some at some stage in every cycle. However, given that errors in minichromosome transmission are rare, it seems more likely that the minichromosome behaves differently than the endogenous chromosomes at some stage of the cell cycle in only a small fraction (0.7%) of the cell divisions.

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Intracellular Topography of Rhodopsin Bleaching

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In a vertebrate eye, the photoreceptor cells are aligned so that most of the light passes through them lengthwise. At the light-transducing outer segment region of the photoreceptor, photons are absorbed in a time-varying, spatially dependent fashion. Because the transduction event is spatially localized around the site of photon absorption, the spatiotemporal patterns of light absorption in outer segments are an important receiver input characteristic. This aspect of receptor biophysics has now been measured; the results were consistent with a theoretical model proposed for bleaching of a pigment in an unstirred layer.

THE ROD PHOTORECEPTORS IN VERtebrate eyes are oriented toward the incoming light (*1–3*). As a result, most of the light passes axially through the light-absorbing region, the rod outer segment (ROS). This feature, along with the highly organized ultrastructure of the ROS (*4*), plays an important role in rod function.

The ROS contains numerous rows of vesicular disks, in register. The disk membranes are packed with the photosensitive rhodopsin molecules. Although rhodopsin moves freely within the disk membranes (*5–8*), it does not "hop" from disk to disk (*5*).

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In a dark-adapted ROS, all disks contain the same concentration of rhodopsin (*9*). Axial passage of the light will bleach more pigment at the base, near the inner segment, than at the sclerad tip. Initially, the axial pattern of bleaching will show an exponential decrease, but because bleaching changes the light-filtering characteristics of the disk surfaces, the patterns of light absorption change with time in the light. This phenomenon can be demonstrated in single ROSs with microspectrophotometry.

The tropical toad *Bufo marinus* was used in this study because of the large size of its rods (6 by 60 μm ROSs were not uncommon). Toads were kept on a 12:12 light: dark cycle but were dark-adapted for 12 to 14 hours before use. During the final 30 minutes of dark adaptation, the toad was immersed in a 10% aqueous solution of MS-222 (Sandoz). The eyes were enucleated under dim red light, with care being taken to avoid disruption of the physiological optics. One eye from each animal was placed in Ringer solution (*10*), covered, and refrigerated. The other eye was exposed to a diffuse field of 500-nm monochromatic light (*11*) for several minutes. Then, the anterior segment was quickly removed, and 50 m*M* NH_2OH in Ringer solution was poured into the eyecup. After 2 to 4 minutes, the eyecup was transferred into 10 m*M* NH_2OH. The MS-222, the enucleation, and the hydroxylamine served to "freeze" the bleaching pattern in the ROSs by inhibiting rhodopsin regeneration (*12–14*) (the physiological mechanism whereby the animal restores its bleached rhodopsin to the prebleached state). Small pieces of retina were cut off and dabbed onto a polylysine-coated cover slip (*15*) to isolate single ROSs. The cover slip was encircled with silicone oil, and another, untreated cover slip was placed on top. This preparation was mounted onto the stage of a photon-counting, single-beam, MacNichol-type microspectrophotometer (*16*). With a 2 by 6 μm measuring beam, absorbance spectra were measured at various distances from the base of each outer segment.

After a light exposure, an ROS always contained less rhodopsin at its base than at its tip (Fig. 1). Measurements made on ROSs from the other eye, which had been kept in the dark, were used to estimate the dark-adapted concentrations of rhodopsin (c_0 values) in the light-exposed ROSs. This allowed the data to be expressed in terms of dimensionless numbers: the fraction of unbleached rhodopsin remaining versus normalized distance from the base (Fig. 2A). The observed, intracellular distributions of rhodopsin remaining were fitted with the relation (*17*) (Fig. 2, A and B):

$$\frac{c_l}{c_0} = \frac{\exp(\alpha c_0 l)}{[\exp(\alpha c_0 l) + \exp(\alpha\gamma I_0 t)] - 1} \quad (1)$$

where c_l is the concentration of rhodopsin at distance l from the base, α is the molar

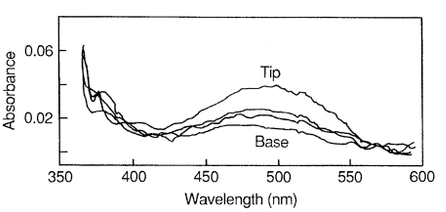

Fig. 1. Absorbance spectra from a bleached ROS. Each spectrum was measured at a different axial position in this ROS. The amplitude of each curve is proportional to the concentration of unbleached rhodopsin. The amplitude differences, which were most noticeable at 503 nm, the lambda max of rhodopsin, indicated that the rhodopsin concentration increased from base to tip. All curves approached a baseline level near 600 nm.

extinction coefficient of rhodopsin, γ is the quantum efficiency of bleaching, I_0 is the photon flux at the base of the ROS, and t is the duration of light exposure. For the derivation of Eq. 1, it was assumed that the light destroyed the pigment and that the pigment did not diffuse through the medium. The region below the curve represents the distribution of rhodopsin that was not bleached during the light exposure, whereas the region above represents the pattern of bleaching.

These measurements reveal the way in which toad ROSs receive the light stimulus in situ. A photon absorbed by a rhodopsin molecule results in the localized closure of Na^+ channels at the plasmalemma; one photon results in the closure of the channels in a ring around the rhodopsin that absorbed it (*18, 19*). As the light intensity increases, more rings of plasmalemma are recruited. The model for the spatial pattern of bleaching, as stated in Eq. 1, helps to quantify the recruitment aspect of the photoreceptor cell response. It may then become possible to predict the responsivities of single rod photoreceptor cells during illlumination through the intact physiological optics of the eye.

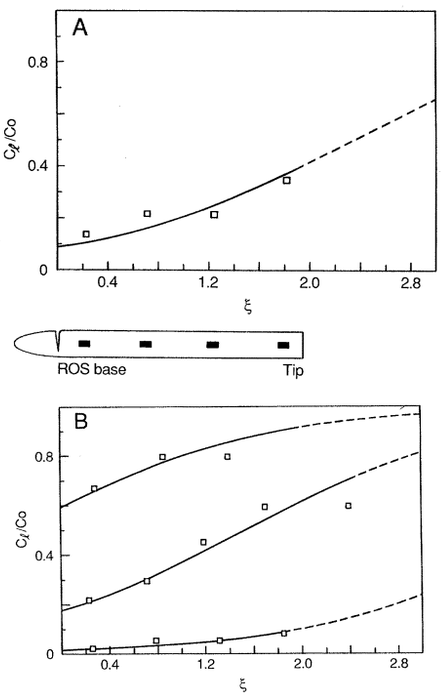

Fig. 2. Fraction of rhodopsin remaining as a function of ξ. The data are plotted as squares. The lines represent the best fits to Eq. 1. The lines are continuous to the end of the ROS; broken line extensions are included for illustrative purposes. (**A**) Each square represents a curve from Fig. 1, plotted here in terms of the fraction of the dark-adapted rhodopsin content remaining as a function of $\xi = \alpha c_0 l$, normalized distance from the base. In the ROS drawn beneath the graph, filled rectangles show where the absorbance spectra were measured. The photon dosage received by the ROS was 1.49×10^8 photons per square micrometer (*20*). (**B**) The bleaching patterns after light exposures of different strengths are illustrated by the results from three ROSs. From the top curve downward, the photon dosages were 3.26×10^7, 1.07×10^8, and 2.59×10^8 photons per square micrometer (*20*), respectively.

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A Novel Putative Tyrosine Kinase Receptor Encoded by the *eph* Gene

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Growth factors and their receptors are involved in the regulation of cell proliferation and also play a key role in oncogenesis. In this study, a novel putative kinase receptor gene, termed *eph*, has been identified and characterized by molecular cloning. Its primary structure is similar to that of tyrosine kinase receptors thus far cloned and includes a cysteine-rich region in the extracellular domain. However, other features of the sequence distinguish the *eph* gene product from known receptors with tyrosine kinase activity. Thus the *eph* protein may define a new class of these molecules. The *eph* gene is overexpressed in several human carcinomas, suggesting that this gene may be involved in the neoplastic process of some tumors.

SEVERAL VIRAL ONCOGENES AND CELLULAR proto-oncogenes comprise a gene family whose products exhibit tyrosine-specific protein kinase activity and are associated with the plasma membrane (*1*). Such features are shared by several genes encoding polypeptide hormone receptors, including epidermal growth factor receptor (EGF-R-1)/c-*erb*B (*2*), EGF-R–related receptor (EGF-R-2)/*neu*/*erb*B-2 (*3*), insulin receptor (IR) (*4*), insulin-like growth factor–I receptor (IGF-I-R) (*5*), platelet-derived growth factor receptor (PDGF-R) (*6*), and colony-stimulating factor–1 receptor (CSF-1-R)/c-*fms* (*7*). In addition, several viral oncogenes appear to encode mutated tyrosine kinase receptors whose cellular homologs have not yet been identified. The importance of tyrosine kinase activity in both normal and abnormal growth regulation suggests that additional tyrosine kinases also exist.

We searched a human genomic library for gene sequences homologous to the tyrosine kinase domain of the viral oncogene v-*fps* (legend to Fig. 1A). A 12.5-kb Eco RI fragment (Cl-7) was found to be homologous to the v-*fps* probe under relaxed hybridization conditions (Fig. 1A). The 0.6-kb Hind III–Sst I fragment (HS fragment) from Cl-7, including the sequence homologous to the v-*fps* probe, was used as a probe in subsequent hybridization experiments. In an erythropoietin-producing human hepatocellular carcinoma cell line (ETL-1), the

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