The STAT3 Transcription Factor Is a Target for the Myc and Riboblastoma Proteins on the Cdc25A Promoter*

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The STAT3 (signal transducer and activator of transcription) transcription factor functions as downstream effector of growth factor signaling. Whereas STAT3 activation is transient in normal cells, constitutively activated forms of the transcription factor have been detected in several cancer cell lines and primary tumors. Through the up-regulation of cell cycle and survival genes, STAT3 plays important roles in cell growth, anti-apoptosis, and cell transformation yet the molecular basis for this behavior is poorly understood. In this study, we show that STAT3 and its transcriptional cofactors are recruited to the promoter of the Cdc25A gene to activate its expression. Using chromatin immunoprecipitations, we observed that Myc is recruited to this promoter following STAT3 DNA binding. Moreover, small interfering RNA-mediated knockdown of Myc specifically inhibits the STAT3-mediated activation of Cdc25A. Reduction in Myc protein level results in defective recruitment of the CREB-binding protein, Cdk9, and RNA polymerase complexes, indicating that Myc is necessary for STAT3 transcription. Surprisingly, the association of STAT3 with the Cdc25A promoter does not necessarily lead to transcriptional induction because this protein also functions as a transcriptional repressor of the Cdc25A gene. Following hydrogen peroxide stimulation, STAT3 forms a repressor complex with the retinoblastoma (Rb) tumor suppressor to occupy the Cdc25A promoter and block its induction. In coimmunoprecipitations and ChIP experiments, Rb was found to associate with STAT3 on DNA and we provide evidence that Rb binds directly to the transcription factor. Thus, we propose that Myc and STAT3 cooperate to induce the expression of Cdc25A and that their transcriptional activity is normally regulated by the Rb tumor suppressor gene.

Cell cycle progression relies on the activation of cyclins and cyclin-dependent kinases (Cdk),1 which successively act in G1 to initiate S phase and in G2 to initiate mitosis. Two classes of cyclins get activated during the G1 phase of the cell cycle, D-type cyclins and cyclin E that assemble with their catalytic partner (Cdk4 or Cdk6 for cyclin D and Cdk2 for cyclin E) (1, 2). Active cyclin/Cdk complexes phosphorylate members of the pocket protein family (Rb, p107, and p130) to release and activate the E2F transcription factor and induce cell cycle progression. Cyclin/Cdk complexes are inhibited by phosphorylation at two sites (Thr-14 and Tyr-15 in Cdk2) that are dephosphorylated by members of the Cdc25 phosphatase family. There are three members of this family designated Cdc25A, Cdc25B, and Cdc25C that can be distinguished by their activity profile. Cdc25A has been described as an oncogene because the protein is overexpressed in a wide variety of tumors and cooperates with Ras to induce cell transformation and tumors in mice (4, 5).

Following mitogen stimulation of quiescent cells, Cdc25A gets activated by E2F but also by other DNA-binding proteins involved in cell cycle progression such as STAT3. STAT proteins are a family of transcription factors that are normally inactive within the cytoplasm and that accumulate in the nucleus upon growth factor stimulation. Whereas STAT3 activation is transient in normal cells, the transcription factor is persistently activated in a large number of tumors as a consequence of aberrant growth factor or tyrosine kinase signaling (6–9). In vivo, it has been recently suggested that the transcription factor plays an important role in epithelial carcinogenesis because STAT3-deficient mice are resistant to skin tumor (10). In addition, a constitutively active form of the transcription factor induces cell transformation of human breast epithelial cells and rodent fibroblasts (11, 12). Through a combined inhibition of apoptosis and activation of cell cycle progression, this protein is believed to play a critical role in cell oncogenesis (8, 12–15). The transcription factor activates several genes involved in cell cycle progression such as fos, cyclin D, or myc. It up-regulates anti-apoptotic genes such as Bcl-2 and Bcl-XL (12–14, 16, 17), and it also induces the expression of genes involved in invasion and metastasis such as the matrix metalloproteinase-9 (11). Using the p21uaF1 and myc genes as a model, we have recently shown that STAT3 recruits histone acetyltransferases such as NCoA1/SRC-1a or CBP. In addition, it also associates with BRG1, the ATPase subunit of the SWI/riboblastoma; GST, glutathione S-transferase; ChIP, chromatin immunoprecipitation assay.
SNF chromatin-remodeling complex, and with Cdk9, the elongating kinase of the P-TEFb complex (18–20). Despite our current understanding (21), the STAT3-mediated regulation of the Cdc25A promoter remains to be elucidated. In this study, we have found that STAT3 and its transcriptional cofactors are recruited to the Cdc25A promoter upon IL-6 stimulation. Interestingly, this cytokine also induced the association of Myc with this promoter. Moreover, small interfering RNA-mediated down-regulation of Myc prevented the activation of Cdc25A by STAT3, suggesting that the two transcription factors synergize to induce the expression of the phosphatase. Surprisingly, we found that the association of STAT3 with the Cdc25A promoter does not necessarily lead to transcriptional induction. Whereas cytokine stimulation leads to the activation of the gene, reactive oxygen species (ROS) activate STAT3 DNA binding but down-regulates the expression of Cdc25A. Under these conditions, we surprisingly observed that the retinoblastoma protein Rb associates with STAT3 on DNA to form a repressor complex. Thus, we propose that Myc and STAT3 cooperate to induce the expression of Cdc25A and that this effect is inhibited by the Rb tumor suppressor gene to prevent cell cycle progression.

MATERIALS AND METHODS

**Antibodies**—Polyclonal anti-STAT3 (C20), anti-phospho-STAT3-Tyr705 (B7), anti-CBP (A-22), anti-Rb (C15), c-Myc (N262), anti-Cdk9 (C20), and type II RNA polymerase (N-20) were obtained from Santa Cruz Biotechnology. Anti-α-tubulin (T9026) was obtained from Sigma.

**Luciferase Assays**—Transient transfections were done using the calcium phosphate precipitation method and repeated at least five times. Cells were plated at a density of 8 × 10^4 in 6-well plates 24 h prior to transfection. After 36–48 h post-transfection, cells were stimulated with 1% formaldehyde at room temperature for 10 min at 4 °C, diluted 3–10 times in dilution buffer (1% Triton X-100). Luciferase activity was normalized based on protein concentrations and then measured using a Packard Topcount scintillation counter.

**Immunoprecipitation and Western Blot Analysis**—Immunoprecipitations were performed overnight at 4 °C with nuclear or whole cell extracts (1–5 mg) in the presence of 1% Nonidet P-40. Cell extracts were precleared with 40 μl of protein A-Sepharose (10% slurry in phosphate-buffered saline) for 2 h at 4 °C, and cleared extracts were immunoprecipitated with 1 μg of the indicated antibodies overnight at 4 °C followed by the addition of 20 μl of protein A-Sepharose for 1 h at 4 °C. Immunoprecipitates were washed three times in lysis buffer (25 mM HEPES, pH 7.9, 300 mM KCl, 0.2 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotonin, 1 mM dithiothreitol) and one time with 20 mM Tris, pH 8, prior to the addition of sample buffer. Following electrotransfer, membranes were analyzed by Western blot with the indicated antibodies diluted in Tris-buffered saline buffer (10 mM Tris, pH 8, 150 mM NaCl). Western blot and luciferase assays were performed as described previously (18). Northern blot were performed with 20 μg of RNA using human cDNA probes labeled with [32P]dCTP using the random-priming labeling kit from Amersham Biosciences.

**Pull-down Experiments**—Pull-down reactions were performed by incubating purified histidine, His-STAT3 full length, or His-STAT3(T767–770) (200 ng) with purified GST or GST-Rb proteins (200 ng) in binding buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 1% Brij 96). After a 30-min incubation at 4 °C, the beads were washed once with binding buffer, twice with binding buffer containing 0.5 mM NaCl, and once with 20 mM Tris-HCl, pH 8. Samples were then analyzed by Western blot.

**Chromatin Immunoprecipitation Assay (ChIP)**—Cells grown to 60% confluence were serum-starved for 2 days. Following stimulation, cells were washed and then cross-linked with 1% formaldehyde at room temperature for 10 min. Cells were washed sequentially twice with 1 ml of ice-cold phosphate-buffered saline, centrifuged, and then resuspended in 0.5 ml of lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotonin) and sonicated three times for 15 s each. Supernatants were recovered by centrifugation at 12,000 rpm for 10 min at 4 °C, diluted 3–10 times in dilution buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 8.1), and subjected to one round of immunoclearing for 2 h at 4 °C with 2 μg of sheared salmon sperm DNA, 2.5 μg of preimmune serum, and 20 μl of protein A-Sepharose (of 50% slurry). Immunoprecipitation was performed overnight with specific antibodies and then 2 μg of sheared salmon sperm DNA and 20 μl of protein A-Sepharose (of 50% slurry) were further added for 1 h at 4 °C. Note that the immunoprecipitations are performed in the presence of 1% Nonidet P-40. Immunoprecipitates were washed sequentially for 10 min each in TSE I (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), TSE II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), and buffer III (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1). Bead precipitates then were washed three times with Tris.
STAT3 Regulates the Expression of Cdc25A

Fig. 2. Myc is recruited to the Cdc25A promoter upon IL-6 stimulation. A, HepG2 were stimulated as described in the legend to Fig. 1A. mRNA levels were analyzed by RT-PCR with primers specific for the Myc, Cdc25A, or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs. B, ChIP analysis of the recruitment of Myc on the Cdc25A promoter. Soluble chromatin was immunoprecipitated (IP) with Myc antibodies, and DNA samples were amplified using two pairs of primers that cover either the proximal Cdc25A promoter (lanes 1–4) or a distal region of the p21waf1 promoter (lanes 5–8). In parallel (lanes 9–12), ChIP assays were analyzed by real-time PCR as described in Fig. 1B, quantification represents the average of three independent experiments.

RESULTS

STAT3 and Myc Are Recruited to the Cdc25A Promoter upon IL-6 Stimulation—To determine the effect of STAT3 on Cdc25A, HepG2 cells were serum-starved and left untreated or stimulated with IL-6 for 4 h. As described previously (21), Northern blot analysis showed that cytokine or serum stimulation induced the expression of the Cdc25A mRNA (Fig. 1A, lanes 1–3). In parallel, Western blot analysis also indicated that IL-6 up-regulates the expression of the Cdc25A protein (Fig. 1A, lanes 4–6). Because several STAT3 binding sites are located within the Cdc25A proximal promoter, ChIP experiments were then performed. As compared with untreated cells, ChIP experiments indicated that STAT3 was recruited to the proximal Cdc25A promoter upon IL-6 stimulation (Fig. 1B, lanes 1–4). Using real-time PCR analysis, we found that DNA binding reached a maximum after 30 min and then gradually declined (Fig. 1B, lanes 9–12). As a control of DNA sonication efficiency, PCR analysis did not detect any occupancy of a control region located on the p21waf1 promoter (Fig. 1B, lanes 5–8).

During the course of these experiments, we noticed that Myc...
was also induced by IL-6 and that its mRNA became apparent as early as 30 min after cytokine stimulation. By contrast, the Cdc25A mRNA was significantly expressed only after 90–120 min (Fig. 2A, compare middle and top panels). Because Myc binds to the Cdc25A promoter to induce its expression (22, 23), this result suggested to us that this transcription factor was involved in the STAT3-mediated activation of Cdc25A. Effectively, ChIP experiments indicated that IL-6 induced the association of Myc with the Cdc25A promoter (Fig. 2B, lanes 1–4). As a control, PCR analysis did not detect any occupancy of the control region (Fig. 2B, lanes 5–8). Importantly, real-time PCR analysis indicated that Myc DNA binding reached a maximum after 90 min when STAT3 binding and nuclear expression had already declined (compare Figs. 1B and 2B, lanes 9–12). We have been unable to detect any association between STAT3 and Myc, further suggesting that the two proteins are not associated on DNA. Altogether, these results indicate that STAT3 and Myc are both recruited to the Cdc25A promoter to induce its activation.

**Myc Is Involved in the STAT3-mediated Activation of Cdc25A**—We then investigated the effect of Myc knockdown on the IL-6-mediated activation of Cdc25A. To this end, we used a doxycycline-inducible expression vector that drives Myc small interfering RNA expression in the LS174T colorectal cell line (24). As expected, IL-6 induced the expression of Myc in this cell line but this effect was inhibited upon the addition of doxycycline (Fig. 3A, lanes 1–8). To evaluate the effect of Myc on the activation of Cdc25A, LS174T cells were serum-starved, treated or not with doxycycline, and further stimulated with IL-6. Under these conditions, reverse transcription (RT)-PCR and Western blot analysis indicated that IL-6 had no significant effect on the expression of Cdc25A in the absence of Myc (Fig. 3B, lanes 1–8 and 9–16). Importantly, the expression of p21waf1 was slightly enhanced, indicating that the inhibition was specific to the Cdc25A gene and confirming that Myc is involved in the down-regulation of the cell cycle inhibitor (Fig. 2B, lanes 1–8, middle panel) (25).

We have previously reported that STAT3 activates its target genes through its association with the CBP coactivator and the recruitment of the RNA polymerase and the Cdk9-elongating kinase (18, 19). To determine whether Myc affects the association of these proteins with the Cdc25A promoter, their recruitment was analyzed by Chip in LS174T cells pretreated or not with doxycycline. In control conditions, STAT3 DNA binding was associated with the recruitment of Myc, CBP, Cdk9, and RNA polymerase II (Fig. 4A, lanes 1–3). As expected, the association of Myc with the Cdc25A promoter was inhibited when cells were pretreated with doxycycline (Fig. 4A, lanes 4–6). Whereas Myc down-regulation did not affect STAT3 DNA binding, the association of CBP, Cdk9, and RNA polymerase II with the Cdc25A promoter was significantly inhibited (Fig. 4A, lanes 4–6). Moreover, using a pair of primers that covers the exon 13 of the gene, we observed that Myc down-regulation prevented the elongating form of the polymerase from reaching the 3′-region of the gene (Fig. 4B, compare lanes 1–3 and 4–6). In each condition, no occupancy of a control region of the promoter was observed (data not shown). Taken together, these results suggest that the binding of Myc to the Cdc25A promoter is necessary for STAT3 to induce the activation of this gene.
STAT3 Does Not Activate the Cdc25A Gene upon Oxidative Stress—To extend our results, we then determined whether the STAT3-mediated activation of Cdc25A was only related to IL-6 stimulation or whether this effect could also be observed with other signaling pathways that induce STAT3 activation. Besides cytokines and growth factors, ROS also function as signal transducers in several systems and can also trigger STAT3 phosphorylation and nuclear translocation (26, 27). To determine whether the transcription factor activates Cdc25A upon oxidative stress, asynchronously growing HepG2 cells were treated with hydrogen peroxide, nuclear extracts were prepared and processed to measure luciferase activity. The mean of five transfections ± S.D. is shown. C, ChIP analysis of the recruitment of Rb on the proximal promoter of the Cdc25A gene after hydrogen peroxide treatment (lanes 1–3) or IL-6 stimulation (lanes 4–6). Results were analyzed by real-time PCR as described above and represents the average of three independent experiments. IP, immunoprecipitation.

Rb Is Recruited to the Cdc25A Promoter to Down-regulate Its Expression—During the course of these experiments, we were able to confirm the effect of IL-6 or ROS on Cdc25A expression in several cell lines such as glioblastoma or colorectal cancer cells (data not shown). However, we noticed that oxidative stress did not inhibit the expression of Cdc25A in human osteosarcoma Saos-2 cells (Fig. 6A, lanes 1–3). Importantly, ROS stimulation induced the phosphorylation of STAT3 and its recruitment to the proximal Cdc25A promoter in this cell line (Fig. 6A, lanes 4–6 and 7–8). Moreover, p21waf1 was induced as expected under these conditions, indicating that this effect was specific to the Cdc25A gene (Fig. 6A, lanes 1–3, bottom panel).

Because Saos-2 cells lack a functional Rb protein, these results suggested to us that Rb might be involved in the down-regulation of the Cdc25A promoter. To verify this finding, HepG2 or Saos-2 cells were cotransfected with a reporter construct containing the Cdc25A promoter (28) in the presence or absence of a vector expressing Rb. Following transfection, cells were treated or not with hydrogen peroxide for 8 h and cytoplasmic extracts were prepared, and STAT3 activation was analyzed. As expected, ROS induced the nuclear translocation of STAT3 and its phosphorylation on the Tyr-705 and Ser-727 residues (Fig. 5A, lanes 1–4). Moreover, ChIP experiments and real-time PCR analysis indicated that STAT3 binds to the Cdc25A promoter upon ROS stimulation (Fig. 5A, lanes 5–7). However, opposite to the effect of IL-6, we surprisingly observed that hydrogen peroxide induced a down-regulation of the Cdc25A mRNA and protein (Fig. 5B, lanes 1–3 and 4–6). Because Myc is involved in the regulation of Cdc25A, we also characterized the effect of ROS on its expression. As expected, Myc was expressed in growing cells but its mRNA was down-regulated by hydrogen peroxide (Fig. 5C, lanes 1–2). Consequently, ChIP experiments indicated that Myc was not associated with the Cdc25A proximal promoter upon oxidative stress (Fig. 5C, lanes 4–6). Thus, we concluded from these results that the binding of STAT3 to the Cdc25A promoter does not necessarily lead to transcriptional activation.
effect was not observed when cells were stimulated with IL-6, suggesting that the binding of Rb to the Cdc25A promoter is ROS-specific (Fig. 6C, compare lanes 1–3 and 4–6). Taken together, these results indicate that Rb is recruited to the Cdc25A promoter to repress its activity upon oxidative stress.

**Rb Interacts with STAT3 on the Cdc25A Promoter**—Because Rb associates with several DNA-binding proteins, we asked whether the tumor suppressor interacts with STAT3 upon ROS stimulation. To this end, HepG2 cells were treated with hydrogen peroxide, nuclear extracts were recovered, and coimmunoprecipitations were performed using polyclonal antibodies directed against Rb proteins (lanes 3–4) or a control serum (lanes 1–2). Samples then were separated by SDS-PAGE, transferred to a nitrocellulose filter, and probed with polyclonal antibodies directed against STAT3 proteins (lanes 1–4, top panel) or Rb (lanes 1–4, bottom panel). Reciprocal immunoprecipitations were performed with polyclonal antibodies directed against Rb (lane 5), STAT3 proteins (lanes 6–7), or a control serum (lane 8) followed by membranes blotting with polyclonal antibodies directed against Rb (lanes 5–8). Note that the lower bands correspond to the IgG signal.

**Fig. 7.** Rb is recruited by STAT3 to the Cdc25A promoter. A, HepG2 cells were treated with hydrogen peroxide for 30 min or left untreated. Nuclear cell extracts were immunoprecipitated (IP) with polyclonal antibodies directed against Rb proteins (lanes 3–4) or a control serum (lanes 1–2). Samples then were separated by SDS-PAGE, transferred to a nitrocellulose filter, and probed with polyclonal antibodies directed against STAT3 proteins (lanes 1–4, top panel) or Rb (lanes 1–4, bottom panel). Reciprocal immunoprecipitations were performed with polyclonal antibodies directed against Rb (lane 5), STAT3 proteins (lanes 6–7), or a control serum (lane 8) followed by membranes blotting with polyclonal antibodies directed against Rb (lanes 5–8). B, soluble chromatin was prepared from HepG2 cells treated or not with hydrogen peroxide for 30 min and immunoprecipitated (1st IP and 2nd IP) with the indicated antibodies. The final DNA extractions were amplified using a pair of primer that covers the proximal Cdc25A promoter. C, Saos-2 cells were transfected with vectors encoding Rb or STAT3-β expression vectors as indicated. Following 48 h, cells were treated with hydrogen peroxide for 30 min and soluble chromatin was prepared and immunoprecipitated with STAT3 (lanes 1–6) or Rb antibodies (lanes 7–11). STAT3 antibodies were either directed against the tyrosine-phosphorylated form of the protein (lanes 1–3) and hence recognized STAT3 and STAT3-β or directed against the C-terminal (Cter) domain of the transcription factor (lanes 4–6) and recognized only STAT3. Results were analyzed by real-time PCR as described above and represent the average of three independent experiments.
FIG. 8. Rb interacts directly with STAT3. A and B, schematic representation of the various fusion proteins used in the pull-down experiments and their interaction with His-STAT3. C, purified GST-Rb proteins (50 ng) were incubated with histidine, His-tagged STAT3 C-terminal domain (STAT3cter), or the constitutively activated form of STAT3 (His-STAT3) immobilized on nickel-agarose beads (100 ng). Samples then were separated on polyacrylamide gels, and Rb binding was detected by Western blot using anti-Rb polyclonal antibodies (lane 1–4). His-tagged STAT3 fusion proteins corresponding to the activation domain of STAT3 (His-STAT3Cter, 100 ng) were tested for binding to GST or to GST-Rb (50 ng) immobilized on Sepharose beads (lanes 5–8). Samples were then separated on polyacrylamide gels, and STAT3 binding was detected by Western blot using anti-STAT3 polyclonal antibodies (lanes 5–8). D, the binding of his-STAT3 or His-STAT3Cter was tested by pull-down experiments using various fragments of Rb immobilized on Sepharose beads as described in C.
conditions, STAT3 antibodies were able to immunoprecipitate the Cdc25A promoter, whereas this was not the case with the CBP antibody (Fig. 7B, lanes 1–4). In each condition, PCR analysis did not detect any occupancy of a control DNA region (data not shown).

To extend this result, we made use of a dominant negative form of STAT3, STAT3β, a splice variant that functions in a competitive manner to block STAT3 DNA binding (13, 29, 30). Transient transfectants of Saos-2 cells were generated, and ChIP experiments were conducted to determine whether the inhibition of STAT3 DNA binding also prevents the association of Rb with DNA. As expected, we observed that STAT3β prevents the association of the endogenous STAT3 with the Cdc25A promoter upon ROS stimulation (Fig. 7C, lanes 4–6). Note that two different antibodies were used. One (lanes 1–3) is directed against the tyrosine-phosphorylated form of STAT3 and recognizes both STAT3 and STAT3β, and the second (lanes 4–6) binds to the activation domain of STAT3 and does not recognize STAT3β. Confirming the above results, ROS induced the association of transfected Rb with the Cdc25A promoter upon ROS stimulation of Saos-2 cells. However, this binding was significantly inhibited in the presence of STAT3β (Fig. 7C, lanes 10–11).

We then investigated which domain(s) in Rb mediated the interaction with STAT3. To this end, pull-down experiments were performed using various forms of GST-Rb or His-STAT3 proteins expressed in bacteria (Fig. 8, A and B). Results indicated that GST-Rb interacts with His-STAT3 and C-terminal His-STAT3 but not with histidine alone (Fig. 8C, lanes 2 and 4). Additionally, His-STAT3Cter interacts with GST-Rb but not with GST alone (Fig. 8C, lanes 7–8). Using various fragments of Rb, we also observed that STAT3 did not interact with amino acids 646–926 of GST-Rb or with the 646–772 fragment. By contrast, the transcription factor was able to bind to amino acids 379–572 of Rb (Fig. 8D, lanes 10–12). Taken together, these results suggest that Rb binds directly to STAT3 and is recruited by the transcription factor to the Cdc25A promoter to repress its expression.

DISCUSSION

The results presented in this study extend the functions of STAT3 and indicate that Myc and Rb regulate the activity of the transcription factor. We have found that STAT3 cooperates with Myc to induce the expression of Cdc25A and that the association of Myc with the phosphatase promoter is necessary for STAT3 to activate this gene. Importantly, STAT3 is probably not directly involved in the recruitment of Myc because the association of this transcription factor with the Cdc25A promoter is maximal when STAT3 DNA binding starts to decline. Moreover, we were not able to detect any association between the two transcription factors. Although the two proteins did not interact, RNA interference experiments clearly indicate that Myc activation is an essential step in the STAT3-Cdc25A pathway. Therefore, further experiments are needed to understand the molecular mechanisms by which Myc regulates the transcriptional activity of STAT3 on the Cdc25A promoter. On the cyclin D2 promoter, recent results have reported that Myc is probably not involved in the formation of the preinitiation complex containing the RNA polymerase and the TFIIH/TFIIB complexes (31). In our hands, we also observed that the CBP and RNA polymerase proteins were recruited by STAT3 before Myc DNA binding, suggesting that this protein is not involved in the initial steps of the preinitiation complex loading. Interestingly, it has been reported that Myc is necessary for the loading of TFIIH, Cdk9, and mediator complexes on its target promoters (31, 32). In addition, Myc interacts with the TIP60 histone acetyltransferase and with the IN1 subunit of the SWI/SNF complex (32–34). Therefore, one potential explanation is that the STAT3-CBP complex might be involved in the loading of the RNA polymerase on the Cdc25A promoter, whereas Myc may confer a permissive state on chromatin, allowing the recruitment of the P-TEFb complex and transcriptional elongation. In line with this hypothesis, we have recently shown that STAT3 regulates chromatin remodeling and transcriptional elongation through its interaction with BRG1 and Cdk9 (19).

These results also indicate that STAT3 may function not only as a transcriptional coactivator but also as a transcriptional repressor when associated with the Rb tumor suppressor gene. The interaction between STAT3 and Rb is probably not only induced by ROS stimulation but also by other signaling pathways that rely on STAT3 to repress cell proliferation. For instance, oncostatin M, an interleukin-6 family cytokine, inhibits cell cycle progression through STAT3 activation, yet the molecular basis for this behavior remains to be characterized. It will be interesting to determine whether this cytokine induces the association between Rb and STAT3 to repress the expression of cell cycle genes. It is well known that Rb is associated with proteins such as histone deacetylases or methyltransferases to repress the E2F family of transcription factors (35). Thus, these corepressors are probably also recruited by Rb to the STAT3-dependent promoters and one hypothesis would be that Rb prevents STAT3 activity by recruiting remodeling factors such as HDAC1, DNMT1, and members of the SWI/SNF complex (36–38). This would lead to transcriptional repression of STAT3 target genes, thereby inhibiting the effect of the oncoprotein on cell proliferation. Because the capacity of Rb to repress transcription is a function of its phosphorylation state, these observations also raise the interesting hypothesis that its interaction with STAT3 might be regulated by cyclin/Cdk and that it could fluctuate during cell cycle progression. Because STAT3 is present in the nucleus in early G1 and since we have previously shown that the transcription factor interacts with cyclin D1 (39), we speculate that Cdk4 and not Cdk2 might regulate the interaction between these two proteins.

Besides their normal functions, the STAT3 and Myc transcription factors play an important role in tumorigenesis (10, 12, 40). Myc has already been reported to cooperate with an activated Ras allele in cell transformation (41, 42), and our results indicate that this protein might also cooperate with the STAT3 oncoprotein. Although previous results have already reported that Myc is required for the transforming activity of STAT3 (43), it remains to be determined whether there is a significant overlap between the set of target genes activated by the two transcription factors. A comprehensive analysis of their common targets should give insights into the genes that are necessary for the two proteins to induce cell transformation, and our results already suggest that Cdc25A is one of these important targets. Therefore, we propose that the STAT3-Myc oncopgenic pathway induces the expression of Cdc25A to allow cell transformation and that this is normally controlled by the Rb tumor suppressor gene. This result extends previous studies by our laboratory and others (44, 45), showing that both p53 and p21waf1 prevent STAT3 activation. This leads us to propose a model by which the two main tumor suppressor pathways, p53 and INK4, prevent the effect of STAT3 and Myc on cell transformation through the activation of p21 and Rb. The frequent inactivations of p53 or Rb observed in cancer cells might therefore not only induce cell cycle progression but also favor STAT3 transcriptional activity toward the activation of growth-promoting genes such as Cdc25A.

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