Activation of STAT Signaling Pathways and Induction of Suppressors of Cytokine Signaling (SOCS) Proteins in Mammalian Lens by Growth Factors

Samuel Ebong, Cheng-Rong Yu, Deborah A. Carper, Ana B. Chepelinsky, and Charles E. Egwuagu

PURPOSE. This study was conducted to examine whether the effects of growth factors are mediated in the lens by Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways and whether they induce expression of suppressors of cytokine signaling (SOCS), a novel family of feedback regulators of cytokine and growth factor activities.

METHODS. STAT activation and SOCS expression were analyzed in transgenic or wild-type mouse lens and lens epithelial cells stimulated with growth factors by immunohistochemistry, RT-PCR, Northern, Western, proliferation, or transient reporter assays.

RESULTS. STATs were constitutively expressed at low levels and activated by insulin-like growth factor (IGF)-1, platelet-derived growth factor (PDGF)-aa, and FGF-1 or -2 in the lens. The intensity of STAT signaling increased at high FGF-2 concentration and FGFs act in synergy with IGF-1 or PDGFA to enhance STAT signaling and SOCS expression. Growth factor-induced proliferation of lens cells is inhibited by AG-490, a specific inhibitor of JAK2/STAT3.

CONCLUSIONS. This is the first report that FGFs activate STAT pathways in the lens and that SOCS proteins are constitutively expressed and upregulated by growth factors in this tissue. Physiological relevance of STAT pathways in the lens is underscored by inhibition of lens cell proliferation by inhibitors of JAK/STAT pathways and by the aberrant proliferation of lens epithelium in the posterior pole of transgenic mice with constitutively activated STAT1 in the lens. Common activation of STAT pathways by FGF-1, FGF-2, IGF-1, or PDGFA and their synergistic activation of STATs and SOCS in lens cells suggest that activities and crosstalk between these factors are sensitive to the steady state levels of activated STATs in the lens and may be under feedback regulation by SOCS family proteins. (Invest Ophthalmol Vis Sci. 2004;45:872–878) DOI:10.1167/iovs.03-0511

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) signal-transduction pathway is an evolutionarily conserved signaling mechanism in species as diverse as insects, slime molds, and mammals. JAKs comprise four non–receptor tyrosine kinases (JAK1, JAK2, JAK3, TYK2), and STATs consist of seven structurally and functionally related latent cytoplasmic transcription factors. Signaling through the JAK/STAT pathway is initiated when a cytokine or growth factor binds to its cognate receptors on a target cell and induces a cascade of events that result in phosphorylation of the receptor and one or more JAKs. The activated receptor/JAK complex serves as a docking site for recruitment of specific STATs, leading to phosphorylation at a tyrosine residue in the STAT-SH2 domain. Homo- and/or heterodimers of activated STATs translocate to the nucleus where they bind to members of the gamma-activated site (GAS) family of enhancers, to activate or repress gene transcription. STATs regulate cellular functions as diverse as cellular proliferation, differentiation, death, and embryonic development, and the importance of regulating the duration and intensity of STAT signaling is underscored by the diverse array of pathologic conditions that arise from disruption or aberrant activation of STATs. These include a number of human neoplastic and autoimmune diseases and developmental anomalies such as dwarfism. The strength and duration of STAT signals has recently been shown to be under stringent regulation by a family of endogenous negative feedback regulators, generically called suppressors of cytokine signaling (SOCS). SOCS proteins bind to tyrosine-phosphorylated receptors and non–receptor tyrosine kinases and prevent recruitment of STATs to the activated receptor complex. In addition to functioning in a classic feedback regulatory loop, SOCS proteins inhibit responses of factors that are different from those that induce their expression. Interest in SOCS proteins stems from the belief that they may serve to integrate multiple extracellular signals that converge on a target cell.

Activation of STAT proteins has been detected in the chick lens and the developing mouse eye. However, constitutive activation of STAT1 in the mouse lens by ectopic lens expression of IPN results in the inhibition of lens differentiation and cataract in transgenic mice, suggesting that perturbation of STAT signaling can induce pathologic changes in the eye. Although the role of insulin-like growth factor (IGF)-1, platelet-derived growth factor (PDGF), and FGFs in proliferation and differentiation of the lens is well established, it is not known whether the effects of these growth factors in the lens are mediated through activation of STAT pathways or whether their activities in this ocular tissue are under feedback regulation by SOCS. In this study, STAT1 and -3 signaling pathways were activated in the lens by FGF-1, FGF-2, IGF-1, or PDGF and that the intensity of STAT signaling in the lens was regulated in part through synergistic interaction between these growth factors. Furthermore, these growth factors induced expression of SOCS and FGFs acted in synergy with IGF-1 and PDGF to enhance the level of SOCS induction, suggesting that growth-factor-induced signals in the lens may be under negative feedback regulation by SOCS.

From the Laboratories of 1Immunology, 2Mechanisms of Ocular Diseases, and 3Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, Maryland.

Submitted for publication March 25, 2003; revised August 15 and October 21, 2003; accepted October 28, 2003.

Disclosure: S. Ebong; None C.-R. Yu; None D.A. Carper; None A.B. Chepelinsky; None C.E. Egwuagu; None

© Association for Research in Vision and Ophthalmology
MATERIALS AND METHODS

Cell Culture and Growth Factor Treatment

The murine lens epithelial cell lines CRLE2, 1AML6, αTN4-1, and NKR-11 were cultured as previously described.6 Cells were stimulated in serum-free medium (SM) supplemented with 30 ng/ml IGF-1 (Roche Molecular Biochemicals, Indianapolis, IN), 30 ng/ml PDGFαα (Biosource International, Camarillo, CA), 100 ng/ml FGF-1 (Roche Diagnostics, Indianapolis, IN), and 5 or 100 ng/ml FGF-2 (Roche Diagnostics) for various amounts of time, as indicated in the figure legends.

Lens Organ Culture

Lens organ cultures were prepared from 6-week-old BALB/c mouse or Wistar rat (Charles River Laboratories, Wilmington, MA) eyes, as previously described.8 The lenses were cultured for 24 hours and examined for integrity and transparency. Suitable lenses were cultured in normal medium or medium containing growth factor. In some experiments, the lenses were separated into lens epithelium and fibers under a dissecting microscope and analyzed separately.

Immunohistochemistry

Embryonic day (E)17 wild-type (WT) or IFNγ transgenic (TR) BALB/c mouse eyes were fixed for embedding in paraffin wax, and sections of eyes were deparaffinized and subjected to immunohistochemical analysis using antibodies specific to proliferating cell nuclear antigen (PCNA; Calbiochem, Cambridge, MA), as previously described.6

Reverse Transcription–Polymerase Chain Reaction

RNA isolation and cDNA synthesis were performed as described.9 Hot-start PCR was performed with DNA polymerase (Amplitaq Gold; Applied Biosystems, Foster City, CA) for 25 cycles at 94°C for 45 seconds, 63°C for 45 seconds after an initial denaturation step at 95°C for 10 minutes. PCR primers used were: mouse-β-actin, 5'-GGGAGATGTCCTGGCAACCA-3' and 5'-TCGTTGCAATTGATGACACACTTGGC-3'; JAK1, 5'-GGGAAACATTGACACACAGGTC-3' and 5'-CAGGAATGACACTATGGTACATTTG-3'; STAT1, 5'-TTTTCGAGCTGCTCTCAAC-3' and 5'-GATTTCCAAGGAGGCGAGTC-3'; STAT2, 5'-TTGAGGAGCACTGCCTATGCTG-3' and 5'-CCCTCGTTCCACTCCAGAGTC-3'; STAT3, 5'-TTGAGGGAAGGTGCCAAGCTG-3' and 5'-CCTCCACGCTAATCTGCTGCAGGT-3'; STAT4, 5'-TGCGCCATGGTCACCCACAGCAAGTTT-3' and 5'-GGAGAGCTGCCTATGAGTTC-3'; STAT5a, 5'-TTTTCCGTAGCAACCGTCTCAAC-3' and 5'-CAACTACCTAATGCATGGGCATCC-3'; STAT5b, 5'-CTCGAGTAGGATTTCTAGTGAC-3' and 5'-CTCGAGTAGGATTTCTAGTGAC-3'; SOCS1, 5'-CTCTTGACGACTGATGTTGAGG-3' and 5'-TTCTGCGAGTCTAATCTGCTGCAGGT-3'; SOCS3, 5'-CTTGGGAGCACGCTGCCTATG-3' and 5'-ACCACATCGTTCCACTCCAGAGTC-3'; and STAT6, 5'-CCTCCACGCTAATCTGCTGCAGGT-3'. SOCS1 or SOCS3 promoter sequences ligated to pGL3 basic reporter plasmid (Promega) were transfected into 1AMLE6 lens epithelial cells (10⁴ cells) using transfection reagent (Lipofectamine 2000; Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. SOCS1 and -3 constructs were kindly provided by Hong Wu and Shlomo Melmed, respectively (UCLA School of Medicine, Los Angeles, CA). Transfection of the cells with 0.1 μg of pRL-TK (Promega), which encodes Renilla luciferase, was used as an internal control for normalizing transfection efficiency. Transfected cells were cultured for 2 hours in SFM supplemented with or without 100 ng/ml IGF-1, FGF-1, or FGF-2, as indicated in the figures or figure legends. Firefly and Renilla luciferase activities of the cell lysates were analyzed with a reporter assay system (Dual-Luciferase; Promega) and luminometer (Lmax; Molecular Devices, Sunnyvale, CA). The indicated relative reporter activities take into account normalized transfection efficiency.

Results

Activation of STAT1 and -3 Signaling Pathways in the Lens

We determined the repertoire of JAKs and STATs expressed in mammalian lens by RT-PCR analysis of RNA derived from four lens epithelial cell lines. In all cell lines examined, we detected expression of STAT1, -3, -5b, and -6 and JAK1 and -2, (Table 1) and proteins corresponding to some of these STATs (STAT1

---

Table 1. Transcription of STATs, JAKs, and TYK2 Genes in Lens Cells

<table>
<thead>
<tr>
<th>Mouse Lens Epithelial Cell Lines</th>
<th>αTN4-1</th>
<th>NKR11</th>
<th>CRLE2</th>
<th>1AML6</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAT1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>STAT2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>STAT3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>STAT4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>STAT5a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>STAT5b</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>STAT6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JAK1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JAK2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JAK3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TYK2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

mRNA transcripts were detected by RT-PCR analysis. +, present; −, not detected; ND, not examined.

Proliferation Assay

For the cell proliferation assay, 5000 1AML6 cells were seeded per well in 96-well plates in SFM or medium supplemented with IGF-1, FGF-1, or FGF-2, with or without genistein (100 μM). For rat or mouse assays, whole lenses in 24-well plates were incubated for 24 hours in serum-free M199 medium or medium supplemented with IGF-1, FGF-1, or FGF-2, with or without genistein (100 μM) or AG490 (100 μM). For some lenses, lens epithelia, and fiber tissues were separated under microscopes. In whole-lens experiments, the contralateral lens from each served as the control. For inhibition studies, 1AML6 cells were pretreated with inhibitors for 30 minutes, and lens explants and whole lens were pretreated for 2 hours. [3H]Thymidine (2 μCi/well) was added to 1AML6 cultures in the final 6 hours of stimulation and to lens cultures after 6 hours of growth factor treatment. Each data point represents an average of three lenses or quadruplet cultures.

---

Notes:

1. This study was supported by National Institutes of Health Grants EY12021, EY13773, and EY14520 and an unrestricted grant from Research to Prevent Blindness, Inc.

2. Address correspondence to A. C. Bertoleiro, Department of Ophthalmology, University of California, 419 Medical Plaza, B727, MAJ 520, Los Angeles, CA 90095-7122. E-mail: bertoleiro@mednet.ucla.edu

3. The authors have no proprietary or financial interest in any of the materials or methods in this paper.

4. This work was supported by the National Institutes of Health (P30 EY014801, P50 EY016702). This work was also supported by a grant from the Research to Prevent Blindness, Inc. to the UCLA Jules Stein Eye Institute.

5. This work was supported by the National Institutes of Health (P30 EY014801, P50 EY016702). This work was also supported by a grant from the Research to Prevent Blindness, Inc. to the UCLA Jules Stein Eye Institute.

6. SOCS Regulation of STAT Signaling Pathways in the Lens

873

---

Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933435/ on 04/27/2017
and -3) and JAKs (JAK1 and -2) were detected in the mouse lens by Western blot analysis (data not shown). These results are in concert with a previous report of STAT1 and -3 detection in the chick lens. However, unlike the mouse (Table 1), STAT5 was not detected in chick lens. To examine whether growth factors that influence the growth and differentiation of lens cells activate STAT signaling pathways in the lens, we stimulated lens epithelial cells with IGF-1 or PDGFaa and analyzed whole-cell extracts from the cells by Western blot analysis. STAT3 was constitutively activated and both STAT1 and -3 were activated by IGF-1 (Fig. 1A, 1B) and PDGFaa (data not shown). To confirm these observations in the whole lens, we established 24-hour mouse lens organ cultures and, after stimulation with IGF-1, the lenses were separated into lens epithelium and fibers. Western blot analysis of protein extracts from the cells reveal that STAT3 was constitutively activated in the mouse lens, as evidenced by detection of phosphorylated STAT3 in the freshly isolated lenses (Fig. 1C). In concert with results from lens epithelial cell lines, both STAT1 and -3 were activated in response to growth factor signaling, and detection of activated STATs was restricted to the lens epithelium. Constitutive activation of STATs and enhancement of STAT activation in the lens by IGF-1 provide empiric evidence for a role of STAT pathways in transducing growth-factor-induced signals in the lens.

**Effect of FGFs on STATs and Synergy with IGF-1**

Experiments using transgenic mice and rat lens explants have established the mitogenic and morphogenic effects of FGFs on the lens. Because STAT1 and -3 are important regulators of growth and differentiation in numerous cell types and tissues, it was of interest to determine whether the effects of FGFs in the lens are mediated through STAT pathways. Stimulation of lens epithelial cells with FGF-1 or -2 at concentrations that induce proliferation (5 ng/mL) or differentiation (>40 ng/mL) shows that FGF-2 induced significant levels of pSTAT1 and -3 (Figs. 2B, 2C), whereas in cells stimulated with FGF-1 only pSTAT3 was detectable (Fig. 2A). Because transduction of IGF-1 and FGF signals requires tyrosine phosphorylation, we also examined whether concurrent stimulation of lens cells by IGF-1 and FGF-1 would have antagonistic or synergistic effects on STAT activation. Although pSTAT1 is undetectable by Western blot analysis after FGF-1 stimulation, costimulation of lens cells with FGF-1 and IGF-1 induces significant activation of STAT1 that exceeds stimulation by IGF-1 alone (Fig. 2D). Because the doses of FGFs used to stimulate the mouse lens epithelial cells were based on those known to induce proliferation of rat cells, we repeated these experiments in the rat and mouse lens to verify that STAT signaling is indeed induced in vivo under these established FGF doses. Similar to results in the lens epithelial cell line (Figs. 2B, 2C), we found that at the concentrations used in this study, both STAT1 and -3 were activated by FGF-2 in freshly isolated mouse (Fig. 2E) or rat (Fig. 2F) lens. Significantly, the increase in STAT1 activation by FGF-2 in the rat lens was inhibited by genistein, a tyrosine protein kinase inhibitor and potent inhibitor of STAT signaling. In contrast to the 1AMLE6 cell line, which activated only STAT3 in response to FGF-1 (Fig. 2A), both STAT1 and -3 were activated by FGF-1 in fresh lens tissue (Fig. 2E). The observed difference in the pattern of STAT activation in the whole lens and cell lines was similar to findings in the chicken, in which expression of STAT1 and -3 was detected mainly in freshly isolated lens epithelial cells but not in 4-hour-old chick lens epithelial explants, and it underscores the importance of verifying results obtained in lens cell lines in primary lens organ cultures.

**Repression of Growth Factor–Induced Lens Cell Proliferation**

To establish a possible link between STAT activation and lens proliferation, we examined whether inhibiting STAT signaling pathways would affect growth factor-induced proliferation in the lens. The proliferation assays in Figure 3 show significant increases in cell proliferation after stimulation of lens epithelial cells with the IGF-1 or FGF-2. Although FGF-1 did not induce significant proliferation of the 1AMLE6 lens cells, it acted synergistically with IGF-1 to enhance IGF-1–induced proliferation (Fig. 3A). We then examined whether genistein, a broad-spectrum inhibitor of most tyrosine kinases, inhibits the proliferation induced by IGF-1. In these experiments, 100 μM genistein was used, because this concentration inhibited growth factor signaling in pilot studies without affecting viability of the cells. Figure 3A shows that addition of genistein induced a slight inhibition of lens cell proliferation in the absence of the growth factor. However, a more dramatic effect was observed in cells stimulated with IGF-1, as genistein inhibited more than 80% of the IGF-1–induced proliferation (Fig. 3A).
3A). To further characterize the role of JAK/STAT pathways in IGF-1 or FGF-2–induced proliferation in the lens, we stimulated whole rat or mouse lenses or explants with IGF-1 or FGF-2 and analyzed the effects of AG-490 or genistein on growth factor–induced proliferation. Unlike genistein, which inhibits tyrosine-phosphorylated receptors and non–receptor-associated kinases, AG-490 is a specific inhibitor of JAK kinases and STAT pathways.19,20 Similar to the effects of genistein on the lens epithelial cell line, both AG-490 and genistein inhibited proliferation of the lens cells induced by IGF-1 or FGF-2 (Figs. 3B, 3C). Furthermore, that AG-490 reduced the level of IGF-1–or FGF-2–induced STAT activation in lens cells (Fig. 3D), providing supportive evidence for a role of JAK-STAT signaling pathways in lens proliferation.

Growth Factor–Induced Expression of SOCS Proteins in the Lens

Even though much is known about the effects of IGF-1, PDGF-Faa, and FGFs on the lens, little is known of the mechanisms that attenuate or terminate the signals they induce. To examine the possibility that signaling pathways activated by these growth factors are under feedback regulation by SOCS, we stimulated lens epithelial cells and whole rat or mouse lenses with various combinations of IGF-1, PDGF-Faa, FGF-1, and FGF-2 and assessed their effects on induction of SOCS expression by Northern blot (Fig. 4A), promoter reporter (Fig. 4B), and Western blot (Fig. 4C) assays. Together, these results indicate that SOCS1 is constitutively and inducibly expressed in the mouse fiber cells, and expression of SOCS3 is upregulated by FGF-2 in the mouse lens epithelium but not in fibers (Fig. 4C). In addition, SOCS3 is upregulated by FGF-1 or IGF-1 in rat epithelium but not fibers (data not shown). Furthermore, we observed synergistic induction of SOCS3, and to a lesser extent SOCS1, by IGF-1 and FGF-1 or PDGF-Faa and FGF-1 (Fig. 4A).

STAT-Induced Aberrant Proliferation of Lens Epithelial Cells

Assessment of the role of STATs in a tissue is complicated by the fact that STATs are latent cytoplasmic transcription factors that become active only after ligand-induced phosphorylation of the SH2 domain.21 Because IFNγ mediates its biological effects through activation of STAT1, we used a transgenic (TR) mouse model with constitutive expression of IFNγ in the lens,6,7 to further investigate the functional link between lens proliferation and STAT activation in the lens. Suitability of these mice for investigating effects of STAT signaling in the lens is underscored by the relatively high levels of activated STAT1 in the TR lens (Fig. 5A). In addition, we detected high levels of SOCS1 and -3 expression in the lens of these mice (Figs. 5B, 5C), suggesting that STAT signaling in the lens is under feedback regulation by SOCS. Histologic analysis of eye sections of WT (Figs. 5D, 5E, 5F) and TR (Figs. 5G, 5H, 5I) mice revealed aberrant extension of lens anterior epithelium to the posterior pole of the TR lens (compare Figs. 5D, 5G). Immunohistochemical analysis of the sections using PCNA-specific antibodies confirmed the presence of proliferating cells in the posterior pole of the TR lens (Figs. 5H, 5I) compared with their WT counterparts (Figs. 5E, 5F). These results, which have also been confirmed in BrdU labeling experiments (data not shown).
shown), suggest that enhanced activation of STAT1 in the lens induces aberrant proliferation of lens epithelia.

**DISCUSSION**

Maintaining homeostatic balance between proliferating and differentiated cells is critical to normal development of the lens and the decision to divide or differentiate is regulated by a plethora of factors present in the ocular media.\(^2\)\(^2\) However, not much is known about the signals induced by the diverse growth factors in the lens or how their activities are integrated into the lens developmental program. In this study, the effects of four growth factors (FGF-1, FGF-2, IGF-1, and PDGF), which regulate growth and differentiation of the lens, were shown to

**FIGURE 3.** Growth-factor-induced lens proliferation was repressed by inhibitors of JAK/STAT pathways. (A) 1AML6 lens epithelial cells were stimulated with IGF-1 (30 ng/mL), FGF-1 (100 ng/mL), FGF-2, or IGF-1 and FGF-1 for 24 hours in the presence or absence of genistein. Cells were pulsed with \(^3\)H-thymidine for 6 hours, and the mean proliferative responses of three quadruplet cultures are presented as counts per minute. Whole lens from rat (B) or mouse (C) were stimulated with IGF-1 (30 ng/mL) or FGF-2 (100 ng/mL) in the presence or absence of AG-490 (specific inhibitor of JAK/STAT pathways) or genistein, as indicated. The whole lens or lens epithelial explant was pulsed with \(^3\)H-thymidine for 18 hours, and the histograms represent the ratio of proliferation between growth factor-stimulated and control untreated cells. The results shown are the mean of three lenses and the multiple of increase or decrease in proliferation after treatment is based on comparison with the untreated contralateral lens. (D) Western blot analysis of whole-cell extracts (40 \(\mu\)g/lane) from rat lens epithelia using pSTAT1 or -3 antibodies. Blots were stripped and reprobed with \(\beta\)-actin antibodies.

**FIGURE 4.** Expression of SOCS was upregulated in lens cells by growth factors. (A) Northern blot analysis of RNA (20 \(\mu\)g/lane) from 1AML6 cells stimulated with various combinations of growth factors for 15 minutes. (B) 1AML6 lens cells transfected with SOCS1 or -3 promoter/luciferase constructs were stimulated with IGF-1, FGF-1, or FGF-2. Histograms represent the ratio of luciferase activity between cells treated with growth factor and control untreated cells. (C) Western blot analysis of whole-cell extracts (40 \(\mu\)g/lane) from mouse lens epithelium or fibers using SOCS1- or -3-specific antibodies after a 2-hour incubation of whole lens with growth factors.
be mediated in part through activation of the JAK/STAT signal-transduction pathway. This is the first time that members of the FGF family have been shown to activate STAT signaling in the mammalian lens, and it is interesting that optimal activation of STAT1 and -3 occurs at FGF concentrations that induce lens differentiation. We also show for the first time that IGF-1 acts synergistically with FGF-1 to further enhance STAT1 activation. These results raise the possibility that the induced increase in STAT1-STAT1 homodimers or STAT1-STAT3 heterodimers resulting from elevated pSTAT1 may provide additional levels of transcriptional regulation in lens cells. Synergistic activation of STAT1 by growth factors implicated in lens differentiation further suggests that STAT1 signals may regulate proteins that influence the decision of the lens cell to divide or differentiate. However, it is important to emphasize that the role of STAT1 in development is complex and may depend on the cell type or its state of differentiation. For example, FGF inhibits chondrocyte proliferation and regulates bone development through the STAT1 pathway, whereas in the lens, sustained STAT1 activation induces aberrant proliferation of the anterior lens epithelium (Fig. 5) and inhibits lens differentiation in IFNγ TR mice.5,7

In the context of the role of IGF-1 in lens differentiation, analyses of transgenic mice with targeted overexpression of IGF-1 in the lens has revealed that a major function of IGFs is to provide spatial cues that restrict proliferation of lens epithelial cells to germinative and transition zones of the lens.23 Our results showing that activation of STAT1 and -3 by IGF-1 is confined to the epithelial compartment of the lens (Fig. 1C) is therefore in concert with the notion that IGF-1 activities are spatially restricted in the lens. The need to segregate cellular processes that regulate lens growth is further reflected by the spatial regulation of the expression of a number of developmental genes in the lens, and this in turn suggests that activation of transcription factors that control expression of these genes may also be spatially regulated.26 An immediate and primary target of activated STATs are members of the interferon regulatory factor (IRF) family of transcription factors.27 The most abundant IRFs in the lens are two cell-cycle-regulatory IRFs: IRF-1 and -2.6 IRF-2 is the most abundant. It is a potent inducer of cell proliferation and, if unregulated, it induces oncogenic transformation in many cell types. In contrast, IRF-1 is a potent growth inhibitor and a tumor suppressor that negatively regulates the transcriptional activities of IRF-

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933435/)
In most cell types, the ratio of IRF-1 to -2 is fixed, and whether the cell divides or differentiates depends in part on a net increase of IRF-1 or -2. In the mouse lens, expression of IRF-1 and -2 is spatially regulated, with IRF-1 expression restricted to the lens fiber, whereas IRF-2 is present in the anterior epithelium and fibers. In this study, we provide evidence to suggest that spatial segregation of proteins whose expression is activated by STATs may derive in part from active suppression by SOCS family proteins. We show that SOCS expression is inducible in the mouse lens (Figs. 5B, 5C) and that lens cells respond to FGF-1, FGF-2, or IGF-1 signaling by upregulating expression of SOCS1 and -3 (Fig. 4). We further note that costimulation of the lens cells with FGF-1 and IGF-1 upregulating expression of SOCS1 and -3 (Fig. 4). We further note that lens cells respond to FGF-1, FGF-2, or IGF-1 signaling by expression of SOCS expression by these factors may serve to provide mechanistic basis for their synergistic activation of FGF-2, IGF-1, and PDGF-B enhances expression of SOCS1 or -3 above the levels induced by either factor alone (Fig. 4A). Of particular interest was the differential pattern of SOCS expression and localization in the lens, with relatively high levels of SOCS1 in the lens fibers, whereas SOCS3 was preferentially induced in the lens epithelium (Fig. 4C). These results, taken together with the spatial pattern of expression in the lens of IGFs, SOCS, and activated STATs, suggest that activities of growth factors in the lens epithelial and fiber compartments may be under feedback regulation by different SOCS proteins.

Because initiation of growth factor signaling is highly dependent on tyrosine phosphorylation, regulating the availability of tyrosine-phosphorylated signaling intermediates such as tyrosine-kinases and STATs may constitute an important means of controlling the intensity and duration of extracellular signals in the lens. It is therefore interesting that targeted overexpression in the lens of growth factors or cytokines that activate STATs, invariably results in similar abnormal lens phenotypes. Results from these overexpression transgenic models suggest that one of the consequence of prolonged signaling by any growth factor or cytokine in the lens is alteration of the homeostatic levels of STATs and other tyrosine-phosphorylated signaling intermediates that are critical to many growth factor-induced signaling pathways. Common activation of STAT pathways by a number of factors (FGF-1, FGF-2, IGF-1, and PDGF) implicated in lens development may provide mechanistic basis for their synergistic activation of developmental pathways in the lens, whereas the ensuing upregulation of SOCS expression by these factors may serve to curtail unmitigated signaling by any of these growth factors. More information on how the bioavailability of tyrosine-phosphorylated signaling molecules is regulated in the lens by SOCS and other feedback regulators of growth factor signaling will undoubtedly further our overall understanding of the molecular basis of cataract and other age-related lens diseases.

References