Photoreceptor differentiation of isolated retinal precursor cells includes the capacity for photomechanical responses

DEBORAH L. STENKAMP AND RUBEN ADLER*

Department of Neuroscience and Retinal Degenerations Research Center, The Wilmer Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21287-9257

ABSTRACT   Isolated retinal precursor cells, grown without pigment epithelial or glial cells and in the absence of intercellular contacts, develop a complex set of photoreceptor-specific properties, including polarized structural and molecular organization and opsin immunoreactivity. We report here that these isolated embryonic photoreceptors are also capable of responding to light. Sequential photography showed that 50% of the photoreceptors grown in a light cycle elongate when exposed to light and contract in response to darkness. A smaller population (20%) showed the opposite response. Responses of individual cells could be observed during several sequential light cycles and resemble photomechanical movements in vivo [Ali, M. A. (1971) Vision Res. 11, 1225–1288]. The differentiation program expressed by isolated precursor cells, therefore, includes the capacity for highly complex functional activities that require light sensitivity. These observations raise challenging questions regarding the nature of the chromophore and pigments that mediate light-regulated behaviors of cultured photoreceptors.

In addition to visual transduction, light regulates a variety of retinal metabolic functions, including synthesis and distribution of cell-specific macromolecules (1, 2), shedding of photoreceptor outer segments (3), and photomechanical movements (for review, see ref. 4). Photomechanical movements occur in many vertebrates in response to daily light cycles. In the light-adapted state, rod photoreceptors are elongated and cone cells are contracted along their long axes, and the opposite movements occur in the dark-adapted state. The inner-segment myoid regions of these cells show the most evident changes in length. Retinal neuromodulators, apparently regulated by circadian or light-stimulated mechanisms, are also involved in some of these phenomena (for review, see ref. 5). For example, in some species dopamine synthesis (6) and release (7) increase at light onset, with a concomitant decrease in the synthesis and release of other neuromodulators, such as melatonin and γ-aminobutyric acid, from photoreceptor cells and/or other cell types (5). Light offset reverses these effects, and it is thought that dopamine and melatonin function as reciprocal antagonists in the regulation of photomechanical movements because in many systems dopamine can mimic the effects of light (for review, see ref. 8), and melatonin can mimic the effects of darkness (9).

The respective regulatory contributions of light and neuromodulators to photomechanical movements remain undefined, as do the cell type or types that drive retinal cyclic metabolism. The sequence of events and mechanisms involved in the establishment of light-dependent cyclic events during retinal development and the possible effects of light on retinal development, in general, have not been explored systematically. Further investigation of these issues has been difficult due to the lack of experimental systems that permit recurrent observation and manipulation of these events.

We are now using an in vitro system that allows direct analysis of some of these experimental questions. In this system, embryonic day 8 chicken retinal cells, cultured at low density in the absence of glia and retinal pigment epithelium, differentiate after 4–7 days in vitro (DIV) into multipolar neurons and opsin-immunoreactive photoreceptors. This in vitro system has been used for the investigation of various aspects of retinal development and differentiation (10), and many elements of in vivo cyclic metabolism are present in these cultures (11). We report here further exploitation of the system as a tool for the investigation of effects of light on photoreceptor metabolism.

METHODS

Cell Culture. Protocols for the culturing of embryonic retina have been described (12). Retinas of embryonic day 8 chicken were dissected free of pigment epithelium, dissociated after mild trypsinization, and seeded at low density (8×10^5 cells) in medium 199/10% fetal bovine serum/linoleic acid-bovine serum albumin at 110 μg/ml on polyornithine-coated 35-mm dishes, and maintained in a humidified incubator at 37°C in a 5% CO_2/9% O_2/86% N_2. For some experiments, cells were seeded on engraved glass coverslips affixed to the bottom of culture dishes to allow identification of cells based on their position relative to fixed landmarks (13).

Light Treatments. Light was supplied by a 30-W fluorescent General Electric "cool white" circular tube with a Plexiglas diffuser and neutral density filter. For some experiments, a 15-W tungsten bulb was used instead. Culture dishes positioned ≈20 cm beneath either light source received 5–25 lux. Experimental cultures were maintained on a timed cycle of 12 hr light and 12 hr dark (L/D), beginning approximately at the time of seeding. Free-running control cultures were maintained in constant light (L/L) with the same light source or in constant darkness (D/D) by placing dishes on trays loosely covered with aluminum foil or in a sealed incubator. Temperature in incubators and in culture medium was monitored regularly and found not to vary by >1°C and with no correlation to light conditions.

Microscopy/Morphometry. Cultures were photographed by using a Nikon camera on an inverted phase-contrast microscope equipped with an environmental chamber to regulate temperature and CO_2 during the 2- to 10-min photography period. Photographic fields were initially chosen at random from the cultures grown on glass coverslips, and sequential photographs of the same field were obtained to assess changes in morphology of identified photoreceptor cells. Comparative measurements of cell length were taken

---

Abbreviations: DIV, day in vitro; D/D, constant darkness; L/L, constant light; L/D, 12-hr light/dark cycle.
*To whom reprint requests should be addressed at: Retinal Degenerations Research Center, The Wilmer Institute, The Johns Hopkins University School of Medicine, Maumenee 519, 600 North Wolfe Street, Baltimore, MD 21287-9257.

Neurobiology: Stenkamp and Adler

Proc. Natl. Acad. Sci. USA 90 (1993) 1983

by projecting these sequential images onto a screen. For some experiments, cultures were fixed with 1% glutaraldehyde, using dim red illumination for processing cultures during a dark period.

More detailed descriptions of methods used for individual experiments are in *Results*.

RESULTS

Qualitative Description of the Cultures. Embryonic retinal cultures contain morphologically undifferentiated cells and principally two differentiated cell types (Fig. 1 *A* and *B*). Neurons are multipolar and have been biochemically characterized as kainate-sensitive and as having high-affinity uptake systems for γ-aminobutyric acid, aspartate, and glutamate (14). Photoreceptors develop a polarized and compartmentalized morphology, having one short neurite, a nuclear compartment, an inner-segment region containing a lipid droplet and enriched for Na^+/K^+-ATPase, and a rudimentary outer-segment-like structure that is immunoreactive for the visual pigment opsin (10). Photoreceptors are kainate-resistant and have a high-affinity-uptake system for glutamate but not for γ-aminobutyric acid (14).

Effects of Light on Photoreceptor Morphology *in Vitro*. We observed significant differences in the morphology of photoreceptors from cultures exposed to light (L/L cultures or L/D cultures examined during the light phase of a cycle) as compared with photoreceptors grown in D/D conditions. As shown in Fig. 1 *A* and *B*, analysis under phase-contrast optics revealed that light-exposed photoreceptors were generally more elongated and compartmentalized than their D/D counterparts. A distinctive characteristic of these elongated photoreceptors was a marked constriction of the inner-segment myoid region to a diameter of <1.5 μm (Fig. 1*B*; see also Fig. 4*A*). Presence of this constriction was subsequently used as

a criterion to identify "elongated photoreceptors" for additional quantitative studies (see below).

We quantified these observations, determining photoreceptor length by measuring the distance from the base of each cell's nuclear compartment, near the origin of the neurite, to the center of the inner-segment lipid droplet. For these studies, cultures maintained in darkness were subject to a brief light exposure during photography; control experiments with cultures fixed at different intervals during the dark phase of the cycle indicated that this brief light exposure did not affect cell behaviors in any detectable manner. In these cultures the average photoreceptor length was 12.5 ± 4 μm, whereas retinal cultures maintained in L/L contained photoreceptors with an average length of 20 ± 4 μm (Fig. 1*C*). Cultured L/D photoreceptors had an average length of 18.5 ± 4 μm, when examined during the light period, and 16 ± 4 μm, when measured at the beginning of the light period. These morphological differences were also evident in histograms of the distribution of lengths for photoreceptors grown in D/D, L/D, and L/L conditions, normalized for number of cells measured (≈100 for each condition) (Fig. 1*D*). In D/D cultures, 50% of the photoreceptors were shorter than 12 μm, whereas in L/L and L/D (measured in light) cultures, >75% of the photoreceptors were longer than 12 μm. Furthermore, L/L and L/D cultures showed a much broader distribution of photoreceptor lengths, with L/L cultures having slightly greater numbers of photoreceptors longer than 30 μm.

Photoreceptor Responses to Cyclic Light. A more detailed analysis of changes in photoreceptor morphology over several sequential L/D cycles was undertaken to examine possible cyclic components of the response to light. Photoreceptor lengths in L/D cultures were measured at 12-hr intervals over a period of 2 days, and the resulting histograms showed regular changes in the distribution of photoreceptor length (Fig. 2*A*). Most notably, the frequency of photoreceptors longer than 20 μm was higher in cultures observed at the end of a light period than in those observed at light onset (end of a dark period), and this shift in distribution of length was repeated during two or more light cycles.

An additional assay was developed to allow direct comparison of light- and dark-"adapted" photoreceptors. The number of elongated photoreceptors (defined by the presence of an elongated and constricted myoid region, see above) was determined for L/L, D/D, and L/D cultures fixed at selected times over a 3-day period (Fig. 2*B*). Cultures maintained in L/L showed consistently higher percentages of elongated photoreceptors than those in D/D. Additionally, in both L/L and D/D cultures there was very little change in the frequency of elongated photoreceptors for the duration of the experiment. In cultures maintained on a 12-hr light cycle, however, there were cyclic changes in the frequency of elongated photoreceptors, ranging from a maximum of 20–30% near the end of the light period to a minimum of 5–10% shortly before light onset. These changes were repeated over two subsequent cycles. One notable feature of these experiments, however, was the presence of some elongated photoreceptors at the end of the dark period and the presence of some short photoreceptors at the end of the light period. This apparent heterogeneity in photoreceptor behavior suggested the need to investigate the responses of individual cells to light cycles, which is described below.

Serial Analysis of Individual Photoreceptor Cells. To investigate morphological changes of individual photoreceptors, cell behavior was monitored by using sequential photography of cells identified according to their position relative to coded grids on glass-coverslip substratum. Possible heterogeneity of photoreceptors regarding their responses to light was investigated with a photographic schedule involving photography at light onset (to represent cell appearance during the dark period) and again before light offset (to represent cell
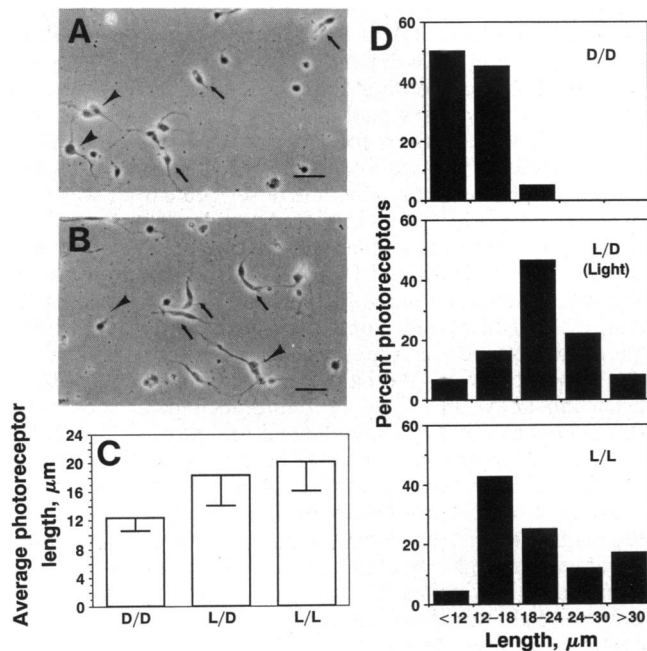

FIG. 1. Effects of light on morphology of isolated photoreceptors in embryonic retina cultures. (*A*) Culture maintained in D/D. (*B*) Culture grown for 6 days on a 12-hr L/D cycle, photographed at the end of the light period. Many photoreceptors (arrows) appear longer in *B* than their counterparts in *A*. Multipolar neurons (arrowheads) are also present. (Bar = 20 μm.) (*C*) Average length (± SD) of photoreceptors from cultures photographed after 6 days in D/D, L/L, or a 12-hr L/D cycle; the latter were analyzed at the end of the light period. (*D*) Distribution of photoreceptor cell lengths in cultures grown under conditions similar to those described for *C*.

FIG. 3. Identification of photoreceptor subpopulations on the basis of their responses to light cycles. Cells were categorized as elongating (>10% length increase) (open bars), contracting (>10% length decrease) (solid bars), or not changing (<10% length change) (hatched bars), over a 12-hr interval.
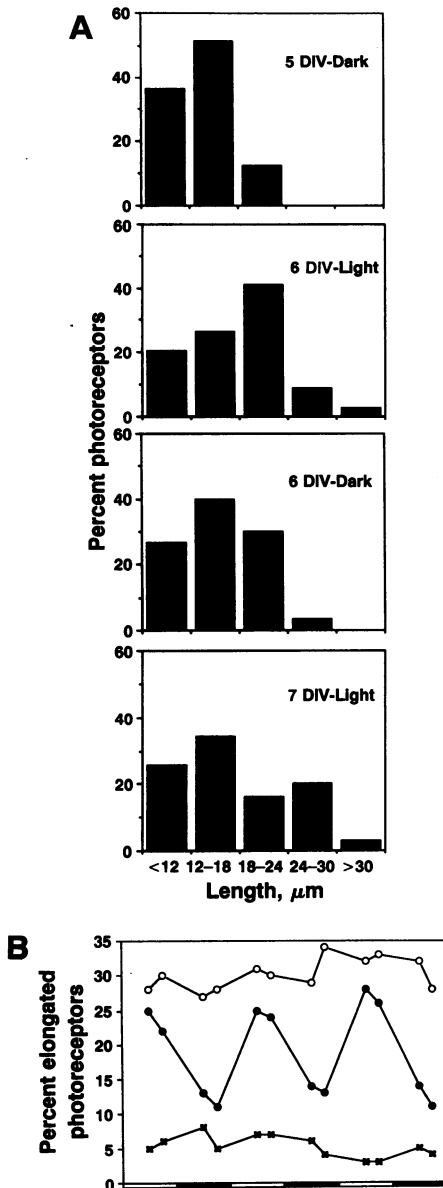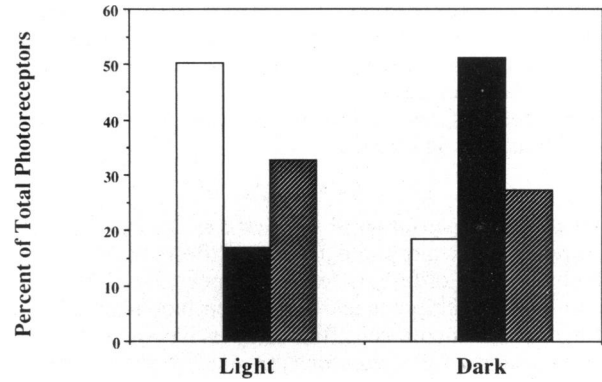

FIG. 2. Cyclic changes in photoreceptors exposed to light cycles. (A) Distribution of photoreceptor cell length in cultures grown in L/D, analyzed at light onset (representing the end of the dark period) and before light offset (representing the end of the light period). (B) Frequency of elongated photoreceptors (defined by the presence of an elongated and constricted inner segment) in cultures maintained in D/D (×), L/D (●), and L/L (o) and fixed at various times over several light cycles. Light and dark periods over the 3-day experiment (4 DIV–7 DIV) were 12 hr long and are indicated by x-axis shading.

appearance during the light period). Free-running control cultures were photographed at the same time. This photographic schedule was usually followed for 2–3 days (4–7 DIV), to collect data from several light cycles. Changes in length were calculated for each photoreceptor over each experimental interval, and the cells were categorized as elongating (>10% length increase), contracting (>10% length decrease), or unchanged (<10% length change) for that time interval. Length changes for cells in each of these subcategories were then averaged for four to six microscopy fields (20–30 photoreceptors).

As shown in Fig. 3, the greatest proportion of cells elongated during the light period and contracted during the dark period. These cells shall be referred to as L[+] photorecep-

tors. However, not all photoreceptors showed this same behavior. A smaller subpopulation was seen to contract during the light period and to elongate in darkness (L[−]), and the remainder showed no significant changes (L[0]). The relative frequencies of L[+], L[−], and L[0] subpopulations (≈50%, 20%, and 30%, respectively) were fairly reproducible from experiment to experiment.

The majority of D/D and L/L photoreceptors showed <10% change in length over a 12-hr period (data not shown). There were, however, occasional L/L photoreceptors that did contract or elongate by as much as 20–60%. In these cases, contraction and elongation were unpredictable and apparently occurred at random.

Characterization of Responses to Light for L[+] Photoreceptors. A more detailed analysis of *in vitro* photoreceptor behaviors was undertaken to quantitate the magnitude and kinetics of light-cycle-dependent responses. The heterogeneity of photoreceptor light responses suggested the need to characterize responses at the level of individual cells, and L^{+} cells were chosen for these studies because of the experimental advantage due to their abundance in the cultures. The photographic schedule described earlier was used to monitor behavior of identified photoreceptors for at least two consecutive light cycles. An example of morphological changes for an identified L[+] photoreceptor is presented in Fig. 4A, in which an individual cell appears elongated at the end of the light period, contracted at the end of the subsequent dark period, and repeats this pattern of changes during the next light cycle. We have observed that many of these cells can show this type of photomechanical movement throughout a 2- or 3-day sampling period. Seventy percent of the photoreceptors that elongated during the light period (and remained viable over the next 24 hr) were observed to contract during the subsequent dark period. Quantification of the magnitude of these length changes showed that between light onset and light offset (light period), L[+] photoreceptors elongated by an average of 45% (i.e., 14 μm–21 μm) (Fig. 4B). Between light offset and onset (dark period), L[+] photoreceptors contracted by an average of 40% (i.e., 21 μm–11 μm). Length changes of the same photoreceptors were calculated over subsequent experimental intervals, and although the average changes decreased from the initial observation, the magnitude of these changes remained greater than that of D/D cells monitored in parallel (data not shown), and the type of movement (elongation vs. contraction) remained of the L[+] type.

Kinetics of elongation of L[+] photoreceptors in response to light was more closely investigated using an alternative photographic schedule, in which individual cells were repeat-
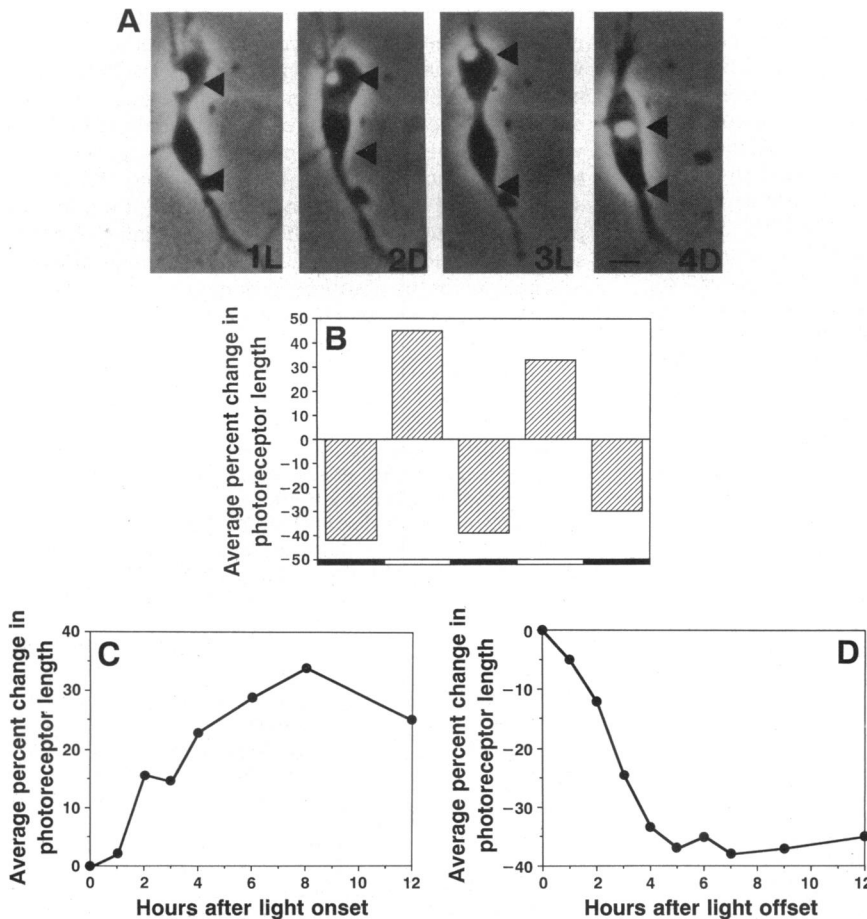
Neurobiology: Stenkamp and Adler

Proc. Natl. Acad. Sci. USA 90 (1993) 1985

FIG. 4. (*A*) Sequential photographs of a cell maintained on a 12-hr light cycle: *1L*, 4 DIV, 10 hr after light onset; *2D*, 5 DIV, 1 hr after light onset; *3L*, 5 DIV, 10 hr after light onset; *4D*, 6 DIV, 1 hr after light onset. Arrowheads indicate cellular reference points for length measurement (see *Results*). (Bar = 5 μm.) (*B*) Average length changes for 40 identified L[+] photoreceptors over several consecutive 12-hr intervals beginning at 4 DIV. *x*-axis shading denotes light-cycle interval over the 2.5-day experiment (black indicates darkness; white indicates light). The *y*-axis shows average length differences during each 12-hr period. (*C*) Kinetics of L[+] photoreceptor elongation during the light phase of the cycle (t_0, light onset on DIV 6) (n = 21). (*D*) Contraction of L[+] photoreceptors in response to darkness (t_0, 1 hr before light offset on DIV 5) (n = 25). Note that each cell appeared to have a characteristic "robustness" to its response: percent changes in length differed markedly from photoreceptor to photoreceptor. In spite of the variability in magnitude of individual responses, shape of the curves, rates of elongation/contraction, and average percent changes were reproducible from experiment to experiment.

edly photographed during the light period for comparative measurements of photoreceptor length. As shown in Fig. 4*C*, maximum elongation typically required 6–8 hr of light and was followed by a slight contraction before light offset. Kinetics of L[+] photoreceptor contraction in response to light offset was also investigated photographically, but a different approach was needed to avoid exposing cells to light during the dark phase of the cycle. For these studies, several culture dishes were photographed at the end of the light period and returned to a dark incubator. At selected times during the dark period, some of the cultures were fixed, and the same fields were rephotographed to obtain comparative measurements of photoreceptor length (separate controls showed that photoreceptor length was not affected by chemical fixation). Average photoreceptor contraction reached a maximum 5 hr after light offset, with little variation thereafter (Fig. 4*D*). Photoreceptors cultured in constant light, which happened to contract over the same time interval, did so by a much smaller magnitude (data not shown), indicating that light offset (or entrainment to a light cycle) triggers a more substantial contraction than may occur spontaneously.

DISCUSSION

The findings outlined above indicate that isolated embryonic chicken photoreceptors can respond morphologically to light and light cycles in a quantifiable manner. We have observed that exposure to light results in the presence of longer photoreceptors *in vitro* and that, when maintained on a L/D cycle, cultured photoreceptors can respond with morphological changes that resemble photomechanical movements (4). The majority of photoreceptors showing these movements elongate in light, contract in darkness, and have a capacity to repeat these movements for several light cycles.

The observation that cultured photoreceptors can sense changes in light conditions and repeat their morphological responses implies the presence of photosensitive metabolic machinery. This capacity was unexpected because the cells are grown in the absence of pigment epithelium and glia (10). Cultured photoreceptors are known to contain materials immunoreactive with antibodies against opsin, the visual pigment apoprotein (10). However, light sensitivity requires the presence of the chromophore 11-*cis*-retinaldehyde, covalently bound to the opsin apoprotein (15). When visual pigments absorb light, their chromophore is isomerized to all-*trans*-retinaldehyde and then reduced to all-*trans*-retinol; the 11-cis chromophore must be regenerated to restore light sensitivity. This regenerative step of the visual cycle is thought to take place in the retinal pigment epithelium, and newly made 11-*cis*-retinaldehyde then diffuses or is transported to the photoreceptors (16). The neural retina of the chicken contains large stores of 11-*cis*-retinyl esters (17), but whether the pigment epithelium is necessary for the synthesis of active chromophore in this species is unknown. It is possible, therefore, that the cultured cells contain adequate deposits of 11-*cis*-retinal or can generate the 11-cis chromophore from the retinyl esters and retinol present in culture medium and serum. Although it seems unlikely that the responses in our cultures are mediated by chromophore/pigment systems different from those used by adult photoreceptors for phototransduction, it is worth noting that *in vitro* responses to light have been reported for cell types that are not known to contain rhodopsin-like pigments (18, 19).

The observation that L[+] photoreceptors show rod-like photomechanical behaviors (elongate in light), whereas L^{−} cells act as cones (contract in light) raises questions about the cellular identity of the various subpopulations present in the cultures because both cell types contain lipid droplets that are

considered cone-cell markers (20). It is possible, however, that oil droplets and other "cone markers," such as receptors for peanut lectin (20), are only transiently expressed by some of the developing photoreceptors during their differentiation (for review, see ref. 21). Distinguishing between rods and cones has been frequently difficult in many species, including diurnal birds (for review, see ref. 22). For that matter, a comprehensive study of photomechanical movements in chicken retina does not appear to be published (but see ref. 23), and investigators have proceeded under the assumption that rods and cones move as they do in better-studied species, such as teleosts. Cohen (24) summarized many examples of ambiguities in photoreceptor properties, warning against premature tendencies to establish rigid correlations among morphological, biochemical, and physiological phenotypes. In this context, it is of interest that we recently cloned from chicken retina libraries a visual pigment that, although resembling "chicken green" in spectral properties, is, in fact, closer in homology to rhodopsin than to other cone pigments (28).

In vivo photomechanical movement has been shown to require the participation of a functional cytoskeleton (4). The morphological resemblance of *in vitro* responses to *in vivo* photomechanical movements (4) suggests that similar forces may be involved in the photomechanical responses of isolated photoreceptors in culture. Consistent with this possibility is the previous finding in this laboratory that the development and maintenance of the elongated, polarized organization of cultured photoreceptors result from an equilibrium between constantly active microtubule-dependent elongating forces and actin-dependent contracting forces (13). Studies with cytoskeletal inhibitors support this idea because actin-depolymerizing drugs, such as cytochalasin D, inhibit contraction of L[+] photoreceptors in darkness, and microtubule-depolymerizing drugs, such as nocodazole, block their elongation in light (unpublished work). Despite this similarity to the *in vivo* situation, the rate of photoreceptor contraction (0.2 μm/min) is substantially slower than that seen in other systems (1.0 μm/min, ref. 8). Several possible explanations include lower activities of regulatory pathways, delays in autocrine and/or paracrine communication *in vitro*, or differences in cell–substrate interactions. The latter are suggested by the observation that *in vivo* photoreceptors elongate apically, with a stationary nuclear compartment (4), whereas cultured chicken photoreceptors elongate proximally, with a stationary distal inner segment.

Several neurotransmitter/neuromodulator and second-messenger systems implicated in the regulation of photomechanical movements *in vivo* (for review, see ref. 8) have also been documented in the cultures used for these experiments. Particularly noteworthy is the presence of serotonin *N*-acetyltransferase, the rate-limiting enzyme for the synthesis of melatonin (11). This enzyme has been shown to be regulated by cyclic nucleotides in the chicken retina both *in vivo* (25) and in our dissociated cell culture (11). The presence of light-regulated responses and of neuromodulator metabolism indicates that this developmental system may be ideal for further investigation of the respective roles of light and neuromodulators in the regulation and the development of photomechanical movements and other cyclic retinal events. We have recently observed that dopamine, which mimics the effects of light upon photoreceptor length *in vivo*, also mimics the *in vitro* effects of light (26), suggesting that dopamine and/or related neuromodulators may participate in the regulation or development of photomechanical responses not only *in vivo* but also in dissociated culture.

The results summarized in this report add further support to the contention that many aspects of photoreceptor differentiation are regulated by a "master program" expressed by retinal precursor cells even when developing *in vitro*, in isolation, in the absence of contacts with other retinal cells or with the retinal pigment epithelium (27). Previous studies have shown that this master program regulates not only the expression of cell-specific genes, such as those coding for the visual pigment protein opsin, but also complex phenotypic behaviors, such as the development and maintenance of structural and molecular polarity (10). Some functional aspects of photoreceptor differentiation have also been described, including the presence of a high-affinity-uptake mechanism for the photoreceptor neurotransmitter glutamate (14). We have presented here evidence indicating that the photoreceptor master program also includes the development of a very complex physiological activity, the capacity of the cells to respond to light with photomechanical movements.

1. Korenbrot, J. I. & Fernald, R. D. (1989) *Nature (London)* **337**, 454–457.
2. Uehara, F., Matthes, M. T., Yasumura, D. & Lavail, M. M. (1990) *Science* **248**, 1633–1636.
3. Bok, D. (1985) *Invest. Ophthalmol. Visual Sci.* **26**, 1660–1694.
4. Burnside, B. & Dearry, A. (1986) in *The Retina: A Model for Cell Biology Studies*, eds. Adler, R. & Farber, D. (Academic, Orlando, FL), pp. 152–206.
5. Besharse, J. C., Iuvone, P. M. & Pierce, M. E. (1988) in *Progress in Retinal Research*, eds. Osborne, N. N. & Chader, G. J. (Pergamon, Oxford), Vol. 2, pp. 67–110.
6. Iuvone, P. M. (1984) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **43**, 2709–2713.
7. Kramer, S. G. (1971) *Invest. Ophthalmol.* **10**, 438–452.
8. Dearry, A. & Burnside, B. (1988) in *Dopaminergic Mechanisms in Vision*, ed. Bodis-Wollner, I. (Liss, New York), pp. 109–135.
9. Dubocovich, M. L. (1983) *Nature (London)* **306**, 782–784.
10. Adler, R. (1986) *Dev. Biol.* **117**, 520–527.
11. Iuvone, P. M., Avendano, G., Butler, B. J. & Adler, R. (1990) *J. Neurochem.* **55** (2), 673–682.
12. Adler, R. (1990) in *Methods in Neuroscience*, ed. Conn, P. M. (Plenum, New York), pp. 152–206.
13. Madreperla, S. A. & Adler, R. (1989) *Dev. Biol.* **131**, 149–160.
14. Politi, L. E. & Adler, R. (1986) *Invest. Ophthalmol. Visual Sci.* **27**, 656–665.
15. Jones, G. J., Crouch, R. K., Wiggert, B., Cornwal, M. C. & Chader, G. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9606–9610.
16. Bernstein, P. S., Law, W. C. & Rando, R. R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1849–1853.
17. Bridges, C. D. B., Alvarez, R. A., Fong, S.-L., Liou, G. I. & Ulshafer, R. J. (1987) *Invest. Ophthalmol. Visual Sci.* **28**, 613–617.
18. Giebultowicz, J. M., Riemann, J. G., Raina, A. K. & Ridgway, R. L. (1989) *Science* **245**, 1098–1100.
19. Albrecht-Buehler, G. (1991) *J. Cell Biol.* **114** (3), 493–502.
20. Adler, R., Lindsey, J. D. & Elsner, C. L. (1984) *J. Cell Biol.* **99** (3), 1173–1178.
21. Grun, G. (1982) *The Development of the Vertebrate Retina: A Comparative Survey* (Springer, Berlin).
22. Stell, W. K. (1972) in *Handbook of Sensory Physiology*, ed. Fourtes, M. G. F. (Springer, New York), Vol. 7, pp. 111–213.
23. Ali, M. A. (1971) *Vision Res.* **11**, 1225–1288.
24. Cohen, A. (1972) in *Handbook of Sensory Physiology*, ed. Fourtes, M. G. F. (Springer, New York), Vol. 7, pp. 63–109.
25. Iuvone, P. M. (1990) *J. Neurochem.* **54**, 1562–1568.
26. Stenkamp, D. L. & Adler, R. (1991) *Invest. Ophthalmol. Visual Sci.* **32**, 848 (abstr.).
27. Adler, R. & Hatlee, M. (1989) *Science* **243**, 391–393.
28. Wang, S.-Z., Adler, R. & Nathans, J. (1992) *Biochemistry* **31**, 3309–3315.