We are interested in identifying the transcriptional targets of the Myc oncoproteins. To this end, we have fused Myc of the MC29 retrovirus with the rat glucocorticoid receptor. This chimeric protein requires dexamethasone to undergo nuclear translocation and achieve an active conformation. We employed a differential hybridization approach to identify mRNAs that are induced or repressed in infected avian fibroblasts in response to dexamethasone. This screen yielded one mRNA underrepresented in the dexamethasone-treated cells. In Myc-transformed cell clones, its level decreases 6-fold as early as 4 h and more than 30-fold after 32 h of exposure to the hormone. This mRNA was also down-regulated by recombinant Myc retroviruses in rodent fibroblasts, including those refractory to transformation.

Sequence analysis revealed that it is homologous to the 3’ untranslated regions of the mammalian thrombospondin-1 genes. Using an anti-thrombospondin antibody, we confirmed that rodent cells overexpressing Myc produce very small amounts of this protein. Also, they do not support efficient expression of a reporter gene driven by the thrombospondin-1 promoter. Thus, thrombospondin-1 is a bona fide target of Myc. Moreover, its silencing might pertain to the transforming activity of Myc, since in several systems thrombospondin-1 exhibits tumor suppressor properties, presumably due to its negative effect on neovascularization.

v-myc was first identified as a transforming gene of the MC29 retrovirus (1, 2). It has been since shown to be an integral component of other acutely transforming retroviruses of both avian and mammalian species (Ref. 3 and references therein). Furthermore, its cellular homolog c-myc is frequently amplified or rearranged in human malignancies, such as Burkitt’s lymphomas and mammary adenocarcinomas (Refs. 4 and 5 and references therein). All myc genes encode nuclear phosphoproteins capable of transcription regulation through a somewhat poorly characterized activation domain at the N-terminus of the proteins (for a recent review see Ref. 6). Gene activation is thought to also involve sequence-specific binding, through the basic region at the C terminus of Myc, to a simple DNA element CACGTG, termed the E-box. Consequently, transient expression of a reporter gene placed downstream of tandemly repeated E-boxes is positively, if weakly, regulated by overexpressed c-Myc (7–9). Furthermore, transiently expressed c-myc positively influences transcription of several E-box-containing genes, such as protothymosine (10, 11), ornithine decarboxylase (12), and cyclin A (13); but these studies are complicated by the fact that a variety of abundant cellular transcription factors (e.g. USF) as well as endogenous c-Myc also bind to the E-box (14). This makes activation of a suspected target gene difficult to confirm (15).

More recently, c-Myc has also been shown to behave as a transcriptional repressor (16). One proposed mechanism of repression involves the initiator element found in a variety of cellular and viral promoters (17–19) including that of the cyclin D1 gene (ccnD1). Indeed, overexpression of human c-Myc has been shown to cause significant reduction in the level of the endogenous ccnD1 mRNA (20). Unlike other putative Myc targets, ccnD1 does possess oncogenic activity when coexpressed with ras (21). However, since in proliferating cells Myc represses, not activates, ccnD1, this type of regulation does not seem to bear on the transforming activity of Myc.

To identify transformation-relevant targets of Myc, we have chosen to use a hormone-regulated allele of v-myc. Fusions between hormone-binding domains and oncoproteins, including Myc (22), have been valuable in dissection of molecular mechanisms of neoplastic transformation (Ref. 23 and references therein). We thus generated a conditional mutant of the MC29 v-myc retrovirus (GRIM), which expressed v-Myc as a triple fusion protein with Gag (the retroviral structural protein) and the ligand-binding domain of the rat glucocorticoid receptor (GR) (24). GRIM-encoded oncoprotein (P145GRIM) is only active when dexamethasone, a synthetic ligand for GR, is present in cell culture medium. Indeed, establishment of the transformed phenotype by dexamethasone-inducible v-Myc is strictly hormone dependent. No phenotypical changes are observed when infected cells are maintained in hormone-free media. Furthermore, transformation is absolutely dependent upon DNA binding by Myc since a variant with a mutated basic region (GRIMlost) lacks any transforming potential (24). We concluded that this system was well suited to study the early effects of Myc on gene regulation using differential hybridization techniques. Specifically, we set out to identify mRNA(s) that were induced or repressed after short exposure to dexamethasone in cells infected with the v-Myc-GR chimeric virus.

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The abbreviations used are: GRIM, glucocorticoid receptor-immobilized Myc; GR, glucocorticoid receptor; GEF, quail embryo fibroblasts; 4-OHT, 4-hydroxytamoxifen; Tsp-1, the thrombospondin-1 gene; Tsp-1, the thrombospondin-1 protein; RIP, radioimmunoprecipitation; CAT, chloramphenicol acetyltransferase.
We reasoned that this might lead to identification of new or suspected tumor susceptibility or tumor suppressor genes.

**EXPERIMENTAL PROCEDURES**

**Propagation of Cells**—Primary quail fibroblasts (QF) and clones derived therefrom were maintained in F10 medium supplemented with 10% bovine serum, 1% heat-inactivated chicken serum, and 1% MeSO. All rat cells (PC12, fibroblasts, and aortic smooth muscle cells) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Media for the PC12 pheochromocytoma cell line (25) were also supplemented with 5% horse serum. Human foreskin fibroblasts were maintained in RPMI supplemented with 10% fetal calf serum. Media and sera were purchased from Life Technologies, Inc. Cells to be tested for thrombospondin-1 expression were either kept untreated or exposed to one of the following drugs: 10 μM dexamethasone (Sigma), 10 μM RU486 (UCLAF-Resoull, Romanois, France), 100 μM etimine (Sigma), or 0.25 mM 4-hydroxytamoxifen (Research Biochemicals International, Natick, MA). Preparation of hormone-free media by charcoal/dextran stripping has been described earlier (24).

**Generation and Screening of cDNA Libraries**—A differential hybridization approach was used to identify mRNAs over- or under-represented in thrombospondin-1 riboprobes to screen replica filters representing the original libraries. With respect to individual RNAs, the specific activities of these riboprobes were calculated according to the manufacturer’s recommendations. The radiolabeled RNAs were used as hybridization probes. Several thousand random clones were checked using differential cDNA libraries, no subtractive enrichments were performed. Instead, we reasoned that this might lead to identification of new or suspected tumor susceptibility or tumor suppressor genes.

**Radioimmunoprecipitation**—Approximately 5 × 10^6 cells were left untreated or treated with 4-hydroxytamoxifen for 24 h. They were subsequently labeled for 4 h with 50 μCi of [35S]methionine (DuPont NEN) in Dulbecco’s modified Eagle’s medium/5% dialyzed fetal calf serum. Cells were lysed in the Ab buffer (29), and 2.5 μl of the A4.1 α-anti-Tht-1 monoclonal antibody (Life Technologies, Inc.) were added to cell lysates and incubated overnight at 4°C. Then, 2.5 μl of a secondary goat m- IgM antiserum (TAGO Inc., Camarillo, CA) were added along with 50 μl of Gamma-Bind G Sepharose (Pharmacia Biotech Inc.), and incubation continued at room temperature for another 90 min. Standard collection and washing techniques were applied, and the immunoreactive proteins were run on 7.5% denaturing polyacrylamide gel as described earlier (29).

**RESULTS**

**The Thrombospondin-1 mRNA Is Under-represented in v-Myc-transformed Cells**—We were particularly interested in early targets of v-Myc, rather than genes whose altered expression would merely be symptomatic of transformation. Hence, QEF infected with the wild type GRIM retrovirus (GRIMwild) were maintained in glucocorticoid-free medium until the virus had spread and were then treated with dexamethasone for only 4 h. At this point, no changes in cell morphology or growth rates were apparent. To account for effects induced by the endogenous GR, we used, as a negative control, QEF infected with a transformation-incompetent GRIMlost derivative (24) and treated with dexamethasone for the same interval. Out of several thousand clones checked, only one was reproducibly found to display a differential hybridization pattern. Since it reacted much more strongly with the GRIMlost probe, it represented a potential Myc-repressed gene. The cDNA insert was sequenced and compared with the GenBank data base. It appeared to be almost identical (Fig. 1), with only one gap, to the 3′ terminal gapdh mRNA. The composition of the primer was: GACTATCTAGATGTCTTATTAATATAA (forward) and GTACATAAGAAAAACATATATATAG (reverse). The resulting fragment, 289 base pairs in length, was cloned into the pCRT vector (Invitrogen), and the corresponding riboprobe was synthesized using T7 RNA polymerase. Since the internal primer DraI site was used to prepare the DNA template, the protected fragment was only 289 nucleotides long. The rat gapdh probe was prepared by transcribing the 3′ terminal 211-base pair fragment of the full-length cDNA clone (Ref. 28; downstream from the StyI site) with T7 RNA polymerase. The intensities of gapdh-protected fragments were quantitated using a phosphoimager (Molecular Dynamics).
progressions of primary tumors and metastases (41–43). Thus, its down-regulation would be consistent with the oncogenic activity of Myc.

To confirm that tsp-1 mRNA is indeed down-regulated by P145grim, we performed RNase protection analyses on QEF acutely infected with either GRIMwild or GRIMlost and maintained either in the absence or in the presence of dexamethasone. Molecular clones of tsp-1 and chicken gapdh were used to prepare antisense hybridization probes. In quail fibroblasts (Fig. 2), the tsp-1 riboprobe protects the predicted 304-nucleotide RNA fragment (see “Experimental Procedures”) and also several slightly shorter fragments presumably truncated at cryptic polyadenylation sites in the AT-rich 3′-untranslated regions of tsp-1 (33). gapdh encodes a housekeeping enzyme glyceraldehyde-3-phosphate dehydrogenase whose mRNA does not vary significantly among different tissues (44). Therefore, its 170-nucleotide protected fragment was used as an internal control to which all tsp-1 values were normalized.

We found that when dexamethasone was omitted, GRIMwild- and GRIMlost-infected cells produced similar amounts of tsp-1 mRNA (Fig. 2A). In the presence of dexamethasone, however, tsp-1 mRNA was down-regulated 7-fold, but only in GRIMwild and not in GRIMlost cells. Thus, tsp-1 down-regulation by dexamethasone does require intact Myc and is not due to the endogenous glucocorticoid receptor. To corroborate this conclusion, we treated hormone-starved GRIMwild cells with RU486. RU486 is a synthetic antagonist of dexamethasone, which most likely prevents binding of the GR to the glucocorticoid-responsive elements in DNA (45). However, RU486 is able to serve as a ligand and to support gene regulation by chimeric receptors via non-glucocorticoid-responsive element sequences. Indeed, in the case of GR-Myc chimeras, RU486 triggers transformation as efficiently as does dexamethasone (data not shown). Predictably, RU486-treated QEF contain less tsp-1 mRNA than untreated (Fig. 2B).

We also examined whether down-regulation of tsp-1 was peculiar to the GRIM system or could be observed in cells transformed by naturally occurring Myc variants. To this end, we infected avian fibroblasts with the wild type MC29 (MC29wild) and the MC29lost derivative (46). In MC29wild-but not MC29lost-infected cells, tsp-1 mRNA levels were severalfold lower than in uninfected cells, suggesting that v-Myc exerts the same repression as its artificial GR-Myc counterpart (Fig. 2C). Furthermore, QEF productively infected with a c-Myc-encoding retrovirus exhibited only a very subtle decline in the steady-state level of the tsp-1 mRNA (data not shown). This correlates with the observation that unmutated c-Myc, even when overexpressed, possesses only very weak transforming potential in avian fibroblasts (3).
Silencing of the Thrombospondin-1 Gene by Myc

**ficient and Rapid**—We further used the GR-v-Myc system to assess the extent and kinetics of tsp-1 silencing. We have repeatedly observed that in retrovirally infected cultures, some cells always expressed very little or no v-Myc (immunofluorescence data not shown). This fraction of cells could account for rather high (~15%) residual levels of tsp-1 mRNA in mass cultures expressing P145<sup>trim</sup>. We therefore analyzed several single cell GRIM clones that had been selected for a pronounced transformed phenotype in dexamethasone-containing media and, by inference, for high levels of v-Myc expression (24). The steady-state levels of tsp-1 mRNA were assessed at different times after the addition of dexamethasone. In control uninfected QEF, the expression level of tsp-1 mRNA increases slightly during the first 4 h of exposure to dexamethasone and then levels off (Fig. 2D). This transient phenomenon may reflect the limited responsiveness of the tsp-1 promoter to glucocorticoids, previously reported for mouse Swiss 3T3 fibroblasts (negative response) (47) and cultured human umbilical vein endothelial cells (positive response) (48). However, we never observed any significant alterations in tsp-1 mRNA expression after prolonged (>24 h) stimulations (Fig. 2A and data not shown). In contrast, in the GRIM-producing clonal cell line B6 (Fig. 2E), the level of tsp-1 mRNA decreases more than 30-fold after 32 h of exposure to dexamethasone. Similar results were obtained for two other independent GRIM-producing clones (data not shown).

Importantly, in GRIM clones tsp-1 mRNA levels decreased at least 6-fold as early as 4 h post-induction (Fig. 2E, two leftmost lanes). This implied that tsp-1 is an early or even an immediate early target of Myc, and its regulation might not require de novo macromolecule synthesis, as is the case during induction of tsp-1 by platelet-derived growth factor (48). If this scenario is correct, then down-regulation of tsp-1 by Myc could occur even in the presence of protein synthesis inhibitors, such as emetine. We thus treated the GRIM/B6 clone with dexamethasone for 6 h in the presence and in the absence of emetine (Fig. 2F). While dexamethasone alone caused a sharp reduction in the tsp-1 mRNA level (compare lanes “−” and “Det”), no inhibition was apparent when both drugs were present in the cell culture medium (lane “D+E”). We thus concluded that protein synthesis is required for GR-Myc to function as an inhibitor. Still, this observation is hard to interpret unambiguously. Myc proteins are extremely short-lived, and treatment with protein synthesis inhibitors dramatically reduces their steady-state levels (50). Hence, we could not determine whether de novo protein synthesis is required to silence tsp-1 or merely to maintain an adequate level of P145<sup>trim</sup>.

**c-Myc Down-regulates Thrombospondin-1 mRNA in Rodent Fibroblasts Including Those Refractory to Transformation**—Our finding that tsp-1 mRNA levels are lowered by P145<sup>trim</sup> in transformed avian fibroblasts prompted us to investigate the effect of other alleles of Myc in other cell types. To extend the correlation between the down-regulation of tsp-1 and neoplastic transformation, we examined rodent Rat-1A cells, the only tissue culture system in which c-Myc itself can induce complete morphological transformation (51). Probes specific for the rat tsp-1 and gapdh genes were designed to detect RNA fragments 260 and 211 nucleotides long, respectively (see “Experimental Procedures”). They were used to analyze RNAs from Rat-1A cells infected with either the empty LXSN retroviral vector or the LMycSN retrovirus bearing the human c-myc gene. Rat-1A cells (panel B) were represented by untransfected cells (“control”) or by a subclone expressing the c-Myc/estrogen receptor chimera (Myc-ER<sup>TM</sup>). − and + refer to cells maintained in the absence and in the presence of 4-OHT, respectively. For each experiment, the ratio of tsp1-to-gapdh, adjusted to the same ratio in uninfected/unreated cells, is shown below as a bar. End-labeled Map1 fragments of pBR322 were used as molecular weight markers (M.W.).

The positive correlation between the transforming potential of Myc and its ability to down-regulate tsp-1 raised the formal possibility that silencing of thrombospondin-1 is merely a marker of transformation, rather than a direct consequence of v-Myc activation. To address this concern, we used a second rodent cell line, Rat-1. Unlike its derivative Rat-1A, Rat-1 is not susceptible to transformation by c-Myc alone (51). The ability of c-Myc to silence tsp-1 in Rat-1 cells was determined using a subclone expressing a fusion between c-Myc and the estrogen receptor (20). This fusion utilizes a particular estrogen receptor variant (ER<sup>TM</sup>), which is refractory to endogenous estrogens often present in the serum but sensitive to the synthetic ligand 4-hydroxytamoxifen (4-OHT). In addition, ER<sup>TM</sup> conveniently lacks the transcription activation domain present in the wild type receptor (53). We observed (Fig. 3B) that inclusion of 4-OHT in the cell culture medium for 24 h caused a profound decrease in the tsp-1 mRNA level in cells expressing Myc-ER<sup>TM</sup> (two leftmost lanes) but not in the control Rat-1 cells (two rightmost lanes). We thus concluded that down-regulation of tsp-1 by Myc does not require establishment of the transformed phenotype and can be directly attributed to the transcriptional activity of Myc.

**Down-regulation of tsp-1 mRNA by Myc Results in Diminished Synthesis of the Thrombospondin-1 Protein**—To extend our observation that tsp-1 mRNA is down-regulated in Myc-
Silencing of the Thrombospondin-1 Gene by Myc

Expression of Myc in Rat-1 Cells Promotes the Formation of a Specific Complex with the Tsp-1 Promoter

Fig. 4. Down-regulation of the thrombospondin-1 protein in Myc-infected cells. RIP were performed on cell lysates obtained from human foreskin fibroblasts (HFF, lanes 1 and 2), rat smooth muscle cells (SMC, lane 3), PC12 cells (lane 4), and either Myc-ER™-infected (lanes 5 and 6) or control (lanes 7 and 8) Rat-1 cells. α refers to RIP performed on human foreskin fibroblasts in the absence of the primary antibody. – and + refer to Rat-1 cells maintained in the absence and in the presence of 4-OHT, respectively. Thrombospondin-1 expression levels relative to those observed in untreated Rat-1 cells are shown below the last four lanes as bars. Migration of MultiMark™ multi-colored protein standards (Novel Experimental Technologies, San Diego, CA) is indicated on the left.

Infected cells, we assessed the levels of its translation product. For this purpose, we carried out radioimmunoprecipitation (RIP) experiments using a commercially available monoclonal antibody to human thrombospondin-1 (see “Experimental Procedures”). Although the predicted molecular mass of the Tsp-1 polypeptide chain is 140 kDa, thrombospondin-1 usually migrates as a protein of almost 180 kDa due to extensive glycosylation (54). Consistent with this, the α-Tsp-1 antibody precipitates one major protein of approximately 170 kDa from primary human foreskin fibroblasts (Fig. 4, lane 1). This band does not appear when the primary antibody is omitted from the RIP (lane 2). We thus conclude that it represents Tsp-1. The same protein is present in immunoprecipitates from primary rat aortic smooth muscle cells (lane 3) but not from rat PC12 cells (lane 4) lacking tsp-1 mRNA (data not shown). This indicates that the antibody specifically recognizes both human Tsp-1 and its rat homolog.

We used this antibody to assess thrombospondin-1 levels in Rat-1 cells under different conditions. We found that uninfected Rat-1 cells express high levels of thrombospondin-1 characteristic of primary cells both in the absence and in the presence of 4-OHT (Fig. 4, lanes 7 and 8). Consistent with our RNase protection data (Fig. 3B), in untreated Rat-1/Myc-ER™ cells thrombospondin-1 levels are reduced approximately 2.5-fold, but the protein is readily detectable (Fig. 4, lane 5). However, 24-h treatment with 4-OHT reduces thrombospondin-1 expression to barely detectable levels (Fig. 4, lane 6). Thus, sharp reduction in the tsp-1 mRNA levels accurately translates into diminished protein synthesis.

Silencing of the tsp-1 Gene by Myc Can Be Faithfully Reproduced in a Transient Expression System—Since Myc is a DNA-binding protein, it is likely to repress tsp-1 mRNA at the transcription level. However, we could not rule out the possibility that Myc increases the rate of tsp-1 mRNA turnover. To distinguish between these two possibilities, we examined the effect of Myc on transient expression of a reporter gene linked to the tsp-1 promoter. For these studies we used a construct containing approximately 2.75 kilobases of the tsp-1 upstream regulatory sequences (including untranslated exon I and intron I) driving the CAT gene (Ref. 55, construct TSPCat1A). This plasmid was transfected into parental Rat-1A cells and three independent Rat-1A subclones transformed by Myc (see above).

To account for variations in transfection efficiencies, a plasmid expressing the luciferase gene from a cytomegalovirus promoter (30) was used as an internal control, and the same number of arbitrary β-galactosidase units was used in each CAT reaction. For comparison, we also used a CAT reporter driven by the murine sarcoma virus long terminal repeat (MSVcat) (56), which is not known to be influenced by Myc.

As expected, the expression levels of the MSVcat did not vary significantly between parental Rat-1A cells and Myc-producing subclones (Fig. 5, four leftmost lanes). However, TSPcat produced much less chloramphenicol acetyltransferase when Myc was present (Fig. 5, four rightmost lanes). Hence, a transiently expressed hybrid tsp-cat gene is subject to the same silencing by Myc as the endogenous tsp-1. It is unlikely that mRNA turnover is involved in this process since in our transient expression assays all coding sequences are derived from the cat gene, not tsp-1. This further implicates the tsp-1 promoter as a target for repression by Myc.

DISCUSSION

We have demonstrated that in fibroblasts activation of a transformation-competent Myc protein results in efficient and rapid reduction in tsp-1 mRNA levels. Nuclear run-off experiments will be required in the future to conclusively demonstrate that this down-regulation occurs at the transcription level. However, we are inclined to believe that silencing occurs through interactions that take place on the tsp-1 promoter since replacement of the tsp-1 coding sequences by a reporter gene does not abolish the regulation. The results of the experiment with protein synthesis inhibitors hint that this regulation may be complex and involve labile co-repressors and/or intermediate regulators. These regulators can be either Myc-controlled transcription factors or secreted proteins. The latter scenario implies that in vivo Myc could act in a paracrine fashion, that is by suppressing thrombospondin production not only by the transformed cells but also by neighboring stromal or endothelial cells.

On the other hand, despite the apparent requirement for de novo protein synthesis and given the promptness of tsp-1 response to dexamethasone, it is still plausible that Myc acts directly through binding to the tsp-1 regulatory sequences. Potentially, this effect could be accomplished by Myc alone; however, no canonical Myc-binding sites can be found in the tsp-1 promoter. Thus, Myc is likely to rely on cooperation with other proteins.

It has been proposed that Myc can disrupt transcription by interacting with the initiator element (Inr). Inr is a binding site for the transcription factor TFII-I, which positions RNA polymerase on a template to ensure correct initiation from promoters lacking the canonical TATA-box (Ref. 19 and references therein). Binding of Myc to Inr is thought to prevent the activity of
Silencing of the Thrombospondin-1 Gene by Myc

**Fig. 5. Transient expression of CAT reporter constructs in Rat-1A cells.** Transfections and β-galactosidase and CAT assays were performed on parental cells (Rat-1A) and three subclones transformed by LMyecSN (see legend to Fig. 3) as described under “Experimental Procedures.” Transient expression experiments were repeated three times and yielded consistent results; data from one of the experiments is shown. For this experiment, 5 μg of MSVcat and 20 μg of TSPcat per transfection were used. For each cell line, ratios of TSP-driven to MSV-driven CAT activities are shown below as bars. Since different amounts of TSPcat and MSVcat DNAs were used, these numbers do not reflect the relative strengths of the two promoters.

TFII-I and thus transcription from Inr-containing promoters (17, 18). Therefore, the presence of an Inr element in the tsp-1 promoter would support the direct involvement of Myc in thrombospondin regulation. While the sequences of neither the rat nor quail tsp-1 genomic loci are available, the human tsp-1 promoter (55, 57) contains a trinucleotide CCA at position -2 → +1 followed by a pyrimidine-rich stretch of DNA, both characteristic of Inr. Thus, the primary structure of the human tsp-1 promoter is consistent with that of a Myc target.

Moreover, it has been recently shown that Myc also forms complexes with another transcription factor, YY1 (58). Dimerization of Myc with YY1 impedes gene activation by YY1 and thus silencing of numerous YY1-activated genes, ranging from c-myc to dihydrofolate reductase (59). Provocatively, the human thrombospondin-1 promoter contains, at position -1216, the nucleotide sequence CGGcCCATTTTTCTT, which bears strong resemblance to the YY1 recognition site CGGCCATCTTGNCT (60). Thus, it is conceivable that Myc represses the itp-1 promoter. Future experiments will show whether these interactions are important for down-regulation by Myc.

Regardless of the exact molecular mechanism, tsp-1 mRNA down-regulation, at least in rat fibroblasts, translates into profoundly reduced protein levels. Indeed, in 4-hydroxytamoxifen-treated Rat-1/Myc-ERTM cells, thrombospondin-1 levels barely exceed those observed in the rat cell line PC12 lacking tsp-1 mRNA, and these residual amounts may reflect weak cross-reactivity of the antibody with other members of the thrombospondin family. But whether or not repression of tsp-1 by Myc is exhaustive, it might represent a crucial step in, or even a prerequisite for, Myc-induced transformation of fibroblasts.

The contribution of thrombospondin-1 to carcinogenesis is somewhat controversial. On one hand, Tsp-1 is thought to promote adhesion, migration, and invasion of cells comprising the tumor (61). On the other hand, Tsp-1 and closely related Tsp-2 have been shown to inhibit angiogenesis (62, 63). This latter property is commonly attributed to its inhibitory effect on migration and, perhaps, proliferation and adhesion of endothelial cells of blood vessels (61). Thus, its expression in transformed cells would be incompatible with tumorigenicity, which inevitably relies on neovascularization (64, 65). Indeed, tsp-1 is not expressed in a majority of tumors and transformed cell lines (66, 67), and at least in some of endothelial and mammary epithelial cells forced re-expression of thrombospondin-1 abolishes the tumorigenicity (42, 43). Thus, tsp-1 may be regarded as a tumor suppressor gene (39).

Although the exact molecular basis for its regulation remains unclear, several tumor suppressors and oncogenes have been implicated in this process. Provocatively, tsp-1 is transcriptionally activated by p53, another tumor suppressor frequently deleted in human malignancies (68), and two oncoproteins, Src and Jun, have been reported to have a negative effect on tsp-1 expression (67, 69). However, it has not been determined how quickly tsp-1 responds to these proteins.

The data presented in this paper suggest that at least in fibroblasts Myc is directly involved in tsp-1 silencing. This outcome might be very beneficial for an oncogenic v-Myc encoding virus such as MC29 since it would help infected cells survive and grow in the host organism. Still, it comes as a surprise that tsp-1 silencing is such an early effect of Myc activation in cell culture. Perhaps, besides suppressing the growth of endothelial cells, thrombospondin-1, at least at some steps of immortalization and neoplastic transformation, negatively regulates proliferation of fibroblasts as well. This hypothesis is strengthened by the fact that thrombospondin-1 interacts functionally, and perhaps physically, with two growth factors implicated in fibroblast proliferation: bFGF (39) and TGFβ (70). Such interactions could impede fibroblast proliferation, and thus silencing of tsp-1 by Myc might be crucial for the outgrowth of virally infected cells. Finally, given the simplicity of the putative Myc-binding sites, it seems likely that, besides tsp-1, Myc regulates other proteins that directly contribute to cell proliferation. Identification of these additional target genes will undoubtedly provide further insights into mechanisms of transformation by viral oncogenes.

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