coding properties[13]. All these properties strongly suggest that the unique structure of $tRNA_f^{Met}$ has been specially adapted for its role in the initiation of protein synthesis. The presence of this unusual molecule in close association with tumour virus nucleic acid is therefore somewhat surprising. 4S RNA molecules are required as primers for reverse transcriptase which synthesises viral DNA[19]. Although viral $tRNA_f^{Met}$ seems to be identical to the host cell molecule both in its structure and its ability to function as an initiator tRNA, it is possible that $tRNA_f^{Met}$ is also involved as a primer in the transcription process. It is equally possible that another of the 70S-associated 4S RNA molecules, including the other methionine accepting species, might be involved as a primer. The role of those tRNA species that are not primer molecules is unclear, but they may be necessary to maintain the structure of the genome, or be an example of tRNA modulation[21]. A definite answer to these questions must await further analysis of the structure of the primer molecule and the role of virus-associated tRNAs in the translation of tumour virus messenger RNA.

Contaminating degradation products of the high molecular weight RNA may also be released by denaturation of 70S RNA. Hence, it is difficult to calculate the number of molecules of $tRNA^{Met}$ associated with the 70S RNA. A crude estimate indicates that there may be about one mol of methionine acceptor activity per mol of 70S RNA. Since the amount of free 4S RNA in different virus preparations is variable, a calculation of the amount of free $tRNA^{Met}$ has little meaning. In general, however, there is usually five to ten times more $tRNA^{Met}$ free in the virion than associated with 70S RNA. The significance of the distribution of tRNAs between viral compartments is, as yet, unexplained.

The origin of the viral tRNAs is also uncertain but the similarity in structure and function between host and both free and associated viral methionine accepting tRNAs strongly suggest that the viral tRNAs are of host cell origin and are not virus coded.

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Lateral diffusion of rhodopsin in the photoreceptor membrane

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Rhodopsin undergoes rapid lateral diffusion in the disk membranes of isolated frog and mudpuppy rods. The rate of lateral diffusion is consistent with the rapid rotational diffusion of rhodopsin: both indicate the disk membrane is highly fluid with a viscosity of ~1P.

High-speed flash photometry has shown that rhodopsin undergoes rapid rotational diffusion in the disk membrane of frog rod outer segments[1]. Rotational diffusion about an axis perpendicular to the membrane occurs with a relaxation time about 20 μs at 20°C. Rhodopsin is deeply embedded in the membrane, and this relaxation time indicates that the site it occupies is highly fluid, with an effective viscosity for rotational diffusion comparable with that of a light oil. Rhodopsin molecules appear from X-ray evidence to be distributed in a liquid-like array in the plane of the membrane[2], so they may also be free to undergo rapid lateral diffusion in the membrane. Recently, evidence has been reported for several types of cells that membrane proteins can undergo rapid lateral motion[3–6,6a], and it is clear that a general understanding of molecular events within cell membranes will require more knowledge of the diffusional motions of membrane proteins. We report here microspectrophotometric observations of rapid lateral diffusion of rhodopsin in freshly isolated rod outer segments.

Rod outer segments were obtained by gently shaking retinas dissected under dim red light from the eyes of frog (*Rana catesbeiana*) and mudpuppy (*Necturus maculosus*) which had been dark-adapted for more than 10 h. The rods were shaken into a microchamber containing a standard Ringer solution and examined in a Shimadzu 50L microspectrophotometer (MSP) fitted with a high quantum efficiency photomultiplier (Hamamatsu type R375). Single rods which appeared intact and which lay flat on the bottom of the chamber were selected for observation, and all observa-

tions were completed within 30 min after the rods were isolated. The rhodopsin in isolated rods, once bleached, does not regenerate, hence dim red light was used for selection. focusing, and alignment.

The measuring beam of the MSP was limited by an aperture and a condensing lens to form a rectangle about 2 × 20 μm in cross section. The long axis of the rectangle was aligned with the long axis of the rod and a simple motor-driven 'alternator' optically shifted the rectangular measuring beam back and forth between the two sides of the rod. Thus the absorbance of the rhodopsin on each side of the rod could be compared directly. The wavelength of the measuring beam was set at the absorption peak of the visual pigment: 500 nm for frog, 530 nm for mudpuppy.

With suitable alignment and focusing the absorbance was essentially equal on both sides of unbleached rods, as shown by the first pair of measurements on an unbleached rod at the beginning of each of the two recordings in Fig. 1. The alternator was then stopped momentarily and the intensity of the measuring beam was increased about 1,000-fold to bleach some pigment on one side of the rod. The exponential decrease in absorbance during the bleach was recorded, and then the intensity was dropped to the original level and the alternator turned on again. Figure 1 shows that immediately after the bleach the absorbance on the unbleached side was little changed, but there was a marked drop in absorbance on the bleached side. Within the next few seconds, however, the absorbance of the unbleached side decreased while that on the bleached side increased, and within less than 1 min the absorbance of the two sides became equal, reaching a final level midway between that of each side immediately after the bleach. This is expected if rhodopsin molecules can diffuse laterally in the membrane: the total pigment present after the bleach remained constant and only its distribution changed, approaching uniform distribution with an exponential time course. The half-time of the final approach to uniform distribution has been observed in more than ten rods each for frog, and mudpuppy. At 20°C, it was 35 ± 7 s for frog rods 8 μm in diameter, and for mudpuppy rods 23 ± 5 s for rods 12 μm in diameter. In addition, half-times measured at temperatures in the range 17°–24°C decreased with temperature with a Q_{10} of 3.7 ± 0.7 in frog rods and 3.1 ± 1.4 in mudpuppy rods (apparent energy of activation 24 and 21 kcalories mol^{-1} respectively).

Control experiments were performed to help ensure that the observed redistribution of rhodopsin was the result of diffusion of the entire rhodopsin molecule and not simply energy transfer between chromophores or chromophore transfer between protein molecules (opsin). As Fig. 2 shows, there was no redistribution of rhodopsin in rods fixed with glutaraldehyde before bleaching. In contrast, fixation with the same amount of formaldehyde had no effect on the rate of redistribution. These results are consistent with the findings that glutaraldehyde fixation blocks rotational diffusion of rhodopsin whereas formaldehyde fixation has no effect[1,7]. Glutaraldehyde seems to form crosslinks between rhodopsin molecules whereas formaldehyde does not, for rhodopsin can be extracted with digitonin after formaldehyde fixation but cannot be extracted after glutaraldehyde fixation[7]. Chromophore transfer between opsin molecules seems unlikely given the nature of the covalent linkage between retinal and opsin, but as a further check experiments were performed in the presence of 50 mM hydroxylamine which rapidly converts free chromophore to the stable compound retinal oxime (λ_{max} = 363 nm)[8]. When one side of the rod was bleached, all chromophores from the bleached pigment converted immediately to retinal oxime. The redistribution of rhodopsin, however, proceeded as usual, no further oxime was formed and the total remaining pigment stayed constant. Thus the chromophores of the unbleached pigment did not react with hydroxylamine, as might be expected if transfer of free chromophores accounted for the redistribution of pigment. Energy transfer between rhodopsin molecules is also improbable since the distance between chromophores is large[9,10], and the fluorescence yield is low[11]. Moreover, energy transfer cannot explain the redistribution of pigment since pre-lumi rhodopsin, the first photoproduct after bleaching, forms within a few picoseconds at room temperatures[12]. Thus energy transfer between rhodopsin molecules, if it occurs, would have to take place on a sub-picosecond scale, whereas the observed redistribution takes place in seconds. Finally, as a further test for the plausibility of energy or chromophore transfer, we investigated whether redistribution of pigment occurs along the axis of the rod. Rods were bleached with a black and white 'grating' pattern which alternated in intensity along the axis of the rod with a repeat distance of 8 μm. Rhodopsin absorbance was monitored by scanning the length of the rod with the measuring beam in this case being limited to a circular spot 4 μm in diameter. After the bleach the resulting 'grating' pattern in absorbance persisted unchanged for as long as stable recordings could be made (~10 min)[13]. Thus the redistribution of rhodopsin seems to be confined to the plane of the disk membrane, since no migration along the length of the rod was detected. This result is consistent with the autoradiographic investigations reported by Young and his co-workers[14] which show that once rhodopsin is inserted into the membrane of a disk, it does not migrate away from that disk. From all these considerations, lateral diffusion of entire rhodopsin molecules within the disk membrane seems to be the only reasonable explanation for the redistribution of pigment shown in Fig. 1.

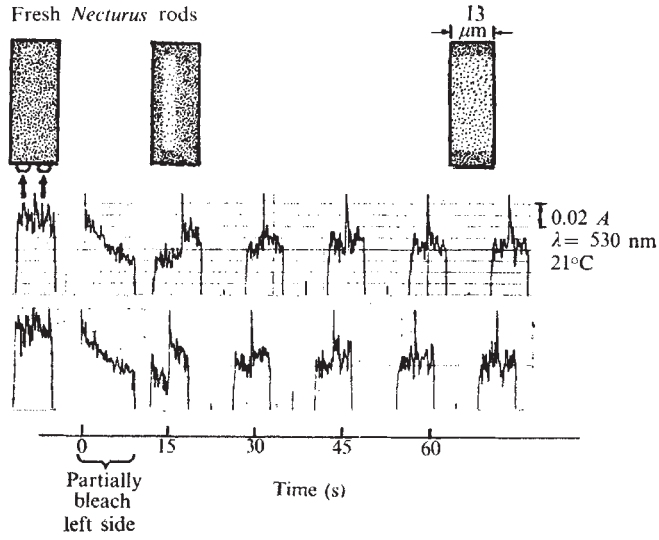

FIG. 1 Lateral diffusion of rhodopsin in the disk membrane. The diagrams of a rod depict the pigment distribution corresponding in time with the absorbance measurements shown below. The arrows indicate the location on the rod at which each absorbance measurement was made. Recordings made from two different rods are shown to give an indication of the repeatability of the measurements. In each experimennt the chart recorder was run continuously, as shown by the time base. The alternator also ran continuously except during the bleach. The records thus consist of a repeated pattern in which absorbance measurements were made first on the left side, then the right side of the rod. Between each pair of measurements baseline measurements were also made to ensure that no drifts occurred (for clarity, these were omitted from the figure). The spikes on the traces were caused by switching transients in the alternator. The diameter of a disk is essentially equal to the width of the rod, and the width was measured in the MSP after completing each experiment. In a separate experiment described in the text[13], it has been shown that no diffusion of rhodopsin occurs along the length of the rod. Thus in the diagrams of a rod, the rhodopsin concentration is depicted as unchanged at the ends, where no bleaching occurred.

The half-time for redistribution of rhodopsin did not seem to depend on the amount of uniform bleaching which in some cases occurred before starting the diffusion experiments. Redistribution proceeded as usual even when two-thirds of the pigment was previously bleached. Since rhodopsin molecules occupy a major fraction ($\sim\frac{1}{3}$ to $\frac{2}{3}$) of the total membrane area, redistribution can only occur if rhodopsin molecules displace bleached molecules (opsins). Thus opsin molecules appear to have essentially the same diffusion constant.

The diffusion constant, D, for lateral diffusion of rhodopsin in the disk membrane can be obtained by solving the two-dimensional diffusion equation for the closed boundary defined by the edge of the disk. The result is, to first order of accuracy,

$$D = (0.69bL^2)/(\pi^2 t_{1/2}) \quad (1)$$

where L is the diameter of the disk $t_{1/2}$ is the half-time with which pigment distribution approaches final uniform distribution, and b is a geometry factor included to account for the irregular boundary of the disk, especially the fissures which extend well in toward the centre. To obtain the value for b, we performed an analogue experiment in which the rate of diffusion of heat was observed in thin sheet-metal disks with the boundary cut to simulate the shape of typical disks in frog and mudpuppy rods as observed by electron microscopy[15]. The value obtained was 2.7 ± 1.0 for frog disks which have numerous deep fissures, and 0.9 ± 0.2 for mudpuppy disks which have numerous but very shallow fissures. For these values of b, and the observed values of L and $t_{1/2}$, equation (1) yields

$$D = (3.5 \pm 1.5) \times 10^{-9}\ \mathrm{cm^2\,s^{-1}} \quad \text{for frogs}$$

$$D = (3.9 \pm 1.2) \times 10^{-9}\ \mathrm{cm^2\,s^{-1}} \quad \text{for mudpuppy}$$

Viscosity of membrane

The diffusion constant is a measure of diffusion rate, depending not only on the viscosity of the medium in which diffusion occurs, but also on the size of the diffusing particle. Therefore, even within a given medium diffusion constants for various particles cannot be compared directly. However, as Einstein showed, the viscosity of a solution can be predicted by observing the diffusion constant of a large solute molecule in the solution and by assuming that in undergoing thermal motions the solute molecule experiences a viscous drag given by Stoke's law[16]. Later theoretical treatments have shown that only a minor numerical correction is needed to extend this procedure to molecules with dimensions comparable with those of the solvent molecules[17]. Thus, although viscosity is usually observed macroscopically, it is a property of solutions which can be observed equally well using small molecules, even molecules as small as the solvent molecules. In membranes, diffusion constants have been observed for various probes using different techniques. It seems reasonable to expect that regardless of the size and shape of a probe molecule or structure, as long as a significant portion of it spans a major fraction of the membrane thickness, its rate of diffusion in the plane of the membrane, when properly interpreted, should yield the same viscosity. Hence diffusion constants for different probes can be compared by comparing the predicted viscosities.

The rotational diffusion of rhodopsin, as previously reported[1], indicates the viscosity, η, of the membrane site occupied by frog rhodopsin is about 2 P at 20°C. The calculation was based on the relationship derived by Einstein for Brownian rotation of a spherical particle about one axis in a isotropic medium. A similar calculation can be made for lateral diffusion by using the Einstein-Sutherland diffusion equation[17]

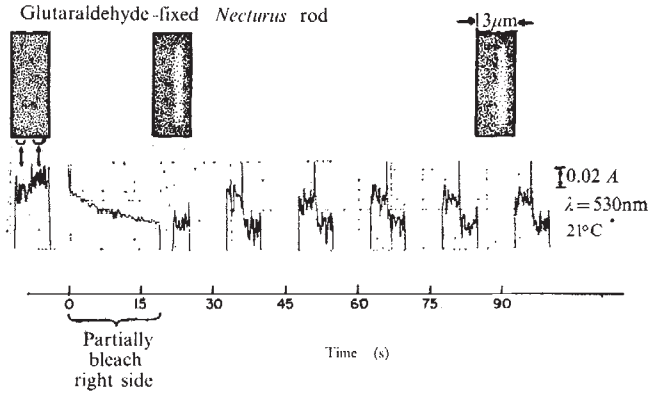

FIG. 2 Glutaraldehyde fixation blocks lateral diffusion of rhodopsin in rod disks. Isolated mudpuppy rod outer segments were soaked in 1% glutaraldehyde in 0.1 M phosphate buffer (*p*H 7.1) for 15 min before starting the experiment. The diagrams again depict the distribution of pigment at the time of measurements shown below. Although the recording procedures were the same as described in Fig. 1, after glutaraldehyde fixation the unequal distribution produced by the bleaching persisted as long as stable recordings could be made. It may be noted in this experiment that stray light caused negligible bleaching on the unbleached side. Hence in the first recordings after the bleach in Fig. 1, the small decrease in absorbance on the unbleached side is the result of some diffusion having already occurred.

$$\eta = (kT)/(a\pi Dr) \quad (2)$$

where k is the Boltzman constant, T is the absolute temperature, r is the radius of a spherical diffusant, and $a = 6$ for diffusants large compared with the solvent molecules, while $a = 4$ for diffusants with dimensions comparable with the solvent molecules. Recent evidence indicates that the rhodopsin molecule spans the membrane[18,19] (see also discussion of ref. 13) and on the basis of molecular weight[20] and X-ray evidence[9], the effective radius of rhodopsin for lateral diffusion is probably in the range 15–30 Å. This is significantly larger than the effective radius of phospholipids for lateral diffusion. In this case equation (2) yields a viscosity of about 1–4 P. This is in the same range indicated by the rotational diffusion of rhodopsin, 0.7–6 P[1]. Thus if it is assumed that rhodopsin has an effectively circular cross section, the viscosity of the disk membrane indicated by lateral diffusion is in good agreement with the viscosity indicated by rotational diffusion. This is not surprising since lateral diffusion in the membrane and rotational diffusion about an axis perpendicular to the membrane surface both depend on the same 'slippage' motion between the surface of the rhodopsin molecule and the molecules in the surrounding membrane environment. Moreover, as we show when, each rhodopsin molecule undergoes numerous collisions with other rhodopsin molecules during its rotational relaxation time. Thus a rhodopsin molecule samples essentially the same membrane environment for both its rotational and translational motions.

Evidence that other membrane proteins may also exist in such highly fluid environments has been reported by Frye and Edidin who observed, using fluorescent antibodies, the translational diffusion of cell surface antigens in membranes of mouse and human cell hybrids[3]. The rate at which the surface antigens diffuse along the cell surface indicates the membrane viscosity for lateral diffusion of these proteins is on the order of 1–10 P at 20°C. (For a recent discussion of membrane fluidity, see ref. 21.) As pointed out previously[1], lipid soluble spin labels seems to encounter a viscosity of 0.1–1 P at 20°C in the lipid phase of mitochondria and nerve axons[22,23]. Spin label studies by Scandella *et al.* on lateral diffusion of phospholipids in sarcoplasmic reticulum

vesicles from rabbit muscle[24] have shown the diffusion constant to be about 6×10^{-8} cm^2 s^{-1} at 40°C. Using the Einstein-Sutherland equation for self-diffusion, a reasonable extrapolation of this result to 20°C indicates that membrane viscosity falls within 0.5–5 P. Thus inspite of differences in probes and techniques the viscosity of each of these membranes seem to lie within an order of magnitude of 1 P. That is, the viscosity for lateral motion in each membrane is between 10 and 1,000 times the viscosity of water. Differences in the composition of each membrane and in the membrane layer sampled by the probe molecule, together with possible systematic experimental errors, can account for the variations in viscosity so far observed. Rhodopsin can now be incorporated into model membrane systems[25–27] with which it should be possible to study quantitatively the effects of each membrane component on membrane viscosity.

Rhodopsin collision frequency

X-ray evidence indicates that the average nearest-neighbour distance between rhodopsin molecules in the disk membrane is about 70 Å (refs 9 and 10). The average time τ, between collisions with another rhodopsin can be estimated with the following equation in which s is the distance rhodopsin travels between collisions:

$$s^2 = 4D\tau.$$

If the effective diameter of rhodopsin is about 45 Å (ref. 9), then s is 25 Å, and the average time between collisions is about 4 μs at 20°C. This is considerably less than the 20 μs relaxation time for rotational diffusion of rhodopsin, and indicates that the collision frequency between rhodopsin molecules is in the range 10^5–10^6 collisions per second.

The rapidity of rhodopsin-rhodopsin collisions points out an important consequence of the highly fluid nature of cell membranes, namely, the potential rapidity of reactions between membrane proteins. By partitioning into the membrane, protein molecules may achieve much higher effective concentrations and can in addition maintain favurable orientations as a result of protein-lipid interactions. Thus, since the viscosity for lateral motion in the membrane exceeds that of water by only a factor of 10–1,000, reaction rates both within and on the surface of a mebrane may potentially exceed by many orders of magnitude the rates which would occur if the reactants were dispersed throughout the aqueous phase.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Limits on variation of G from clusters of galaxies

If G decreases by a sufficient amount a cluster of particles will expand. In some cases the particles may have sufficient velocity to escape from the cluster as the gravitational binding decreases. Since it is known that clusters of galaxies and globular clusters currently exist with finite dimensions, limits can be set on the magnitude of the variation of G. In order to set a limit on the rate of change of the gravitational constant ($\dot{G}/G$), the dynamical effects on an isolated cluster of galaxies caused by non zero values of $\dot{G}/G$ were studied numerically. For a range of initial conditions, the maximum rate of change in G was determined which would still allow the existence of clusters of galaxies at the current epoch. A similar study was made for globular clusters and the results were found to be comparable.

The gravitational fields of both the cluster of galaxies and the globular cluster were simulated by Plummer potentials[1], and the effect on a particle (galaxy/star) of various rates of G variation were observed. The distribution of galaxies in a cluster at the time of formation is unknown, but if gravitational forces were dominant, a Plummer potential should provide a reasonable approximation to the cluster's field. The Plummer potential was initially designed to simulate the potential field of a globular cluster, and has the form

$$\Psi = \Psi_0/[1 + (r/r_0)^2]^{1/2}$$

where $\Psi_0 = M_0G/r_0$ and r_0 and M_0 are chosen using a method given by Henon[2]. We assumed that both the globular