in humans, for example, large accumulation of that pigment means that you are really sick. That's what I started studying. It didn't have to do immediately with coloration of frogs; it was more a physiological question.

Carlos Taboada. Image credit: Laura Andrade (photographer).

PNAS: In trying to isolate the protein that binds to biliverdin, how did you choose to focus on the polka dot tree frog, *Boana punctata?*

Taboada: I had some spots in Argentina where I would go every summer and stay there for weeks, following the populations of these frogs. I decided to continue with that species, in particular, because I had a deep knowledge about their chromatophores and experience working [with captive frogs], too.

What really surprised me was, at the beginning, I had this isolated protein, but I didn't know what it was. It was a. . . slow process trying to identify the protein because these are nonmodel animals. Normally, for nonmodel animals we don't have access to [protein and nucleotide] sequences. Databases are pretty sparse. I had to do a manual inspection of every single mass spectrometry dataset. It took months and months.

PNAS: In the long search for the protein's identity, you uncovered another interesting feature of *B. punctata*: They fluoresce in UV light (2). How did that happen?

Taboada: While isolating the blue protein from the polka dot tree frog, I found that it was fluorescent in the far-red portion of the spectrum. So it was natural to try to elicit that fluorescence in live animals. Instead of turning red, the animals emitted green light, way stronger than I expected. That is how we discovered that the polka dot tree frog was fluorescent and that the underlying chemistry had to be different from what I thought. That opened a new research avenue that led us to the identification of a fluorophore and the study of the photophysics and biology of frog biofluorescence (2). It was a discovery we made by chance while looking for something else.

PNAS: You determined that the proteins binding biliverdin in these frogs are members of the serpin superfamily of proteins. Was that expected?

Taboada: It was surprising. No other serpins are known to carry biliverdin. But what is really shocking is that the sequences of all of the serpins were so different. Between the different frogs, the sequence identities were only around 50%. If we compare any of those sequences with the most similar human serpin, the protease inhibitor antitrypsin, we find that they have around 45% identity.

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We are talking about proteins that change. Either they changed rapidly, or the frogs coopted different genes to produce the same function over and over. It's an amazing example of convergence. In fact, as opposed to other cases in nature in which the convergence of a trait relies on the substitution of the same amino acid in the same site of a protein, in this [case] it is [possible] that the atomic explanation, when we solve the crystals, may show that the thermodynamic stability of the biliverdin–serpin binding depends on different intermolecular interactions that evolved independently.

PNAS: How do these biliverdin-binding serpins create the frogs' color?

Taboada: These proteins are blue, and blue normally is a really difficult color to find in nature. Mostly, when we see blue it's not based on pigments; it is based on structure. It's a physical thing. In the case of these frogs, blue and green have a chemical origin.

When bound, biliverdin changes its absorbance. When we see the biliverdin alone, it's greener, but it also absorbs less light. When it's bound to the protein, it changes color, and it gets trapped inside the protein. This also prevents the biliverdin from being excreted. The blue color of the protein is responsible for the blue regions of the body, but when there's yellow pigment in the skin, we see green.

But that's not all. What is really amazing is that the blue serpins, in combination with deeper connective tissues, in this case full of guanine crystals, not only explain the leaf-like coloration of the animals under visible light, but also how the frogs can mimic the vegetation in infrared.

There's a well-known phenomenon in leaves known as red-edge reflectance. This is a really sharp increase in the amount of light that plants reflect in the region between red and infrared and depends on their chlorophyll content. We knew that leaf-dwelling frogs also have this red-edge profile, but we didn't know how. Now we know that the serpins tune this reflectance and match the plants' red edge. In this way, the frogs and plants have the same spectral properties but [through] a different mechanism. If it were only biliverdin without the protein, we would not get the same match.

1 C. Taboada *et al.*, Multiple origins of green coloration in frogs mediated by a novel biliverdin-binding serpin. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 18574–18581 (2020).
2 C. Taboada *et al.*, Naturally occurring fluorescence in frogs. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 3672–3677 (2017).