Interferon consensus sequence-binding protein is constitutively expressed and differentially regulated in the ocular lens

(Received for publication, October 27, 1998, and in revised form, December 21, 1998)

Wenmei Li, Chandrasekharan N. Nagineni, Hong Ge, Bassey Effioe, Ana B. Chepelinsky, and Charles E. Egwuagu

From the Laboratories of Immunology and Molecular Developmental Biology, NEI and the Laboratory of Molecular Hematology, NHLBI, National Institutes of Health, Bethesda, Maryland 20892

Interferon signaling is mediated by STATs and interferon regulatory factor (IRF) families of transcription factors. Ten distinct IRFs have been described and most are expressed in a variety of cells except for interferon consensus sequence-binding protein (ICSBP) and lymphoid-specific IRF/Pip that are thought to be exclusively expressed in lymphoid cells. We show here for the first time that ICSBP is constitutively and inducibly expressed in the mouse lens. In contrast to lymphoid cells with exclusive expression of ICSBP in the nucleus, ICSBP is present in both the cytoplasm and nucleus of the lens cell. However, ICSBP in the nucleus is of lower apparent molecular weight. We further show that the ICSBP promoter is constitutively bound by lens nuclear factors and that its activation requires binding of additional factors including STAT1. Furthermore, transcriptional activation of ICSBP gene by interferon γ is accompanied by selective nuclear localization of ICSBP in proliferating epithelial cells but not in the nuclei of nondividing cells in the lens fiber compartment. Constitutive and inducible expression of ICSBP in the ocular lens and differential regulation of its subcellular localization in the developing lens suggest that ICSBP may have nonimmunity related functions and that the commonly held view that it is lymphoid-specific be modified.

Interferons (IFNs) are a family of secreted proteins that are involved in the regulation of diverse cellular processes (1). In addition to their well-defined roles in host defense against infectious agents, they have been associated with the regulation of cellular immunity, cell growth (1–3), and epithelial cell differentiation (4). The importance of IFNs is underscored by the expression of their receptors in virtually all mammalian cell types and by the fact that they regulate the expression of more than 50 cellular genes (1, 5). Interaction of IFNs with their cell surface receptors leads to activation of protein tyrosine kinases, JAK1, JAK2, or Tyk2, which in turn phosphorylate and activate members of a family of latent cytoplasmic transcription factors called STATs (signal transducers and activators of transcription) (6, 7). Phosphorylated STATs form homo- or heterodimers that translocate to the nucleus where they bind to well defined DNA sequences called GAS (gamma interferon activation site) or ISREs (IFN-stimulated response elements) and activate the transcription of genes coding for members of the interferon regulatory factor (IRF) family of transcription factors (8, 9).

IRFs are important mediators of transcriptional activation or repression of IFN-regulatable genes. They are characterized by a 115-amino acid N-terminal DNA-binding domain that interacts with ISRE motifs of IFN-regulatable genes (9). Direct and indirect evidence indicate that the C-terminal portion of IRFs contains a protein-protein interaction domain able to function as transcriptional activators and/or repressors (9, 10). Ten members of the IRF family have been identified, and they include ICSBP, ISGF3γ/p48, IRF-1, IRF-2, IRF-3, IRF-4, lymphoid-specific IRF/Pip/ICSAT, IRF-5, IRF-6, IRF-7, and vIRF (9). IRF-1 and IRF-2 are the best characterized members of this family and were initially identified by studies of the transcriptional regulation of the human IFNβ gene (11, 12). They have subsequently been shown to be key factors in the regulation of cell growth through their effects on the cell cycle (2, 3). IRF-1 is a tumor suppressor (13), whereas IRF-2 is oncogenic (14).

In contrast to IRF-1 and IRF-2, which are expressed in a variety of cell types, two IRF members, interferon consensus sequence-binding protein (ICSBP) (15) and lymphoid-specific IRF/Pip (Pu1 interaction partner) (16–18) are thought to be expressed exclusively in cells of macrophage and lymphocyte lineages. Constitutive expression of ICSBP is thought to be limited to B lymphocytes, and mice with null mutation for the ICSBP gene develop myelogenous leukemia-like syndrome, suggesting that ICSBP activities may be restricted to lymphoid cells (15, 19).

We have previously reported the generation of transgenic mice with targeted ectopic expression of IFNγ in the lens under the direction of the αa-crystallin promoter (20, 21). In these mice, the normal pattern of endogenous lens gene expression is perturbed, and the developmental fate of cells destined to become lens fiber cells is altered. It was during the course of studies to establish a biological link between expression of IFNγ and the observed developmental defects that we discovered that several IRFs are constitutively expressed in the mouse lens. In this report, we present evidence that ICSBP is constitutively and inducibly expressed in the mouse lens.

EXPERIMENTAL PROCEDURES

Animals—BALB/c wild type (WT) mice were purchased from Jackson Laboratories (Bar Harbor, ME). CD-1 WT mice were from Charles River (Raleigh, NC). Generation of the BALB/c IFNγ transgenic (TR) mice has previously been described (20, 21). All animal procedures conformed to Institutional Guidelines and the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

Cell Culture and IFNγ Treatment—The murine lens epithelial cell line, aTN4–1 (22), kindly provided by Dr. Paul Russell (NEI, NIH, Bethesda, MD), was grown in Dulbecco’s modified Eagle’s medium

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Lab. of Immunology, National Eye Institute, 10/10N116, 10 Center Dr., 1858, NIH, Bethesda, MD 20892.

§ The abbreviations used are: IFN, interferon; IRF, interferon regulatory factor; ICSBP, interferon consensus sequence-binding protein; WT, wild type; TR, transgenic; CHX, cycloheximide; PCR, polymerase chain reaction."
supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. The CRL226 and 1AMLE 6 mouse epithelial cell lines (23), kind gifts from Dr. Christina M. Sax (NEI, NIH), were propagated in minimum essential medium supplemented with 5% rabbit serum, 5% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. These cells were treated with murine recombinant IFNγ (Life Technologies, Inc.) at a concentration of 100 units/ml for 2 h at 37°C, 5% CO2. Some cells were propagated in medium containing the protein synthesis inhibitor, cycloheximide (CHE) (Sigma) at 35 μg/ml for 30 min followed by addition of IFNγ and incubation for 2 h.

Reverse Transcript-PCR—Lenses from 6-week-old WT or TR mouse littersmates were carefully dissected and washed before RNA isolation to avoid any possible contamination by other tissues. Total RNA was isolated from the lenses or cultured lens cells as recommended for the TRizol Reagent (Life Technologies, Inc.). All RNA samples were digested with RNase-free DNase I (Life Technologies, Inc.) for 30 min and purified by phenol/chloroform extraction and precipitation in 0.4 × LiCl. CDNA synthesis was performed at 42°C for 1 h with 10 μg of total RNA, 0.3 μg of oligo(dT), 1,10, and 1000 units Reverse Transcriptase II (Life Technologies, Inc.) in a final volume of 50 μl. For each RNA preparation, a negative control reaction was performed without reverse transcriptase. After purification of the cDNA, hot start PCR assays were performed with AmpliTaq Gold DNA polymerase (Perkin-Elmer) or Pwo polymerase (Roche Molecular Biochemicals) incubated in a 10 μl reaction. AmpliTaq Gold, and amplification was carried out for 25 cycles at 94°C for 45 s, 63°C for 45 s, and 72°C for 45 s, and this was followed by a final 10-min extension at 72°C. All the primer pairs used for PCR amplifications spanned at least one intron, making it possible to distinguish between amplification products derived from cDNA and those resulting from any contaminating genomic DNA templates. The sequence of the PCR primers used are for mouse G3PDH, 5'-TGAAGGTCGGTGTTGACAGCGGATTTGGC-3' and 5'-CATGATTAGCGCATGAGTGC-3 (24), and for mouse ICSBP, 5'-GCTGGCGCAGTGTGCGC-3 and 5'-AGTTGGGCGCTACTGGGTCTGCTG-3 (25). For Southern blot analysis, the amplified fragments were electrophoresed in agarose gels, transferred onto Hybond N+ nylon membrane (Amersham Pharmacia Biotech), and probed with fluorescein-in-DTP 3'-end-labeled oligonucleotides, internal to the corresponding PCR primers. Probe labeling and signal detection were performed with the ECL 3'-oligolabeling and detection system (Amersham Pharmacia Biotech).

Northern Blot Analysis—Total RNA (30 μg) was fractionated on a 0.8% agarose-formamide gel, transferred to Hybond N+ membrane (Amersham Pharmacia Biotech), and hybridized for 12 h at 65°C in hybridization solution containing 5 × 106 cpm/ml of probe as described (26). ICSBP or β-actin-specific cDNA fragments were labeled to high specific activity (>106 cpm/μg) with [α-32P]dCTP by random priming (oligolabeling kit; Amersham Pharmacia Biotech) and used as hybridization probes. After two high stringency washes in 0.1 × SSC, 1% SDS at 65°C, signals were detected by autoradiography at −70°C with Kodak XAR-5 and Cronex intensifying screens.

Western Blot Analysis—Lenses derived from 6-week-old WT or TR mouse littersmates were disrupted in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxylcholate, 2 μM leupeptin, 2 μM pepstatin, 0.1 μM aprotinin, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride, 0.5 mM phenylmethylsulfonyl fluoride, and 1 μM EDTA on ice. Extracts were clarified by centrifugation, and protein levels were determined by Coomasie Blue dye binding method as recommended for Coomasie Plus Protein Assay Reagent (Pierce). For analysis of the lens epithelial cell lines, cells were cultured for 2 h in medium alone or medium containing 100 units/ml IFNγ. The cells were lysed and fractionated to cytosolic and nuclear fractions as described previously (27). Nuclear and cytosolic fractions were also obtained from the EL4 lymphoma cell line (ATCC TIB-39) (American Type Culture Collection, Manassas, VA) and BALB/c mouse spleen cells. All samples were heated for 10 min at 95°C in 1× sample buffer and electrophoresed in 10% SDS/polyacrylamide gel. The gel was electrophoresed onto polyvinylidene fluoride membrane, blocked with 5% nonfat milk, and probed with either goat anti-mouse ICSBP polyclonal antibodies (1: 20000) (Santa Cruz Biotech, Santa Cruz, CA) or a rabbit anti-ICSBP polyclonal antibody (1:2000) from Zymed Laboratories Inc. (San Francisco, CA). Mouse α-crystallin-specific peroxidase-conjugated secondary P(3) antibodies was kindly provided by Sam Zigler (NEI, NIH), Preimmune serum was also used in parallel as control. Signals were detected with horseradish peroxidase-conjugated secondary P(3) antibodies using the ECL system (Amer sham Pharmacia Biotech).

Immunohistochemistry—Seventeen day mouse embryos were fixed in 4% paraformaldehyde and embedded in Ameraffin tissue embedding medium (Baxter). Tissue sections (5 μm) were deparaffinized in xylene, rehydrated through a graded alcohol series, and used for immunostaining by the avidin-biotin-peroxidase complex method (Vector Laboratories, Burlingame, CA). After preincubation for 30 min with 2% gelatin and 0.1% bovine serum albumin, sections were incubated for 2 h at room temperature with antibodies (2 μg/ml) specific to mouse ICSBP (Santa Cruz). Control sections received the appropriate normal serum. In addition, antibody specificity control experiments were carried out by incubating the primary antibody with a 10-fold excess of a blocking peptide specific for the immunogenic epitope (ICSBP amino acid 407–425) for 2 h. The neutralizing peptide was then used for immunostaining reactions with control tissue sections. All sections were subsequently incubated with biotinylated secondary antibody for 30 min at room temperature, and signal was visualized with diaminobenzidine-H2O2 as recommended (Vector). In some experiments, sections were counterstained with hematoxylin.

Electrophoretic Mobility Shift Assay—Lenses nuclear extracts were prepared either from 1–3-day-old WT CD-1 mouse lenses or cultured lens epithelial cells as described previously (28). Buffer used for nuclear protein extraction contained the following protease inhibitors: 2 μM leupeptin, 2 μM pepstatin, 0.1 μM aprotinin, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride, 0.5 mM phenylmethylsulfonyl fluoride, and 1 μM EDTA. Protein concentration was determined by the method of Bradford (1976), with BSA recommended for Coomassie Blue dye binding as recommended for Coomassie Plus Protein Assay Reagent.

RESULTS

Constitutive and Inducible Expression of ICSBP in the Mouse Lens—We had previously generated TR mice with ectopic expression of IFNγ in the lens to study the paracrine effects of IFNγ in the eye (20, 21). In this study, we examined mRNA and protein levels of IFNγ-inducible transcription factors in the lenses of WT and TR mouse littersmates to determine whether there is any correlation between enhanced expression of members of the IRF family of transcription factors and the abnormal lens phenotype observed in our TR mice. We found that both IRF-1 and IRF-2 are constitutively expressed in the lens and the levels of IRF-1 is markedly enhanced in the TR mouse lens (data not shown). Most surprising, we found ICSBP to be constitutively and inducibly expressed in the mouse lens as indicated by reverse transcribed PCR and Western blot analyses (Fig. 1). These results have been confirmed by six independent experiments, and the authenticity of the ICSBP transcripts has been verified by cDNA sequencing; the nucleotide sequence of the ICSBP transcripts isolated from the lens is identical to published sequences reported for ICSBP derived from mouse hematopoietic cell lines. Detection of ICSBP transcripts in the WT lens was unexpected, as constitutive transcription of the ICSBP gene is thought to be restricted to B-lymphocytes (15, 19, 27). Analysis of rat and bovine lenses reveals that the ICSBP protein and mRNA are also expressed in these species (data not shown). This is the first time that constitutive expression or transcriptional activation of the ICSBP gene has been demonstrated in mammalian cells that are not directly involved in immunological responses.
Spatial Localization of ICSBP Is Differentially Regulated in the Lens by IFNγ—As the vertebrate lens is comprised of undifferentiated, proliferating lens epithelial cells and terminally differentiated fiber cells, we sought to determine the spatial localization of cells expressing ICSBP in the lens. Fig. 2 shows ICSBP localization in embryonic day 17 TR and WT mouse eye sections using polyclonal antibodies specific to mouse ICSBP. In these experiments, three serial sections were fixed onto the same glass slide; one section served as a negative control and was incubated with normal preimmune serum, another section received the primary antibody, and the third section received the primary antibody and 10-fold excess of a neutralizing peptide specific to an immunogenic epitope of mouse ICSBP. In all six independent experiments performed, the experimental sections showed identical antibody-staining patterns, whereas the negative control section consistently showed no immunological reactivity. The section containing 10-fold molar excess of the peptide consistently showed no significant immunoreactivity. In some experiments the amount of the peptide was varied, and the neutralizing effect of the blocking peptide was found to be dose-dependent. As shown in Fig. 2, ICSBP protein is present in both the cytoplasm and nuclei of lens cells. In the TR mouse lens, intense nuclear localization of ICSBP is observed in cells at the lens equator (Fig. 2, C and D) and anterior epithelium (Fig. 2F) but not in nuclei of the cells at the lens fiber compartment (white arrows in Fig. 2, D and F). In the WT lens, the amount of ICSBP in the nucleus is very low and not easily detectable. However, cytoplasmic ICSBP is easily detectable, and the level in the lens epithelia appears to be higher compared with that of the fiber compartment (arrowhead in Fig. 2E).
similar results using eye sections of mice at days 16–20 of embryonic development (data not shown).

Cultured Lens Epithelial Cells Constitutively and Inducibly Express ICSBP—As indicated by our immunolocalization studies, a significant amount of ICSBP expression occurs in the WT lens epithelia, and there is selective accumulation of the ICSBP protein in the nuclei of epithelial cells of the TR mouse lens. To confirm these results we examined well characterized lens epithelial cell line for ICSBP expression. Three lens cell lines, aTN4–1, CRLE2, and 1AMLE6 were therefore treated with mouse IFNγ in either the presence or absence of CHX and analyzed for constitutive or inducible expression of ICSBP. RNA was isolated from various treatment groups and used for Northern analyses. In each of the cell lines, two ICSBP mRNAs of 3.0 and 1.7 kilobases are detected (Fig. 3A), and their sizes are similar to those of mouse ICSBP transcripts in hematopoietic cells (15, 25). In cells treated with IFNγ, a significant increase in ICSBP is observed, indicating activation of the gene by IFNγ. Treatment with CHX prior to addition of IFNγ had no effects on the level of ICSBP transcripts, suggesting that inducible transcription of lens ICSBP mRNAs does not require de novo protein synthesis. In addition, cells that were treated for 2 h with IFNγ and untreated cells were fractionated into cytoplasmic and nuclear fractions and analyzed for the presence of the ICSBP protein by Western blotting. As indicated by our immunolocalization studies, IFNγ-activated STAT1 homodimers in the nucleus to the conserved cis regulatory palindromic IFNγ-responsive GAS element, pIRE/GAS, present in the ICSBP gene at positions −147 to −175 (25). We therefore tested by electrophoretic mobility shift assay whether endogenous lens nuclear factors are able to bind pIRE/GAS. Analysis performed using nuclear extracts derived from WT mouse lens is shown in Fig. 4A. Two prominent DNA-protein complexes are formed with the pIRE/GAS probe, and formation of the complexes is competed by the unlabeled probe (Fig. 4A, lane 3), indicating that the interaction is specific. A DNA element located in the ICSBP gene at positions −191 to −217 and 22 base pairs upstream from the mouse pIRE/GAS site (25) contains a minimum ISRE motif (GAAANN) resembling the IRE or Pu box. This sequence, referred to as ICSBP-IRE (25), was used as a competitor to further characterize the ICSBP/GAS binding activities in the lens. The ICSBP-IRE probe allowed us to detect lens factors that bind to other DNA elements in the ICSBP promoter besides its GAS site. As shown in Fig. 4A, the IRE probe competed for the complex labeled b but not with the a complex (lane 4), indicating that there are factors in the lens that constitutively interact with GAS, as well as non-GAS elements of the ICSBP promoter.

To further characterize the interaction between the ICSBP/ pIRE and ICSBP-IRE elements with lens nuclear factors, we analyzed nuclear extracts derived from the various lens cell lines before and after treatment with IFNγ. Typical results obtained from these studies are shown in Fig. 4B. Either probe formed a common complex (d) that is competed away by a 100-fold excess of either probe. Similar to results obtained using lens nuclear extracts, formation of this complex is also observed in extracts from cells that were not treated with IFNγ (lanes 2 and 6), confirming that lens nuclear factors constitutively bind to these elements. An additional retarded band (e) was detected with the pIRE/GAS probe after treatment of the cells with IFNγ (Fig. 4B, compare lanes 6 and 7), suggesting that these DNA binding activities are IFNγ-inducible factors. pIRE/GAS-binding factors are also detected in cells treated with IFNγ and CHX, indicating that expression of the binding activities does not require de novo protein synthesis (Fig. 4B, lane 8). Competition experiments with 100-fold excess of unlabeled probes revealed that the complex formed with the ICSBP pIRE motif after induction by IFNγ is specific (lanes 13 and 14); the ICSBP-IRE probe is neither able to form the c complex nor compete in the formation of the c complex (lanes 10 and 11).

In hematopoietic cells, activation of ICSBP by IFNγ is me-
ICSBP Expression in the Ocular Lens

**DISCUSSION**

Members of the IRF family of transcription factors differ in the range of cell types they are normally expressed in, their physiological inducers, and the distinct biological processes they affect (9). The IRF proteins identified to date include transcriptional activators (IRF-1 and ISGF3γ), transcriptional repressors (IRF-2 and ICSBP), and other members (lymphoid-specific IRF, IRF-3, IRF-5, IRF-6, IRF-7, and vIRF) whose functions are less well understood (1, 9). With the recent demonstration of a virally encoded homologue of cellular IRFs (29), it is likely that more IRFs will be identified and that previously described members would be found to possess new functions. The results of this study provide the first demonstration that the IRF member, ICSBP, is constitutively and inducibly expressed in the embryonic and adult mouse lens. Expression of ICSBP in the lens is unequivocally demonstrated at the RNA and protein levels, and its authenticity has been confirmed by cDNA sequencing. The ICSBP protein and mRNA are also expressed in rat and bovine lenses, suggesting that other mammals constitutively express ICSBP in their lenses.

Constitutive expression of ICSBP is thought to be limited to B lymphocytes and is not observed in virgin or resting T cells, macrophages, bone marrow, or thymus (15, 27, 30). However, transcription of the ICSBP gene has been shown to be inducible by either IFNγ or antiotic stimulation in T cells and macrophages but not nonhematopoietic cells (27, 30). As part of the immunologically privileged environment of the anterior chamber of the eye, the avascular adult lens has no interactions with the immune system and thus would not be expected to come in direct contact with immunological effector molecules. What then is the functional relevance in the lens of ICSBP, a transcription factor whose functions are thought to be restricted to the ontogeny and regulation of immunological responses by macrophages and lymphocytes? It is of note that ICSBP expression has been reported in the chicken (31, 32). The avian protein is expressed not only in cells of the lymphoid system but also in fibroblasts, suggesting that the transcriptional regulatory activities of ICSBP may not be restricted to lymphocytes and hematopoietic cells.

The data presented here suggest that the expression of the ICSBP gene in the lens may be under regulation by endogenous lens nuclear factors that constitutively bind cis regulatory DNA elements present in the mouse ICSBP promoter. The data further reveal that transcriptional activation of the ICSBP gene in lens cells is mediated by the additional binding of IFNγ-activable factors to the GAS element in the proximal ICSBP promoter. Supershift assays using antibodies specific to various members of the STAT family of transcription factors revealed that STAT1 is one of the IFNγ-inducible binding activities. Although our results indicate that the ICSBP protein in the lens possesses essential characteristics previously described for ICSBP in hematopoietic cells, electrophoretic mobility shift assay analyses using ICSBP-pIRE/GAS or ICSBP-IRE probe reveal subtle differences in the nuclear factors that interact with the ICSBP gene in lymphoid and lens cells. Whereas in EL4 lymphoid cells specific binding to either the pIRE/GAS or IRE probe was observed only after treatment with IFNγ (25), lens nuclear factors bind to either element constitutively, as well as, after IFNγ treatment. Furthermore, the IFNγ-inducible binding activity in lens cells appear to be distinct from those in lymphoid cells because the former is resistant to CHX and consists of multiple supershifted bands (data not shown), whereas the latter is sensitive to CHX, consists of a single band, and requires de novo protein synthesis (25). Differential sensitivity to CHX of IFNγ-inducible factors that interact with the ICSBP-GAS element suggests that distinct factors regulate ICSBP gene in different cell types.

In hematopoietic cells, expression of ICSBP is primarily localized in the nucleus (27). Here we show that ICSBP is present in both the cytoplasm and nucleus of lens epithelial cells. In fact, in the unstimulated lens cell, the level of the ICSBP protein in the cytoplasm is higher than in the nucleus. Our immunolocalization studies on embryonic WT mouse eye sections further show that the level of ICSBP expression is significantly higher in the epithelial compartment of the lens, which exclusively contains undifferentiated, proliferating cells. Similar to lens epithelial cells in culture, the ICSBP protein is predominantly localized in the cytoplasm. However, significant nuclear localization of the ICSBP protein is observed in response to IFNγ signaling. This occurs exclusively in the proliferating lens cells but not in nondividing cells at the lens fiber compartment, suggesting that response to STAT1 signaling in the lens may be restricted to the lens epithelia. Interestingly, the ICSBP proteins in the cytoplasmic and nuclear compartments differ in size. The ICSBP protein in the cytoplasm is of a higher apparent molecular weight, suggesting that nuclear localization of ICSBP is accompanied by post-translational modification. We are currently examining whether the lower molecular weight ICSBP species detected in the lens cell nucleus derives from dephosphorylation of ICSBP or if it is the product of an alternatively spliced ICSBP transcript.

Previous studies have shown that ICSBP is constitutively phosphorylated and that phosphorylation events can modulate...
the ability of ICSBP to bind DNA; ICSBP can bind DNA either through its association with other IRFs or directly when it is not tyrosine-phosphorylated (33, 34). Cytoplasmic expression of ICSBP in the normal lens and differential regulation of its spatial and subcellular localization in response to extracellular stimuli suggest that ICSBP may have distinct functions at different subcellular and/or spatial locations. Thus, the nuclear ICSBP may function as a transcription factor in the proliferating epithelia, whereas the ICSBP in the cytoplasm may have regulatory roles through protein-protein interactions with other IRFs, such as IRF-1 and IRF-2, which are also present in the cytoplasm of lens cells. It is important to note that selective localization to either the cytoplasm or nucleus has also been observed for other lens proteins. For example, the cyclin-dependent kinase 5, a protein associated with the elongation machinery by which specific biochemical pathways are temporally and spatially segregated in the vertebrate lens. Additionally, ICSBP and the other IRFs present in this tissue. It is equally important to elucidate the functional roles of nuclear and cytoplasmic ICSBP and the regulatory mechanisms that may control its sequestration in the cytoplasm and subsequent translocation to the nucleus in response to extracellular stimuli.

Acknowledgments—We are grateful to Drs. Peggy Zelenka, Graeme Wistow, and Jen-Yue Tsai for critical reading of the manuscript; Drs. Paul Russell and Christina M. Sax for providing the lens epithelial cell lines; Dr. Samuel Zigler for providing the mouse αA-crystallin-specific antibody; Nicole Newman for preparation of the histological sections; Riccardo Dreyfus, Shauna Everett, Wayne Randolph, John Ward, Gary Best, and Hassan Ennaciri for photographic assistance; and Rashid Mahdi for assistance in DNA and reverse transcribed PCR analysis.

REFERENCES
1. Boehm, U., Klamp, T., and Howard, J. C. (1997) Annu. Rev. Immunol. 15, 749–795
2. Taniguchi, T., Harada, H., and Lamphier, M. (1995) J. Cancer Res. Clin. Oncol. 121, 516–520
3. Vaughan, P. S., van Wijnen, A. J., Stein, J. L., and Stein, G. S. (1997) J. Mol. Med. 75, 348–359
4. Saunders, N. A., and Jetten, A. M. (1994) J. Biol. Chem. 269, 2016–2022
5. Valente, G., Ozen, L., Novelli, F., Geuna, M., Palestro, G., Furni, G., and Garotta, G. (1992) Eur. J. Immunol. 22, 2403–2412
6. Darnell, J. E., Jr. (1997) Science 277, 1630–1635
7. Dosch, E., Zoller, B., Redmann-Muller, I., Nanda, I., Schmid, M., Dars, B.–Z., Flanagan, J. R., Appella, E., and Ozato, K. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3743–3747
8. Eisenbeis, C. F., Singh, H., and Storb, U. (1995) Gene Des. 9, 1377–1387
9. Matsuyama, T., Grossman, A., Mittrucker, H.–W., Siderovski, D. P., Kiefer, F., Kawakami, T., Richardson, C. D., Taniguchi, T., Yoshihara, S. K., and Mak, T. W. (1985) Nature 315, 636–635
10. Sharf, R., Arzeli, A., Lejbkowicz, F., Winograd, S. S., Ehrlich, R., and Levi, B.–Z. (1995) J. Biol. Chem. 270, 13063–13069
11. Menz, M., Fujita, T., Kinura, Y., Narumaya, M., Harada, H., Suzo, Y., Miyata, T., and Taniguchi, T. (1988) Cell 54, 903–913
12. Harada, H., Fujita, T., Miyamoto, M., Kinura, Y., Narumaya, M., Furia, A., Miyata, T., and Taniguchi, T. (1989) Cell 58, 729–739
13. Taniguchi, T. (1997) Cell Physiol. 173, 128–130
14. Vaughan, P. S., Aziz, F., van Wijnen, A. J., Wu, S., Harada, H., Taniguchi, T., Soprano, K. J., Stein, J. L., and Stein, G. S. (1995) Nature 377, 362–365
15. Driggers, P. H., Ennist, D. L., Gleason, S. L., Mak, W.–H., Marks, M. S., Levi, B.–Z., Flanagan, J. R., Appella, E., and Ozato, K. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3743–3747
16. Eisenbeis, C. F., Singh, H., and Storb, U. (1995) Genes Des. 9, 1377–1387
17. Matsuyama, T., Grossman, A., Mittrucker, H.–W., Siderovski, D. P., Kiefer, F., Kawakami, T., Richardson, C. D., Taniguchi, T., Yoshihara, S. K., and Mak, T. W. (1985) Nature 315, 636–635
18. Yamagata, T., Nakamura, T., Westphal, H., and Russell, P. (1990) Curr. Eye Res. 9, 31–37
19. Sax, C. M., Dziedzie, D. C., Piatigorsky, J., and Reddan, J. R. (1995) Exp. Eye Res. 61, 125–127
20. Sabath, D. E., Broome, H. E., and Prystowsky, M. B. (1990) Gene (Amst.) 91, 155–191
21. Kanno, Y., Kozak, C. A., Schindler, C., Driggers, P. H., Ennist, D. L., Gleason, S. L., Darnell, J. E., Jr., and Ozato, K. (1993) Mol. Cell. Biol. 3, 3951–3963
22. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1995) Current Protocols in Molecular Biology, pp. 4.9–4.10. John Wiley & Sons, Inc., New York
23. Politis, A. D., Ozato, K., Coligan, J. E., and Vogel, S. N. (1994) J. Immunol. 152, 2270–2278
24. Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419
25. Moore, P. S., Bonhoff, C., Weiss, R. A., and Chang, Y. (1996) Science 274, 1743–1744
26. Nelson, N., Kanno, Y., Hong, C., Cantursi, C., Fujita, T., Fowlkes, B. J., O’Connell, E., Hu-li, J., Paul, W. E., Jankovic, D., Sher, A. F., Coligan, J. E., and Sher, A. F. (1996) J. Immunol. 156, 3711–3720
27. Junwirth, C., Rebbert, M., Ozato, K., Degen, H. J., Schull, J. H., and Duda, D. I. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 3105–3109
28. Dosch, E., Zoller, B., Redmann-Muller, I., Nanda, I., Schmid, M., Viciano-Goffere, A., and Jungwirth, C. (1998) Gene (Amst.) 210, 265–275
29. Sharf, R., Meraro, D., Azriel, A., Thornton, A. M., Petricoin, E. F., Lerner, A. C., Schaper, F., Hauser, H., and Levi, B.–Z. (1997) J. Biol. Chem. 272, 9785–9792
30. Bovolenta, C., Driggers, P. H., Marks, M. S., Medin, J. A., Politis, A. D., Vogel, S. N., Lery, D. E., Nakaguchi, K., Appella, E., Coligan, J. E., and Ozato, K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5046–5050
31. Gao, C. Y., Zakeri, Z., Zhu, Y., He, H., and Zelenka, P. S. (1997) Dev. Biol. 190, 185–194
32. Gao, C. Y., Bassnett, S., and Zelenka, P. S. (1995) Dev. Biol. 169, 185–194

W. Li, C. N. Nagineni, H. Ge, B. Efok, A. B. Chepelinsky, and C. E. Egwuagu, unpublished data.