Scotopic rod vision in tetrapods arose from multiple early adaptive shifts in the rate of retinal release

Yang Liu\textsuperscript{a,1}, Yimeng Cui\textsuperscript{a}, Hai Chi\textsuperscript{b}, Yu Xia\textsuperscript{a}, Haonan Liu\textsuperscript{a}, Stephen J. Rossiter\textsuperscript{b,1}, and Shuyi Zhang\textsuperscript{a,1}

\textsuperscript{a}Key Laboratory of Zoonosis of Liaoning Province, College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110866, China; and \textsuperscript{b}School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, United Kingdom

Edited by W. Ford Doolittle, Dalhousie University, Halifax, Canada, and approved May 17, 2019 (received for review January 11, 2019)

The ability of vertebrates to occupy diverse niches has been linked to the spectral properties of rhodopsin, conferring rod-based vision in low-light conditions. More recent insights have come from nonspectral kinetics, including the retinal release rate of the active state of rhodopsin, a key aspect of scotopic vision that shows strong associations with light environments in diverse taxa.

We examined the retinal release rates in resurrected proteins across early vertebrates and show that the earliest forms were characterized by much faster rates of retinal release than more recent ancestors. We also show that scotopic vision at the origin of tetrapods is a derived state that arose via at least 4 major shifts in retinal release rate. Our results suggest that early rhodopsin had a function intermediate to that of modern rod and cone pigments and that its well-developed adaptation to low light is a relatively recent innovation since the origin of tetrads.

Author contributions: Y.L. designed research; Y.C., H.C., Y.X., and H.L. performed research; Y.L. and S.Z. contributed new reagents/analytic tools; Y.L., Y.C., H.C., and S.J.R. analyzed data; and Y.L., S.J.R., and S.Z. wrote the paper.

The authors declare no conflict of interest.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

\textsuperscript{1}To whom correspondence may be addressed. Email: yliu@syau.edu.cn, s.j.rossiter@qmul.ac.uk, or szhang@baylor.edu.cn.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1900481116/-/DCSupplemental.

Published online June 10, 2019.

www.pnas.org/cgi/doi/10.1073/pnas.1900481116

PNAS | June 25, 2019 | vol. 116 | no. 26 | 12627–12628
earliest vertebrates. By widening taxonomic breadth, we are able to show that these early taxa were poorly adapted to scotopic vision. Thus, well-developed scotopic vision at the origin of tetrapods may represent a relatively recent innovation.

Materials and Methods

Vertebrate RH1 genes were obtained from GenBank. We reconstructed ancestral RH1 sequences under amino acid (LG+Γ) model using a published tree topology in CODEML (12) and used these to express proteins for functional analyses (Datasets S1–S4).

Briefly, RH1 was expressed, regenerared (adding 11-cis-retinal), and purified in an elution buffer (40 μM RH1 epotope, 50 mM NaCl, 3 mM MgCl2, 0.1% n-dodecyl-β-maltoside, 20% glycerol, 140 mM NaCl, 3 mM MgCl2). The half-life of retinales was measured every 30 s at 20 °C (13). Curves were fitted using \( y = y_0 + a(1 - e^{-bt}) \), and the half-lives of retinal release were calculated (\( t_{1/2} = \ln(2)/b \)) (4). The \( r^2 \) for all fitted curves was >0.99, except for Vertebrata (0.87 to 0.98), as also reported for the cone pigment that shows much faster retinal release (6). We compared \( t_{1/2} \) values between pairs of ancestral pigments (adjacent nodes) in the tree with 2-tailed \( t \) tests. As a control, we synthesized the bovine RH1 (4, 5). We consider the cow to be better adapted to low light than other day-active species; they are diurnal but also show crepuscular activity, and they possess duplex retinas with rods that show intermediate patterns between those of nocturnal and diurnal species (14).

To ensure that our results were robust to model choice, we reanalyzed and measured the \( t_{1/2} \) of RH1 of 4 focal ancestral taxa using sequences from the codon (“free-ratio”) model, which differed by 4 to 17 residues from the amino acid model. For Tetrapoda and bovine RH1, we also estimated the Arrhenius activation energies (\( E_a \)) of Schiff base hydrolysis (6) as 24.38 and 22.93 kcal/mol, respectively, based on retinal release rates at 15, 20, 25, and 30 °C.

ACKNOWLEDGMENTS. We thank R. Crouch (Medical University of South Carolina) and L. Neuhold (NIH) for the 11-cis-retinal. This work was funded by National Natural Science Foundation of China grants to Y.L. (31601855) and S.Z. (31570382) and by a European Research Council Starting grant (310482) to S.J.R.