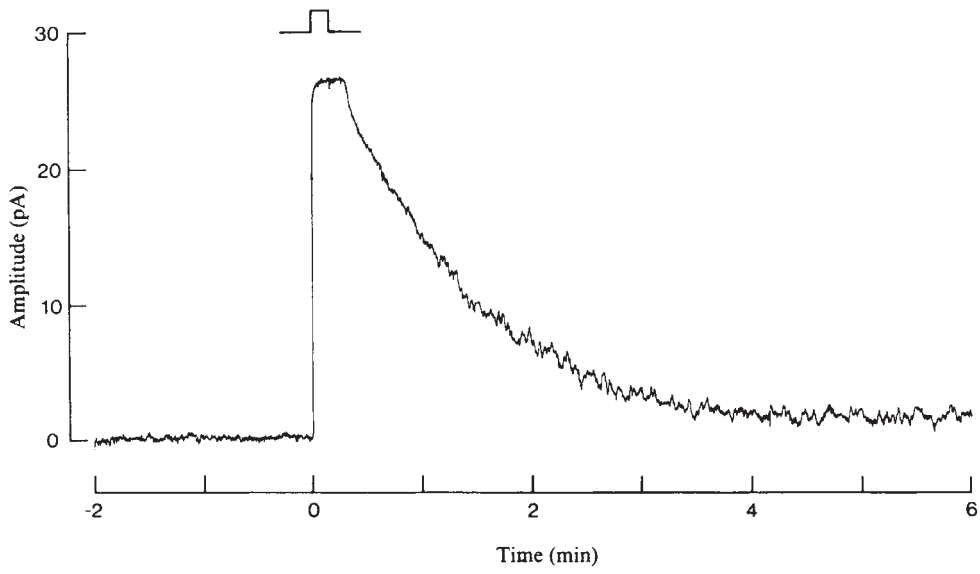

Fig. 3 Response of another cell to a bleach of about 0.7% (7.2×10^5 photons μm^{-2}, calculated to isomerize 1.8×10^7 rhodopsins). Quantal events before the bleach are not visible as the cell was relatively insensitive. Bicarbonate/CO_2–Ringer; 23.6 °C; low pass filtered at 5 Hz.

equivalent isomerizations per s. This is about 2.2 log units greater than the spontaneous rate in the fully dark adapted state (0.02 isomerizations per s, ref. 7).

A similar effect from a cell recorded in bicarbonate/CO_2–Ringer is shown in Fig. 3. Before the bleach the baseline was relatively quiet, but during recovery the trace became quite noisy, despite a slight reduction in its flash sensitivity. The power spectrum of these fluctuations was again well fitted by the form predicted from the average post-bleach flash response (not shown). This cell was less sensitive than the previous one and the post-bleach quantal event amplitude was estimated to be $r_{peak} = 0.12$ pA per isomerization. The zero frequency asymptote $S(0)$ of the spectrum was 0.7 $pA^2\,Hz^{-1}$ and substitution in equation (2) gave an estimated mean rate of $\nu = 11$ isomerizations per s for this bleach of 0.7%, about 2.7 log units higher than in absolute dark adaptation.

The conclusion from these results is that, as proposed by Barlow[2], spontaneous photon-like events occur in the rod outer segments at a much higher rate after a bleach than in absolute dark adaptation. This phenomenon seems to explain qualitatively the existence of the psychophysical equivalent background[14] or dark light[1,2] which limits visual detection, and the extent to which it may quantitatively account for dark adaptation behaviour is under investigation (D. A. Baylor and T.D.L.). It is interesting that the situation appears similar in invertebrates, where exposure of a dark-adapted locust retinula cell to intense light causes an increased rate of occurrence of quantal events[15].

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Light isomerizes the chromophore of bacteriorhodopsin

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The primary photochemical event in the two light-transducing pigments whose chromophore is retinal, rhodopsin or bacteriorhodopsin, is a source of controversy. It was originally proposed that the primary photoevent in the bleaching of rhodopsin is the photoisomerization of the chromophore from 11-*cis* to all-*trans* retinal[1,2]. Photochemical considerations suggested that a photoisomerization is the primary event in both rhodopsin and bacteriorhodopsin[3–7]. However, this description of bacteriorhodopsin's photochemistry has been questioned[8–10]. To elucidate this problem, we determined the isomeric conformation of retinal for two of the photolytic intermediates of bacteriorhodopsin, using a method that enables us to extract chromophores from the photocycle intermediates L and M at low temperatures (−74 °C), and have determined the isomeric conformation of the extracted retinals by HPLC[11,12]. Here we provide direct evidence that isomerization of the chromophore has taken place in two of the early photocycle intermediates (L and M) of bacteriorhodopsin.

The absorption of a photon by light-adapted bacteriorhodopsin (bR^{LA}), the purple membrane protein of the *Halobacterium halobium*, initiates a series of transformations[13], reminiscent of the bleaching sequence of rhodopsin. The primary photochemical product, K, is a bathochromically shifted species; it decays to the photocycle intermediate L and then to M, which eventually returns to bR^{LA} through the intermediates N and O. bR^{LA} slowly converts to the dark-adapted bacteriorhodopsin (bR^{DA}). It has been determined that the isomeric form of chromophore of bR^{LA} is almost completely all-*trans* retinal, whereas bR^{DA} has a mixture of 13-*cis* and all-*trans* retinals with a ratio of 1:1. The isomeric compositions were

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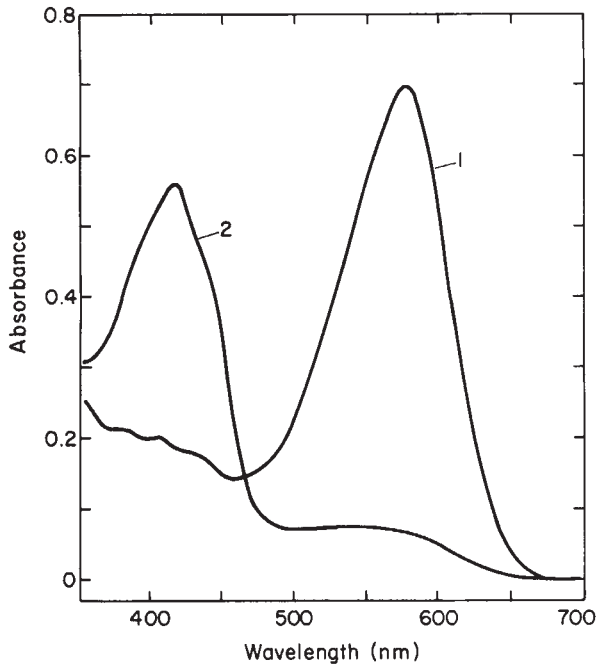

Fig. 1 Photochemical conversion of bR^{LA} to M at −74 °C. Curve 1, bR^{LA} in a 25% NaCl solution adjusted to *p*H 9.5 (0.1 M carbonate buffer) and mixed with glycerol (1:2) at −74 °C. Curve 2, the photosteady-state mixture produced by irradiating with $\lambda > 580$ nm light (Corning filter 2–73) for 15 min at −74 °C. The sample was irradiated with the light from a 200-W tungsten lamp using a glass filter. The light was routed to the sample cell through an optical glass fibre (5-mm diameter).

originally determined by chromophore extraction methods[11,14,15]. The validity of determining the isomeric composition by the extraction technique was later verified by several non-destructive spectroscopic techniques[16–19].

To extract chromophores from the L and M intermediates at low temperatures, the extraction solvent should not freeze at low temperatures (−74 °C), it should denature the pigments at the low temperature, and the retinal should not be isomerized by the solvent itself. We found an extraction solvent composed of buffer/glycerol/dichloromethane/hexane (1.6:3.3:1:4) which satisfied the above conditions. The solvent was first emulsified by sonication (30 s). Then a syringe cylinder with the tip blocked by a Teflon plug, containing the bR sample in 67% glycerol was inserted into a test tube containing the solvent. This in turn was immersed in a dry ice–ethanol bath (−74 °C) and the bR irradiated in the syringe with an optical light guide. The plunger was then inserted into the syringe cylinder and the irradiated bR injected into the extraction solvent. Next, the bR sample was mixed thoroughly with the extraction solvent, using a precooled (−74 °C) spatula. The temperature of the sample was monitored with a thermocouple. This procedure denatured the irradiated bR sample at −74 °C as shown by the total fading of the colour to pale yellow. The mixture was then warmed to 0 °C and chromophores extracted by sonication. Extraction by the sonication method gave a substantially greater yield (60±20%) than the syringe method (<10%) and exhibited no preferential extraction of either isomer.

To insure that the isomeric composition of the extracted retinals represented that of the intact pigment and that no isomerization had taken place during our extraction procedure, we extracted the chromophores of bR^{LA} and bR^{DA}. Table 1 shows that isomerization of bR^{LA} and bR^{DA} extracted in our conditions are basically the same as the compositions previously reported. The *p*H, glycerol and temperature we used affected the isomeric composition of the chromophore slightly.

bR^{LA} suspended in a 25% NaCl solution adjusted to *p*H9.5 was mixed with glycerol (1:2) to prevent crystallization at low temperatures. The extraction of retinals from bR^{LA} at 0 °C yielded 94±3% all-*trans* retinal and 6±3% 13-*cis* retinal. When bR^{LA} was cooled to −74 °C (Fig. 1, curve 1) and irradiated with light of wavelengths greater than 580 nm ($\lambda > 580$ nm light), the M intermediate was formed (Fig. 1, curve 2). In the dark, the absorption spectrum was stable. Extraction of the chromophores from this preparation yielded 14±3% all-*trans* retinal and 86±3% 13-*cis* retinal. The amount of M, based on the decrease in absorption at 570 nm, was estimated to be 91% (Fig. 1, curve 2). Therefore, our results suggested that the isomeric form of retinal in M was at least 95% 13-*cis* retinal. After making the M intermediate at −74 °C, the sample was warmed to 0 °C, and after waiting for 10 min to ensure that M had returned to bR^{LA}, the chromophores were extracted. This preparation yielded 93±4% all-*trans* retinal and 7±4% 13-*cis* retinal, which corresponds to the composition in bR^{LA} before making M. These results suggested that light isomerized the chromophore in the conversion of bR^{LA} to M and the chromophore was thermally re-isomerized in returning from M to bR^{LA} in the dark.

The intermediate L, which is the precursor of M, is produced as a member of a photosteady-state mixture containing L, M and bR^{LA} when bR^{LA} (in a 25% NaCl solution adjusted to *p*H 7.2) is irradiated with $\lambda > 630$ nm light as shown in Fig. 2, curve 2. Table 1 shows that before making L, bR^{LA} in glycerol contains 92±2% all-*trans* retinal and 8±2% 13-*cis* retinal; after making L and warming the sample to 0 °C for 10 min it contains 92±2% all-*trans* retinal and 8±2% 13-*cis* retinal. Denaturation of the photosteady state formed at −74 °C and extraction of its chromophores yields 53±4% all-*trans* retinal and 47±4% 13-*cis* retinal. The content of L, M, and bR^{LA} in this preparation is difficult to determine precisely because two different extinction coefficients for L have been proposed[20,21]. The amounts of L, M and bR^{LA} in the mixture shown in spectrum 2 of Fig. 1 are calculated to the 57%, 13% and 30%, respectively, assuming $\varepsilon_L = 61{,}000$ (ref. 20) (without consideration of bypass of L→bR^{LA}) and as 29%, 13% and 58%, respectively, assuming

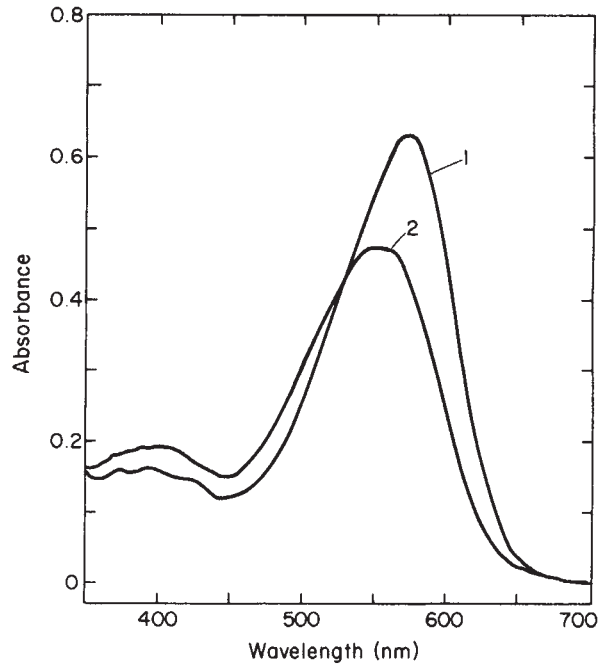

Fig. 2 Photochemical conversion of bR^{LA} to L at −74 °C. Curve 1, bR^{LA} in a 25% NaCl solution adjusted to *p*H 7.2 (0.1 M imidazole-HCl) and mixed with glycerol (1:2) at −74 °C. Curve 2, the photosteady-state mixture produced by irradiating with $\lambda >$ 630 nm light (Corning filter 2–59) for 15 min at −74 °C. Irradiation of sample was carried out as shown in Fig. 1.

Table 1 Retinal isomers extracted from bR^{LA}, bR^{DA}, M and L

Species	Conditions	% Composition 13-*cis*	All-*trans*	Yield (%)
a pH 9.5, 25% NaCl				
bR^{D}		55±2	45±2	43±13
bR^{DA} (glycerol)		54±2	46±2	45± 9
bR^{DA} (glycerol)	denatured at −74 °C and extracted at 0 °C	54±3	45±3	56± 4
bR^{LA}		6±3	94±3	57± 8
bR^{LA} (glycerol)		7±4	93±4	45±12
bR^{LA} (glycerol)	denatured at −74 °C and extracted at 0 °C	11±3	89±3	55± 9
M (glycerol)	bR^{LA} irradiated with $\lambda >$ 580 nm light for 15 min, denatured at −74 °C and extracted at 0 °C	86±3	14±3	55±15
bR^{LA} (glycerol)	M was warmed up to 0 °C and kept 10 min at 0 °C	7±4	93±4	46± 8
b pH 7.2, 25% NaCl				
bR^{DA}		52±2	48±2	59± 8
bR^{DA} (glycerol)		54±2	46±2	65±20
bR^{DA} (glycerol)	denatured at −74 °C and extracted at 0 °C	56±2	44±2	70±15
bR^{LA}		3±2	97±2	56±10
bR^{LA} (glycerol)		8±2	92±2	53±10
bR^{LA} (glycerol)	denatured at −74 °C and extracted at 0 °C	11±2	89±2	60± 8
L	bR^{LA} irradiated with $\lambda >$ 630 nm light for 15 min, denatured at −74 °C and extracted at 0 °C	47±4	53±4	52±10
bR^{LA} (glycerol)	L was warmed up to 0 °C and kept for 10 min at 0 °C	8±2	92±2	60±12

Each experiment was repeated at least five times. Dark adaptation (bR^{DA}) was assured by allowing the bR sample to stand overnight at room temperature for each set of conditions (*p*H, glycerol). Light adaptation (bR^{L}) was accomplished by irradiating the sample with a slide projector for 20 min at 0 °C, followed by immediate extraction. Denaturation and extraction were performed at 0 °C unless otherwise described. An attempt to quantify the efficiency of retinal extraction was made by measuring the A_{365} in hexane solution after extraction. The retinal concentrations in the solution were determined by the retinal composition and using the following extinction coefficients: (4.58×10^4 for all-*trans*, 3.84×10^4 for 13-*cis* at 365 nm)[24]. Separation and analysis of retinals in the extract were carried out by HPLC with micro-Porasil column (Waters Associates). The sample was eluted at a constant flow rate (2 ml min^{-1}) with 4% ether–hexane (v/v). Two well resolved peaks were detected spectrophotometrically ($\lambda = 365$ nm). They were identified as the 13-*cis* and all-*trans* isomers of retinal by a comparison of their retention times with those of authentic samples.

$\varepsilon_L = 49{,}000$ (ref. 21) (with consideration of bypass of L → bR^{LA}). Taking bR^{LA} as all-*trans* and M as 13-*cis* (see above) there is a reasonable correlation between the per cent L and M present spectroscopically from (42–70%) and the per cent of the 13-*cis* isomer present (47 ± 4%) if we assume that chromophore of L is 100% 13-*cis* retinal.

Thus, the present results suggest that light has initiated an event which results in the chromophore of bR^{LA} being isomerized from the all-*trans* to 13-*cis* conformation by the time of the appearance of the L intermediate, and that the 13-*cis* chromophore is thermally re-isomerized to the all-*trans* form in going from M to bR^{LA} in the dark. By far the simplest explanation of our results is the general hypothesis, based on photochemical studies by Rosenfeld *et al.*[3] and Hurley *et al.*[4], that the primary event in bacteriorhodopsin is a photoisomerization requiring that the isomeric conformation of retinal in bR^{LA} be different from its conformation in L and M. Some indications of an isomerization step have also been noticed on the basis of resonance Raman measurements[22,23] although there is still controversy about the interpretation of these spectra.

Our results may be compared with the work of Pettei *et al.*[11], who first suggested the isomerization event was from the all-*trans* to the 13-*cis* isomer. These authors extracted chromophores from 'M-like intermediates' in ether/high salt preparations and guanidinum HCl/high *p*H preparations at 0 °C and concluded that M yields the 13-*cis* isomer, although the correlation between M and the 13-*cis* isomer of the former preparation was rather poor. We find a quite good correlation between the amount of 13-*cis* retinal present and the amount of the M intermediate present. It is possible that the nature of M in ether/high salt and guanidinum HCl/high *p*H at 0 °C is different from M at normal conditions. More likely, as Pettei *et al.* did not actually measure the absorption spectra of their solutions, they had not achieved full photoconversion of bR to M.

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Leukaemia virus infection promotes fibroblast transformation by normal BALB/c mouse DNA

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All normal cells are thought to carry genetic information for oncogenic transformation, which, on activation to continuous expression, might make the cell cancerous[1]. The presently known transforming retroviruses contain transforming genes which were probably derived by recombination of a slow oncogenic retrovirus with cellular sequences closely related to these genes[2]. It was recently reported[3] that cellular DNA fragments from normal tissue culture cells could transform mouse fibroblasts *in vitro* with a low efficiency. High efficiency of transformation was observed in secondary transfections only when high molecular weight DNA from transformed recipient cells was used as the transforming agent[3]. We observed that DNA isolated from different BALB/c mouse organs can transform both NIH/3T3 and BALB/3T3 cells, although at a low frequency[4]. In attempts to increase the initial efficiency of transformation, we have found that preinfection of recipient 3T3 cells with murine leukaemia viruses markedly enhances focus formation by normal BALB/c DNA fragments.

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