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Short Communication

c-*fos* Gene Expression in Postnatal Rat Retinas with Light/Dark Cycle

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We examined the diurnal variation of c-*fos* gene expression during a 12:12 light/dark cycle in developing rat retinas by *in situ* hybridization histochemistry. c-*fos* Gene was not expressed before postnatal day 10 (P10) but was expressed on P15 in the outer nuclear layer throughout the dark period and in the inner nuclear layer and the ganglion cell layer during the light period. These results demonstrated that the earliest c-*fos* gene expression occurred between P11 and P15. The good correlation between the expression of c-*fos* gene and the genes coding for proteins involved in phototransduction, in terms of their diurnal variation and in their development, suggested that c-*fos* gene may play a role in the regulation of these genes in retinal cells during the light/dark cycle.

c-*fos* Postnatal development Diurnal change *In situ* hybridization Retina

INTRODUCTION

Environmental signals of light and darkness are not only carried from retina to brain but also involved in the transcriptional regulation of the retinal cell. For example, the levels of mRNA coding for proteins which are involved in phototransduction in photoreceptor cells, such as opsin, transducin and the 48 kDa protein, reflect the light/dark cycle: high during the night and low during the day (Bowes *et al.*, 1988; Brann & Cohen, 1987; Korenbrot & Fernald, 1989). A molecular correlate of the mechanism that links the light/dark cycle to the transcriptional regulation of the genes encoding these proteins has yet to be determined. We have recently suggested the involvement of proto-oncogene c-*fos* in the regulation of transcription during the light/dark cycle in retinal cells (Yoshida *et al.*, 1993, 1995a, b). In rat retinal cells, c-*fos* mRNA was transiently expressed in the inner nuclear layer (INL) and ganglion cell layer (GCL) after the onset of the light period, and expressed in the outer nuclear layer (ONL) continuously throughout the dark period under a light/dark (12/12 hr) cycle (Yoshida *et al.*, 1993). The good correlation in the diurnal changes of mRNA level between c-*fos* in the ONL and the genes coding for the phototransduction proteins suggests that c-*fos* may play a role in the transcriptional regulation of the genes encoding these proteins during the light/dark cycle. Thus, we expected that the control of the diurnal variation of c-*fos* gene expression in the developing retina induced the beginning of the cyclic change in the level of the genes encoding these proteins, and we examined the expression of c-*fos* mRNA in the developing rat retina under the light/dark (12/12 hr) cycle by *in situ* hybridization histochemistry.

MATERIALS AND METHODS

Experimental animals

Experiments were carried out on rats (Wistar King Aptakeman/HKM) at postnatal ages P5, P10, P15 and adult (around P100). The day of birth was recorded as P0. Rats were housed in a room with controlled temperature and fixed lighting schedule (lights on from 08:00 to 20:00). After weaning the rats were fed *ad libitum* and had free access to tap water. The light intensity inside the cages during the light period was approximately 500 lux. Because our previous paper described c-*fos* induction peaked at 08:30 in the INL and at 02:30 in the ONL (Yoshida *et al.*, 1993), rats were sacrificed either during the dark period (02:30) or 30 min after the beginning of the light period (08:30). Four different animals were used in each experiment.

Tissue preparation

Rats were anesthetized with diethylether and perfused transcardially with 10 ml of 4% paraformaldehyde in 0.1 M phosphate buffer containing 0.5% picric acid at room temperature. (During the dark period, anesthesia

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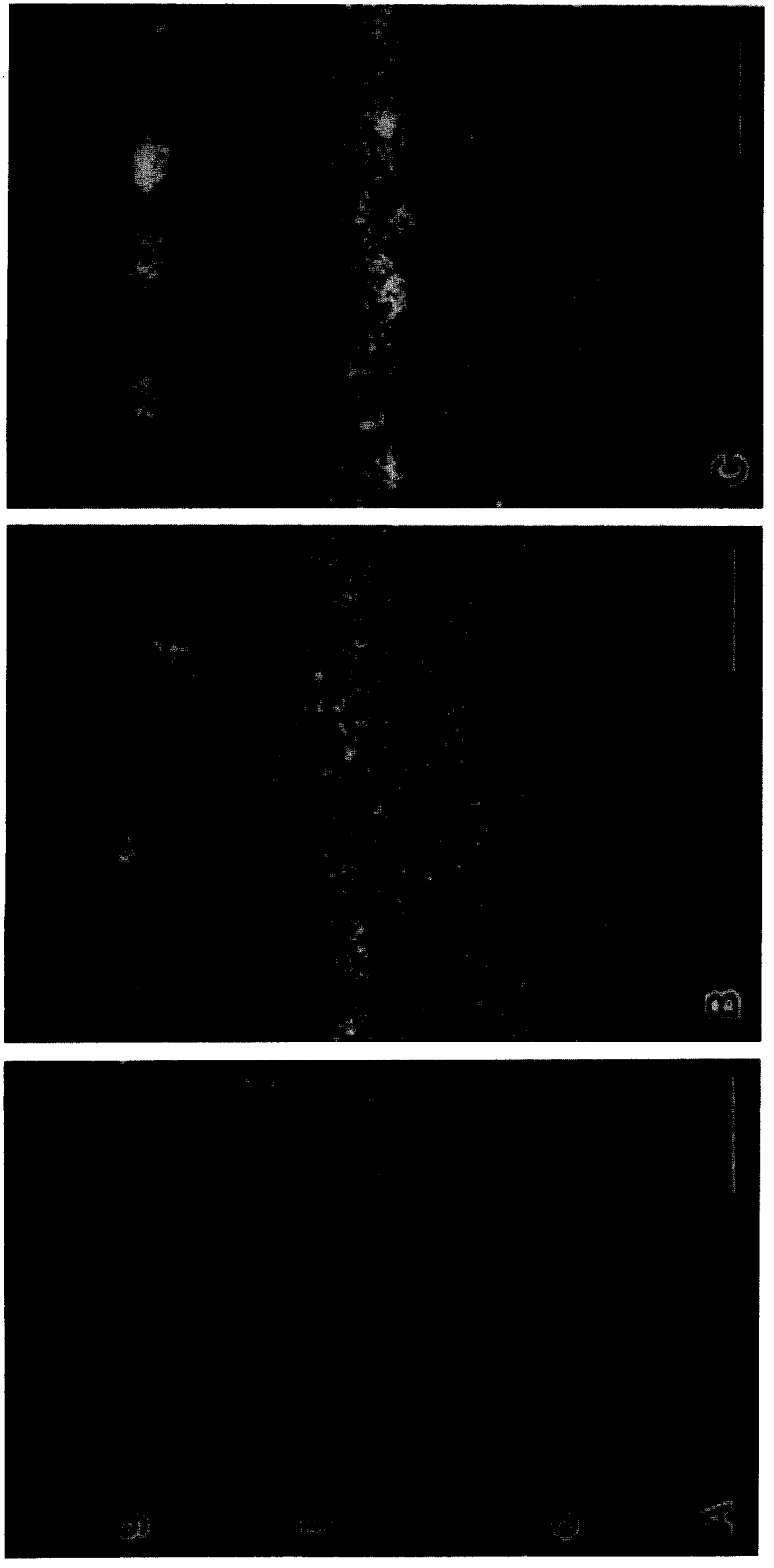

FIGURE 1. *In situ* hybridization analyses of c-*fos* gene expression in the rat retina at 30 min after the onset of light period (08:30). c-*fos* mRNA was not expressed on postnatal day 10 (P10) (A). On P15 (B) and adults (C), c-*fos* mRNA was expressed in the inner nuclear layer and the ganglion cell layer. g, ganglion cell layer; i, inner nuclear layer; o, outer nuclear layer. Bar = 50 μm.

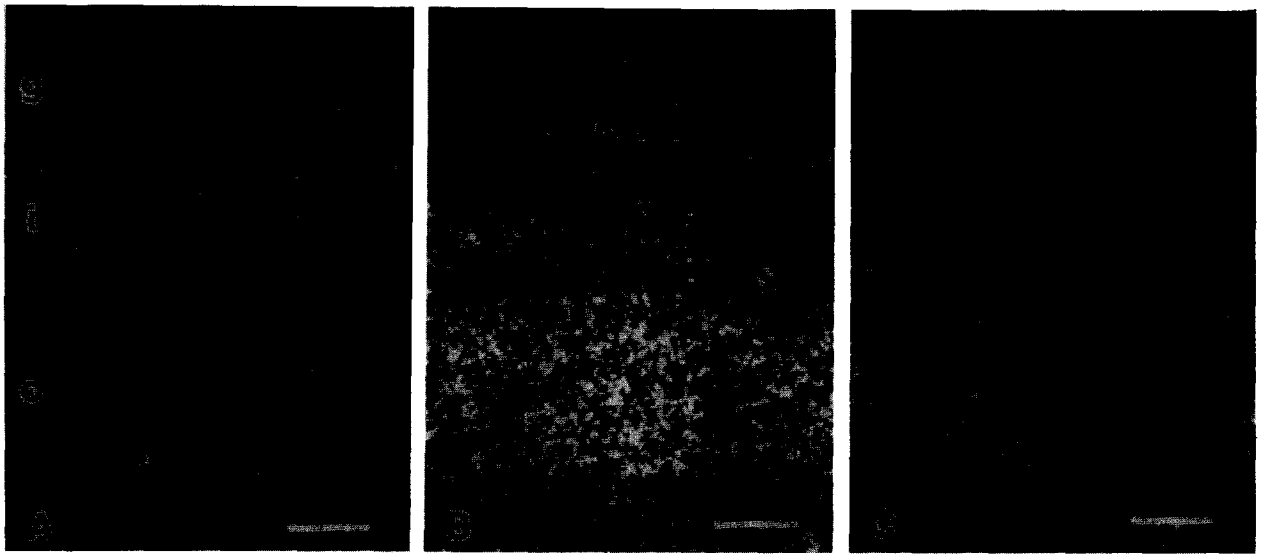

FIGURE 2. *In situ* hybridization analyses of c-*fos* gene expression in the rat retina during dark period (02:30). c-*fos* mRNA was not expressed on postnatal day 10 (P10) (A), but was expressed diffusely in the outer nuclear layer of P15 (B) and adults (C). g, ganglion cell layer; i, inner nuclear layer; o, outer nuclear layer. Bar = 50 μm.

was accomplished in complete darkness, and the rat head was enclosed in a black bag that was impervious to light.) The eyes were removed, opened by a cut in the sagittal plane, and post-fixed overnight in the same fixative containing 20% sucrose at 4°C. They were cut into 10 μm thick frozen sections on a cryostat and were mounted onto gelatin and poly-L-lysine-coated slides.

In situ *hybridization*

In situ hybridization was carried out as described by Simmons *et al.* (1989). Briefly, sections were dried under vacuum and digested by proteinase K (10 μg/ml) for 5 min and acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine. The sections were dehydrated in an ascending ethanol series and air-dried. The probe (3×10^6 dpm/ml) was dissolved in a buffer containing 50% formamide, 10% dextran, 1 × Denhart's solution, 12 mM EDTA (pH 8.0), 10 mM Tris/HCl (pH 8.0), 30 μM NaCl, 0.5 mg/ml yeast tRNA, and 10 mM dithiothreitol (DTT); 100 μl of probe solution was applied to each slide. Slides were cover-slipped and incubated at 55°C overnight. Coverslips were then removed and the slides were rinsed in 4 × SSC, digested with RNase A (20 μl/ml) for 30 min at 37°C, and rinsed sequentially in 2 × SSC, 1 × SSC, 0.5 × SSC, then for 30 min in 0.1 × SSC at 55°C, before finally being dehydrated again. The sections were exposed to X-ray film (Kodak) for 1 day, then dipped in NTB2 nuclear emulsion (1:1 with water, Kodak), and exposed for 3 weeks before being developed. Counterstaining of cells was done with 0.001% bisbenzimide. Radioactive cRNA copies were synthesized using T7 polymerase and full-length rat c-*fos* cDNA (nucleotide No. 142-1211) (Curran *et al.*, 1987) inserted in pBlue KS+ plasmid (gift from Dr. I. Verma) with [α-^{35}S] UTP (Yoshida *et al.*, 1993). The specific activity of the probe was approximately 1.0×10^8 dpm/mg. The specificity of this probe has been previously demonstrated (Yoshida *et al.*, 1995a, b). As a control for nonspecific labeling, a sense-orientated probe generated by T3 polymerase was applied to adjacent sections.

RESULTS

The expression of c-*fos* mRNA was not found in the P5 (data not shown) and the P10 [Fig. 1(A)] rat retinas taken 30 min after the onset of the light period (08:30). On P15 [Fig. 1(B)], c-*fos* mRNA was expressed in the INL and GCL during this time period. Similar but more conspicuous expression was noted in adult retinas [Fig. 1(C)].

The usual expression of c-*fos* mRNA at the end of the dark period (02:30) in adults (Yoshida *et al.*, 1993) was not found in the P5 (data not shown) and the P10 [Fig. 2(A)] rat retinas. From P15 [Fig. 2(B)] onward to adults [Fig. 2(C)], the c-*fos* mRNA was expressed diffusely in the ONL.

The c-*fos* mRNA induction in these analyses had no significant difference between the posterior pole and the peripheral retina, We have shown the results of the midperipheral retina in the figure.

DISCUSSION

In this study, we have demonstrated that the diurnal variation of c-*fos* expression in rat retinal cells was not detected before P10 but was observed on P15 rat retinas, suggesting that the beginning of this diurnal variation was between P11 and P15.

c-*fos* gene transcription has been shown to be induced by the cell depolarization in hippocampal neurons (Dragunow & Robertson, 1987) and PC12 cells (Morgan & Curran, 1986). This relationship between depolarization and transcription can then account for our observa-

tion of c-*fos* expression in the ONL during darkness, as the photoreceptor cells in the ONL are partially depolarized in the dark (Tomita *et al.*, 1967; Hagins *et al.*, 1970). c-*fos* Expression in the INL and GCL during the light period can be accounted for by the depolarization of rod bipolar cells (most of the cells in the outer half of the INL) and of ON center cells in the GCL with illumination (Ehinger & Dowling, 1987; Griferath *et al.*, 1990; Wässle *et al.*, 1990). The beginning of depolarization in each retinal cell during the developmental period, which responds to light/dark change, is unknown. However, a previous study showed that the first electroretinogram (ERG) in rats was recorded on P12 rat retinas and the ERGs were adult-like on P14 (Weidman & Kuwabara, 1968). A more recent electrophysiological study reported that the rat retinal ganglion cells first respond to light stimulation by P11 (Rolig & Grantyn, 1993). In view of the present results, we suggest that the beginning of c-*fos* gene expression in developing retinal cells was induced by the occurrence of electrophysiological neural activity with the light/dark change.

Fos protein produced by c-*fos* gene is a transcription factor which regulates the expression of the late response genes containing AP-1 binding site in their regulatory regions (Sheng & Greenberg, 1990), and it appears likely that c-*fos* gene participates as a third messenger in controlling the transcription of a set of genes in retinal cells. It was reported that the level of mRNA coding for proteins which are involved in phototransduction in photoreceptor cells, such as opsin (Bowes *et al.*, 1988) and transducin (a and b subunits) (Bowes *et al.*, 1988; Brann & Cohen, 1987), increased during the night and decreased during the day, and this diurnal change was synchronous with the cyclic change of the c-*fos* gene level in the ONL (Yoshida *et al.*, 1993). The diurnal variation of the expression of mRNA coding these proteins was first recorded on P13 in the mouse retina (Bowes *et al.*, 1988), and our results revealed that the age of onset of the diurnal change in c-*fos* gene expression was similar to that of the diurnal variation of the expression of mRNA coding these proteins. In addition, a recent study reported that the bovine transducin gene has an AP-1 binding site in its 5′-untranslated leader segment (Tao *et al.*, 1993). Taken together, it is possible that c-*fos* gene plays a role in the regulation of the synthesis of these proteins in retinal cells during the light/dark cycle. It has been reported that the Fos protein induces transcription of the genes encoding enkephalin (Sonnenberg *et al.*, 1989) and dynorphin (Naranjo *et al.*, 1991). Similarly, genes encoding neurotransmitter synthetic enzymes, synaptic vesicle proteins, and metabolic enzymes, may require similar regulation in the cells in the GCL.

REFERENCES

Bowes, C., Veen, T. V. & Farber, D. B. (1988). Opsin, G-protein and 48-kDa protein in normal and rd mouse retinas: Developmental expression of mRNAs and proteins and light/dark cycling of mRNAs. *Experimental Eye Research, 47*, 369–390.

Brann, M. R. & Cohen, L. V. (1987). Diurnal expression of transducin mRNA and translocation of transducin in rods of rat retina. *Science, 235*, 585–587.

Curran, T., Gordon, M. B., Rubino, K. L. & Sambucetti, L. C. (1987). Isolation and characterization of the c-*fos* (rat) cDNA and analysis of post-translational modification *in vitro*. *Oncogene, 2*, 79–84.

Dragunow, M. & Robertson, H. A. (1987). Kindling stimulation induces c-fos protein(s) in granule cells of rat dentate girus. *Nature, 329*, 441–442.

Ehinger, B. & Dowling, J. E. (1987). Retinal neurocircuitry and transmission. *Handbook of Chemical Neuroanatomy, 5*, 389–446.

Griferath, U., Grünert, U. & Wässle, H. (1990). Rod bipolar cells in the mammalian retina show protein kinase C-like immunoreactivity. *Journal of Comparative Neurology, 301*, 433–442.

Hagins, W. A., Penn, R. D. & Yoshikami, S. (1970). Dark current and photocurrent in retinal rods. *Biophysics Journal, 10*, 380–412.

Korenbrot, J. I. & Fernald, R. D. (1989). Circadian rhythm and light regulate opsin mRNA in rod photoreceptors. *Nature, 337*, 454–457.

Morgan, J. I. & Curran, T. (1986). Role of ion flux in the control of c-*fos* expression. *Nature, 322*, 552–555.

Naranjo, J. R., Mellstrom, B., Achaval, M. & Sassone-Corsi, P. (1991). Molecular pathways of pain: Fos/Jun-mediated activation of a noncanonical AP-1 site in the prodynorphin gene. *Neuron, 6*, 607–617.

Rolig, B. & Grantyn, R. (1993). Glutamatergic and GABAergic synaptic currents in ganglion cells from isolated retinae of pigmented rats during postnatal development. *Developmental Brain Research, 74*, 98–110.

Sheng, M. & Greenberg, M. E. (1990). The regulation and function of c-*fos* and other immediate early genes in the nervous system. *Neuron, 4*, 477–485.

Simmons, D. M., Arriza, J. L. & Swanson, L. W. (1989). A complete protocol for *in situ* hybridization of messenger RNAs in brain and other tissues with radiolabeled single-stranded RNA probes. *Journal of Histotechnology, 12*, 169–181.

Sonnenberg, J. L., Raucher III, F. J., Morgan, J. I. & Curran, T. (1989). Regulation of proenkephalin by Fos and Jun. *Science, 246*, 1622–1625.

Tao, L., Pandey, S., Simon, M. I., Henry, K. & Fong, K. W. (1993). Structure of the bovine transducin g subunit gene and analysis of promoter function in transgenic mice. *Experimental Eye Resarch, 56*, 497–507.

Tomita, T., Kaneko, A. & Pautler, E. L. (1967). Spectral response curves of single cones in the carp. *Vision Research, 7*, 519–531.

Wässle, H., Yamashita, M., Greferath, U., Grünert, U. & Müller, F. (1990). The rod bipolar cells of the mammalian retina. *Visual Neuroscience, 7*, 99–112.

Weidman, T. A. & Kuwabara, T. (1968). Postnatal development of the rat retina. *Archives of Ophthalmology, 79*, 470–484.

Yoshida, K., Imaki, J., Fujisawa, H., Harada, T., Ohki, K., Matsuda, H. & Hagiwara, M. (1995a). Differential distribution of CaM kinases and induction of c-fos expression by flashing and sustained light in rat retinal cells. *Investigative Ophthalmology and Visual Science, 37*, 174–179.

Yoshida, K., Imaki, J., Matsuda, H. & Hagiwara, M. (1995b). Light-induced CREB phosphorylation and gene expression in rat retinal cells. *Journal of Neurochemistry, 65*, 1499–1504.

Yoshida, K., Kawamura, K. & Imaki, J. (1993). Differential expression of c-*fos* mRNA in rat retinal cells: Regulation by light/dark cycle. *Neuron, 10*, 1049–1054.

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