If we had performed only single-donor crosses, we would have concluded that environmental conditions during pollen development are unimportant in determining fruit set and seed set. Single-donor pollinations are appropriate only when it is known that, under natural conditions, stigmas receive pollen from just one donor. Multiple paternity is common in wild *Raphanus* populations (*22*). Potential pollen performance in mixtures need not parallel performance in isolation, just as competition between species in mixtures is difficult to predict from growth characteristics when species are grown alone (*23*). It is therefore important to determine the types of pollen loads occurring under natural conditions to understand the potential effect of environmental variation on male mating success.

Typically, analyses of phenotypic variation consider variance due to nuclear genetic, maternal genetic (cytoplasmic), maternal environment, and environmental variation. Any differences between paternal half sibs are considered to result solely from additive genetic variation (*24*). We suggest that paternal environment effects may have important fitness consequences, especially with regard to mating success. Paternal success variation may have a genetic component, but our study demonstrates the existence of strong nongenetic components. It is thus essential to control conditions of pollen development in experiments testing for genetic variation in paternity.

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25. We thank D. Stratton, B. Nakamura, L. Kohorn, and J. Antonovics for comments on an earlier version of this paper. This work was supported by NSF grants BSR-8561333 and BSR-8817813 to M.L.S.

16 January 1990; accepted 25 April 1990

Light-Evoked Changes in the Interphotoreceptor Matrix

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The normal function of vertebrate photoreceptor cells depends on multiple interactions and transfer of substances between the photoreceptors and the retinal pigment epithelium (RPE), but the mechanisms of these interactions are poorly understood. Many are thought to be mediated by the interphotoreceptor matrix (IPM), a complex extracellular matrix that surrounds the photoreceptors and lies between them and the RPE. Histochemical, immunocytochemical, and lectin probes for several IPM constituents revealed that components of the IPM in the rat undergo a major shift in distribution or molecular conformation after the transition between light and dark. In the light, various IPM constituents concentrated in bands at the apical and basal regions of the outer segment zone; in the dark, they distributed much more uniformly throughout the zone. The change in IPM distribution was triggered by the light-dark transition; it was not a circadian event, and it was not driven by a systemic factor. The light-evoked change in IPM distribution may facilitate the transfer of substances between the photoreceptors and the RPE.

THE NORMAL FUNCTION AND METABolism of vertebrate photoreceptor cells depend on numerous interactions with the RPE. These interactions include exchange of metabolites and catabolic by-products (*1*), water and ion transport, retinoid transfer between photoreceptors and RPE during the visual pigment cycle, control of the proper ionic composition in the external milieu, alignment and adhesion of photoreceptor outer segments to the RPE, and possible signaling from the retina to the RPE for the regulation of outer segment disk shedding (*2–7*). Several of these events follow the environmental light-dark transition or follow a light-entrained circadian rhythm (*2–4*). Because there are no direct intercellular connections between

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photoreceptor and RPE cells, many of the interactions between the cells require the transfer of materials through the IPM, which lies between the photoreceptors and the RPE. For this reason, it has been assumed by most workers that the IPM plays a crucial role in photoreceptor-RPE cell interactions (*6, 8, 9*); however, no function for the IPM has yet been firmly established and little is known about the means of transfer of substances through the IPM. The most well-defined macromolecule of the IPM, interphotoreceptor retinoid-binding protein (IRBP), is thought to shuttle retinoids between the photoreceptors and the RPE during different phases of the light cycle (*10*), but even this role has been challenged (*11*). We have discovered a light-evoked change in the IPM that may provide at least some of the motive force for the transfer of substances between the photoreceptors and the RPE and may be involved in other interactions between these cells.

The polyanionic IPM that fills the interphotoreceptor space can be visualized histochemically with cationic reagents, such as colloidal iron (CI), which stains virtually all polyanions (*12*). When we stained retinal sections from normal, light-adapted rats with CI (*13*), we confirmed earlier findings (*14*) that the IPM was most concentrated at the interface with the apical surface of the RPE (apical photoreceptor outer segment zone) and at the photoreceptor inner segment–outer segment junction (basal outer segment region) (Fig. 1A). The IPM was less concentrated in the intervening interstitial zone, that is, the region alongside the outer segments, particularly in the most basal half of the interstitial zone (*15*). Some stainable IPM was usually present between the photoreceptor inner segments reaching inward to the outer limiting membrane (Fig. 1A), although staining in this region was somewhat variable.

The distribution of the IPM changed dramatically in the dark phase of the light cycle. In eyes taken from rats kept two or more hours in the dark, the interstitial zone was much more heavily and uniformly stained than in the light (Fig. 1B), and, although a concentrated apical band of IPM staining was sometimes seen, it was often obscured by the heavily stained interstitial zone. By contrast, a concentration of staining in the basal outer segment zone was seldom seen. Consequently, the overall IPM had a much more homogeneous staining pattern in the dark than in the light. In addition, virtually no stained IPM was present between the photoreceptor inner segments or at the outer limiting membrane in the dark-adapted animals (Fig. 1B).

Immunocytochemical and lectin probes that are more specific for individual IPM constituents than CI confirmed the histochemical findings. These included monoclonal antibodies to chondroitin 6-sulfate (Fig. 1, C and D); the plant lectin wheat germ agglutinin (WGA) (Fig. 1, E and F), which binds specifically to sialyl and *N*-acetylglucosaminyl residues of glycoconjugates (*16*); and a polyclonal antibody to IRBP (Fig. 1, G and H). Thus, the chief components of the IPM apparently undergo a major shift in distribution or molecular conformation after light-dark transition (*17*).

Using CI staining, we explored the time

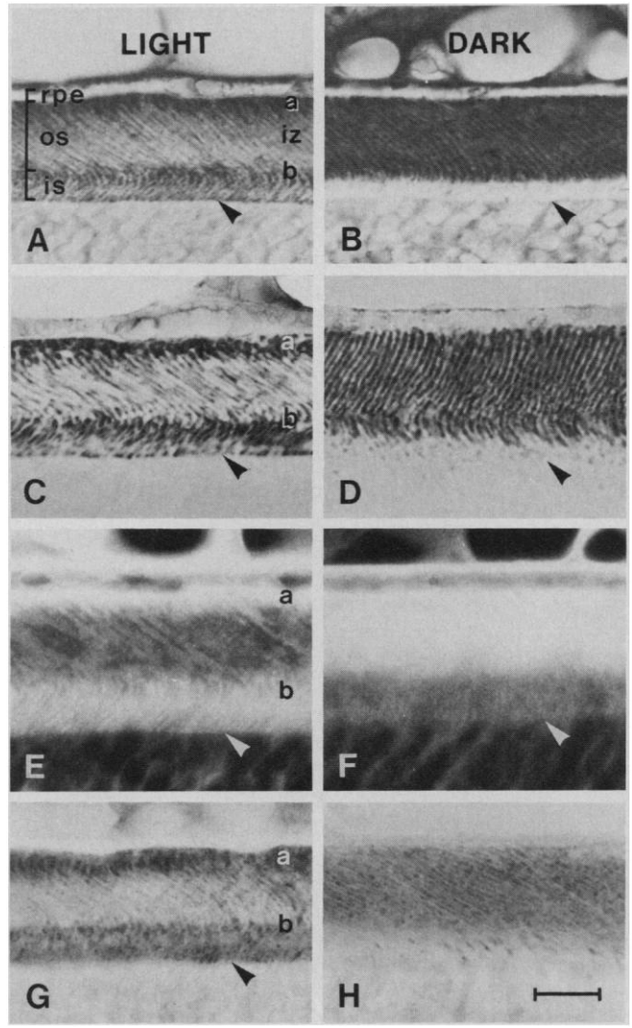

Fig. 1. Light micrographs of polyester wax-embedded sections stained to demonstrate various constituents of the IPM of albino rat eyes taken either in the light (one or more hours into the light cycle) (A, C, E, G) or in the dark (two or more hours into the dark cycle) (B, D, F, H). The retinal pigment epithelium (rpe), the photoreceptor outer segments (os) and inner segments (is) are labeled in (A), and the outer limiting membrane (arrowheads) are shown in other micrographs. (**A** and **B**) CI histochemical staining. In retinas taken in the light (A), there is a strong concentration of densely stained IPM in the apical outer segment zone (a, at the interface with the RPE) and in the basal outer segment zone (b, at the junction with the inner segments), but a less intensely stained interstitial zone (iz, alongside the outer segments) than in the apical and basal zones. There is also some CI-stained IPM between the inner segments, reaching inward as far as the outer limiting membrane (arrowhead). In dark-adapted retinas (B), the interstitial zone is much more intensely stained with CI than in the light-adapted retinas; an apical band of staining is less obvious; and there is no conspicuous basal outer segment concentration. Inner segment staining is virtually absent in the dark. (**C** and **D**) Chondroitin 6-sulfate immunoperoxidase. The distribution of densely stained reaction product in the light-adapted (C) and dark-adapted (D) retinas is almost identical to that seen with CI histochemical staining. Control experiments in which either the pretreatment of the sections with chondroitinase ABC was omitted or nonimmune mouse serum was substituted for the primary antibody (*30*) eliminated staining in both lighting conditions (not shown). (**E** and **F**) FITC-WGA lectin fluorescence. With this procedure, the FITC-WGA binding patterns in the light-adapted (E) and dark-adapted (F) retinas are similar to those obtained with CI and chondroitin 6-sulfate, although the fluorescent labeling appears bright in the micrographs. The near absence of lectin binding in the interstitial zone in the light-adapted retinas (E) contrasts sharply with the intense binding of this zone in the dark-adapted retinas (F). Neuraminidase treatment of the sections before application of FITC-WGA significantly reduced fluorescence in the IPM (not shown), indicating that much of the fluorescence in (E) and (F) represents binding to sialoglycoconjugates. (**G** and **H**) IRBP immunoperoxidase. The IRBP immunoreactivity patterns in the light-adapted (G) and dark-adapted (H) retinas are similar to those obtained with CI, chondroitin 6-sulfate, and FITC-WGA. Similar IRBP staining was seen with immunofluorescence methods (not shown). All IRBP immunoreactivity in the IPM of both lighting conditions was abolished when the primary antiserum was preabsorbed with IRBP (not shown), indicating that the binding without preabsorption is specific. The patterns of staining in the light-adapted retinas are similar to those shown in light-adapted rodent retinas by methods used to demonstrate CI (*8, 14*), IRBP (*20, 31, 32*), and chondroitin 6-sulfate (*30, 33*); although a strong apical concentration of chondroitin 6-sulfate was not conspicuous in previous light microscopic immunocytochemical staining (*30*), it was in electron microscopic immunogold labeling (*33*). Bar, 10 μm.

required to complete the change in the IPM distribution in three separate groups of rats at each of the normal light-dark transition times, that is, light onset in the morning and light offset in the evening. Before and immediately after light onset in the morning (for example, 1 min), the retinas still showed the diffuse pattern of IPM staining characteristic of eyes fixed in the dark. However, as early as 5 min after light onset and thereafter (10, 15, 30, 60, and 120 min), the banded light pattern was clearly evident. The transition to the diffuse IPM pattern after light offset in the evening was more gradual and required 1 to 2 hr to complete, although this was less consistent from animal to animal than the rapid morning transition from dark to light. In one group of rats, we used antibodies to IRBP and confirmed the same transition times observed after CI staining. Thus, the dark to light changes in the IPM occur rapidly, within 5 min, and the light to dark changes require significantly longer to complete, between 1 and 2 hr.

Because photoreceptor outer segment disk shedding in the rat follows a circadian rhythm and is independent of the onset of lighting (*4, 18*), we carried out several experiments to determine whether the IPM change is light-triggered or follows a circadian rhythm. When the dark phase of the light cycle was extended for different periods (up to 2 hr) in the morning, the retinas still displayed the dark pattern. When the light phase was extended for up to 2 hr in the evening, the IPM still showed the light pattern. Likewise, when rats were exposed to room light for 15 to 60 min at different times of the dark cycle (2, 4, or 11 hr into the 12-hr dark cycle), they showed the light pattern. Conversely, when rats were placed in the dark at different times of the light cycle (3 or 6 hr into the 12-hr light cycle), they switched to the dark pattern, but full transition required 1 to 2 hr as it did at the normal time of the light offset. Moreover, if rats were placed in the dark in the middle of the light cycle, were kept there for 2 hr (where they presumably switched to the dark pattern), and then were returned to the light for 5 min or more, the IPM showed the light pattern. Thus, the results of each of these experiments demonstrate that the change in IPM distribution is triggered by the change in lighting and is not a circadian event.

We also patched one eye of the rats with black opaque tape at 2 to 4 hr after light onset and killed the animals 2 hr later (*19*). In each case, the patched eye showed the diffuse dark pattern whereas the open eye retained the banded light pattern. These results add further evidence that the change in IPM distribution is light-evoked and argue against the involvement of a systemic factor, which presumably would affect both eyes equally. Moreover, it demonstrates that the IPM change can readily occur in anesthetized animals (*19*).

We suggest that light-evoked changes in the IPM may facilitate the transfer of substances between photoreceptors and the RPE by changing the gross distribution of its proteoglycans and acidic glycoproteins. Several events occur after the light-dark transition that are consistent with this notion. For example, retinoids move from the photoreceptor outer segments to the RPE after the bleaching of visual pigment by light and move in the reverse direction in the dark to allow the regeneration of visual pigment (*2, 3*). After the onset of light, many IPM constituents shift rapidly away from the outer segment interstitial zone toward the RPE and inner segments; after light offset, the IPM components assume a more uniform distribution across the outer segment zone (Fig. 1). The time course for the light-evoked change (less than 5 min) slightly precedes the movement of vitamin A from the RPE during light adaptation in the rat (*2*). Likewise, the much slower change to the dark pattern (1 to 2 hr) closely matches the rate of vitamin A movement back to the photoreceptors during dark adaptation (*2*). Thus, there is a close temporal relation between the light-evoked IPM changes and the partitioning of vitamin A in the photoreceptor-RPE complex in the visual pigment cycle. Regardless of whether IRBP actively transports retinoids across the IPM (*10*) or simply binds them to allow gradual exchange between the photoreceptors and RPE (*11, 20*), the concentration of IRBP at the apical zone in the light may serve to accumulate retinoids adjacent to the RPE and thereby facilitate their transfer to the RPE cells.

Light-induced changes in the IPM distribution may also influence the transfer of substances across the interphotoreceptor space in other ways. For example, the concentration of chondroitin 6-sulfate in the basal outer segment zone during the light may have an effect on the diffusion of substances [electrical potentials that influence diffusion rates across extracellular matrices are generated by concentration gradients of chondroitin sulfates in other systems (*21*)]. In addition, proteoglycans such as those in the IPM immobilize water and resist its flow (*22*), and they alter the osmotic pressure of their environment as a function of their concentration (*22*). A change in the osmotic pressure would presumably change the state of hydration of the proteoglycans, which would thereby influence the diffusion rates of other substances within the IPM (*22*). Thus, the rate of water flux across the interphotoreceptor space from retina to choroid (*23*) may be altered by the light-evoked generation of concentration gradients of IPM constituents.

Clues about the underlying mechanism of the light-evoked IPM changes come from studies on changes of ion fluxes after light transition. Light causes (i) a release of Ca^{2+} by the photoreceptor into the interphotoreceptor space (*24*), (ii) a decrease in extracellular K^+ surrounding the photoreceptors (*25, 26*), (iii) an increase in pH in the interphotoreceptor space (*27, 28*), and (iv) a predicted reduction in the release of lactic acid by photoreceptors (*28*). Glycosaminoglycans in other systems undergo conformational changes (reversible extension and retraction) when the pH or ionic composition of their milieu is changed (*22*). Such conformational changes of the IPM after photoreceptor illumination may underlie the changes we have demonstrated and would alter the diffusional and transport properties of the matrix (*22*). The changes in extracellular Ca^{2+}, K^+, and pH peak at less than 4 min (mostly less than 1 min) after light onset (*25, 26*), immediately before the rapid light-evoked IPM redistribution. Likewise, depending on the degree of visual pigment bleach, the recovery during dark adaptation may require many minutes for pH (*27*), and up to 80 min for K^+ (*25*), similar to the slow IPM changes during dark adaptation. Thus, the time course for each of these changes is consistent with its involvement in the distributional changes of the IPM.

It remains to be shown experimentally that the light-evoked changes we have demonstrated are directly related to changes in the transport or diffusion of substances across the IPM. However, it is clear that the further analysis of light-dark changes will be an important key for understanding the vital interactions between photoreceptors and the RPE. Moreover, it is remarkable that such dynamic, light-dependent changes occur extracellularly in the IPM, while even more rapid, light-evoked movement of photoreceptor-specific proteins thought to function in phototransduction (for example, α-transducin, β-transducin, 33-kD protein, and 48-kD protein) occurs intracellularly between the photoreceptor inner and outer segments (*29*).

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13. For this and all other experiments, albino (F344), pink-eyed (RCS-*rdy*$^+$) or black-hooded, black-eyed (RCS-*rdy*$^+$*p*$^+$) rats were maintained in cyclic lighting (12 hr on: 12 hr off) at an in-cage illuminance of less than 20 footcandles. Albino and pink-eyed rats were used for most of the experiments, because the absence of melanin in the RPE enhanced the visibility of the apical band of stained IPM. However, each type of experiment was done with several pigmented rats, and these produced the same results as in albino and pink-eyed rats. Except for one experiment (*19*), the rats were killed with an overdose of carbon dioxide, after which they were immediately perfused intravascularly with a mixed aldehyde fixative, and their eyes were embedded in polyester wax (*8*). Sections cut along the vertical meridian of the eye were visualized either (i) histochemically with the CI reaction (*8*); (ii) with immunoperoxidase after the application of previously characterized mouse monoclonal immunoglobulin M antibody to chondroitin 6-sulfate (ICN Immunobiologicals, Lisle, IL) (*30*); (iii) with the fluorescein isothiocyanate (FITC)–labeled lectin wheat germ agglutinin [F. Uehara, M. Sameshima, T. Muramatsu, N. Ohba, *Exp. Eye Res.* **36**, 113 (1983)]; or (iv) with immunoperoxidase (*30*) after the application of previously characterized rabbit polyclonal immunoglobulin G to bovine IRBP (*31*) or by immunofluorescence with the use of a FITC-labeled secondary antibody to IRBP. The appropriate controls were used with each reagent as noted. Each experiment was repeated two or more times.
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26 September 1989; accepted 26 March 1990