Table 2 Genetic transmission of M-MuLV sequences and incidence of viraemia in second backcross generation

M-MuLV-specific *Eco*RI fragment in father	Locus	M-MuLV-specific sequences in DNA of N-2 mice/ total tested	Viraemic N-2 animals/ total tested	Percentage
18 Kbp	Mov-3	14/28	125/255*	49
21 Kbp	Mov-2	6/14	14/147†	9.5
9 Kbp	Mov-4	1/5	0/60	0

N-1 males of genotype Mov-2, Mov-3 and Mov-4 were identified after partial hepatectomy as described in Table 1. They were bred with normal females and some of the offspring were again partially hepatectomized and the presence of M-MuLV-specific sequences in the liver DNA determined by quantitative hybridization[22]. All animals were repeatedly tested for viraemia using the RIA for p30 (ref. 13).

* Animals segregating the *Mov-3* locus were viraemic at the youngest age tested (3 weeks).

† Animals segregating the *Mov-2* locus were non-viraemic at 3 weeks of age; some mice developed viraemia at later ages (between 2 and 4 months of age).

Having become viraemic, Mov-2 animals develop leukaemia quickly. The disease is indistinguishable by histological and molecular parameters from the leukaemia occurring in Mov-3 animals. The host range of the activated virus was determined. The virus grew equally well on NIH 3T3 and BALB 3T3 cells but did not replicate in CCl 64 cells. This phenotype bred true when cloned on either of the permissive cell lines. Thus the virus was NB tropic like the original virus used for infection of the embryos.

The sequence homology of Mov-2 and Mov-3 virus was analysed by molecular hybridization. RNA was isolated from NIH 3T3 cells which were infected with Mov-2 virus and from spleens of viraemic Mov-2 and Mov-3 animals. Each RNA preparation annealed to the same extent (95%) to M-MuLV cDNA at low C_rt values. In contrast, RNA of spleens from non-viraemic Mov-2 animals annealed to the same extent as RNA from control animals (15% annealing at a C_rt value of 10,000). Furthermore, when DNA from NIH 3T3 cells infected with virus activated from Mov-2 animals was digested with *Pvu*II, no differences in the cleavage pattern of integrated viral sequences were observed in comparison to Cl 1A virus, BALB/Mo virus or Mov-3 virus (data not shown). This suggests that Mov-2 animals activate NB tropic Moloney virus integrated into their germ line.

We chose Moloney leukaemia virus as a model system to establish the experimental conditions for integrating genetic material into the germ line of mice. The animal exposed to virus was a mosaic carrying viral genomes in part of his somatic and germ cells and genetically defined mouse strains were only obtained after segregating the genes in the next generation. As preimplantation embryos are not permissive for expression of viral functions[15], our experimental approach can be used to introduce other than viral genetic information into the germ line.

The M-MuLV genome carried at the *Mov-1, Mov-2* and *Mov-3* locus, respectively, was indistinguishable by all criteria used. Virus activation in the respective mice, however, was different. Thus, cellular sequences flanking an integrated viral genome may influence its expression by a *cis*-acting mechanism, as has been suggested[24,25]. The mechanisms by which animals have acquired endogenous viruses and the evolutionary forces that influenced virus expression are not known. Our results indicate that the endogenous viruses of mice may have entered the germ line by infecting early embryos and that the different phenotypes of virus expression observed in inbred mouse strains may depend on the chromosomal integration site of the virus. Our model system should facilitate investigations to understand the regulation of C-type virus expression during cellular differentiation and its possible role in evolution.

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T-DNA of a crown gall teratoma is covalently joined to host plant DNA

N. S. Yadav*, K. Postle†, R. K. Saiki†§, M. F. Thomashow† & M.-D. Chilton*

*Department of Biology, Washington University, St Louis, Missouri 63130
†Department of Microbiology & Immunology, University of Washington, Seattle, Washington 98195

Agrobacterium tumefaciens **strains containing tumour-inducing (Ti) plasmids[1–3] incite cancerous growths called crown galls when inoculated into wounded dicotyledonous plants. Tumour tissue can be cultured axenically *in vitro*, and exhibits a transformed phenotype in the absence of the inciting bacterium. Transformed cells grow autonomously, are auxin and cytokinin autotrophic *in vitro*[4] and synthesize opines[5–8], novel amino acid derivatives dictated by Ti plasmid genetic information[9–11]. A small segment of the Ti plasmid, termed T-DNA is maintained in axenic tumour cells[12–19]. Mitochondrial and chloroplast DNA from a crown gall teratoma are free from T-DNA, whereas nuclear DNA contains T-DNA in amounts similar to that in total tumour cell DNA[20,21]. T-DNA appears to be attached to what is presumably plant DNA in the crown gall tumour cell: Southern blot analysis[22] of tumour DNA digested with restriction endonucleases reveals T-DNA fragments that are not fully homologous to Ti plasmid DNA[14,17,21]. We report here the isolation by molecular cloning of a 'border fragment' T-DNA and flanking plant DNA from the crown gall teratoma BT37 and show that T-DNA is covalently joined to a repeated DNA element of the tobacco nuclear genome.**

‡Present address: Department of Microbiology, University of California, Irvine, California 92717.
§Present address: Cetus Corporation, 600 Bancroft Way, Berkeley, California 94710.

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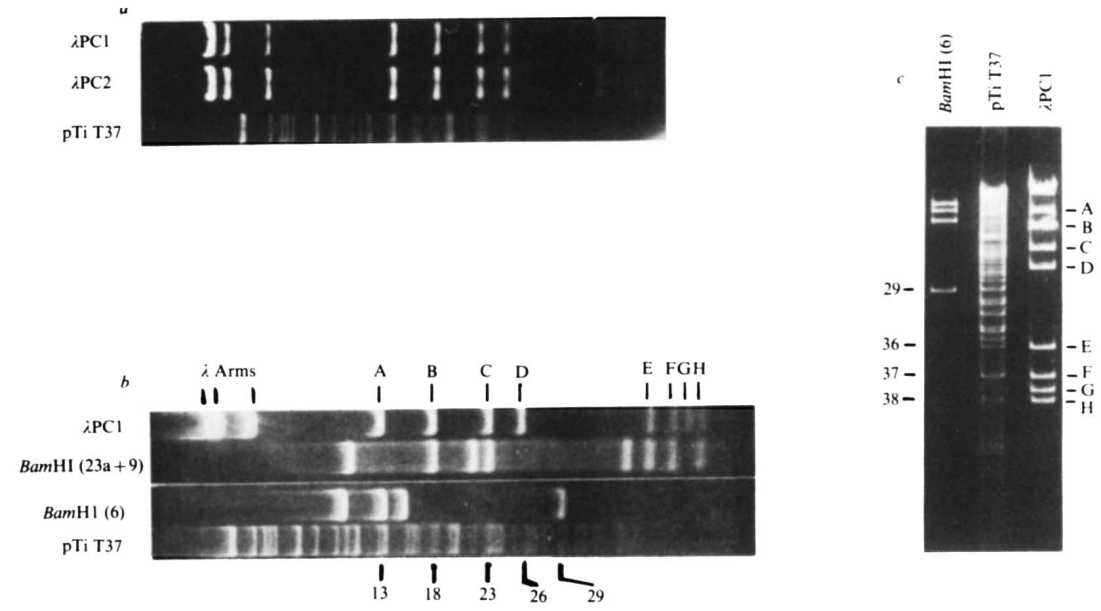

Fig. 1 *Eco*RI digests of DNA from λPC1 and λPC2, pTi T37 and cloned fragments of pTi T37. Charon 4A bacteriophage[30] DNA was digested with *Eco*RI and the vector arms separated from internal fragments by sedimentation through 5–20% NaCl linear gradients. Partial and complete *Eco*RI digests of BT37 tumour DNA, isolated as described in ref. 12, were fractionated in the same manner (F. Blattner, personal communication). Fragments 10–20 kilobases in size (correct for Charon 4A cloning inserts) were pooled. Phage arms (20 μg) and target fragments (7 μg) were incubated with T4 DNA ligase for 16 h at 4 °C (50 μl reaction volume). Packaging was performed by published procedures[23,24]. Phages were plated on 9.2 cm^2 Petri dishes at 20,000 plaques per plate. Lifts were prepared[25] and hybridized with pTi T37 DNA labelled *in vitro* by nick translation[26], in order to detect plaques containing T-DNA inserts. Two positive plaques, λPC1 and λPC2, were detected and purified. *a*, *Eco*RI digests of λPC1 and λPC2 DNA (isolated from CsC1 banded phage particles by phenol deproteinization and dialysis) and of pTi T37 DNA were fractionated by electrophoresis in a horizontal slab gel of 0.7% agarose as described previously[31]. The three largest molcular weight fragments in the phage DNA digests are Charon 4A arms. *b*, *Eco*RI digests of λPC1 DNA, pTi T37 DNA and two recombinant plasmids containing the left end of T-DNA from pTi T37 (see text and Fig. 2) were fractionated as described above in 1% agarose. c, *Eco*RI digests of λPC1 DNA, pTi T37 DNA and (as molecular weight marker) one recombinant plasmid from the left end of T-DNA were fractionated by vertical 1.5% agarose slab gel electrophoresis in TB buffer (10.8 g Tris base, 0.93 g disodium EDTA and 5.5 g boric acid per litre). Fragments of pTi T37 were numbered starting from fragment 29, which is also indicated in *b*. Fragment G is not an internal pT1 T37 fragment.

For molecular cloning of T-DNA fragments from BT37 tobacco teratoma DNA, we used the strategy of F. Blattner *et al.*[23]: shotgun cloning of a mixture of *Eco*RI partial and complete digest fragments of total tumour DNA into Charon 4A bacteriophage. Ligated DNA (see legend to Fig. 1 for details) was packaged *in vitro* into phage coats[23,24] and approximately 10^6 plaque-forming units were plated and screened by hybridization of plaque 'lifts'[25] with Ti plasmid DNA, labelled *in vitro* by nick translation[26] with [α-^{32}P]deoxynucleotide triphosphates. Two positive plaques were picked and purified. The *Eco*RI cleavage patterns of the resulting two recombinant phage DNAs are presented in Fig. 1*a*. λPC2 possesses six of the eight *Eco*RI insert fragments contained in λPC1; the latter are designated A–H in decreasing order of molecular weight (Fig. 1*b*). To determine how many of the insert fragments were homologous to Ti plasmid DNA, a Southern transfer prepared from them was hybridized with ^{32}P-labelled[26] pTi T37 plasmid DNA. Autoradiography revealed that all insert fragments except the G fragment of λPC1 hybridized strongly (data not shown). Further studies used only λPC1 to determine whether any of its insert fragments were border fragments containing both Ti plasmid and plant DNA sequences.

A map of the part of pTi T37 detected in plant tumour line BT37[16,19–21] is presented in Fig. 2. Part of *Bst*I fragment 6 as well as the whole of 23A, 9 and 14A are detectable in the tumour DNA by Southern blot analysis[21]. Eight intact *Eco*RI fragments of the Ti plasmid are internal to this region and are also detectable by Southern blot analysis of BT37 tumour DNA[19,21]. To compare the insert fragments of λPC1 with the *Eco*RI fragments that map in T-DNA, we compared the single and multiple cleavage products of Ti plasmid fragments with those of λPC1. Because of the complexity of total Ti plasmid DNA, we used two clones of Ti plasmid DNA in the vector pBR322. One contained *Bst*I fragment 6, and the other contained a partial digest product, *Bst*I fragments 9 plus 23A plus the unnumbered small fragment between them. Figure 3 shows that *Eco*RI fragment 13 (cut from the appropriate *Bst*I clone) co-migrates with A, 18 with B, and 23 with C. Also, the two subfragments of D generated by *Bst*I/*Eco*RI double digestion of λPC1 correspond to *Bst*I/*Eco*RI double digest fragments of the appropriate *Bam*HI clones (data not shown). Confirmation of D as *Eco*RI fragment 26 was achieved by recloning D into pBR325 (ref. 27), labelling this recombinant plasmid and hybridizing it to a Southern blot of pTi T37 *Eco*RI fragments as well as λPC1 *Eco*RI fragments. The probe hybridized with D in the phage DNA digest as expected and with fragment 26, of identical electrophoretic mobility, in the Ti plasmid digest (data not shown).

The low molecular weight inserts E, F and H of λPC1 co-migrate with subfragments of the (23A+9) *Bst*I clone (Fig. 1*b*) and in the Ti plasmid *Eco*RI digest, fragment 36 co-migrates with E, 37 with F and 38 with H (Fig. 1*c*). Clearly fragment G is not an intact *Eco*RI fragment of the Ti plasmid: no *Eco*RI fragment of pTi T37 co-migrates with it (Fig. 1*c*).

Fragment G could be either a plant DNA fragment included in the original phage clone by coincidence, or a border fragment abutting A on the left or (less plausibly) F on the right. For DNA hybridization analysis, G was recloned into the *Eco*RI site of pBR325 (ref. 27), and an *Eco*RI digest of this clone, containing vector and G fragments, was used to prepare Southern blots. When duplicate tracks were hybridized with labelled pTi T37 DNA (Fig. 3*a*), fragment G hybridized weakly. G thus

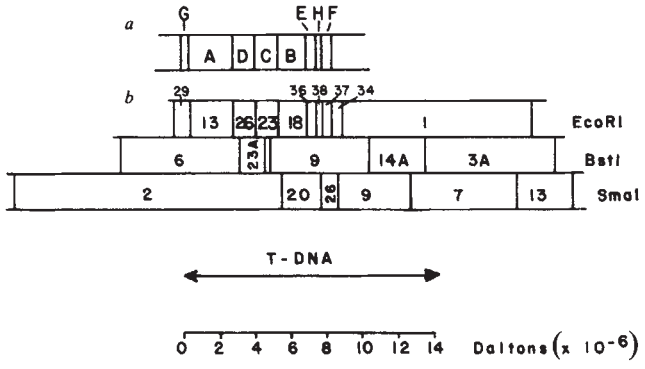

Fig. 2 Map of the T-DNA portion of pTi T37 and of the inserts of λPC1. For the *Sma*I fragment map and evidence see ref. 16. The *Bst*I fragments were mapped hybridizing isolated *Sma*I and *Hpa*I fragments to Southern blots[22] of *Bst*I digest fragments of pTi T37 as described previously[32]. *Bam*HI (a *Bst*I isoschizomer) fragments of partially digested pTi T37 were cloned into the *Bam*HI site of pBR322, and clones containing the following inserts were picked from a bank of 200 clones by colony hybridization[33]: fragment 6, fragments 9 and 23A and the small unnumbered fragment between them, fragment 14A and fragment 3A. Digestion of these recombinant plasmids with *Sma*I, *Hpa*I, *Bst*EII and combinations of these enzymes produced the fragments predicted by the map presented here and the published *Hpa*I and *Bst*EII maps[16]. Digestion of these recombinant plasmids with *Eco*RI alone and in combination with the other enzymes mentioned above allowed us to determine the *Eco*RI fragment map of pTi T37 shown above. The numbers assigned to the small fragments (34–37) were determined by the method described in Fig. 1*c* legend. *a*, The fragment map of the inserts in λPC1; *b*, the fragment map of pTi T37. Evidence for the arrangement of these fragments is based on the identification of their counterparts in the Ti plasmid digest as described in the text. Assignment of G to the left edge of the phage insert is based on its hybridization to Ti plasmid fragments mapping in this region (see text).

appeared to contain a short piece of Ti plasmid DNA. To determine whether G derived from the left edge of T-DNA, we hybridized Southern blots of Ti plasmid fragments generated by four different restriction enzymes (Fig. 3*b*). The G probe hybridized to *Bst*I fragment 6, *Eco*RI fragment 29, *Sma*I fragment 2 and *Hpa*I fragment 5 (see map in ref. 16), evidence that G is indeed from the left edge of T-DNA. *Eco*RI fragment 29 was recloned from *Bst*I fragment 6, and this recombinant plasmid also hybridized with G weakly (Figure 3*a*). We conclude that fragment G has weak homology with the leftmost edge of the T-DNA portion of the Ti plasmid. Much of G is not Ti plasmid DNA, for its hybridization with the Ti plasmid fragments is poor compared with homologous reactions run simultaneously.

Next we tested fragment G for homology with tobacco DNA. Normal tobacco leaf DNA and BT37 tumour DNA were cleaved with *Eco*RI or *Bst*I and the fragments immobilized by Southern blotting[22]. The cloned G fragment in the vector pBR325 was labelled by nick translation and used as probe. Figure 4 shows that this fragment hybridizes to a series of bands in the tobacco DNA digests. Two other fragments of λPC1 (A and D) cloned in the same vector and used as probes in parallel hybridization studies with tobacco DNA did not hybridize to such bands (data not shown). We conclude that fragment G is homologous to a family of repeated DNA sequences in the tobacco genome. The prominent pattern of *Eco*RI fragments of tobacco DNA to which G hybridizes suggests that this family is a 'clustered repeat'[28,29]. The smaller and simpler *Bst*I cleavage products to which G hybridizes suggest that there are *Bst*I sites internal to the repeat element. The role of such repeated DNA sequences in the eukaryotic genome is unknown. We note no significant difference in the intensity or pattern of bands in the tumour DNA digest compared with normal tobacco leaf DNA digest (Fig. 4 and other data not shown). Thus, the organization and copy number of the repeated DNA family to which G hybridizes is not altered grossly during tumorigenesis. Tumour DNA must contain one altered member of this family—the one into which T-DNA is inserted; however this member is not noticeable in Fig. 4, presumably because of the abundance of unaltered family members.

We conclude that the T-DNA found in BT37, a tobacco crown gall teratoma, is covalently joined at its left edge to plant DNA. Apart from this, the T-DNA fragments that we have isolated from this tumour line appear to be colinear with the corresponding region of the Ti plasmid: no noticeable alterations have occurred in this region of T-DNA whilst in the plant cell. Consistent with the location of T-DNA in the nuclear rather than chloroplast or mitochondrial DNA fraction[21], its left border fragment hybridizes to repeated DNA chracteristic of eukaryotic genomic DNA. It is not clear from this study whether T-DNA is an integral part of a tobacco chromosome in the transformed plant cell; it could constitute an extrachromosomal autonomously replicating DNA element. There is, however,

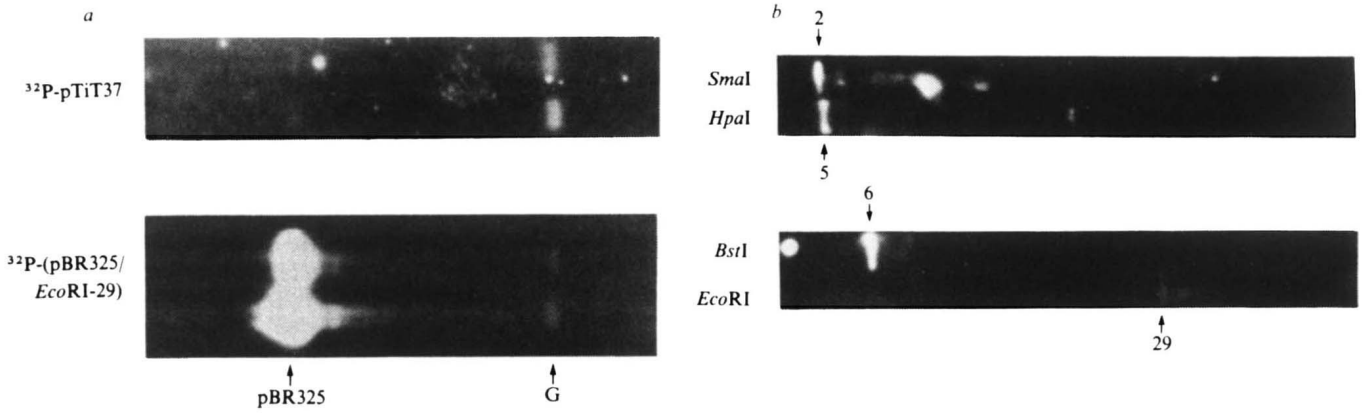

Fig. 3 Evidence for homology between fragment G and pTi T37. *a*, Southern blots containing fragment G and pBR325 (cut from the recombinant plasmid pBR325/*Eco*Ri-G) were prepared in duplicate with the immobilized fragments at two loading levels (0.5 μg for the upper track and 1.0 μg for the lower track in each experiment). Blots were hybridized by the procedure of Thomashow *et al.*[14] with either 1 μg of pTi T37 DNA (2.6×10^7 c.p.m. μg^{-1}) or 0.5 μg of recombinant plasmid pBR325/*Eco*RI–29 DNA (1.66×10^8 c.p.m. μg^{-1}) in 5 ml of reaction medium[14]. Probes were labelled by nick translation[26] with ^{32}P-labelled deoxynucleotide triphosphates. *b*, Southern blots[22] of pTi T37 digests produced by four restriction endonucleases (*Sma*I, *Hpa*I, *Bst*I and *Eco*RI) were hybridized[14] with 0.5 μg of recombinant plasmid pBR325/*Eco*RI-G (1.26×10^8 c.p.m. μg^{-1}). The numbers of the digest fragments to which the probe hybridized are indicated.

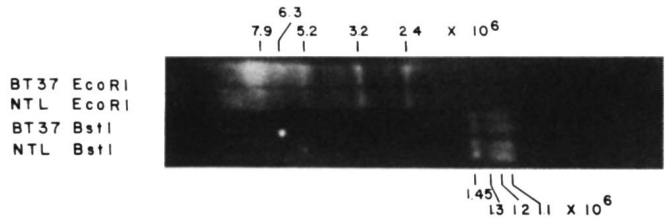

Fig. 4 Evidence for homology between fragment G and tobacco DNA. *Bst*I digests of 2.8 μg of normal tobacco leaf DNA isolated as described elsewhere[34] and of BT37 tumour DNA, in addition to *Eco*RI digests of 5.0 μg of the same two DNAs, were fractionated in horizontal slab gels of 0.7% agarose and 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

Motoyuki Tsuda*, Fumio Tokunaga*‡, Thomas G. Ebrey*, Kwok To Yue†, Jeffrey Marque† & Laura Eisenstein†

* Department of Physiology and Biophysics, 524 Burrill Hall, University of Illinois, Urbana, Illinois 61801
† Department of Physics, Loomis Laboratory, University of Illinois, Urbana, Illinois 61801

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

‡ Present address: Deparment of Physics, Tohoku University, Sendai, Japan.

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