long the suggestion that India should host a future IOC – maybe in the year 2014 or 2018. While this may seem a long way to go, most often it has turned out that preparations for inviting the IOC may itself take as many as eight years. As an elected member of the International Ornithological Committee for the years 2002–2006, I hope that we might be able to organize a symposium or round table on 'Recent advances in ornithological research in South Asia'. This might provide a forum where we could highlight the long-standing commitment that we have had in India for the study of birds, and also our scientific capabilities. The Government of India, through MoEF, CSIR, DBT, DST and UGC, should consider sponsoring a large number of Indian ornithologists to participate in the 24th and 25th IOCs (the 25th IOC is likely to be in Brazil). While these ideas are open to discussion, let us look forward to a good beginning.

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RESEARCH NEWS

Cocking the gun – First steps in the bacteriorhodopsin photocycle

M. K. Mathew

Bacteriorhodopsin (bR) served as the archetype for membrane proteins for two decades, before gracefully making way for relative newcomers like potassium channels and visual rhodopsin. Structure determination of bR was greatly aided by its organization into a hexagonal lattice in the purple membrane of the archaebacterium *Halobacterium salinarium*. Structures of a few other membrane proteins have since been determined, but mechanism in detail available for many soluble proteins remains elusive. bR remains the paradigm here with its well-characterized photocycle leading to the pumping of a proton from one side of the membrane to the other[1].

Members of the large family of seven transmembrane receptors respond to ligand binding by undergoing a series of structural transitions, leading to the formation of a conformational state capable of activating a transducing second messenger. The best-characterized example is visual rhodopsin where an intramolecular ligand, retinal, absorbs a photon and forms metarhodopsin II or MII. MII can then activate the G-protein transducin and thereby initiate a signal transduction cascade.

Light-adapted bR has all-trans retinal attached covalently to a lysine side chain via a protonated Schiff's base[1]. All-trans retinal is yellow, absorbing maximally at 480 nm. Interactions within the binding pocket shift its absorbance spectrum to 568 nm – the opsin shift. bR is purple-coloured, absorbing at 568 nm (bR_{568}), while the different cone pigments absorb at different wavelengths due to this opsin shift. Absorbing a photon initiates a photocycle, with a series of optically distinct intermediates in all rhodopsins[2]. The intermediates in bR are I, J_{610}, K_{625}, L_{550}, M_{412}, N_{520} and O_{640}, with the numbers in subscripts referring to the wavelength of maximum absorption of each intermediate. These intermediates had been identified by flash spectrometry – monitoring absorbance changes at each of several wavelengths after initiating the

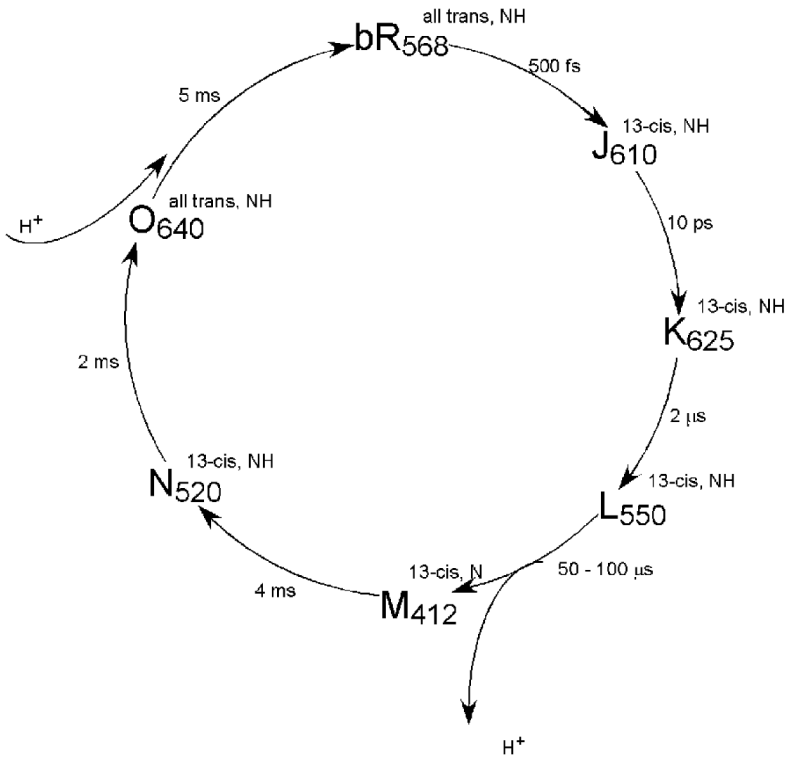

Figure 1. Photocycle of bacteriorhodopsin initiated on absorbing a photon of visible light. Ground state, light adapted bR absorbs maximally at 568 nm (bR_{568}) and its chromophore is all-trans retinal attached through a protonated Schiff's base (all-trans, NH). Intermediates J, K, L, M, N and O are listed with subscripts denoting the wavelengths of maximal absorption and superscripts indicating the configuration of the retinal chromophore and the protonation state of the Schiff's base linkage. A proton is released from bR on the extracellular side of the membrane on the same timescale as the formation of M, while uptake from cytoplasm occurs with kinetics similar to the reformation of bR.

photocycle with a brief flash of actinic light[3]. Clearly, the duration of the flash has to be very short to monitor early intermediates like K which are formed in picoseconds, whereas even microsecond flashes suffice to characterize later intermediates such as M which rise in 50–100 μs. Flashing membrane sheets containing bR (the famous purple membrane sheets) release protons into the medium with the same kinetics as the rise of M (50–100 μs), while the re-uptake of the proton follows the kinetics of regeneration of bR (5–10 ms).

Photoisomerization to 13-*cis* retinal generates strain in a binding pocket optimized for all-trans retinal. Formation of M involves loss of a proton on the extracellular side, while thermal relaxation back to bR requires re-uptake of a proton from the cytoplasmic side of the membrane[4]. The M intermediate was shown to have a deprotonated Schiff's base and a 13-*cis* chromophore by IR and resonance Raman spectroscopy in the eighties[5]. The crystal structures of many of the later intermediates have since been determined in the late nineties[6–8], leading to a delineation of the pumping pathway[9], which amounts to a bucket brigade relaying the proton from side chain to side chain across the membrane. However, the initial steps by which absorption of the photon sets-off the whole train of events – the cocking of the gun, as it were – are only now becoming amenable to experimental characterization.

Photoexcitation of bR occurs on a time scale faster than nuclear rearrangements can occur and the formation of a ground state, isomerized intermediate occurs later. The K state, which forms in a few picoseconds, has recently been crystallized and shown to have a 13-*cis* chromophore[10]. Theoretical studies predict the formation of a 13-*cis* ground-state intermediate in under 500 fs, corresponding to the J intermediate[11]. However, studies with sterically constrained retinal analogues appear inconsistent with this expectation[12]. The early intermediates had previously been characterized in kinetic experiments using a brief laser pulse to initiate the photocycle and then probing the system with brief interrogating pulses. These experiments require a very stable protein, and thus can be carried out with bR, but have not yet been extended to visual rhodopsin. The femtosecond experiments required to detect I and J had thus far been carried out at visible wavelengths, which report on the electronic state of the system but not directly on the configuration of the chromophore. Herbst and co-workers[13] have now carried out pump-probe experiments at infrared wavelengths with 200 fs resolution and have shown that all photoproduct vibrational modes rise with time constants of around 500 fs and are consistent with a 13-*cis* configuration.

The strain generated by isomerization is subsequently relieved by protein relaxation, leading to very different results in different proteins–proton pumping in bR; chloride translocation in halorhodopsin, and photoperception in visual rhodopsin. bR thus remains the beacon, guiding research into the mechanisms by which the signal transduction machinery is primed.

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COMMENTARY

Animal experimentation rules – Separating the reality from the rhetoric

Saionton Basu

An attempt has been made to analyse the Guidelines proposed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and how they affect scientific research in India. An analysis of the conditions imposed by the CPCSEA has been made to see whether they fulfil the 'test of reasonableness' on whose touchstone every action of a statutory authority, in this case the CPCSEA, must be evaluated to be valid. The sole national regulatory and monitoring body has created several administrative bottlenecks, which are delaying the progress of scientific research, which will blunt the competitive edge of India in the 'pharma-biotech' sector.

There can be few more emotive issues in science than animal experimentation. To the researchers involved, such research is vital for continued scientific progress, while animal-rights activists term them as the worst kind of animal abusers, deserving public vilification[1]. This paper seeks to examine the guidelines (Breeding of and Experiments on Animals [Control and Supervision] Rules, 1998 framed under Section 17(1) of the Prevention of Cruelty to Animals Act, 1960) proposed