STAT3 Is Required for the gp130-mediated Full Activation of the c-myc Gene

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Summary

The signal transducers and activators of transcription (STAT) family members have been implicated in regulating the growth, differentiation, and death of normal and transformed cells in response to either extracellular stimuli, including cytokines and growth factors, or intracellular tyrosine kinases. c-myc expression is coordinately regulated by multiple signals in these diverse cellular responses. We show that STAT3 mostly mediates the rapid activation of the c-myc gene upon stimulation of the interleukin (IL)-6 receptor or gp130, a signal transducing subunit of the receptor complexes for the IL-6 cytokine family. STAT3 does so most likely by binding to cis-regulatory region(s) of the c-myc gene. We show that STAT3 binds to a region overlapping with the E2F site in the c-myc promoter and this site is critical for the c-myc gene promoter-driven transcriptional activation by IL-6 or gp130 signals. This is the first identification of the linkage between a member of the STAT family and the c-myc gene activation, and also explains how the IL-6 family of cytokines is capable of inducing the expression of the c-myc gene.

Key words: signal transducer and activator of transcription 3 • c-myc • promoter • interleukin 6 • gp130

Signal transducers and activators of transcription (STAT) proteins have been shown to play pivotal roles in cytokine signaling pathways, which are involved in regulating cell growth and differentiation in systems ranging from Dictyostelium to mammals (1–4). STAT proteins are activated not only by receptor-associated Janus tyrosine kinases (JAK) (5–9), but also by receptor type tyrosine kinases (10–13) and by the oncogenic tyrosine kinases v-src and v-abl (14–16). The involvement of STAT proteins in cell survival, growth, transformation, and differentiation has been reported in a number of instances. A role for STAT5 has been suggested in IL-2-mediated cell growth signals in murine pro-B BAF/B03 cell lines expressing a variety of mutant IL-2 receptors (17), and it is also partially responsible for IL-3-induced cell growth in a pro-B BAF/B03 cell line (18). STAT3 activity plays a critical role in mediating gp130 signals leading to both growth arrest and macrophage differentiation in M1 leukemic cells (19, 20) as well as to cell survival in BAF/B03 cells stably expressing chimeric gp130 receptors (21). Lymphocytes from mice with a disruption in their stat5 or stat4 genes lose their proliferative responses to IL-4 and IL-12, respectively, indicating critical roles for these STAT molecules in cytokine-induced cell growth (22–26). Moreover, a disruption of the stat3 genes causes embryonic lethality around embryonic day E7.5 (27), suggesting a role for STAT3 in cell proliferation or survival in early embryonic stages. Recently, a comparison of the responses of lymphocytes from normal and gene-disrupted mice deficient in STAT6 or STAT4 led to the suggestion that STAT6 and STAT4 control lymphocyte proliferation by downregulating the levels of p27Kip1 protein (28). However, STAT5a is also involved in IL-2-induced lymphocyte proliferation via induction of the IL-2 receptor α chain, as shown by comparing lymphocytes from STAT5a null mice with those from normal mice (29). Regarding the roles of STAT proteins in oncogenesis, STAT1 activation is correlated with cellular transformation by Eeyk (30), and recently STAT3 was shown to be involved in the transformation of NIH3T3 cells by v-src (31, 32). In other cases, STAT1 protein plays a role in IFN-γ-induced growth arrest and apoptosis (33). All of these indicate the complexity of the

Abbreviations used in this paper: Ad, adenovirus; APRE, acute phase response element; EMSA, electromobility shift assay; G-CSF, granulocyte colony-stimulating factor; G-CSFR, G-CSF receptor; HA, hemagglutinin; JAK, Janus tyrosine kinases; PDGF, platelet-derived growth factor; pRb, products of the retinoblastoma gene; SH2, SH3 domain containing phosphatase; STAT, signal transducers and activators of transcription.
multiple roles of STAT family proteins. However, regarding the roles for STAT family proteins in cell proliferation, there is no report showing a linkage between STAT proteins and the direct regulation of genes critically involved in the cell cycle progression or in cellular transformation.

The product of the c-myc gene has been shown to be a critical regulator of cell growth, especially for cell cycle progression from the G1 to S phase (for review see reference 34) and for the induction of cdc25A (35). The c-myc gene is commonly activated during responses to the proliferative signals elicited by extracellular stimuli such as serum, epidermal growth factor, platelet-derived growth factor (PDGF) (36), nerve growth factor (37), colony stimulating factor 1 (38), and a variety of cytokines including IL-1, IL-3, GM-CSF, IL-5, IL-2, IL-4, IL-6, IL-9, and IL-12 (39-45). Among the molecules known to be involved in growth factor and cytokine signaling, the nonreceptor tyrosine kinases c-src (46), syk (47), and JAK (48-50) have been shown to activate the c-myc gene. N oncogenic nonreceptor tyrosine kinases, v-src and v-abl, bcr-abl, and the oncogenic serine/threonine kinase myc have also been shown to induce c-myc mRNA expression (51-54). STAT (the signal transducing adaptor molecule), which is phosphorylated on tyrosine residues after stimulation with a variety of cytokines such as IL-2, IL-3, GM-CSF, and epidermal growth factor appears to be involved in IL-2- and GM-CSF-induced activation of the c-myc gene promoter (55). However, very little is known about the mechanisms by which these different effector molecules activate the c-myc gene. Only E2F molecules, composed of members of the E2F family and DP1 or DP2, have been identified as the final common target molecules that affect transcriptional activation of the c-myc gene upon stimulation with serum, PDGF (56), v-abl (57-59), bcr-abl (54), and phosphatidyl inositol 3-kinase/c-akt (60, 61).

The IL-6 family of cytokines, which includes IL-6, ciliary growth, spreading, differentiation, and survival in a variety of tissues and cell lines (62). In particular, IL-6 and IL-11 are potent growth factors for many cell types, including T lymphocytes, B lymphocytes, and macrophages. IL-6 and IL-11 are also involved in the regulation of the immune system, and they are used as therapeutic agents in various diseases, such as inflammation and tissue repair. IL-6 and IL-11 are produced by a variety of cell types, including fibroblasts, endothelial cells, and epithelial cells. They are also produced by immune cells, such as macrophages and dendritic cells.

The product of the c-myc gene is a member of the c-myc family of transcription factors, which are involved in cell growth, proliferation, and differentiation. The c-myc gene is activated in many tumors and is a target of oncogenic activation in the development of a variety of human malignancies, including lymphoma, leukemia, and breast cancer. The activation of c-myc is often associated with the development of resistance to apoptosis, which is a hallmark of cancer cells. The c-myc gene is also involved in the regulation of the cell cycle, and its activation can lead to the G1/S transition and the entry of cells into the S phase of the cell cycle.

Plasmid Constructions. PHXL (55), a gift from Dr. K. Sugamura and T. Arita (Tohoku University School of Medicine, Sendai, Japan), is a luciferase reporter plasmid containing the human c-myc gene with the region spanning from −2309 to +532 bp relative to the transcription initiation site of the P1 promoter. This construct was called −2309/P1 in this paper for simplification. A series of 5′ deletion mutants were made as follows. To make −1398/+532 and −349/+532 Luc, respectively, fragments extending from a BglII site in the PHXL multicloning site to a SpeI or Pvull site in the c-myc promoter were deleted. The resultant constructs were then made blunt-ended and religated. For −101/+532 Luc and +68/+532 Luc, SmaI and the XhoI fragments, respectively, were deleted from PHXL. To make −102/+532 constructs, the region containing −102/+532 was amplified by PCR using the oligonucleotides 5′-AATCTGAGAAGAAA-GAAGCGAGGAGGGA-3′ and 5′-GCGGCGCTTTTCTTTT-GTTT-3′ as primers. The XhoI-HindIII fragment of the PCR product was subcloned into the XhoI, HindIII site of PHXL, from...
which the longer XhoI-HindII fragment, containing the c-myc region upstream of the HindII site had been deleted. To make 3 × c-myc E2F Luc and 3 × adenovirus (Ad) E2F Luc, three repeats of c-myc E2F and Ad type E2F oligonucleotides were inserted in front of the minimal mouse junB promoter Luc (66, 67). The sequences of the oligonucleotides were as follows: c-myc E2F 5′-TTGGCGGGAAAAA-3′ and Ad E2F promoter E2F 5′-GGTTTCGCCCTTCTCCTA-3′ (68) with an additional SacI and KpnI site at each end.

Site-directed mutagenesis by PCR. Mutations were introduced in the c-myc E2F binding site in the context of the intact c-myc promoter with the upstream region to −2309 bp (−2309/+532 mE2F Luc) and with that to −101 bp (−101/+532 mE2F Luc) by the overlap extension technique, using PCR. The primers used for these mutations were 5′-AGGTTGGAGTTAAAAGAACCAGGAGGAGGAAGGCCT-3′, 5′-TTTAACTCCAGGCCCTGAGAAGCT-G-3′, and reverse and universal primers for pBluescriptII (Stratagene). The underlined bases contain the XhoI-HindIII fragment of the c-myc promoter was used as a template. After the PCR products were subcloned into pBluescriptII SK+ (Stratagene), their sequences were verified by DNA sequencing. The PCR product digested with XhoI and HindIII was then inserted at the proper position of −2309/+532 Luc and −101/+532 Luc to make −2309/+532 mE2F Luc and −101/+532 mE2F Luc.

Transient Transfection Assay. For transfection experiments, HEpG2 cells were transfected with DNA mixtures using the calcium phosphate coprecipitation method. Typically, 1.2 μg of one of the reporter plasmids containing the firefly luciferase gene, and 1 μg of pEFlα2, a pEF-BOS expression vector containing the β-Iα2 gene encoding β-galactosidase as an internal control for transfection efficiency, were used. 3 μg of either pCAGGS-Neo, an expression vector without an insert (control), or pCAGGS-NeoHAStat3F, an expression vector containing a cDNA encoding HA-STAT3F (20) was cotransfected in some experiments. Cells were incubated with DNA precipitates for 12 h, washed with PBS, fed with DMEM containing 0.1% FCS for 20–24 h, and stimulated with 100 ng/ml of IL-6 for the last 6 h. Approximately 42 h after transfection, cells were collected in 120 μl lysis buffer and subjected to assays for luciferase and β-galactosidase activity as described (69).

Electromobility Shift Assay (EMSA).

Figure 1. Induction of c-myc mRNA expression via gp130 signaling in BAF-G277 cells (A) Northern blot analysis for c-myc mRNA expression in G-CSF-stimulated BAF/B03 transfectants expressing a chimeric receptor consisting of the G-CSFR extracellular domain and the transmembrane and cytoplasmic domains of gp130, including the full-length, 277 amino acid-long cytoplasmic domain (BAF-G277). Total RNA was extracted from BAF-G277 cells (lanes 1–7) and parental BAF/B03 cells (lanes 8–10) which had been deprived of IL-3 for 12 h and then stimulated with 100 ng/ml of G-CSF for up to 15 h. The levels of mRNAs for the c-myc gene and a housekeeping gene, CHO-B, are shown (Fig. 1 A). The c-myc mRNA levels normalized with those of CHO-B were plotted (Fig. 1 B). G-CSF increased immediately the levels of c-myc mRNA by about sixfold with a peak at 1 h after stimulation, followed by a gradual decrease until 12 h and a slight increase at 15 h (Fig. 1 A, lanes 1–7; Fig. 1 B). This induction is due to the activation of the chimeric receptor since G-CSF did not increase c-myc mRNA level in the parental BAF/B03 cells (Fig. 1 A, lanes 8–10). Pretreatment with D N ovo Protein Synthesis. We first characterized the nature of the gp130-mediated c-myc mRNA induction in BAF-G277, a BAF-B03 pro-B cell line expressing the chimeric receptor containing the extracellular domain of the G-CSF receptor and the transmembrane and cytoplasmic domains of gp130 (21). Total RNA was obtained from BAF-G277 cells which had been deprived of IL-3 for 12 h and then stimulated with 100 ng/ml of G-CSF for up to 15 h. The levels of mRNAs for the c-myc gene and a housekeeping gene, CHO-B, are shown (Fig. 1 A). The c-myc mRNA levels normalized with those of CHO-B were plotted (Fig. 1 B). G-CSF increased immediately the levels of c-myc mRNA by around sixfold with a peak at 1 h after stimulation, followed by a gradual decrease until 12 h and a slight increase at 15 h (Fig. 1 A, lanes 1–7; Fig. 1 B). This induction is due to the activation of the chimeric receptor since G-CSF did not increase c-myc mRNA level in the parental BAF/B03 cells (Fig. 1 A, lanes 8–10). Pretreatment
than that in BAF-G277, whereas BAF-G68 showed c-myc mRNA expression at a much lower level (Fig. 2 B). The STAT3, respectively (21, 72). In BAF-G133 cells, G-CSF responsive observed and estimated as one-seventh and one-fifth of those of BAF-G277 and BAF-G133, respectively. These results suggested that although a poorly characterized and weak signal to the c-myc gene was generated from the membrane-proximal region between 1 and 68, the region between 68 and 133 is necessary for the full activation of the c-myc gene. The use of the other transformants, BAF-G133F2 and BAF-G133F3, which express the truncated chimeric receptors with a tyrosine to phenylalanine mutation at the second (Y759) and third (Y767) tyrosine residues in the cytoplasmic domain, respectively, indicated that the third tyrosine residue, Y767, in G133 was required for the full activation of the c-myc gene (Fig. 2 B). Since this requirement is the same as for STAT3 activation (19, 72), it seemed likely that STAT3 is involved in the full c-myc induction. To test this directly, we used two other BAF transformants expressing both truncated chimeric receptors, G133, and the other two dominant-negative STAT3 mutants (20), BAF-G133 STAT3F and BAF-G133 STAT3D. Expression of either dominant-negative STAT3 almost completely inhibited the gp130-mediated expression of both c-myc and junB mRNA (Fig. 3), the latter of which has been known to be one of IL-6-inducible immediate early response genes (66, 70, 73). The three cell lines, BAF-G133, BAF-G133 STAT3F, and BAF-G133 STAT3D, could respond to IL-3 by expressing c-myc mRNA with levels similar in each cell line but much higher than that of gp130-induced c-myc mRNA, indicating that the poor gp130-mediated induction of c-myc mRNA seen in the dominant-negative STAT3-expressing cell lines was due to the specific effect of the exogenous STAT3F and STAT3D. These results indicated a critical role for STAT3 in the gp130-mediated c-myc and junB gene activation. Considering that the c-myc mRNA induction through gp130 signals occurred without requiring de novo protein synthesis (Fig. 1 C) and that the STAT3 transcription factor was involved, it seemed likely that STAT3 directly activated transcription of the c-myc gene by binding to a cis-regulatory region(s) of the gene.

rapid activation of the c-myc Promoter by STAT3. We tested whether IL-6 or gp130 activation could induce c-myc mRNA expression in cells other than BAF transfor-

mRNA expression. Cell lines tested were as follows: BAF-G277, BAF-G68, BAF-G133 (lanes 1–3), BAF-G133 stably expressing HA-tagged STAT3 (BAF-G133 STAT3D; lanes 7–9).
promoters. IL-6 increased transiently the c-myc mRNA levels by ∼2.5–3-fold in HepG2 at 1 h after stimulation (Fig. 4A), as for BAF-G27. We then took advantage of the easy transfectability of HepG2 cells to test if IL-6 can activate the c-myc gene promoter. First, we transiently transfected HepG2 cells with a luciferase gene construct containing the human c-myc gene with the region spanning from −2.3 kb to +530 bp relative to the P1 initiation site, and tested for IL-6 responsiveness. As shown in Fig. 4B, IL-6 increased the reporter gene expression by ∼3.0-fold. This activation of the c-myc promoter-driven transcription was effectively inhibited by the dominant-negative STAT3F3, indicating that a STAT3-responsive element(s) resides in the upstream or promoter region of the c-myc gene. Fig. 4C also shows that only the chimeric receptors containing the gp130 region capable of activating STAT3, that is, G277, G133, and G133F2, but not G133F3 or G68, could activate the c-myc promoter-driven-reporter gene expression, fully consistent with the c-myc mRNA expression pattern observed in BAF transformants. However, this does not rule out the possible involvement of other region(s) outside the upstream and promoter region of the c-myc gene used in this study. Enhancement of induction was observed with the G133F2 expression vector. This may be explained by an inhibitory effect of SHP-2, which is activated through the tyrosine phosphorylation module at Y759, on the STAT3 activity (74).

STAT3 responsive element resides in the proximal region of the c-myc P2 promoter. To identify a STAT3 responsive element in the c-myc promoter, we made a series of deletion mutants linked to the luciferase gene, as depicted in Fig. 5. We transfected HepG2 cells with these constructs and tested for IL-6 responsiveness. Whereas the promoter constructs bearing deletions −1398, −349, −101, and −68 bp relative to the P1 transcription initiation site responded to IL-6 by 2.7–3.8-fold over baseline transcription levels, the promoter truncated at −102 bp lost IL-6 responsiveness (Fig. 5), suggesting the existence of a STAT3 responsive element just upstream of the P2 promoter.

A region overlapping with the c-myc E2F binding site is responsible for STAT3-mediated activation of the c-myc promoter. Since the promoter region between −68 and −102 bp from the P1 initiation site contained two known functional sites, Me1a2 and part of the E2F binding site, we hypothesized that the major determinant for IL-6-induced and STAT3-mediated activation might be the c-myc E2F site, located at +98 to +106 bp. To test if this site was responsible for IL-6-induced and STAT3-mediated activation of the promoter, we made point mutations at this c-myc E2F site, changing from TTTGCGGGAAA to TTTGGAAGTTAA, in the context of the full-length promoter construct (−2309/+532 mE2F Luc) and in the truncated version of the c-myc promoter construct (−101/+532 mE2F Luc), and tested for IL-6 responsiveness. The mutations severely compromised the IL-6 responsiveness of the c-myc P1 promoter, 3 μg of either an expression vector, pCAGGS-Neo or pCAGGS-Neo bearing cDNA encoding the dominant-negative HA-tagged STAT3F3, pCAGGS-Neo HAStat3F3, and 1 μg of pEFlacZ. Transfected cells were serum starved for 24 h and then stimulated with 100 ng/ml of IL-6 for the last 6 h (black bars) or left unstimulated (open bars). Cells were then harvested and subjected to assays for luciferase and β-galactosidase activity. Values were normalized to transfection efficiency and represent the means of three different experiments. The SDs of the mean values are indicated by error bars. The numbers at the right of the bars indicate the fold increases in response to IL-6.
both constructs (Fig. 6 A), indicating that this site is really required for IL-6 responsiveness in the intact promoter. Then we proceeded to test whether the STAT3-mediated activation can be seen in E2F binding site in general or specifically in the c-myc E2F binding site. We constructed two reporter gene constructs, one containing three repeats of the c-myc E2F site, TTGGCGGGAAAG, and the other containing three repeats of a typical E2F binding site, GTTTCGGCCCTTTCTCAA, from the Ad E2 promoter (68, 75) inserted upstream of the heterologous minimal junB promoter linked to the luciferase gene (66, 67), and tested them for IL-6 responsiveness in HepG2 cells (Fig. 6 B). Interestingly, IL-6–driven transcription, but not the wild-type E2F site–driven expression, and the IL-6–induced activation of the c-myc E2F site was inhibited by the coexpression of the dominant-negative STAT3F (Fig. 6 B). From these results, we concluded that STAT3 activated the c-myc E2F site–driven expression, and the IL-6–induced activation of the c-myc E2F site was inhibited by the coexpression of the dominant-negative STAT3F (Fig. 6 B). From these results, we concluded that STAT3 activated the c-myc E2F binding site or an overlapping region. These results also indicated that the STAT3-mediated activation of the c-myc gene promoter occurs independently of E2F activity. The basal promoter activities of the c-myc promotor with the mutated E2F site (−2309/−532 mE2F Luc and −101/−532 mE2F Luc) were reproducibly higher than those of the corresponding intact c-myc promoters (−2309/+532 Luc and −101/+532 Luc), consistent with the existence of a repressor activity bound to the c-myc E2F site in the serum-starved HepG2 cells.

Direct Binding of STAT3 to the c-myc E2F Binding Site. Since the sequence of the c-myc E2F binding site, TTGGCGGGAAAG, is distinct from the known STAT3 binding sites (76–78), we tested whether STAT3 and other STAT proteins bind directly to the c-myc E2F site or indirectly through interactions with other proteins. To do this, we used EMSAs, testing nuclear extracts from BAF-G277 and HepG2 cells stimulated with either G-CSF or IL-6. The extracts were incubated with labeled oligonucleotides bearing the c-myc E2F site and specific antibodies to each STAT protein in some experiments and unlabeled oligonucleotides as competitors in other experiments. The nuclear extracts from BAF-G277 cells stimulated with G-CSF for 15 min (Fig. 7 A, lanes 1–5) and HepG2 cells stimulated with IL-6 for 15 min (Fig. 7 B, lanes 1–4) have prominent c-myc E2F site binding activity. The IL-6–stimulated HepG2 nuclear extracts were most likely to contain STAT3, STAT3/STAT1, and STAT1 (indicated by arrows in Fig. 7 B), since anti-STAT3 serum shifted the major component bound to the c-myc E2F site in BAF-G277 cells, which have very little E2F activity, either free E2F or complexes of E2F and one of the retinoblastoma protein family members. The inducible complexes in the IL-6-stimulated HepG2 nuclear extracts were most likely to contain STAT3, STAT3/STAT1, and STAT1 (indicated by arrows in Fig. 7 B), since anti-STAT3 serum shifted the two upper bands and anti-STAT1 antibody shifted the lower band. Interestingly, the mobilities of the complexes with the c-myc E2F probe are exactly the same as those of complexes seen using an oligonucleotide probe containing an APRE from a2-macroglobulin, a typical STAT binding site (compare lane 2 with lane 4 in Fig. 7 C), suggesting that these inducible complexes with c-myc E2F probe are dimers of STAT3, STAT3/STAT1, and STAT1, although c-myc E2F probe has less affinity than APRE probe. These inducible complexes of STAT1 and 3 with the c-myc E2F probe were competed by an oligonucleotide containing APRE (Fig. 7 D, lanes 7 and 8) five times as efficiently as by a control c-myc E2F oligonucleotide (Fig. 7 D, lanes 3 and 4), but not by the typical E2F binding site, TTTTGCGC, taken from the adenovirus E2 promoter (Fig. 7 D, lanes 5 and 6). These results suggested that activated STAT proteins may directly bind to the c-myc E2F site with around fivefold less affinity than to APRE and that in spite of the low affinity to the c-myc E2F site, the binding activities of STAT proteins were much stronger than those of E2F-containing complexes.

Binding Specificity of STAT Proteins for the c-myc E2F Site. Next we tested the binding specificity of other
STAT proteins for the c-myc E2F site. For this experiment, we first tested the nuclear extracts from IL-2-stimulated KT-3 cells (79) for STAT5 activity using EMSA with an oligonucleotide containing the STAT5 binding site (71) and a specific antibody to STAT5 that recognizes both STAT5a and 5b. As shown in Fig. 8, IL-2-stimulated KT-3 nuclear extracts showed abundant STAT5 complexes on the STAT5 probe recognizable by anti-STAT5 antibody (lane 10) and a small amount of STAT3-containing complex, which was recognized by an anti-STAT3 antibody (lane 9). The same extracts showed inducible binding activity to the c-myc E2F site (lanes 1 and 2). Interestingly, the anti-STAT5 antibody did not cause any shift of the complexes (Fig. 8, lane 5), whereas most of the complexes were shifted by the anti-STAT3 antibody (Fig. 8, lane 4). These results indicated that the c-myc E2F site binds preferentially to STAT3 and STAT1, but not to STAT5, and that the nuclear extracts from IL-2-stimulated KT-3 cells had STAT3 bound to the c-myc E2F binding site. Although we do not know the role of STAT3 in the IL-2 signaling pathway, this binding specificity is at least consistent with the lack of a role for STAT5 in the IL-2-induced activation of the c-myc gene.

Discussion

Rapid activation of the c-myc gene by STAT3. E2F activity has been shown to be involved in the activation of the c-myc gene in response to serum or PDGF (56). It is also likely that E2F activity is elevated in cycling cells in the G1 to S phase transition, including BAF-G277 cells. However, this mechanism does not account for the immediate induction of c-myc mRNA observed in BAF-G277 and HepG2 cells, since the increases in CDK4/6 kinase activity were only apparent 4 h after gp130 stimulation in BAF-G277 cells (data not shown), and no apparent increase in E2F activity was detected in IL-6-stimulated HepG2 cells, as exemplified by the lack of activation of the reporter gene containing three repeats of the wild-type E2F binding sites upstream of the minimal junB promoter (Fig. 6 B). Instead, we suggested that STAT3 is essential for the rapid IL-6-induced or gp130-mediated induction of the c-myc mRNA expression and showed that STAT3 rapidly activated the c-myc gene promoter by binding to a site overlapping with the c-myc E2F site. However, at the present time we do not know whether STAT3 cooperates with other proteins to activate the c-myc promoter-driven transcription or whether STAT3 synergistically enhances the c-myc mRNA expression with other characterized pathway(s) or mechanisms affecting the levels of transcriptional elongation or mRNA stability. The putative STAT3 binding site and the E2F binding site in the c-myc promoter are depicted and compared with the typical E2F binding site in the adenovirus E2 promoter (Fig. 9). As shown in Fig. 9, a STAT3

Figure 7. The nature of the gp130-signal-induced DNA binding activities at the c-myc promoter E2F site. Nuclear extracts from (A) IL-6-deprived BAF-G277 cells and (B) serum-starved HepG2 cells either untreated (A and B, lane 1) or treated with G-CSF (A, lanes 2–5) or IL-6 (B, lanes 2–4) at 100 ng/ml for 15 min were preincubated without (A and B, lanes 1 and 2) or with anti-STAT1 mAb (A and B, lane 3), anti-STAT3 antibody (A and B, lane 4), or anti-STAT5 antibody (A, lane 5) for 30 min on ice before the addition of 32P-labeled c-myc E2F oligonucleotides, and were then subjected to electrophoresis and autoradiography. The positions of the gp130-signal inducible complexes containing STAT3, STAT3/STAT1, and STAT1 are indicated. (C) Nuclear extracts from serum-starved HepG2 either unstimulated (lanes 1 and 3) or stimulated with IL-6 for 15 min (lanes 2 and 4) were subjected to EMSA with c-myc E2F oligonucleotide probe (lanes 1 and 2) and APRE oligonucleotide probe (lanes 3 and 4). (D) Binding specificity of IL-6-inducible c-myc E2F site-binding complexes. Nuclear extracts from serum-starved HepG2 either untreated (lane 1) or treated with IL-6 (lanes 2–8) were preincubated with unlabeled oligonucleotide competitors (50- or 250-fold molar excess as indicated) for 5 min, followed by incubation with labeled c-myc E2F probes. The competitors used were unlabeled oligonucleotides containing the c-myc E2F site (lanes 3 and 4), the adenovirus E2 E2F site (lanes 5 and 6), or the α2 macroglobulin APRE (lanes 7 and 8).
Cytokine Signals Regulating c-myc Expression. Upon binding to a ligand, most cytokine receptors initiate a variety of signaling pathways by activating JAK tyrosine protein kinases (6, 84, 85). Some signaling pathways appear to activate the c-myc gene. For instance, IL-2 has been shown to activate the c-myc gene through at least three distinct pathways, the tyrosine kinase syk (47), STAM (55), and phosphatidylinositol 3-kinase/Akt, protein kinase B (60, 61). The last pathway was shown to increase the E2F activity by phosphorylating and removing pRb from E2F (61).

As for the STAT proteins, STAT5, activated by IL-2 or by IL-3R βc, has been shown not to be responsible for c-myc mRNA induction (18, 86, 87). Mui et al. showed that carboxy-terminally truncated dominant-negative STAT5 inhibited the IL-3-induced mRNA expression for the ds and pim-1 genes but not that of the c-myc gene (18). The absence of a role for STAT5 in c-myc activation is consistent with our result showing that STAT5 does not bind to the c-myc E2F site (Fig. 8). This is quite a contrast to the critical role for STAT3 in activation of the c-myc gene through gp130 signaling, and shows that each STAT molecule has different target genes. Although STAT1 can bind to the E2F binding site overlapping with E2F site in the c-myc promoter, the contribution of STAT1 in IL-6-induced or gp130-mediated c-myc gene activation may not be important considering the following observations. First, the amount of STAT1 is much smaller than that of STAT3 in BAF-G277 cells. Second, two types of dominant-negative STAT3 efficiently inhibited the gp130-mediated c-myc mRNA induction in BAF-G133 cells. Third, dominant-negative STAT3, but not dominant-negative STAT1 (data not shown), inhibited IL-6-induced activation of the c-myc gene promoter activity in HepG2 cells (Fig. 4B). Our results indicate that different cytokine receptor systems have their own strategy to cause the similar outcomes, survival of cells and induction of the genes required for cell cycle progression, including the c-myc gene. It is also noteworthy that STAT3 activates the c-myc gene in some cells shown here but in other cells, e.g., M1 leukemic cells, the same STAT3 is involved in repression of c-myc gene expression with slower kinetics (20). The role of STAT1 in regulating the c-myc gene expression in response to other cytokines, including IFN-γ, should be examined carefully in this context.

This is the first report showing the linkage between STAT family proteins and c-myc gene activation. This result implies that other growth factor receptors, or nonreceptor type tyrosine kinases, or oncogene products that are capable of activating STAT3 also induce c-myc mRNA expression, at least in part through STAT3 activation.
References


