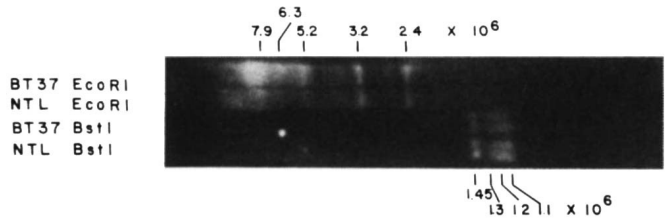

Fig. 4 Evidence for homology between fragment G and tobacco DNA. *Bst*I digests of 2.8 μg of normal tobacco leaf DNA isolated as described elsewhere[34] and of BT37 tumour DNA, in addition to *Eco*RI digests of 5.0 μg of the same two DNAs, were fractionated in horizontal slab gels of 0.7% agarose and blotted by Southern's procedure[22]. The blots were hybridized[14] with 1.5 μg of recombinant plasmid pBR325/*Eco*RI-G (5.4×10^7 c.p.m. μg^{-1}). The autoradiogram shown was exposed for 48 h (with two Dupont Cronex intensification screens) at −80 °C. Molecular weights of the bands in the autoradiogram were estimated from a calibration curve for the original gel based on digest fragments of known molecular weight. NTL, Normal tobacco leaf DNA, BT37, tobacco BT37 tumour DNA.

little precedent for the existence of such an element. Regardless of the physical situation of T-DNA our data make it clear that this foreign prokaryotic DNA element has become joined to eukaryotic DNA. The mechanism and specificity of such novel joining of phylogenetically alien DNAs may be revealed by analysis of DNA sequence at the border regions.

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Note added in proof: Similar results have been obtained for two T-DNA border fragments from an octopine-synthesizing tobacco crown gall tumour[35].

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Behaviour of octopus rhodopsin and its photoproducts at very low temperatures

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Many aspects of light energy transduction in photoreceptors are under study[1,2]. One important problem concerns the light-initiated primary event in rhodopsin and the identity of the primary photoproduct. It is usually assumed that the red-absorbing pigment bathorhodopsin[3] is the first photoproduct. In cattle rhodopsin, it is produced within 6 ps of excitation[4] and is stable at 77 K. However, Yoshizawa *et al.*[5–7] have found a blue-absorbing pigment, hypsorhodopsin, in photosteady-state mixtures formed with orange light at liquid helium temperatures (~5 K). On warming the hypsorhodopsin in the dark to liquid nitrogen temperatures (77 K), it converts to bathorhodopsin. These two results suggested that hypsorhodopsin might be a precursor of bathorhodopsin, but that presents difficulties. For example, hypso-type intermediates have not been seen for all visual pigments[6]. Moreover, when cattle rhodopsin was irradiated with blue light at liquid helium temperatures, it was converted to bathorhodopsin without forming detectable hypsorhodopsin[6,7]. Finally, rapid kinetic experiments to determine the time course of formation of bathorhodopsin and hypsorhodopsin have given ambiguous results[4,8–10]; this may be due to the use of rhodopsins from different species. Because squid rhodopsin seemed to have more hypsorhodopsin in photosteady states than cattle rhodopsin[7], we have studied the photo products of another cephalopod rhodopsin at carefully controlled low temperatures (±0.2 K). We report here that octopus rhodopsin has a hypso intermediate that is easily formed on irradiation but that the batho-product appears before significant amounts of the hypso-product accumulates. Moreover, the thermal conversion of hypsorhodopsin to bathorhodopsin must be fitted with two rate constants, and the activation enthalpy of the faster process is almost zero, that is, it is temperature independent over the range studied.

Octopus (Mizudako, *Paroctopus defleini*) microvillar membranes were prepared as previously described[11]. Rhodopsin was extracted from the membranes with a 2% digitonin solution, glycerol was added to the preparation to give a final concentration of 75%, and the samples were stored at −70 °C. The *p*H of the solution was adjusted to 10.5 with sodium carbonate buffer just before the start of an experiment, so that at room temperature any bleached rhodopsin would go to alkaline metarhodopsin (λ_{max} = 376 nm)[12].

On cooling to 10 K, the λ_{max} of octopus rhodopsin (476 nm at 10 °C) shifted to 486 nm (Fig. 1, curve 1). When the octopus rhodopsin was very briefly irradiated with 480 nm light at 10 K, the spectral change indicated the formation of a bathochromic product (octopus bathorhodopsin) (Fig. 1, curve 2). On further irradiation, the concentration of the bathochromically absorbing product increases (curve 3) and the appearance of a hypsochromically shifted photoproduct (octopus hypsorhodopsin) can also be seen (Fig. 1, curve 4). With further irradiation using 530 nm light, a photosteady state is formed which contains large amounts of hypsorhodopsin (56%), as well as rhodopsin (<4%) and isorhodopsin (>40%), with almost no bathorhodopsin. A detailed analysis of the spectra shows that hypsorhodopsin and bathorhodopsin appear concomitantly. Figure 1 strongly suggests that on irradiation of rhodopsin at

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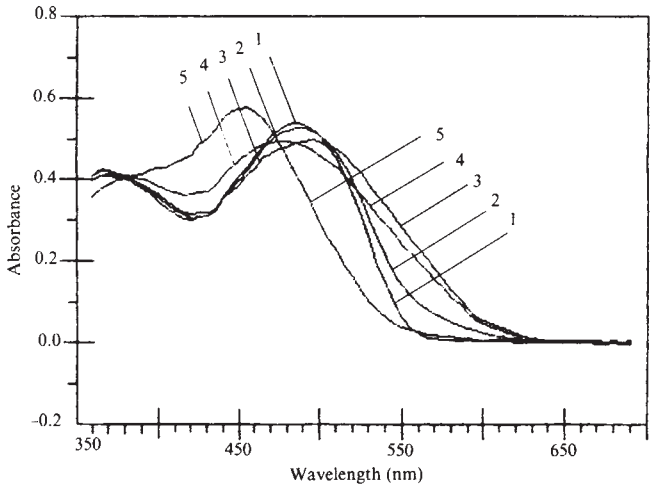

Fig. 1 Spectral changes observed in the conversion of rhodopsin to bathorhodopsin, hypsorhodopsin and isorhodopsin at 10 K. Curve 1, octopus rhodopsin in digitonin (*p*H 10.5) 10 K; curves 2–4, products of irradiation at 480 nm for successive periods of 8, 64 and 2,048 s; curve 5, photosteady state obtained by irradiation at 530 nm for 10 min. Curve 5 represents about 56% hypsorhodopsin, >40% isorhodopsin and <4% rhodopsin. This composition was determined as in ref. 7. All spectra were recorded with a Cary 14 using a Janis 10DT super Varitemp Optical Dewar.

10 K, the stable, blue-absorbing ground state species, hypsorhodopsin, is apparently not the precursor of the bathorhodopsin formed at this temperature.

On slowly warming up from 45 K, the spectrum of the photosteady-state mixture formed by irradiating octopus rhodopsin at 10 K shifted to longer wavelengths with sharp isosbestic points at 479 nm and 374 nm, as shown in Fig. 2. In the insert, the change in absorbance at 540 nm, indicating the formation of bathorhodopsin, is shown as a function of temperature. Judging from the similarity of their absorption spectra, the batho-product obtained by warming is identical to bathorhodopsin produced by irradiation of octopus rhodopsin at 80 K (B. Mao, M.T. and T.G.E., unpublished observations). Thus, the data of the insert show that hypsorhodopsin is converted to bathorhodopsin above 45 K. This transition temperature is much higher than that observed for the bovine[6] and

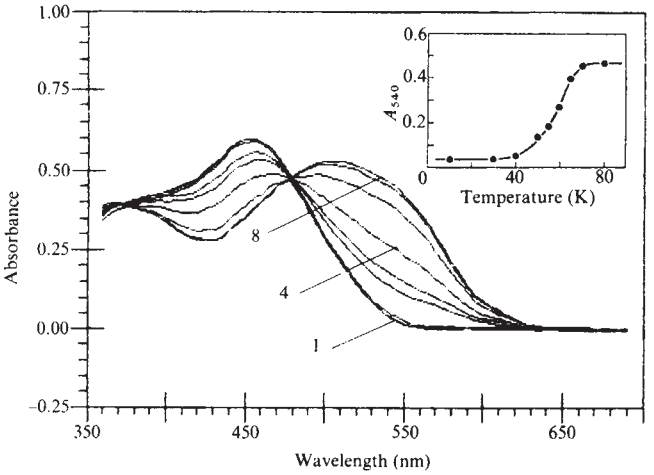

Fig. 2 The transformation of hypsorhodopsin to bathorhodopsin on warming above 10 K in the dark. The photosteady state (curve 1) was produced by irradiating rhodopsin at 10 K with 530 nm light for 5 min. The sample was then slowly warmed and spectra recorded every few minutes. The temperatures of the sample when the spectrum was measured are: (1) 10 K, (2) 40 K, (3) 50 K, (4) 55 K, (5) 60 K, (6), 65 K, (7) 70 K, (8) 80 K. Inset: absorbance at 540 nm (indicating the formation of bathorhodopsin) as a function of temperature.

squid[7] or frog[13] hypsorhodopsin → bathorhodopsin transition. There is no evidence of any stable intermediates between hypsorhodopsin and bathorhodopsin.

We attempted to measure the enthalpy of activation in the thermal conversion of hypsorhodopsin to bathorhodopsin. At a given temperature, rhodopsin was irradiated with continuous yellow light ($\lambda > 490$ nm) for 3 min to reach the photosteady state. Immediately after the light exposure, the decay of hypsorhodopsin was monitored by the absorbance at 440 nm and the formation of bathorhodopsin by the absorbance at 550 nm. For temperatures between 53 K and 73 K, we plotted $\ln(A_t - A_\infty)$ as a function of time, where A_t and A_∞ are the absorbance at t min and infinity, respectively. The rates of both hypsorhodopsin and bathorhodopsin are non-exponential; both curves can be fitted fairly well by the sum of two exponentials. The temperature dependence of the two rates is plotted in an Arrhenius manner ($\ln K$ against $1/T$) in Fig. 3. The temperature dependence of the decay of hypsorhodopsin gives $\Delta H = 0.87 \pm 0.29$ kcal mol^{-1} and $\Delta H = 0.09 \pm 0.28$ kcal mol^{-1}. A

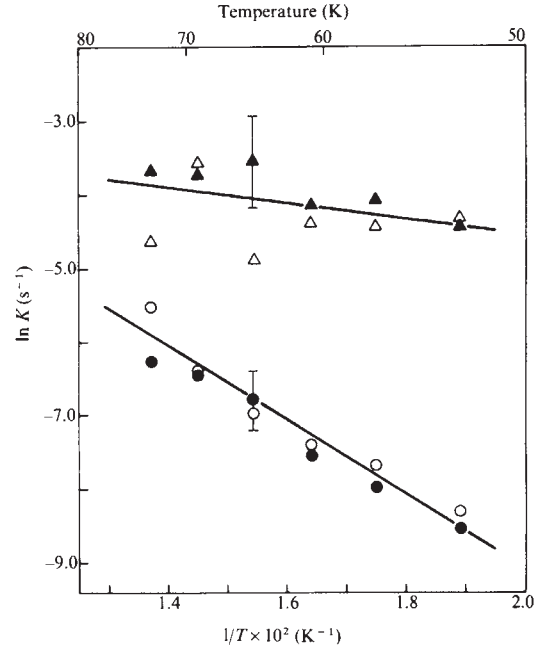

Fig. 3 Arrhenius plot of kinetic data for the decay of hypsorhodopsin (decrease in absorbance at 440 nm) and formation of bathorhodopsin (increase in absorbance at 550 nm). △, Faster phase; ○, slower phase in the decay of hypsorhodopsin. ▲, Faster phase; ●, slower phase in the formation of bathorhodopsin.

similar pair of values are found for the formation of bathorhodopsin. The non-exponential character of the hypsorhodopsin decay processes implies that hypsorhodopsin is not a single homogeneous species. We are now studying the thermal decay process in more detail, in particular the nature of the component which seems to be almost temperature independent.

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