transfer the translocated chromosome into a number of high-yielding durum varieties in order to produce a high-yielding durum wheat with acceptable bread-making quality. It might be feasible to extract other desirable characteristics from established hexaploid-wheat varieties that carry translocations involving the D-genome by crossing them to durum-wheat varieties and by isolating the desirable characteristics in the extracted tetraploid.

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Membrane Origin of the Fast Photovoltage of Squid Retina

Abstract. *When a bright light flash is absorbed by a small region in the outer segments of squid photoreceptors fixed in glutaraldehyde, a brief pulse of membrane current flows locally. The passive spreading of this current along the outer segments produces the photochemical component of the "early receptor potential." The source of the current lies electrically in parallel with the cell membranes and perhaps is located within them. Fixation with glutaraldehyde apparently does not reduce the resistance of the cell membrane to less than 5 percent of its value in live cells.*

Intense flashes of light produce small transretinal voltages of very short latency ("early receptor potentials") in the eyes of many different animals (*1, 2*). Both in vertebrate rods (*2*) and in squid photoreceptors (*3*), photochemical reactions of rhodopsin underlie the generation of these fast photovoltages (FPV's), but the mechanism producing electrical events from chemical reactions is not yet understood. Since drastic changes in the nature and concentration of the electrolytes bathing the retina and even fixation with glutaraldehyde or exposure to temperatures below 0°C do not abolish FPV's (*3–5*), it is generally thought that changes in the membrane permeability, such as produce ordinary electrophysiological responses like the electroretinogram, are not involved. Instead, it has been suggested that the FPV might be a direct external sign of changes in the distribution of charges in rhodopsin molecules as they undergo photochemical reactions (*4, 6*). We now report a test of this idea which leads us to conclude that the photochemical component of the FPV in squid photoreceptors is indeed a charge displacement in the electrical capacitance of the plasma membranes of the outer segments. The thermal component of the FPV previously described (*3*) is small enough in the present experiments to be negligible.

Live squid retinas were prepared as described previously (*7*), all manipulations being carried out in darkness or under infrared illumination with the aid of image converters. A retina was attached by suction to a membrane filter (Millipore Type HA) and cut into rectangular blocks (300 by 700 μm) by cuts made parallel to the long axes of the photoreceptors. A single block consisting of photoreceptor cells and all other retinal layers was fixed for 20 minutes at 0°C in 5 percent buffered glutaraldehyde in seawater. The block was then washed with cold seawater and placed on the cooled stage of an infrared microscope, with the long axes of the photoreceptors parallel to the horizontal plane of the stage. Fluid was blotted away until the block was insulated by moist air on five sides and by the supporting cover slip on the sixth. Light from a xenon flashtube was freed of wavelengths less than 520 nm and focused into a narrow beam whose cross section was a rectangle 16 μm wide and 700 μm long. The beam was directed downward through the block so that the long axis of the rectangle and the direction of propagation of the light were both normal to the long axes of the outer segments. All outer segments were thus illuminated at the same distance from their inner ends. With this arrangement, the light-induced voltage gradients in the interstitial spaces of the block were found to be negligible in all directions save that parallel to the long axes of the photoreceptors. The locations of sources and sinks along the cells could therefore be found by analyzing the voltage gradients in the context of a one-dimensional boundary-value problem.

When two micropipettes filled with seawater and connected to a differential amplifier were placed on the upper surface of the block with their tips in a line parallel to the long axes of the outer segments, the interelectrode voltage waveforms (Fig. 1) produced by repeated flashes and summed in a transient averaging computer closely resembled the externally measured FPV of the light-adapted retina (*3*). But the sizes and polarities of the responses varied according to the location of the light beam relative to the electrode pair. When the beam fell between the black pigment layer and the lower electrode, small waves whose fast phases were in the upward direction (electrode A positive) were seen. As the slit was advanced upward in steps toward the inner ends of the outer segments, the voltage difference between the pipettes first increased, then diminished and reversed in sign, growing to maximum size with the beam at A and finally diminishing as the beam approached the inner limiting membrane (ILM) at the top of the figure. The beam position where reversal of the voltage transients occurred shifted with the electrodes if the latter were moved along the block parallel to the long axes of the outer segments. Thus the pattern of voltage differences depended mainly on the relative positions of light and electrodes, not on their absolute position on the block. The same dependence of FPV form on stimulus position was also seen when the electrodes were thrust into the block, when the cell bodies of the photoreceptors were cut away from the block, or when blocks of live retina were used.

This result is not consistent with any theory which places the current generators responsible for the FPV within the bulk of the interstitial spaces; in the

cytoplasm of the outer segments; or in structures isolated from the plasma membrane, such as mitochondria or the internal sacs of vertebrate rods. It can be accounted for by a localized electric displacement in the illuminated parts of the plasma membranes of the photoreceptors. Suppose that rhodopsin or some molecular system associated with it were to undergo a sudden change in its charge distribution due to a photochemical reaction. If the resulting electrical dipoles were oriented so that their positive ends were preferentially directed toward one surface of the retina (Fig. 2a), a voltage difference would indeed be seen between two electrodes placed at A and B, but it would be largest when the illumination falls between the electrodes, and no inversion of the response would occur as the beam was swept along the outer segments. Dipoles located in the interstitial spaces would give the same result. On the other hand, if dipoles normal to the cell surfaces were produced in a molecular system astride the plasma membranes of the outer segments (Fig. 2b), the transmembrane potentials would change, causing longitudinal currents to flow inside and outside the cells over a region whose length would be comparable with the electrical space constants of the outer segments. This current would produce a longitudinal voltage gradient in the interstitial spaces like that along one conductor in a noninductive coaxial cable with a localized current source across the dielectric at the point where the light falls. The interelectrode voltage would be maximum with the light beam at A, minimum when it was at B, and would reverse at some intermediate point, just as in Fig. 1.

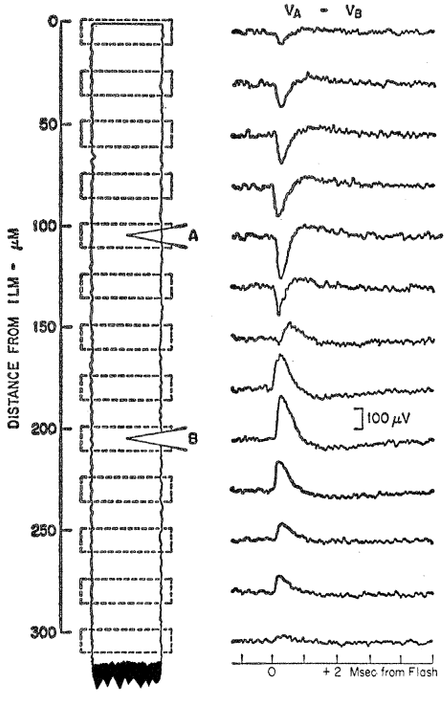

Fig. 1. Fast photovoltages produced by an isolated block of squid retina fixed in glutaraldehyde. Waveforms on right are voltage differences between two micropipette tips placed at A and B on the layer of outer segments at different distances from the inner limiting membrane (ILM) in response to 100-μsec flashes delivered to the outer segments at positions shown at left. Upward deflections indicate A positive relative to B. Each oscilloscope trace is the average response to 16 flashes. The retinal block was 750 μm long and 300 μm thick so that the diagram greatly foreshortens the slice length. The black pigment layer which divides outer segments from cell bodies of photoreceptors is at bottom. Stimulus: xenon flash freed of wavelengths below 520 nm by a Wratten-15 filter. Exposure equivalent to about 10^{16} photon cm^{-2} at 520 nm. Slit: width about 16 μm, length 800 μm.

When the predictions of the membrane polarization hypothesis of Fig. 2b are compared quantitatively with the experiment, three conclusions are evident. First, the plasma membranes of the photoreceptors are the only known structures in the layer of outer segments long enough to account for the ability of flashes falling near the inner limiting membrane in Fig. 1 to produce such relatively large voltage differences between electrodes A and B. Second, the electrical space constants of the outer segment membranes must be at least 100 μm even after fixation with glutaraldehyde. Since living photoreceptors have space constants of only about 400 μm (*7*), fixation does not reduce the membrane resistance of the cells more than 20-fold. Third, the charge displacement responsible for the FPV must be located in the plasma membrane itself and not merely in some structure electrically in series with it, because if the horizontally oriented dipole shown in the membrane in Fig. 2b were detached and moved inside or outside the cell, the eddy currents set up by the polarization would be confined almost entirely to the region immediately around the dipole, and the resulting voltage gradients would be small except within the illuminated zone of the outer segments.

How, then, can the ordinary FPV produced by illumination of the entire outer segments and recorded with external electrodes (the so-called "early receptor potential") be explained by the membrane polarization model? Clearly the structural feature responsible for the transretinal voltage under such conditions must be the asymmetry of the photoreceptor cells themselves, with their current-producing outer segments at only one retinal surface (*8*). Near the inner limiting membrane, the outer segments have closed ends so that a polarization of the plasma membrane can relax only by longitudinal current flow into the deeper layers of the retina, thus producing a net transretinal voltage.

Situating the current source in the plasma membranes of the outer segments not only explains the spatial

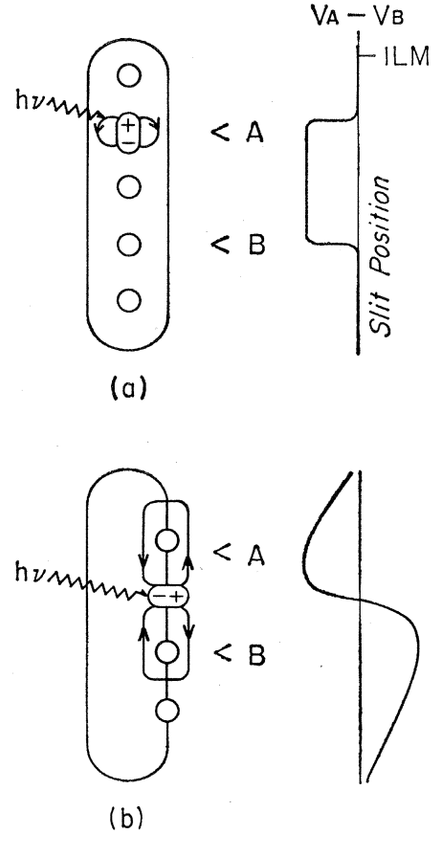

Fig. 2. Two models for the generation of fast photovoltages in a cellular array from intramolecular charge displacements in the individual cells. A hypothetical cell containing unpolarized (circles) and photochemically polarized (oval) rhodopsin molecules is shown at left. The longitudinal voltage gradient produced in the interstices of an array of such cells closely packed side-by-side by currents arising from the charge displacements is at right. (a) Photochemically induced molecular dipoles longitudinally oriented inside each cell. (b) Dipoles located in each cell's plasma membrane and oriented normal to it.

characteristics of the FPV in the squid retina, but it can account for three other observations as well. These are (i) Smith and Brown's (*9*) finding that the short-latency photovoltage of *Limulus* photoreceptors reverses sign when the recording microelectrode is thrust through the receptor cell membrane, (ii) Cone and Brown's report (*10*) that the vertebrate FPV disappears when rhodopsin in rods is disoriented by heat treatment, and (iii) our observation (*3*) that the squid FPV is similar in size and shape to that of vertebrates, even though the cell membranes are oriented and folded quite differently in the two types of retina. What physical process produces the membrane current? The short latency and simple kinetic form of the FPV (*3*) favor the idea that the plasma membranes of the outer segments themselves contain rhodopsin and that a photochemically induced change in its molecular charge distribution constitutes a change in the charge stored in the electrical capacitance of the membranes. For the biphasic FPV's described in our work, the charge displacements are such as to require the membrane's external surface to become relatively positive to its internal surface when rhodopsin is converted to acid metarhodopsin and relatively negative when the latter is reconverted to rhodopsin by the flash.

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11. We thank Dr. R. A. Cone for many stimulating discussions and the staff of the Marine Biological Laboratory, Woods Hole, Massachusetts, for their hospitality during the course of this work.

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Hexokinase Isoenzymes in Human Erythrocytes

Abstract. *The electrophoretic mobility of hexokinase from human erythrocytes and other tissues was studied with a new method that depends on the fluorescence of reduced nicotinamide-adenine dinucleotide phosphate for detecting enzyme activity on starch gel. The hexokinase of cord-blood erythrocytes has slightly different electrophoretic properties from that of adult red cells. Type I enzyme is split into type I_A and type I_F; the latter is more intense in cord blood; in hemolyzates of adult blood, the activity of the two bands is usually about equal. No type II enzyme was found in cord blood. The double type I band was present in red cells from adult rabbits.*

Four hexokinase isoenzymes found in rat tissues (*1–3*) have been numbered I, II, III, and IV in increasing order of electrophoretic mobility at *p*H 8.6 (*1*).

After analyzing the electrophoretic mobility of human erythrocyte hexokinase, Holmes *et al.* (*4*) concluded that normal adult erythrocytes contained only type I and type III enzymes. Red cells from newborns, infants, and adults with persistence of fetal hemoglobin contained an electrophoretically distinct hexokinase that moved somewhat faster than the type I enzyme detected in adult erythrocytes. This was identified as type II enzyme, although it moved more slowly than the type II enzyme of rat liver.

Exact localization of hexokinase when tetrazolium dyes are used in staining is difficult because of the relatively low activity of hexokinase in red cells and other tissues and because of nonspecific reduction of tetrazolium dyes by 2-mercaptoethanol incorporated in the gel to preserve hexokinase activity. In our study after electrophoresis, fluorescence of reduced nicotinamide-adenine dinucleotide phosphate (NADP) was used to reevaluate these findings. Apparently, the enzyme in red cells of newborns (*4*) does not coincide with type II enzyme of either rat liver or human tissues. Rather, comparison of the electrophoretic mobility of this enzyme with type II isoenzyme at different hydrogen-ion concentrations and in different human tissues indicates that it is a separate hexokinase isoenzyme, which we have designated I_F.

We studied human adult and cord-blood hemolyzates and extracts of other human, rat, and rabbit tissues, including leukocytes, liver, muscle, heart, kidney, and fat. Crude extracts were prepared in a 0.01*M* phosphate buffer, *p*H 7.0, containing 5 m*M* 2-mercaptoethanol, 5 m*M* neutralized sodium ethylenediaminetetraacetate (EDTA), and 10 m*M* glucose (*4*). Hemolyzates and leukolyzates were made from one volume of this medium per milliliter of packed, washed cells, frozen and thawed three times, and centrifuged at 30,900*g* for 30 minutes at 4°C. Tissue homogenates were prepared with 1 ml of medium per gram of fresh tissue and were centrifuged under the same conditions. Starch-gel electrophoresis was performed for 4 hours at 16 to 24 volts per centimeter in a horizontal system with a buffer containing 10 m*M* tris(hydroxymethyl)aminomethane (tris), 0.5 m*M* EDTA, 5 m*M* 2-mercaptoethanol; the *p*H was adjusted with HCl or with H_3PO_4 over the range 7.85 and 9.6. In the electrode compartment, the buffer, except for the 2-mercaptoethanol, was ten times more concentrated. Some experiments were made at *p*H 7.5 with 0.01*M* potassium phosphate buffer for the gel and 0.1*M* phosphate buffer in the electrode compartments. Stronger hexokinase bands were found when 1 m*M* glucose was incorporated in the gel, and this was done in most of our studies.

The position of hexokinase on the gel was ascertained by applying to the sliced gel a mixture comprised of 0.1*M* tris (*p*H 7.6), 5 m*M* adenosine triphosphate (ATP), 5 m*M* $MgCl_2$, 1.3 m*M* NADP, 0.4 unit of glucose-6-phosphate dehydrogenase per milliliter (*5*), and either 0.5 m*M* or 0.1*M* glucose at approximately 25°C. At intervals of 30 minutes, the gel was illuminated with ultraviolet light of long wavelength and photographed with a yellow filter.

Hexokinases I, II, and III from human, rat, and rabbit tissues have similar mobility (Figs. 1 and 2). Type III enzyme is strongly inhibited by high concentrations of glucose (0.1*M*), a useful property for identification of the band (*1, 3*). Conversely, type II is stabilized and better visualized in the presence of high concentrations of glucose. Enzyme from striated muscle and adipose tissue serve as reference standards for identifying enzyme bands in human erythrocytes.

In erythrocytes from normal human adults, a double type I band and a much weaker, single type III band are