Selective Transcription Factor Induction in Retinal Pigment Epithelial Cells during Photoreceptor Phagocytosis*

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Expression of early response genes during rod outer segment phagocytosis by normal Long Evans and Royal College of Surgeons-rdy-p* rats and by dystrophic Royal College of Surgeons-p* rat retinal pigment epithelial cells was studied in primary cell culture. Northern analysis revealed that the abundance of zif-268 (egr-1), c-fos, and tis-1 (NGFI-B) mRNA was rapidly and transiently increased in normal retinal pigment epithelial cells during rod outer segment phagocytosis but not during phagocytosis of latex particles. No increase in gene expression was found in Royal College of Surgeons-p* dystrophic retinal pigment epithelial cells challenged with rod outer segments. As shown by electrophoretic mobility shift assay, a prominent short term increase in the intensity of the gel-shifted band was detected using nuclear protein extracts derived from rod outer segment-challenged, control retinal pigment epithelial cells and zif-268, AP-1, AP-2, or tis-1 consensus oligonucleotides. No such increase was detected when using nuclear factor κB consensus oligonucleotide or when the early response gene prostaglandin H synthase-2 mRNA was measured over the time course studied. The results suggest that in retinal pigment epithelial cells, rod outer segment-specific phagocytosis is accompanied by the selective expression of early response genes coding for transcription factors. The specific pattern of the induction of these transcription factors is predicted to modulate the expression of gene cascades.

The retina is a part of the central nervous system directly exposed to external environmental stimuli. The outer segments of photoreceptors contain rhodopsin and other molecules that capture light signals and transmit this information through the retinal synaptic circuitry to the brain. The outer segments are damaged by oxidative stress and need to undergo a renewal process during which the tip is shed daily and then phagocytized by the retinal pigment epithelial (RPE)1 cells. Retinol and docosahexaenoic acid, essential for visual cell function, are recycled to the inner segment by the RPE cells (1–8), which also regulate the intake of nutrients, removal of waste, and maintenance of photoreceptor cells in a daily, rhythmic manner, which is light-regulated in amphibians and circadian in mammals.

The ability of RPE to phagocytize the shed discs of outer segments is essential for photoreceptor integrity and function. If this ability is compromised, as in mutant RCS (Royal College of Surgeons) rats, irreversible degeneration of the photoreceptor cells takes place (9).

RPE cells are the most active phagocytes in the body. In the rat, an RPE cell ingests and degrades 25,000–30,000 membrane outer segment discs each day. Virtually all are degraded efficiently within a few hours after ingestion. In addition, RPE cells sort, process, and retrieve compounds essential for visual cell function, such as vitamin A (1–5) and docosahexaenoic acid (6–8). The RPE cells then prepare for the next day’s cycle of phagocytosis. To perform this task, RPE cells must have tightly regulated intracellular signaling pathways that couple phagocytosis with expression of genes encoding the rod outer segment (ROS)-degradative enzymes, transport proteins, etc. There is no information available on gene expression in RPE cells early during ROS phagocytosis.

Even though RPE cells normally phagocytize the outer segments of photoreceptors, they can also nonspecifically phagocytize particles such as latex beads (10). Phagocytosis of ROS is thought to be mediated by a specific receptor in RPE cells, although identification of this receptor remains elusive, as do the associated signal transduction pathways (11, 12).

The products of early response genes (ERGs) or immediate-early genes are engaged in receptor-mediated transduction events by which extracellular stimuli modulate gene expression. ERGs are a class of genes whose expression is low or undetectable in quiescent cells but whose transcription is activated rapidly after extracellular stimulation, independent of de novo protein synthesis. Several ERGs encode transcription factor proteins that modulate the subsequent opening of gene cascades (13). c-fos proto-oncogene encodes a component of the AP-1 transcription factor (14). Among the external stimuli that turn the expression of c-fos on in mouse peritoneal macrophages is Fc- and C3b-mediated phagocytosis (15). Other ERGs include zif-268, which is a zinc finger-containing transcription factor (also known as tis-8, egr-1, krox24, d2, NGFI-A) (16, 17); tis-1, a transcription factor from orphan steroid/thyroid hormone superfamily (NGFI-B, nur77, N10) (18–20); AP-2 transcription factor (21, 22); and cux-2 (tis-10, pgs-2, ptgs-2, pghs-B), an ERG that codes for the inducible form of prostaglandin synthase (23, 24).

We now report that phagocytosis of ROS by RPE cells selectively activates certain early response gene transcription factors. These genes are not activated by nonspecific phagocytosis or in dystrophic RPE cells. Part of these results have appeared in abstract form (25).
Experimental Procedures

Animals—Long Evans female rats with timed litters (from Harlan Sprague-Dawley) were kept in plastic cages at the LSU Medical Center animal care facility until the pups reached the age of 10 days. The retinal dystrophic RCS-p+ strain and congenic control RCS-rdy-p+ strain of rats (26) were kindly provided by Dr. Matthew M. LaVail (University of California, San Francisco). Breeding pairs of these rats were kept in plastic cages in the animal care facility, and births were monitored daily. All rats were kept on the 12-h dark/12-h light cycle.

RPE Cell Primary Culture—RPE cells were isolated from 10-day-old Long Evans rat pups and grown in primary culture in six-well plates until confluent. ROS were isolated from adult Long Evans rats by centrifugation in a continuous sucrose gradient. 100 μL of ROS suspension (2 × 10^5/mL) in MEM without supplements was layered on top of the RPE cell monolayer in three of six wells in each plate. To the three control wells an equal volume of vehicle (MEM) was added. The cells were incubated in a humidified incubator at 37 °C in 95% air, 5% CO2 for the stated period of time before RNA was isolated or NPXT was prepared. To determine the relative abundance of mRNA or DNA binding activity of transcription factor proteins is plotted as a function of time of incubation with ROS (vertical axis). Left panel, Northern blot analysis of zif-268, c-fos, and tis-1 mRNA expression. Total RPE cell RNA was isolated using the guanidine-thiocyanate-phenol-chloroform method and was subjected to gel electrophoresis (5 μg of RNA/lane) under denaturing conditions on a 1.2% agarose-formaldehyde gel. RNA was transferred to Hybond-N nylon membrane followed by hybridization at 42 °C with [32P]DNA probes for zif-268, c-fos, or tis-1. A [32P]DNA probe for gapdh was used as a reference. mRNA expression was quantified on Northern blots using a Packard Instant Imager. mRNA abundance in each sample was calculated as a ratio of cpm in the zif-268, c-fos, or tis-1 bands to cpm in the gapdh band in each lane. The results are presented as a ratio of mRNA abundance in ROS-treated RPE cells to that in control cells (to which no ROS were added) for each time point (mean ± S.D.). For the measurement of mRNA abundance, experiments were repeated at least twice with two to six plates of RPE cells/time point in each experiment. Right panel, EMSA of RPE nuclear extracts with zif-268, AP-1, and TIS-1 consensus oligonucleotides. Nuclear protein extracts from RPE cells were incubated for 30 min at 0–4 °C with 10,000–50,000 cpm of [32P]-radiolabeled DNA sense oligonucleotides, 22–27 base pairs in length, containing the consensus and mutant binding sequences for AP-1, zif-268, and TIS-1. Protein-DNA complexes were analyzed on a 0.5% acrylamide (acrylamide:bisacrylamide, 40:1), using 0.5 TBE gel matrix and running buffers. After electrophoresis, dried gels were analyzed using either a GS-250 Molecular Imager or an Instant Imager. The results are presented as a ratio of radioactivity of a shifted band in a sample derived from ROS-treated RPE cells to that in a sample from control cells (to which no ROS were added) for each time point (mean ± S.D.). EMSAs were repeated at least twice with two or three plates of RPE cells used for each time point.

RNA Extraction and Northern Blot Analysis—RPE cells were isolated from 10-day-old rats (27). Cells were seeded into six-well plastic tissue culture plates at a density of approximately 10^6 cells/well or into 60-mm plastic tissue culture dishes (2.5 × 10^5 cells/dish) and cultured in minimum essential medium (MEM) (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (Life Technologies, Inc.), 100 units/ml of both penicillin and streptomycin, and 2 mM glutamine. The cells were used when they reached confluence, usually after 7–10 days of growth at 37 °C in 95% air, 5% CO2.

Phagocytosis of ROS by RPE Cells—ROS were isolated from adult Long Evans rats by centrifugation in a continuous sucrose gradient (28) and were resuspended in MEM without supplements at a final concentration of 2 × 10^5/mL. The ROS suspension (100 μL) was layered on top of the RPE cell monolayer, and the medium was mixed with gentle rocking. For controls, an equal volume of vehicle (MEM) was added to RPE cells. The cells were incubated in a humidified incubator at 37 °C in 5% CO2, 95% air for the time indicated in each experiment before RNA was isolated or nuclear protein extract (NPXT) was prepared. As a morphological control, some cell cultures from the same experiments were double immunostained using anti-ROS antisera as described before (28), and phagosomes were visualized with a Odyssey XL confocal laser scanning microscope (Noran Instruments, Middleton, WI). To study the specificity of gene expression for phagocytosis of ROS, Polybead polystyrene latex spheres (1.053-μm outer diameter, Polysciences, Warrington, PA) or fluorescent yellow-green latex microspheres (1.0-μm outer diameter, Molecular Probes, Eugene, OR), were used in the place of ROS (2 × 10^5/mL in 100 μL of MEM layered on top of the RPE cell monolayer). In representative cell cultures, phagocytosed fluorescent beads were observed directly with the confocal scanning microscope to ensure, as with ROS, that phagocytosis of latex beads took place.

RNA Extraction and Northern Blot Analysis—After incubation with ROS or latex beads, the plates were put on ice and washed three times with ice-cold phosphate-buffered saline. The cells were kept on ice until guanidine-thiocyanate extraction buffer was added to isolate total cell RNA (29). Gel electrophoresis of RNA (5 μg/lane) was performed under denaturing conditions on a 1.2% agarose-formaldehyde gel. RNA was transferred to Hybond-N nylon membrane (Amersham Corp.) followed by hybridization at 42 °C with [32P]DNA probes for zif-268 (18, 30), c-fos, tis-1 (18, 21), or coc-x-2 (29). [32P]DNA probe for the glyceraldehyde-3-phosphate dehydrogenase gene (gapdh) (32) was used in each hybridization as a reference. Autoradiography and quantification of Northern blots were performed using GS-250 Molecular Imager (Bio-Rad) or an Instant Imager (Packard, Meriden, CT).

Electrophoretic Mobility Shift Assay (EMSA)—NPXTs and cytoplasmic protein extracts (CPX Ts) were prepared from RPE cells (33–35). DNA sense and mutant oligonucleotides, 21–30 base pairs in length, containing the consensus and mutant binding sequences for AP-1 (consensus, 5'-CGGTGATGACTCGCAGCGGAA-3'; mutant, 5'-CGGTGGATGACTCGCAGCGGAA-3'), AP-2 (consensus, 5'-GATCAGACTGACC-GCCCGGCGCGCCGT-3'; mutant, 5'-GATCAGACTGACC-GCCCGGCGCGCCGT-3'), zif-268 (consensus, 5'-GGATGTCAGCGGGGGGAGCGGGGGGGCCGA-3'; mutant, 5'-GGATGTCAGCGGGGGGA-3'), or GAPDH (consensus, 5'-GATTTTAAAGGTCATGCTCAATTGGAT-3') (36) were obtained from either Santa Cruz Biotechnology (Santa Cruz, CA) or Promega Corporation (Madison, WI), or were synthesized at the LSU Medical Center Core Facility. NPXT and CPXT concentrations were determined by the method of Bradford (37), using whole histones as standards. To calibrate the EMSA system further, purified transcription factors (AP-1) from Promega were used. Protein-DNA complexes were formed in 10–15 μl at 0–4 °C by the tandem addition of 0–10 μg of each NPXT, CPXT, or purified transcription factor into an assembly buffer consisting of 50 mM KCl, 50 mM Tris-HCl, pH 7.5, 6 mM magnesium acetate, 1 mM EDTA, 1 mM β-mercaptoethanol, 5% glycerol, 0.01% Nonidet P-40, and 0.1–1 μg/μl poly(di-dC), followed by the addition of 10,000–50,000 cpm of end-radiolabeled sense or mutant oligonucleotide DNA. Samples were incubated for 30 min in 500-μL
RESULTS AND DISCUSSION

Induction of c-fos, zif/268, and tis-1 mRNA during Phagocytosis of R0S—Primary cultures of rat RPE cells readily phagocytize freshly isolated rat ROS. This was monitored by direct microscopic observation of immunostained phagosomes inside RPE cells (data not shown). Kinetic studies using ROS, compared with nonspecific latex particles, strongly suggest that RPE cells, in this *in vitro* system, phagocytize ROS through a specific receptor-mediated mechanism (11, 38). Resting RPE cells in culture revealed low levels of zif/268 mRNA; c-fos, tis-1, and c-2 mRNA were undetectable. Because ERGs are extremely sensitive to experimental manipulations, ROS were added in small volumes (100 μl) of serum-free medium because larger volumes (e.g. 1 ml) or the addition of serum enhanced ERG expression. To control for other factors that contributed to nonspecific induction of ERGs (e.g. exposure to room air, shaking, low temperature, and/or high pH due to manipulations carried out outside the CO₂ tissue culture incubator), separate six-well plates were used for each time point, three of which received ROS, and the other three wells served as controls.

c-fos, zif/268, and tis-1 mRNA abundance in normal Long Evans rat RPE cells was rapidly and transiently increased during ROS phagocytosis (Fig. 1). After 30 min of incubation with ROS, the c-fos, zif/268, and tis-1 mRNA expression was greatly enhanced compared with controls. Maximum mRNA abundance was observed at 30 min for c-fos, at 45 min for zif/268, and at 60 min for tis-1. The amplitude of responses differed: c-fos and tis-1 mRNA accumulation ranged from 6- to 20-fold, whereas ROS induction of zif/268 was smaller, ranging from 2- to 3-fold, which is similar to the relative induction of these genes in cultured rat astrocytes by mitogens (39). By 2 h, mRNA levels for all three genes decreased but still remained higher than controls. RPE cells from RSC-Rdy *p* strains, which are a normal congenic control for the dystrophic RSC-Rdy/rdy *p* rats, showed similar gene induction during phagocytosis of ROS (data not shown).

Lack of cox-2 Expression during Phagocytosis of R0S—cox-2 is induced in cells in response to diverse stimuli, such as tetrade cycanophbolacetate, platelet-derived growth factor, lipopoly saccharide, or platelet-activating factor (40). However, no expression of cox-2 was observed in RPE cells in response to ROS phagocytosis or to platelet-activating factor (100 μM), although both 50 M tetrade cycanophbolacetate and 10 ng/ml platelet-derived growth factor did induce this gene (Fig. 2a).

Induction of Transcription Factor Proteins in RPE Nuclear Extracts during Phagocytosis of R0S—EMSA using zif/268, AP-1, and TIS-1 consensus oligonucleotides and RPE nuclear extracts showed the appearance of shifted bands during ROS phagocytosis (Figs. 1 and 2b, b and c). Most of the zif/268 shifted bands were observed in nuclear extracts compared with extracts prepared with RPE cytosol. There was some enhancement in the shifted band in RPE cytosol 45 min after the addition of ROS. However, this was much lower than the corresponding change observed in nuclear extracts. Using zif/268 mutant oligonucleotides, no shifted bands were observed under these experimental conditions (Fig. 2c), suggesting that the shifted bands specifically recognized the zif/268 consensus sequence. The maximum intensity of gel-shifted bands occurred at 30 min for AP-1, 45 min for zif/268, and 60 min for TIS-1. This suggests that c-fos, zif/268, and tis-1 mRNAs are translated very rapidly into transcription factor proteins. The short half-life of c-fos mRNA (t1/2 ~ 15 min) suggests that, indeed, the cellular mechanism for translation of c-fos mRNA into c-FOS gene product was very rapid (41).

Detailed analysis of the kinetics of induction of DNA binding activity of transcription factor proteins compared with their respective mRNA levels revealed a seeming disparity between these two patterns of expression. For example, a significant increase in zif/268 protein appears at 15 min after incubation...
with ROS, well before there is a demonstrable increase in zif-268 protein levels (30 min). Similarly, increased mRNA expression for c-fos and tis-1 is evident after 2 h of ROS phagocytosis, whereas increased AP-1 DNA binding activity can only be seen at 30 min and TIS-1 binding activity at 60 and 75 min. This difference in the kinetics of expression suggests the involvement of post-transcriptional and translational control in the expression of these genes. Since the constitutive level of zif-268 mRNA expression is relatively high in REP cells, regulation of zif-268 protein expression may involve the existence of a translational block. This type of zif-268 modulation has been shown in other cells (42). The initial induction of zif-268 by ROS phagocytosis may relieve the translational constraint by specific phagocytosis stimuli and then transcriptionally activate zif-268 mRNA synthesis. The possible involvement of post-transcriptional regulation is even more evident in the case of AP-1 transcription factor. AP-1 is formed as either a homodimer or as a heterodimer by different members of the fos and jun proto-oncogene families. It is well established that AP-1 is not only regulated by transcription of fos and jun genes, but also at the translational level (43) and by post-translational phosphorylation of Jun and Fos proteins (14). The very narrow peak of DNA binding activity of AP-1 protein coexisting with longer induction of c-fos mRNA expression suggests this post-transcriptional regulation in RPE cells.

The transcription factor AP-2 was also rapidly and transiently expressed in RPE nuclear extracts during ROS phagocytosis with a prominent peak at 15–30 min after incubation with ROS (Fig. 3). However, using the NFkB consensus oligonucleotide (5'-AGTTGAGGGGACTTTCCCAGGC-3') in EMSA, no evidence was found of an enhanced expression in the RPE nuclear extract during phagocytosis (Fig. 3). Tate et al. (45) found activation of NFkB DNA binding in cultured human RPE cells after prolonged (4-h) incubation with bovine ROS. This response was not specific for ROS as the same level of induction was found during phagocytosis of latex beads. The foregoing results suggest that initial NFkB induction is not an early event in ROS phagocytosis.

**Specificity of ERG Expression during Phagocytosis of ROS—** Rat RPE cells in primary culture display specificity toward phagocytosis of ROS compared with that of nonspecific particles (11). However, these cells can also ingest large quantities of latex beads (10, 46) presumably through a nonspecific mechanism (44). Therefore, to see if the induction of ERGs by ROS phagocytosis also occurred with latex bead particles, RPE cultures were fed with either ROS or the same number of latex particles that had a diameter similar to ROS. Even though RPE cells phagocytized large quantities of latex beads (as confirmed by visualization of phagocytosed fluorescent latex beads in a confocal scanning microscope; data not shown), only ROS-mediated phagocytosis stimulated expression of c-fos, zif-268, and tis-1 mRNAs (Figs. 2 and 4), as well as AP-1, AP-2, and zif-268 nuclear transcription factor proteins (Fig. 4) in RPE cells.

![Fig. 3. EMSA of RPE nuclear extracts with AP-2 and NFkB consensus oligonucleotides after 0–60 min of incubation with Long Evans RPE cells with ROS. NPXTs were analyzed, and results are presented as described in the legend to Fig. 1. For AP-2, two independent experiments were performed with two plates assayed for each time point; for NFkB three experiments with three plates for each time point were performed.](image109x297 to 237x424)

![Fig. 4. Absence of the expression of immediate-early transcription factors in RPE cells of control (Long Evans or RCS-p°"rdy") rat RPE during phagocytosis of latex, or mutant (RCS-p°) rat RPE challenged with ROS. Left panel, Northern blot analysis of zif-268, c-fos, and tis-1 mRNA expression. Control or mutant RPE cells were incubated with either ROS or latex beads for 45 min as described in Fig. 2c. Relative mRNA abundance (mean ± S.D.) is expressed as described in Fig. 1. Results of duplicate experiments with two to four plates of RPE cells assayed in each experiment for each condition are presented. Right panel, EMSA of control or mutant RPE nuclear extracts with zif-268, AP-1, and AP-2 consensus oligonucleotides after 30 min (for AP-1 and AP-2) or 45 min (for zif-268) of incubation with ROS or latex beads. Nuclear protein extracts were analyzed, and results are presented as described in the legend to Fig. 1. Results of duplicate experiments with two to four plates of RPE cells assayed for each condition are presented.](image40x101 to 464x223)
specific patterns, starting with AP-2 (15 min after the addition of ROS), followed by AP-1 (30 min), zif-268 (45 min), and TIS-1 (60 min). Gene induction was specific for ROS phagocytosis because (a) it occurred only while RPE cells phagocytized outer segments and not when they ingested latex beads; (b) some transcription factors were induced while others were unaffected; and (c) RPE cells from RCS dystrophic rats did not display activation of immediate-early transcription factors upon the addition of ROS. This selective activation of ERGs in RPE cells supports the notion that phagocytosis of ROS by RPE cells is indeed a receptor-mediated process, different from nonspecific phagocytosis of particles such as latex beads. Also, for the first time, this study provides a qualitative assay (ERG expression) to distinguish between specific and nonspecific phagocytosis by RPE cells.

During phagocytosis of ROS, a receptor-mediated event may trigger a signal in RPE cells which, in turn, activates certain ERGs in a specific pattern. Gene cascades may then be opened by activating the subsequent expression of genes that encode proteins necessary for the completion of phagocytosis, preparation of the cell for the next cycle, and for the fulfillment of other functions. Furthermore, signals engaged on this receptor-gene activation cascade may be critical for the survival of photoreceptors, particularly in the aging retina, and may be relevant to the understanding of events crucial to senile macular degeneration.

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REFERENCES
1. Okajima, T. I. K., Pepperberg, D. R., Rippes, H., Wiggert, B., and Chader, G. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6907–6911
2. Pepperberg, D. R., Okajima, T. I. K., Wiggert, B., Rippes, H., Cruach, R. K., and Chader, G. J. (1993) Mol. Neurobiol. 7, 61–85
3. Ong, D. E., Davis, J. T., O’Day, W. T., and Bok, D. (1994) Biochemistry 33, 1835–1842
4. Chen, Y., and Noy, N. (1994) J. Biol. Chem. 269, 21983–21989
5. Okajima, T. I. K., Wiggert, B., Chader, G. J., and Pepperberg, D. R. (1994) J. Biol. Chem. 269, 21983–21989
6. Bazan, N. G., Gordon, W. C., and Rodriguez de Turco, E. B. (1992) Adv. Exp. Med. Biol. 318, 295–306
7. Bazan, N. G., Reddy, T. S., Redmond, T. M., Wiggert, B., and Chader, G. J. (1985) J. Biol. Chem. 260, 13677–13686
8. Chen, H., and Anderson, R. E. (1993) Exp. Eye Res. 57, 369–377
9. Bok, D., and Hall, M. O. (1971) J. Cell Biol. 49, 664–682
10. Edwards, R. B., and Szamier, R. B. (1977) Science 197, 1001–1003
11. Mayerson, P. L., and Hall, M. O. (1986) J. Cell Biol. 103, 299–308
12. Boyle, D. L., Tien, L. F., Cooper, N. G., Shepherd, V., and McLaughlin, B. J. (1991) Invest. Ophthalm. & Visual Sci. 32, 1464–1470
13. Deuet, J. P., Squinto, S. P., and Bazan, N. G. (1990) Mol. Neurobiol. 4, 27–55
14. Kariot, M. (1995) J. Biol. Chem. 270, 16483–16486
15. Collart, M. A., Belin, D., Briottet, C., Thornes, B., Vassali, J. D., and Vassalli, P. (1989) Oncogene 4, 237–241
16. Milbrandt, J. (1987) Science 236, 797–799
17. Christy, E., and Nathans, D. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8737–8741
18. Hazel, T. G., Nathans, D., and Lau, L. F. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8444–8448
19. Milbrandt, J. (1988) Neuron 1, 183–188
20. Lim, R. W., Varrn, B. C., and Herschman, H. R. (1987) Oncogene 1, 263–270
21. Moser, M., Pecherer, A., Bauer, R., Imhof, A., Seegers, S., Kerscher, M., and Buettner, R. (1993) Nucleic Acids Res. 41, 4844
22. Bauer, R., Imhof, A., Pecherer, A., Kopp, H., Moser, M., Seegers, S., Kerscher, M., Tainsky, M. A., Hofstaedter, F., and Buettner, R. (1994) Nucleic Acids Res. 22, 1413–1420
23. O’Banion, M. K., Sadowski, H. B., Winn, V., and Young, D. A. (1991) J. Biol. Chem. 266, 23621–23626
24. Ryseck, R. P., Raynosocek, C., Macdonald-Bravo, H., Dorfman, K., Mattei, M. G., and Bravo, R. (1992) Cell Growth & Differ. 3, 443–450
25. Ershov, A. V., Wiggert, B., and Bazan, N. G. (1995) Invest. Ophthalmol. & Visual Sci. 36, (suppl.) 9815
26. LaVail, M. M. (1981) J. Cell Biol. 87, 2983–2991
27. Mayerson, P. L., Hall, M. O., Clark, V., and Abrams, T. (1985) Invest. Ophthalmol. & Visual Sci. 26, 1599–1609
28. Chaitin, M. H., and Hall, M. O. (1983) Invest. Ophthalmol. & Visual Sci. 24, 812–820
29. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
30. Chaid, B. A., Lau, L. F., and Nathans, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7857–7861
31. Ryseck, R. P., Macdonald-Bravo, H., Mattei, M. G., Ruppert, S., and Bravo, R. (1989) EMBO J. 8, 3327–3335
32. Tso, J. Y., Sun, X.-H., Kao, T.-H., Reece, K. S., and Wu, R. (1985) Nucleic Acids Res. 13, 2485–2502
33. Ceglaek, J. A., and Rezvan, A. (1989) Electrophoresis 10, 360–365
34. Luik, W. J., Rognier, E. I., Wong, L., Vaulin, G., McLaughlan, D. R. C., and St. George-Hyslop, P. H. (1994) Mol. Brain Res. 22, 121–131
35. Zhang, D.-E., Hoyt, P. R., and Papacostanitou, J. (1990) J. Biol. Chem. 266, 3292–3301
36. Wilson, T. E., Paulsen, B. E., Padgett, K. A., and Milbrandt, J. (1992) Science 256, 107–110
37. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
38. Hall, M. O., and Abrams, T. (1987) Exp. Eye Res. 45, 907–922
39. Arenander, A. T., Lim, R. W., Varnum, B. C., Cole, R., de Vellis, J., and Herschman, H. R. (1989) J. Neurosci. Res. 23, 257–265
40. Herschman, H. R. (1994) Cancer Metastasis Rev. 13, 241–256
41. Shyu, A. B., Greenberg, M. E., and Belasco, J. G. (1989) Science 245, 8737–8741
42. Maass, A., Grohe, C., Oberdorf, S., Sukhatme, V. P., Vetter, H., and Neyses, L. (1994) Biochem. Biophys. Res. Commun. 202, 1337–1346
43. Adunyah, S. E., Ceesay, K. J., and Rivero, J. A. (1996) Biochem. Biophys. Res. Commun. 221, 213–218
44. Higo, H., and Hohn, H. M. (1984) Exp. Eye Res. 33, 47–53
45. Tate, D. J., Mizuchi, M., and Newsome, D. A. (1995) Invest. Ophthalmol. & Visual Sci. 36, 1271–1279
46. Seyfried-Williams, R., McLaughlin, B. J., and Cooper, N. G. (1984) Exp. Cell Res. 154, 500–509