

PHYSIOLOGY

Lessons on transparency from the glassfrog

Insert Deck – a summary of the take home message in accessible terms, to fit one line

By Nelly M. Cruz¹ and Richard M. White^{1,2}

An obvious feature of life on Earth is that most animals and plants can be seen. Such visualization is important for predation, feeding, and mating. But this does not apply to all animals – some are transparent. The origins of this phenomenon and how it has been evolutionarily selected are unclear. One such species are the glassfrogs, so called because of their relatively transparent bodies and translucent skin. These nocturnal arboreal frogs sleep during the day on green leaves and appear to camouflage during their rest time. A previous study (1) found that they exhibit dynamic transparency, mainly at night, allowing them to camouflage with the leaves where they sleep and likely escape from predators. On page XXX of this issue, Taboada *et al.* (REF) investigated the distinct optical properties of the glassfrog species, *Hyalinobatrachium fleischmanni*, and uncover the surprising physiological mechanism behind their transparency, which could be informative for...

Taboada *et al.* found that the extent of transparency in the glass frog changes depending on activity, with its highest level of transparency achieved during sleep. One of the barriers to transparency in vertebrates is the presence of respiratory pigments such as hemoglobin. Transmittance and reflectance measurements using optical spectroscopy confirmed that light attenuation during the active state can be attributed to changing levels in hemoglobin. The authors used three modalities of photoacoustic microscopy (PAM), high-resolution, deep-penetrating, and high-speed PAM, to noninvasively measure red blood cell (RBC) perfusion of the whole body and specific tissues during rest and exercise. This allowed Taboada *et al.* to capture dynamic changes in RBC circulation in real-time with precision and high resolution, and to measure oxygenation by measuring saturation of hemoglobin (sO₂). They found that the glassfrogs “hide” RBCs in their liver during sleep, allowing them to achieve high levels of transparency on their ventral side. As the glassfrogs wake and become active, the amount of RBCs in circulation increases considerably. Remarkably, the glassfrog can pack ~89% of their RBCs at high density in the liver without signs of detrimental effects, such as clotting.

The distinct properties of the glassfrog make it an important model to understand the general phenomenon of organismal transparency. In land animals, transparency is extremely rare, with the glassfrog being an exception to the rule. By contrast, transparency is surprisingly common in aquatic species, especially in pelagic (open sea) regions. In a comprehensive review of this phenomenon (2), it was noted that transparent animals cover a wide range of aquatic species, ranging from *Amphogona apicata* (hydromedusa) to *Amphitretus pelagicus* (octopus) to many different species of fish such as the *Ambassidea* (glassfish). This raises the question of what binds these various transparent species together? From an ecological standpoint the range of transparent species is quite diverse, and it appears that transparency has evolved many times over, suggesting that the physiological function of transparency is likely highly adapted to local conditions. A clue to this evolution is that many (but certainly not all) transparent aquatic species are found at specific depths called euphotic or dysphotic zones, where light can penetrate reasonably well and animals cannot hide easily. Unlike other forms of camouflage (such as pigmentation and mirroring), transparency is distinct in that it successfully hides animals from nearly all viewpoints and depths.

How does transparency arise? Generally speaking, transparent substances neither absorb nor scatter light. Thus, one mechanism of opacity is the production of molecules such as melanin or hemoglobin which strongly absorb certain wavelengths of light. These pigments in turn tend to be concentrated in specific cell types: melanin in melanocytes in the skin and hemoglobin in RBCs. Alternatively, light scattering can be due to structural proteins such as collagen, which are highly concentrated in structures such as the sclera, which accounts for the white part of the human eye. Other animals such as fish have reflective cells in their skin called iridophores, which are packed with guanine crystals that scatter light (3), the lack of which makes the skin transparent such as in the zebrafish mutant *casper* (4). These two phenomena – absorption and scattering – have been overcome in a distinct way by the glassfrog to allow transparency. Their ability to concentrate nearly their entire blood volume at night effectively removes the light absorbing hemoglobin from circulation. They also

lack melanin pigmentation and reflective structures in their ventral skin, further improving their transparency. But they are not “perfectly” transparent, because they retain some dorsal pigmentation, raising the question of whether they should be called translucent rather than transparent (1).

Regardless, the adaptations described by Taboada *et al.* make a strong case that it is the RBC “hiding” that predominates in the glassfrog’s unusual optical properties. To further demonstrate how special this really is, the authors investigated whether other opaque tropical frog species can store RBCs in their livers during sleep. They examined three additional species, including the most closely related opaque species *Allophryne ruthveni*. These frogs did not store RBCs in their livers to a great extent, suggesting that the ability to conceal RBCs is distinct to the glassfrog. One of the opaque species, *Agalychnis callidryas*, has reflective guanine crystals throughout its body, precluding imaging of its vasculature. Interestingly, the authors were able to examine a translucent mutant of this species that lacks pigment and guanine crystal layers. This mutant still transmits much lower levels of visible light compared to the glassfrog, demonstrating that transparent skin is not enough to endow organism-level transparency.

The mechanism that drives RBC redistribution in glassfrogs is not understood. It is unclear if the glassfrog can actively manipulate the changes in RBC circulation, for example, in the presence of a predator. Future studies might also investigate how this behavior is influenced by environmental and behavioral factors such as their mating season or food availability. Another intriguing question is how sequestration of RBCs in the liver affects cellular respiration and whether the glassfrogs have a special metabolism that adapts to the drastic changes in RBC circulation. Such studies might also have implications for understanding human vascular disorders. In particular, there might be anti-coagulant factors that allow glassfrogs to redistribute RBCs. The development of genomic and molecular tools will be critical for the successful use of the glassfrog as an unconventional model system for studying why and how transparency evolves in nature.

REFERENCES AND NOTES

1. J. B. Barnett *et al.* *Proc. Natl. Acad. Sci. U.S.A.* **117**, 12885 (2020).

¹Memorial Sloan Kettering Cancer Center

²University of Oxford

Email:

1 2. S. Johnsen. *Biol Bull.* **201**, 301 (2001).
2 3. S. Hozumi, M. Shirai, J. Wang, S. Aoki, Y. Kikuchi.
3 *Biochem Biophys Res Commun.* **502**, 104 (2018).
4 4. R. M. White *et al.*, *Cell Stem Cell* **2**, 183 (2008).

5 **ACKNOWLEDGMENTS (OPTIONAL)**
6 Please add any relevant conflicts of interest here –
7 please keep this concise as it is included in the word
8 limit.

9 PHOTO CREDIT GOES HERE adf7524

10
11 **Figure title**
12 Glassfrogs become transparent due to hiding of
13 RBCs. a) While sleeping on leaves, the glassfrog is
14 almost entirely transparent, which is associated
15 with dense packing of nearly their entire RBC vol-
16 ume in their liver. b) In contrast, upon awakening
17 the animal rapidly becomes opaque and redis-
18 tributes the RBCs into the vasculature. c) This is a
19 dynamic process, such that when the animal
20 again returns to sleep the RBCs return to the liv-
21 er.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59