Phototransduction gain at the G-protein, transducin, and effector protein, phosphodiesterase-6, stages in retinal rods

Martin Heck[a], Klaus Peter Hofmann[a,b], Timothy W. Kraft[c,1], and Trevor D. Lamb[d]

In PNAS, Yue et al. (1) deal with the question of amplification early in the phototransduction cascade. Although their approach is elegant, we cannot agree with some major points in their analysis and conclusions.

The authors suggest that their estimate of "12–14 transducin–PDE effector complexes" per photoisomerization is different from literature estimates that "range from teens to hundreds of G_T*s per Rho*." However, the gain at the stage of the effector protein is an indirect measure of the actual rate (ν_{RG}) of transducin (G_T*) activation, which is the parameter determined in many previous investigations. From the present data, and the authors' assumption that the active PDE binds a single transducin, ν_{RG} would be obtained by dividing the above number of PDE complexes by the R* lifetime. For an R* lifetime of 0.04–0.08 s (references 16–19 in supporting information of ref. 1), this calculation gives a rate ν_{RG} of 150–350 G_T* s^{-1}, close to values obtained in previous studies. Moreover, after correcting for shortcomings in the authors' analysis (enumerated below), this estimate increases considerably, to around 1,000 s^{-1} and is then consistent with the rate obtained from kinetic light-scattering measurements (2, 3).

i) A recent experimental study (4) concluded that the rod PDE is activated only when two transducins are bound. In addition, however, one needs to take account of transducins bound singly (i.e., ineffectively) to PDE, as well as unbound transducins, and transducins that have already been inactivated. These considerations lead to the conclusion that the total number of transducins activated is three to four times the number of elementary PDE events contributing to the single-photon response (SPR) (see p. 7 of ref. 5). On this basis, there would be 40–50 G_T* activated per SPR, at an activation rate of 500–1,250 G_T*·s^{-1}.

ii) In both their approaches for extracting the number of elementary events underlying the SPR, the authors' calculations rely on the unwarranted assumption that those events all have identical amplitude and kinetics.

a) The experimental results in figure 3A (right) show a major difference in shape between the variance and mean square traces, demonstrating variability in the underlying elementary events. If the early rising phases were scaled to match, the peaks would differ by a factor of ∼2. We therefore contend that, in their first method, the corrected mean number of events (*m*) per SPR might double, to 25–30 activated PDEs, taking the number of activated G_T* to 80–100.

b) In their second (noise) method, any stochastic variability in the lifetime of individual PDE molecules would likewise invalidate the estimate of event rate based on their assumption of identical shapes, although the quantitative effect is difficult to estimate.

In summary, the authors' results are consistent with 50–100 G_T* being activated during the SPR, with activations occurring on a millisecond timescale. The authors' results are thus in agreement with kinetic light-scattering measurements (2, 3). We conclude that rod photoreceptors exhibit a very high gain for the receptor to G-protein amplification step.

1 Yue WWS, et al. (2019) Elementary response triggered by transducin in retinal rods. *Proc Natl Acad Sci USA* 116:5144–5153.
2 Vuong TM, Chabre M, Stryer L (1984) Millisecond activation of transducin in the cyclic nucleotide cascade of vision. *Nature* 311:659–661.

[a]Institut für Medizinische Physik und Biophysik der Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, D-10117 Berlin, Germany; [b]Zentrum für Biophysik und Bioinformatik, Humboldt-Universität zu Berlin, D-10115 Berlin, Germany; [c]Department of Optometry and Vision Science, University of Alabama at Birmingham, Birmingham, AL 35294; and [d]Eccles Institute of Neuroscience, John Curtin School of Medical Research, The Australian National University, Canberra, ACT 2600, Australia

Author contributions: M.H., K.P.H., T.W.K., and T.D.L. wrote the paper.
The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. Email: twkraft@uab.edu.
Published online April 30, 2019.

3 Heck M, Hofmann KP (2001) Maximal rate and nucleotide dependence of rhodopsin-catalyzed transducin activation: Initial rate analysis based on a double displacement mechanism. *J Biol Chem* 276:10000–10009.
4 Qureshi BM, et al. (2018) It takes two transducins to activate the cGMP-phosphodiesterase 6 in retinal rods. *Open Biol* 8:180075.
5 Lamb TD, Heck M, Kraft TW (2018) Implications of dimeric activation of PDE6 for rod phototransduction. *Open Biol* 8:180076.