Two Separate Signal Transducer and Activator of Transcription Proteins Regulate Transcription of the Serine Proteinase Inhibitor-3 Gene in Hepatic Cells*

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The serine proteinase inhibitor (SPI-3) gene expression is transcriptionally regulated by interleukin (IL)-6 and glucocorticoids in hepatic cells. To identify the transcription factors involved in regulation of the SPI-3 promoter–chloramphenicol acetyltransferase constructs we overexpressed Signal Transducer and Activator of Transcription (STAT) proteins (STAT1, STAT3, STAT5B, and STAT6) and CAAT enhancer-binding protein β. Specific signaling pathways were activated by coinjected receptors for growth hormone, IL-3, IL-4, or chimeric receptors containing the cytoplasmic domain of gp130. STAT3 and STAT5B induced transcription via the SPI-3 promoter. The STAT5B response was substantially enhanced by truncation of the 5'-flanking region from –1021 to –148. The responsiveness to STAT3 and STAT5B required the STAT binding element at –132 to –124. This element was sufficient to confer regulation onto a heterologous promoter gene construct. In contrast, overexpression of CAAT enhancer-binding protein β reduced the transcriptional activity of the SPI-3 promoter, presumably by interfering with STAT protein binding to the promoter element. The SPI-3 gene is the first example of an acute phase gene that is responsive to both STAT3 and STAT5B.

At least three members of serine proteinase inhibitor gene family (SPI) are synthesized in rat liver (Hill and Hastie, 1987; Pages et al., 1990). These genes code for highly homologous proteins containing different reactive centers resulting in specific inhibitory spectra. In healthy rats only the SPI-1 and SPI-2 are synthesized, and their expression is controlled by growth hormone (Le Cam et al., 1987; Paquereau et al., 1992). The growth hormone-responsive element within promoter regions of both genes has been identified; however, the transactivating proteins have yet to be defined (Yoon et al., 1990; Thomas et al., 1995). The SPI-3 mRNA is barely detectable in healthy rats and is not affected by growth hormone (Paquereau et al., 1992).

The pattern of expression of the SPI genes is profoundly modified during an inflammatory response, with transcription of the SPI-3 gene being greatly stimulated, whereas that of the SPI-1 and SPI-2 genes is suppressed (Hill and Hastie, 1987; Le Cam and Le Cam, 1987). IL-6 has been shown to be the major activator of SPI-3 gene expression in primary hepatocytes and rat hepatoma H-35 cells, and its activity was further enhanced by dexamethasone (Kordula et al., 1994; Kordula and Travis, 1996). The induction of SPI-3 gene expression by IL-6 occurs at the level of transcription. The SPI-3 promoter containing the region from –148 to –3 is sufficient for mediating the regulation by IL-6 and dexamethasone (Kordula and Travis, 1996). Two functional elements located at –132 to –124 and at –58 to –66 are necessary for maximal IL-6 responsiveness. The distal element serves as a binding site for STAT proteins (SBE), whereas the proximal element is recognized by C/EBP isoforms (Kordula and Travis, 1995, 1996). However, binding of C/EBP to the distal element was also detected (Rossi et al., 1992; Kordula and Travis, 1996). The relevance of the SBE element was demonstrated by mutations and in combination with a heterologous promoter. The C/EBP binding site contributes to the magnitude of IL-6 regulation because mutation of this element decreased the response to IL-6.

To define which one of the STAT proteins known to be activated by IL-6 and inflammation in the liver cells is acting on the SPI-3 gene, we reconstituted the regulation pathways in hepatoma cells. The cells were transiently transfected with vectors expressing C/EBPβ, STAT proteins (STAT1, STAT3, STAT5B, and STAT6), and different hematopoietin receptors for the specific activation of the overexpressed STAT isoforms. The data indicate a novel response pattern for the SPI-3 gene consisting of an inducing action of both STAT3 and STAT5B.

EXPERIMENTAL PROCEDURES

Cells and Cytokines—Rat H-35 cells (clone T-7–18) (Baumann et al., 1989) were cultured in Dulbecco’s modified Eagle’s medium, and human HepG2 cells (Knowles et al., 1980) were cultured in minimal essential medium. Media were supplemented with 10% fetal calf serum, penicillin, streptomycin, and gentamycin. All cytokine treatments occurred in serum-free media. The following purified human recombinant cytokines were used at a concentration of 100 ng/ml: IL-6 (Genetics Institute, Cambridge, MA), growth hormone (Genentech, South San Francisco, CA), G-CSF, IL-4 (Immunex Corp., Seattle, WA), and IL-3 (provided by Dr. F. Meyer, Sandoz, Basel, Switzerland). IL-1α (Immunex Corp., Seattle, WA) was used at 0.5 ng/ml. To enhance the cytokine effect 1 μmol/l dexamethasone was included in the treatment media where indicated.

Expression Vectors—Expression vectors for the following receptors...
and transcription factors have been described before: truncated form of chimeric human receptor G-CSFR-gp130(133 m3) (Baumann et al., 1994a), mutated chimeric human receptor G-CSFR-gp130(133 m3) containing alanine at position 125 (Y125A) in box 3a (Lai et al., 1995a), human IL-2R α (Ziegler et al., 1995), human IL-4R α (Mosley et al., 1989), human IL-3R α (Hayashida et al., 1990), human IL-3R γ (Kitamura et al., 1991) (provided by Dr. A. Lopez, Institute of Medical and Veterinary Science, Adelaide, Australia); rabbit GHR (Leung et al., 1987), rat STAT1 (Hayashida et al., 1994a, 1994b) by using the DEAE-dextran method (Kordula and Travis, 1993) and in HepG2 cells by a calcium phosphate procedure (Graham and Van der Eb, 1973). For H-35 cells, 1 ml of transfection solution contained CAT reporter constructs (6 mg), expression vectors for receptors (0.5–1 μg), STATs (3 μg), and plE-MUP (2 μg) as internal marker for transfection efficiency (Prowse and Baumann, 1988) and pUC13 to bring the total amount to 10 μg. For HepG2 cells, 1 ml of solution contained CAT reporter constructs (15 μg), expression vectors for receptors (0.5–1 μg), STATs (3 μg), and plE-MUP (2 μg) as internal marker for transfection efficiency (Prowse and Baumann, 1988) and pUC13 to bring the total amount to 22 μg. Transfected H-35 cells, after a recovery period of 24 h, were subcultured into 6-well cluster plates and 24 h later were treated for an additional 24 h with cytokines. Transfected HepG2 cells were allowed to recover for 48 h and then were treated in the same manner as H-35 cells. The CAT activities were determined in serially diluted cell extracts of both cell lines and were normalized to the amount of MUP secreted into the medium for HepG2 cells. The values were then calculated to the untreated control culture (defined as 1.0), which were neither transfected with STATs nor receptors. Data from representative experiments were shown and each of the experiments was repeated at least in duplicate in order to confirm reproducibility of the results.

RESULTS

C/EBPβ Reduces the SPI-3 Promoter Activity—Recombinant C/EBPβ protein has been shown to bind to multiple sites within the promoters of SPI genes (Rossi et al., 1992; Le Cam et al., 1994). The expression of the two isoforms C/EBPβ α and δ is enhanced in acute phase liver (Alam et al., 1992). Although both proteins are capable of binding and transactivating via the DNA sequences that are utilized by C/EBPα, a predominant role in stimulating transcription of acute phase genes has been ascribed to C/EBPβ in hepatocytes (Alam et al., 1993) and in IL-6-treated hepatoma cells (Baumann et al., 1992). Therefore, we restricted the analysis of SPI gene regulation to C/EBPβ. We cotransfected rat H-35 cells with the SPI-3 promoter-CAT constructs and an expression vector for C/EBPβ. For comparison, we also included the corresponding promoter construct from the SPI-1 gene containing a sequence of different STAT binding element at positions −132 to −124 and three possible C/EBP binding sites at −58 to −66, −107 to −99, and −132 to −124. The cells were stimulated with IL-6, IL-1, and Dex.

The transcription of the −1020 and −148 SPI-3 promoter-CAT constructs was not affected by IL-6 or dexamethasone alone; however, both factors together led to a synergistic activation (Fig. 1). In contrast, the SPI-3 promoter construct containing the mutated C/EBP binding site at −58 to −66 was responsive to IL-6 alone, and this response was further enhanced by dexamethasone. The transcription of the SPI-1 promoter-CAT construct was induced by IL-6 together with dexamethasone, whereas these factors were separately ineffective. Overexpression of C/EBPβ inhibited transcriptional regulation of the SPI-3 promoter-CAT constructs. The activity of the SPI-1 promoter-CAT construct was, however, enhanced (Fig. 1). Both SPI-promoter CAT constructs were not stimulated by IL-1 treatment; in fact their expression was reduced when IL-1 was combined with IL-6. These results suggest that the IL-6 stimulation of SPI-3 gene expression is not likely be mediated by C/EBPβ and thus, this gene falls into the category of the type II acute phase protein genes that are regulated by mechanisms independent of IL-1 and C/EBPβ.

The observed inhibition of the SPI-3 gene expression by C/EBPβ is interpreted to represent binding of C/EBPβ to the SBE at −132 to −124, thereby preventing interaction of STAT with the adjacent SBE. This site contains only two mismatches to the C/EBPβ consensus, and binding of a C/EBPβ protein to this element was demonstrated by DNA footprinting (Rossi et al., 1992; Le Cam et al., 1994) and gel shift analysis (Kordula and Travis, 1996). The second C/EBPβ binding site at −58 to

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activate STAT3 (Lai et al., 1995b), containing the most proximal box 3-motif of gp130 (1336), which represents a C/EBP consensus sequence and is also present at an identical site in the SPI-1 gene promoter, appears to be insufficient to mediate suppression by overexpressed C/EBPβ. The promoter construct containing the mutated C/EBP binding site at −58 to −66 (mut C in Fig. 1) was responsive to IL-6 and still suppressed by C/EBPβ. The opposite effect of C/EBPβ on the SPI-1 promoter-CAT construct likely results from the presence of the additional C/EBP site at −107 to −99 identified by Le Cam et al. (1994). We conclude from these data that STATs rather than C/EBPs are critical for IL-6 regulation of the SPI-3 gene.

Regulation of the SPI-3 Promoter Is in Part Dependent on the Box 3 Signals of gp130—Binding of IL-6 to IL-6Rα triggers association with gp130 and dimerization of this complex into a hexamer structure (Ward et al., 1994). The resulting homodimerization of the cytoplasmic domains of gp130 initiates signal transduction (Kishimoto et al., 1995). Two separate gp130-derived signals were identified that control gene induction. One signal requires the box 3 motif and appears to be correlated with the activation of STAT3, whereas the second signal is box 3-independent and has been in part associated with STAT5 (Lai et al., 1995b). We asked to what extent is the SPI-3 gene promoter activity dependent on the box 3 derived signal. In order to determine the signal specificity without interference by the endogenous receptor subunits, we applied the chimeric receptors G-CSF-gp130. These receptor constructs consist of the extracellular domain of G-CSFR and the transmembrane and cytoplasmic domains of gp130 (Ziegler et al., 1993). The chimeric receptor binds G-CSF and homodimerizes, thereby producing a dimer of cytoplasmic domain of the gp130 equivalent to that in the activated IL-6Rs (Baumann et al., 1994a; Baumann et al., 1994b). H-35 cells were cotransfected with SPI-3-promoter-CAT constructs and either the chimeric receptor G-CSF-gp130(133), containing the most proximal box 3a and thus still capable of activating STAT3, or G-CSF-gp130(133 m3) with mutated box 3 and thus unable to activate STAT3 (Lai et al., 1995b). The chimeric receptor with box 3 activated the expression of the SPI-3 promoter-CAT gene and also enhanced the response mediated by the IL-6R (Fig. 2). The receptor lacking box 3 was still capable of mediating an induction; however, this effect was drastically reduced. These results suggest that the expression of the SPI-3 promoter-CAT construct was primarily sensitive to the box 3-dependent signal but also regulated to a minor extent by the box 3-independent pathway.

Both STAT3 and STAT5B Activate Transcription via the SPI-3 Gene Promoter—To analyze the STAT-specific transactivation via the SPI-3 gene promoter elements, we used human HepG2 cells that permitted complementation of cytokine receptor activation by transfected STAT proteins (Morella et al., 1995a, 1995b; Lai et al., 1995). The experimental approach relies on the properties of nonhepatic hematopoietin receptors such as IL-3R or IL-4R, which when transfected into hepatoma cells can inefficiently recruit endogenous STAT proteins to mediate gene induction. However, in the presence of overexpressed STAT proteins the same receptors achieve sufficient activation of STATs to elicit a transcriptional stimulation via specific gene elements (Morella et al., 1995b; Wang et al., 1995). For instance, IL-4R can activate a broad spectrum of overexpressed STATs including STAT1, STAT3, STAT5B, and STAT6. In contrast, IL-3R, while highly active toward specific gene elements (Morella et al., 1995b), cannot efficiently use endogenous STAT3 unless provided in excess by overexpression (Wang et al., 1995). The response of the SPI-3 gene promoter to the STATs were probed in such a reconstituted system using IL-3R (Fig. 3) and IL-4R (Fig. 4). IL-3R without additional STAT barely stimulated the transcription of the constructs containing the −1020 or −148 fragments of the SPI-3 gene. In the presence of STAT3 or STAT5B, however, the inducing activity by IL-3R was greatly enhanced. The stimulation of the −1020 SPI-3 construct was consistently less than that by endogenous IL-6R. In contrast, the −148 SPI-3 construct showed a more prominent response to IL-3 than to IL-6 (Fig. 3). The same experimental approach was applied to the IL-4R, and the results indicated that the −1020 construct of the SPI-3 gene was strongly transactivated only by STAT3. The transactivation by STAT5B was only evident with the −148 fragment of the SPI-3 promoter. Cotransfection of STAT1 or STAT6 had no stimulatory effect on either of the SPI-3 promoter constructs. These data suggest that both STAT3 and STAT5B can act as mediators of cytokine receptor signals to the SPI-3 gene.

GHR Effect on the SPI-3 Transcription—Recognizing that a STAT5B-sensitive regulation of the SPI-3 gene exists, and considering that growth hormone receptor is known to signal through STAT5 (Gouilleux et al., 1995), as well as control SPI gene expression (Le Cam et al., 1987), we determined the effect

![Figure 2](http://www.jbc.org/)

**Fig. 2. Effect of box 3 motif of gp130 on the SPI-3 gene expression.** The chimeric receptor forms G-CSF-gp130(133) and G-CSFR-gp130(133m3) were cotransfected with pSPI-3(−1020)CAT or pSPI-3(−148)CAT into H-35 cells. Subcultures were treated with G-CSF and/or IL-6 for 24 h in the presence of Dex. The CAT activities were quantified and expressed relative to the control in each experimental series (fold induction indicated by numbers above the bars).

![Figure 3](http://www.jbc.org/)

**Fig. 3. Comparison of the effects of STAT3 and STAT5B on the expression of the SPI-3 promoter-CAT constructs.** HepG2 cells were cotransfected with pSPI-3(−1020)CAT or pSPI-3(−148)CAT, IL-3Rα, IL-3Rβ, and expression vector pSV-SPI-3 or pSV-STAT5B. Cultures were treated with IL-3 (3) or IL-6 (6) in the presence of Dex. The CAT activities were normalized to the internal transfection marker pE-MUP and expressed relative to the control culture (fold induction indicated by numbers above the autoradiogram).
of GHR on regulation of the SPI-3 gene promoter. Expression vectors for GHR, STAT5B, and SPI-3 promoter-CAT constructs were cotransfected into H-35 and HepG2 cells. In agreement with the observed lower sensitivity to STAT5B (Fig. 4), the construct containing the −1020 to −3 fragment of the SPI-3 gene did not respond to the GH alone. However, the stimulation by GH and IL-6 resulted in a synergistic effect (Fig. 5A). Furthermore, a 4-fold induction could be achieved in response to GH in HepG2 cells in the presence of overexpressed STAT5B (Fig. 5B). As already noted for the shorter promoter fragment in the experiments with IL3R and IL-4R (Fig. 3 and 4), the −148 SPI-3-CAT construct displayed a prominent STAT5B response in combination with GHR yielding a 7–15-fold increase in expression in either H-35 or HepG2 cells in comparison with cells transfected with GHR alone (Fig. 5, A and B). From these experiments we conclude that the GHR signal via STAT5B was stimulatory, analogous to the signaling by IL-3R and IL-4R, and that this signaling pathway could not explain the differential GH effect on SPI genes in vivo. The data also confirm the activatory effect of STAT5B on the SPI-3 gene promoter and indicate that this activation is not restricted to a specific hematopoietin receptor.

The SBE of the SPI-3 Gene Is a Target for Both STAT3 and STAT5B—Recently, we identified the SBE as critical for IL-6 response (Kordula and Travis, 1996). To determine whether SBE is the target of STAT3 or STAT5B in the SPI-3 promoter, we tested the SBE independent of the SPI-3 promoter. A reporter gene construct containing four copies of this element linked to the thymidine kinase promoter (p4xStat5CAT) was cotransfected into both H-35 and HepG2 cells together with either STAT5B or STAT3. GHR was used to activate STAT5B and IL-3R to activate STAT3 (Fig. 6). In both cell lines the construct had a 10–15-fold higher basal activity than the SPI-3 promoter constructs. Furthermore, the construct proved to be responsive to GH and IL-3 alone, and this response was enhanced by overexpressed STAT5B (Fig. 6, A and B) and STAT3 (Fig. 6C). This result suggests that the STAT element at −132 to −124 is regulated by both STAT3 and STAT5B and may account for the regulation of the SPI-3 promoter constructs. What is not explained is the difference in STAT5B activity on the −148 and −1020 SPI-3 promoter constructs. Two possible mechanisms may apply, the region between −1020 and −149 may contain either a dominant STAT3 response element or an element that suppresses STAT5B response via SBE at −132 to −124. To test these possibilities we inserted the fragment from −1020 to −155 in front of the tk promoter linked to the CAT gene or in the front of four copies of STAT element 5 to the tk promoter CAT construct. The results of transfection experiments, shown in Fig. 7, indicate that the −1020 to −155 SPI-3 promoter fragment on its own is neither responsive to IL-6 nor inhibited regulatory activity of the SBE-CAT construct.

**Fig. 4.** Effect of STAT proteins on the expression of the SPI-3 promoter-CAT constructs. HepG2 cells were cotransfected with pSPI-3(−1020)CAT, pSPI-3(−148)CAT, IL-2Rα, IL-4Rα, and expression vectors pSV-STAT1, pSV-STAT3, pSV-STAT5B, or pDC-STAT6 as indicated. Cultures were treated with IL-4 (4) or IL-6 (6) in the presence of Dex. The CAT activities were normalized to the internal transfection marker pIE-MUP and expressed relative to the control culture (fold induction indicated by numbers above the bars).

**Fig. 5.** Effect of GHR-mediated activation of overexpressed STAT5B on the SPI-3 promoter-CAT construct expression. H-35 cells (A) or HepG2 cells (B) were cotransfected with pSPI-3(−1020)CAT, pSPI-3(−148)CAT, or pSPI-3(−148)mutStatCAT; GHR, and STAT5B as indicated. Cells were treated with IL-6 or GH in the presence of Dex for 24 h. The CAT activities were normalized to the internal transfection marker pIE-MUP and expressed relative to the control culture (fold induction indicated by numbers above the bars).
The control of expression of the SPI gene family in liver by GH and inflammatory cytokines (i.e. IL-6) represents a striking example of fundamentally different regulatory effect for closely related genes. Although we could reproduce a liver-like regulation of SPI-3 gene by IL-6 and glucocorticoids in tissue culture, the same experimental tools proved not to be optimal to define the molecular basis for the differential effect of GH on the expression of the SPI-3 and SPI-1 genes. As already suggested by the studies of Waxman et al. (1995), hepatic gene regulation by GH may require consideration of frequencies and magnitude of signaling, processes that have not yet been approached in reconstituted systems as applied here.

The main focus of our studies was on the induction of SPI genes by IL-6. We analyzed in detail the regulation of the SPI-3 gene because the pattern of expression of the cloned promoter in transfected hepatoma cells was qualitatively similar to that of the endogenous SPI-3 gene. Previous results (Kordula and Travis, 1996) suggested that STAT isoforms, C/EBP isoforms, and GR may control the SPI-3 gene expression. We did not address the specific role of GR in the induction of the SPI-3 gene expression in this study. However, glucocorticoids are necessary for the maximal expression of the SPI-3 gene in H-35 cells (Fig. 1; Kordula and Travis (1996)). Moreover, the enhancing effect could be demonstrated by overexpression GR in HepG2 cells that resulted in a 30-fold higher expression of the SPI-3 promoter-CAT construct (data not shown). Although the results suggest a direct action of GR via a GRE within the SPI-3 promoter, the mode of action still needs to be proven.

The mutational analysis of the SPI-3 promoter and measurement of function in transfected hepatoma cells implied participation of STAT isoforms (Kordula and Travis, 1996). Both STAT3 and STAT5B are expressed in hepatocytes and hepatoma cells. However, the level of STAT5 protein, detectable by Western blot analysis and EMSA, is severalfold lower than that of STAT3 (Ripperger et al., 1995; Lai et al., 1995b and data not shown). The precise contribution of the individual endogenous STAT isoforms to the stimulation by the IL-6R could not experimentally be determined. By using the alternative approach of ectopically expressed hematopoietin receptors and STATs, the roles of both STAT3 and STAT5B as inducers were demonstrated. The responsiveness to STAT3 and STAT5B was attributed to the STAT element of the SPI-3 gene at −128 to −144 (Fig. 6). This element was recognized by STAT3 and STAT1 (Kordula and Travis, 1996; Kordula and Travis, 1995), but the binding of STAT5B could not be detected (data not shown). This observation suggests that the binding of STAT factors observed in the in vitro experiments may not always correlate with the activation of transcription via STAT elements in cells. Alternatively, the STAT5B-specific induction is achieved by a promoter sequence that is larger than that applied to DNA binding assay. A striking finding is the influence of surrounding promoter sequences on STAT action. The sensitivity to STAT5B is reduced in the presence of a sequence 5' to the STAT site. The (−1020 to +3) fragment from the SPI-3 promoter is strongly STAT3 responsive and only weak to STAT5B. The 5' fragment (−1020 to −155) neither contains any detectable functional STAT3 binding site nor confers responsiveness to IL-6 onto tk promoter (Fig. 7). The deletion of this fragment has been shown to result in the responsiveness of the truncated promoter to GH (Paqueruea et al., 1992). Our data are consistent with this observation since a promoter truncated to −148 is significantly stimulated by STAT5B, which is known to be activated by GHR. However, identity of a suppressive element for response to STAT5B of the −1020 promoter is not yet known. The modulator element is likely located between position −304 and −148 because the response to STAT3 and STAT5B of the 304–base pair-long SPI-3 promoter was similar to that of the 1020–

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**DISCUSSION**

**Fig. 6.** *Analysis of the effect of STAT3 and STAT5B on the expression of p4xStatCAT.* H-35 cells (A) or HepG2 cells (B and C) were cotransfected with p4xStatCAT and vectors expressing GHR or IL-3R and STAT5B or STAT3. Cells were treated with IL-6, GH, or IL-3 in the presence of Dex for 24 h. The CAT activities were normalized to the internal transfection marker pE-MUP and expressed relative to the control culture (numbers above the bars).

**Fig. 7.** *Analysis of the −1020 to −155 fragment of the SPI-3 gene.* HepG2 cells were transfected with p(−1020−155)tkCAT, p(−1020−155)4xStattkCAT, or p(−1020−155)tkCAT and treated with IL-6 in the presence of Dex for 24 h. The CAT activities were normalized to the internal transfection marker pE-MUP and expressed relative to the control culture (fold induction indicated by numbers above the bars). Light bars, control; dark bars, IL-6.
STAT3 and STAT5B Transactivate the SPI-3 Gene

base pair-long promoter (data not shown).

STAT3 has been proposed to be a key signaling molecule controlling expression of the acute phase genes activated by IL-6 (including the SPI-3 gene) (Wegenka et al., 1993; Akira et al., 1995; Kordula and Travis, 1996). Our new data show that STAT5B contributes to the regulation of the SPI-3 gene. Thus, the SPI-3 gene appears to be the first example of a liver gene that is a target of the two separate signaling pathways generated by gp130 (Lai et al., 1995). Be-


tween its sensitivity to STAT3 and STAT5B, the SPI-3 gene differs from other acute phase genes that have been studied for STAT isoforms specificity. The genes coding rat α1- acid glyco-

protein, rat and human haptoglobin, rat hemopexin, β fibrinogen, and human C reactive protein are strictly STAT3 responsive with minor to nondetectable activation by

STAT5B.2

The inhibition of the SPI-3 transcription by overexpressed C/EBPβ suggests that the C/EBP isoforms might significantly modulate regulation by STATs. Both STAT3 and C/EBPβ are activated during inflammation in liver and by IL-6 in hepatoma cells and bind to sequence similar gene elements (Baumann et al., 1992; Alam et al., 1992; Wegenka et al., 1993). However, the two factors are not regulated with similar kinetics. STAT3 is rapidly activated in response to inflammatory factors, whereas activation of C/EBPβ is delayed (Baumann et al., 1992). This difference in activation may represent a mechanism explaining the transition from gene induction to repression, in other words a switch from STAT-mediated activation to C/EBP-mediated inhibition. This transition would equal the course of the SPI-3 gene regulation following inflammation or IL-6 treatment (Hill and Hastie, 1987; Kordula and Travis, 1996). In this report the regulation of the SPI-3 gene transcription emerges as one of the best models to elucidate the processes governing activation as well as deactivation of an acute phase gene in liver cells.

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