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Transactivation of gene expression by Myc is inhibited by mutation at the phosphorylation sites Thr-58 and Ser-62

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ABSTRACT **The product of the human c-*myc* protooncogene (Myc) is a sequence-specific DNA binding protein. Here, we demonstrate that the placement of the specific Myc DNA binding site CACGTG upstream of a luciferase reporter gene conferred Myc-stimulated expression that was inhibited by the overexpression of the basic–helix–loop–helix/leucine zipper protein Max. It was observed that Myc was phosphorylated *in vivo* within the NH_2-terminal domain at Thr-58 and Ser-62. Replacement of these phosphorylation sites with Ala residues caused a marked decrease in Myc-stimulated reporter gene expression. In contrast, the replacement of Thr-58 or Ser-62 with an acidic residue (Glu) caused only a small inhibition of transactivation. Together, these data demonstrate that the NH_2-terminal phosphorylation sites Thr-58 and Ser-62 are required for high levels of transactivation of gene expression by Myc.**

It has been established that the product of the c-*myc* gene (Myc) is a short-lived nuclear phosphoprotein that has an important functional role during cellular proliferation, differentiation, and neoplasia (1). The structural organization of Myc indicates the presence of subdomains that are found in several transcriptional regulators. A transcriptional activation region is located within the NH_2-terminal domain (2), and basic–helix–loop–helix (bHLH) and leucine zipper (Zip) motifs (associated with dimerization and DNA binding activities) are found within the COOH-terminal domain of Myc (1). Furthermore, a specific DNA binding site for Myc has been identified as the E-box sequence CACGTG (3–6). These data suggest that Myc may function as a sequence-specific regulator of gene expression (7).

The presence of dimerization motifs (HLH and Zip) in Myc suggests that this protein may function as a homo- or heterooligomer. A functional assay has failed to detect Myc homooligomers in intact cells (8), but evidence for the formation of heterodimeric complexes of Myc with the bHLH/Zip protein Max has been obtained (9). The overall structure of Max is similar to Myc, except that Max lacks the transcriptional activation domain that is present within the NH_2-terminal region of Myc (9–11). Significantly, the formation of Myc–Max heterodimeric complexes increases the specific binding of Myc to the DNA sequence CACGTG (9–13). It is therefore likely that the Myc–Max complex represents the functional form of Myc *in vivo* (1).

The Max protein has a long half-life and is expressed constitutively (9, 11). In contrast, the expression of Myc is regulated at both transcriptional and posttranscriptional levels. An increase in Myc expression is observed during the growth factor-stimulated transition of growth-arrested cells from the G_0 to the G_1 phase of the cell cycle (14). However, in proliferating cells, the level of Myc expression is invariant during the cell cycle (15). Therefore, Myc activity may be regulated by posttranslational modification in growing cells. The activity of many transcription factors is regulated by phosphorylation. For example, phosphorylation regulates the DNA binding activity of Myb, Max, SRF, Jun, E2F, and E4F (16). Phosphorylation also regulates the transactivation of gene expression by cAMP response element binding protein (CREB) (17) and Jun (18–20). As Myc is a phosphoprotein (21), it is therefore possible that Myc function is also regulated by phosphorylation.

Recently, we have demonstrated that Myc is an *in vitro* substrate for phosphorylation by mitogen-activated protein kinase (MAP kinases) at Ser-62 (22). This phosphorylation site is located within a proline-rich region of the transactivation domain that is conserved in the Myc family. The location of Ser-62 within the transcriptional activation domain of Myc suggests that it may be a regulatory site of phosphorylation. Evidence consistent with this hypothesis was obtained in experiments using an artificial GAL4–Myc fusion protein (23). Thus, phosphorylation may be an important mechanism of regulation of Myc function *in vivo*. Experiments reported here test this hypothesis. We show that Ser-62 is a site of phosphorylation of the Myc protein in intact cells. An additional site of *in vivo* phosphorylation of Myc was identified as Thr-58. Mutational analysis demonstrated that the replacement of Thr-58 and Ser-62 with Ala caused a marked decrease in the Myc-dependent transactivation of gene expression. These data demonstrate that the function of the Myc NH_2-terminal transactivation domain may be regulated *in vivo* by phosphorylation at Thr-58 and Ser-62.

MATERIALS AND METHODS

Plasmids. The plasmids pCH110 and pUC13 were from Pharmacia LKB. The plasmid pGEM-Luc was from Promega. The expression vectors pCMV2 and pCMV5 (24) were from D. Russell (University of Texas Southwestern Medical School). The plasmid pM21 containing the human c-*myc* gene (25) was obtained from C. V. Dang (Johns Hopkins University School of Medicine). The following plasmids were constructed:

(*i*) Point mutations were introduced at codons Thr-58 and Ser-62 by using PCR and the human c-*myc* gene (26). The 526-bp *Dra* III–*Bst*EII restriction fragments (containing the point mutations) were cloned into pM21 at the *Dra* III and *Bst*EII sites. A simian virus 40 origin of DNA replication was cloned into these plasmids by replacing the murine leukemia virus long terminal repeat of pM21 (*Bam*HI fragment) with the simian virus 40 promoter and origin of replication from pCMV2 (1064-bp *Bam*HI fragment) to create pMyc.

(*ii*) The plasmid pG5E1bLuc was prepared from pG5E1bCAT (23) by replacing the chloramphenicol acetyltransferase gene (*Eco*RI and *Sty* I fragment) with the firefly

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Abbreviations: bHLH, basic–helix–loop–helix; Zip, leucine zipper; CREB, cAMP response element binding protein; MAP kinase, mitogen-activated protein kinase.

luciferase gene (from pGEM-Luc) as a 1745-bp *Hin*dIII–*Stu* I blunt-end fragment. The GAL4 binding sites were removed from pG5E1bLuc as a *Pst* I and *Kpn* I fragment and replaced with a double-stranded 20-mer oligonucleotide that contains the E1b promoter TATA element (5′-GAGGGTATATAATGTGGTAC-3′ and 5′-CACATTATATACCCTCTGCA-3′) to create the plasmid pMyc0E1bLuc. Specific Myc binding sites were inserted in pMyc0E1bLuc as double-stranded oligonucleotides at the *Hin*dIII site. To prepare plasmids with one, two, three, and four Myc binding sites (underlined), a 26-mer double-stranded oligonucleotide (5′-AGCTTAACTGAC-CACGTGGTCAACTA-3′ and 5′-AGCTTAGTTGAC-CACGTGGTCAGTTA-3′) was employed in a ligation reaction to obtain the plasmids pMyc1E1bLuc, pMyc2E1bLuc, pMyc3E1bLuc, and pMyc4E1bLuc.

(*iii*) The cDNAs encoding p21 Max and p22 Max (10) were provided by E. Blackwood and R. N. Eisenman (Fred Hutchinson Cancer Center, Seattle). These cDNAs were cloned in the polylinker of the pCMV5 expression vector as a *Hin*dIII–*Sal* I fragment to create the plasmids pCMV-p21Max and pCMV-p22Max.

Cell Culture and Transient Expression Assays. COS-7 and CV-1 monkey cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (GIBCO/BRL). Transfections of COS-7 cells were performed by using the DEAE-dextran method (22) with 2 μg of expression vector (pMyc), 2 μg of control β-galactosidase expression vector (pCH110), and 2 μg of pUC13 carrier DNA. The cells were harvested after 48 hr of incubation at 37°C. CV-1 cells were cotransfected using the calcium phosphate method (23) with 2 μg of a luciferase reporter plasmid, 0–15 μg of the Myc expression vector, and 3 μg of a control plasmid (pCH110). The total DNA was maintained at 20 μg using pUC13 as carrier DNA. In experiments using the Max expression vector, 2 μg of pCMV-Max (or 2 μg of pUC13 carrier DNA) was included in the transfection (a total of 22 μg of DNA). The cells were harvested 36 hr posttransfection, and cell extracts were prepared for measurement of luciferase and β-galactosidase activity (27).

Analysis of Metabolically Labeled Proteins. Metabolic labeling of transfected cells in 100-mm dishes was performed 40 hr posttransfection by transferring the cells into culture medium (Flow Laboratories) supplemented with 1% fetal calf serum. The culture media used were (*i*) methionine-free modified Eagle's medium containing [^{35}S]methionine (Amersham) at 200 μCi/ml (1 Ci = 37 GBq) or (*ii*) phosphate-free modified Eagle's medium containing [^{32}P]phosphate (DuPont/NEN) at 2 mCi/ml. After 3 hr of additional incubation, the cells were lysed in 0.01 M Tris (pH 7.5), 0.05 M NaCl, 0.5% (vol/vol) Nonidet P-40, 0.5% (wt/vol) SDS, 0.5% (wt/vol) sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, and leupeptin at 10 μg/ml. The Myc proteins were immunoprecipitated with 10 μg of sheep anti-Myc IgG prebound to Pansorbin (Calbiochem) and resolved by SDS/PAGE. The sheep anti-Myc IgG was purified from serum that was obtained after immunization with a bacterially expressed human Myc protein (residues 2–243) as an antigen (22). Phosphopeptide mapping was performed by two-dimensional separation on 100-μm cellulose thin-layer plates (Machery & Nagel) as described (22). Phosphoamino acid analysis was performed as described (22).

Immunocytochemistry. Laser scanning confocal microscopy of cells stained with anti-Myc monoclonal antibody (Ab-2; 5 μg/ml; Oncogene Sciences, Mineola, NY) and fluoroscein isothiocyanate-conjugated goat anti-mouse IgG antibody (ICN) was performed as described by Seth *et al.* (27).

RESULTS

The DNA Sequence CACGTG Confers Myc Regulation of Gene Expression. To investigate the transactivation of gene expression by Myc, we used a reporter plasmid containing specific Myc DNA binding sites. The experimental strategy that we employed is illustrated schematically in Fig. 1. A plasmid containing the firefly luciferase gene and a minimal E1b promoter (TATA) element was constructed. Transfection of this plasmid into CV-1 cells caused a very low level of luciferase activity (Fig. 2). However, the insertion of the Myc binding site CACGTG into this plasmid (Fig. 1) resulted in the observation of Myc-stimulated luciferase expression (Fig. 2). Examination of the dose–response indicated that luciferase expression was increased in assays using 2 μg of the Myc expression vector and that 15 μg caused the maximum observed increase (data not shown). The level of Myc-stimulated luciferase activity was increased when multiple binding sites were inserted into the reporter plasmid (Fig. 2*A*). However, the basal luciferase activity was also increased when a larger number of Myc binding sites were present in the reporter plasmid. [The increased basal activity is likely to be caused by endogenous E-box binding proteins (including Myc) present in CV-1 cells.] The fold increase in luciferase activity caused by Myc was similar when different numbers of binding sites (from one to four) were inserted into the reporter plasmid (Fig. 2*B*). Several genes have been identified as putative targets for transactivation by Myc that contain only a single Myc binding site (c-*sis*, mouse *HI9*, and U3B RNA gene; ref. 4). Therefore, in further experiments, we used a reporter plasmid containing one Myc binding site to study Myc-stimulated gene expression.

The active form of Myc has been proposed to be a complex with Max (1). We therefore investigated the effect of the expression of exogenous Max on the Myc-stimulated luciferase expression in CV-1 cells. Expression of p21 Max caused an inhibition of the Myc-stimulated gene expression (data not shown). An alternatively spliced variant of p21 Max, p22 Max (10), also caused an inhibition of Myc-stimulated luciferase activity (data not shown). An inhibition of a different function of Myc (rat embryo fibroblast transformation) caused by the expression of exogenous Max has previously been reported (13, 27). It is likely that the observed inhibition of Myc function is the result of Max homodimers that compete with Myc complexes for binding to the DNA sequence CACGTG (1). Thus, the level of endogenous Max (9, 11) is likely to be sufficient to form complexes with Myc introduced into CV-1 cells (1). Therefore, in further experiments, we examined the transactivation function of Myc without expressing exogenous Max.

Myc Is Phosphorylated at Thr-58 and Ser-62 *in Vivo*. In previous studies we established that the Myc protein is phosphorylated *in vitro* at Ser-62 by MAP kinases (22, 23, 28). Phosphorylation of Myc at this site *in vivo* has not been reported. However, we have investigated the phosphorylation of the Myc transactivation domain in intact cells by using a GAL4–Myc fusion protein (22). Phosphoamino acid analysis and phosphopeptide mapping demonstrated that this

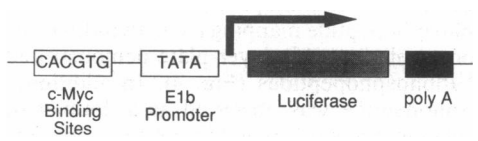

FIG. 1. Schematic illustration of the Myc transactivation assay. The structure of the Myc reporter plasmids [pMyc*x*E1bLuc (*x* = 1–4), where *x* refers to the number of Myc binding sites] employed for transient transfection assays are presented. The Myc binding sites (CACGTG) are located upstream of a minimal E1b promoter (TATA) element and the firefly luciferase gene.

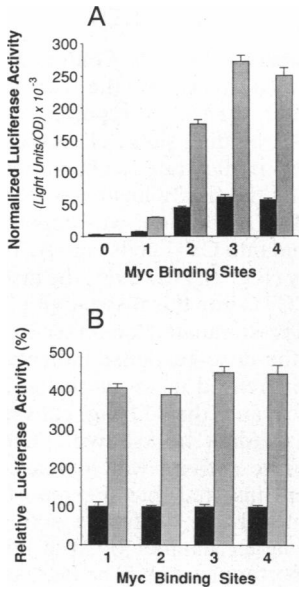

FIG. 2. The DNA binding site CACGTG confers Myc-regulated gene expression. The effect of the number of Myc DNA binding sites (CACGTG) on transactivation of gene expression by Myc was investigated using a transient expression assay employing a luciferase reporter plasmid. CV-1 cells were cotransfected with 15 μg of Myc expression vector (pMyc) or pUC13, 2 μg of luciferase reporter plasmid [pMyc$x$E1bLuc (x = 0–4, where x refers to number of Myc binding sites)], and 3 μg of a control plasmid that expresses β-galactosidase. Cell extracts were prepared and the luciferase activity (light units), and β-galactosidase activity (OD units) were measured. (*A*) To control for the efficiency of transfection, the normalized luciferase activity (light units per OD unit) was calculated. (*B*) The relative luciferase activity was calculated by designating the basal luciferase activity of each reporter plasmid as 100%. The data presented were obtained from triplicate samples in a single experiment (mean ± SE) and are representative of results obtained in three separate experiments. Solid bars, − Myc; shaded bar + Myc.

fusion protein was phosphorylated at Ser-62 and at an additional Thr site. Mutational analysis demonstrated that the replacement of Ser-62 with Ala caused a decrease in the level of both Ser and Thr phosphorylation of the GAL4–Myc fusion protein (22). We therefore examined the sequence surrounding the phosphorylation site at Ser-62 to identify possible Thr residues that could account for the observed Thr phosphorylation. It was noted that a single Thr residue was present in this region, Thr-58 (Fig. 3). This residue is conserved among all members of the Myc family except for some isolates of the viral oncogene v-*myc* (MC29 and MH2; refs. 29 and 30). These considerations indicate that both Ser-62 and Thr-58 represent possible sites of phosphorylation of the Myc protein *in vivo*.

To test this hypothesis, we constructed Myc expression vectors in which Ser-62 and Thr-58 were replaced with Ala residues. The wild-type and mutated Myc proteins were expressed in COS cells and labeled by incubation in tissue culture medium containing [^{32}P]phosphate. The Myc proteins were isolated by immunoprecipitation and SDS/PAGE. Tryptic phosphopeptide mapping using two-dimensional separation on a cellulose thin-layer plate demonstrated several minor [^{32}P]phosphopeptides (Fig. 4). In addition, a major [^{32}P]phosphopeptide was observed as a doublet (indicated with arrows) in maps of the wild-type Myc protein (Fig. 4). We previously demonstrated in experiments using a GAL4–Myc fusion protein that this phosphopeptide doublet corresponds to partial and limit tryptic peptides containing the Myc sequence Phe-Glu-Leu-Leu-Pro-Thr-Pro-Pro-Leu-Ser-Pro-Ser-Arg (residues 53–65) (22). Phosphoamino acid analysis demonstrated the presence of both [^{32}P]phosphoserine

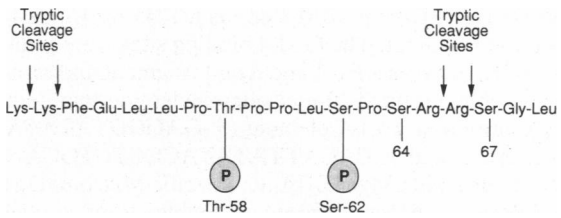

FIG. 3. Sequence surrounding the MAP kinase phosphorylation site (Ser-62) located within the transcriptional activation domain of Myc. This proline-rich region of the transactivation domain containing Ser-62 is highly conserved among members of the Myc family. Mutational analysis has indicated that two additional Ser residues (Ser-64 and Ser-67) are not detectably phosphorylated in intact cells (22). The phosphorylation site Thr-58 is illustrated.

and [^{32}P]phosphothreonine (data not shown). Significantly, this phosphopeptide doublet was absent in maps of [Ala^{58}]Myc, [Ala^{62}]Myc, and [Ala^{58}Ala^{62}]Myc (Fig. 4). In contrast, the mutations at Thr-58 and Ser-62 did not cause marked changes in the phosphorylation of Myc at other minor sites (Fig. 4). These data indicate that both Thr-58 and Ser-62 represent sites of Myc phosphorylation in intact cells. In further experiments, the role of Myc phosphorylation at these sites was investigated.

Mutation at the NH_2-Terminal Sites of Myc Phosphorylation Causes an Inhibition of Transactivation of Gene Expression. The transactivation function of the mutated Myc proteins was investigated by cotransfection of CV-1 cells with a luciferase

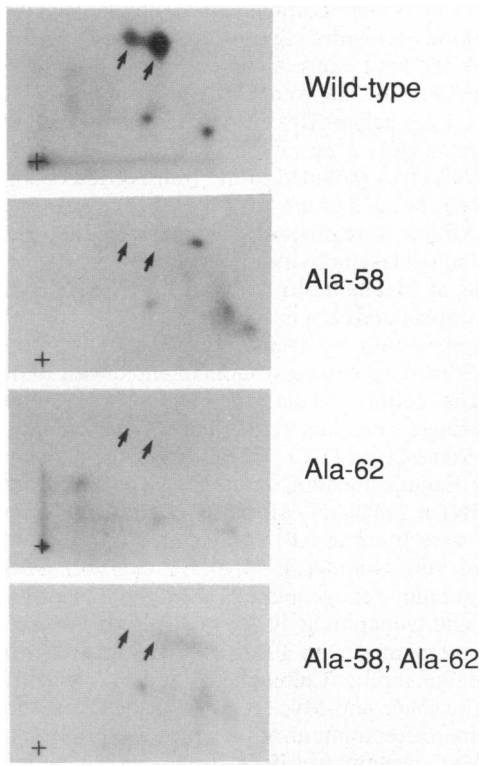

FIG. 4. Identification of Thr-58 and Ser-62 as sites of Myc phosphorylation *in vivo*. Wild-type and mutated Myc proteins were expressed in COS-7 cells, labeled by incubation with [^{32}P]phosphate, and isolated by immunoprecipitation followed by SDS/PAGE. The phosphorylation state of the Myc proteins was analyzed by tryptic phosphopeptide mapping. The origin is marked with a cross at the lower left corner of each map. The horizontal dimension was electrophoresis (cathode at right), and the vertical dimension was chromatography. The exposure time for autoradiography was 72 hr at −80°C with Kodak X-Omat AR film and a DuPont Lightning Plus enhancing screen.

reporter plasmid. Fig. 5*A* shows that the replacement of Thr-58 and Ser-62 with Ala caused a marked decrease in Myc-stimulated luciferase expression. The simultaneous replacement of both Thr-58 and Ser-62 with Ala also caused an inhibition of Myc-stimulated luciferase activity. These data support the hypothesis that the NH_2-terminal region of Myc contains sites of phosphorylation, Thr-58 and Ser-62, that regulate the transactivation function of Myc.

To confirm that the mutation at Thr-58 and Ser-62 directly affects transactivation by Myc rather than altering Myc expression or subcellular localization, we examined the properties of these Myc proteins expressed in COS cells metabolically labeled with [^{35}S]methionine. The Myc proteins were isolated by immunoprecipitation and analyzed by SDS/PAGE. Fig. 6*A* shows that the wild-type Myc protein and the mutated [Ala^{58}]Myc and [Ala^{62}]Myc proteins were expressed at a similar level. Immunofluorescence analysis demonstrated that both the wild-type and mutated Myc proteins were localized within the nucleus (Fig. 6*B*). The observed punctate intranuclear localization (31) was found to be similar for the wild-type and the mutated Myc proteins (Fig. 6*B*). [Under these conditions, the low level of endogenous Myc was not detected in mock-transfected COS cells analyzed by immunoprecipitation and immunofluorescence (data not shown).] These data demonstrate that the reduced transactivation caused by the phosphorylation-defective Myc proteins (Fig. 5) is not accounted for by decreased expression or defective nuclear localization compared with wild-type Myc (Fig. 6), supporting the hypothesis that the mutation of the phosphorylation sites Thr-58 and Ser-62 decreases the transactivation function of Myc.

Effect of the Replacement of the Phosphorylation Sites with Glu. Phosphorylation of a protein causes an increase in negative charge. It is therefore possible that the effects of phosphorylation on protein function could be reproduced experimentally by the addition of a negative charge at the phosphorylation site. To test this hypothesis, we constructed Myc expression vectors in which the phosphorylation sites

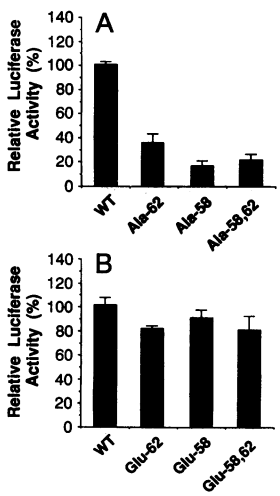

FIG. 5. Mutation of the phosphorylation sites Thr-58 and Ser-62 causes an inhibition of transactivation of gene expression by Myc. Point mutations were introduced into Myc by site-directed mutagenesis to replace Thr-58 and Ser-62 with Ala (*A*; pMyc) or Glu (*B*; pM21). The wild-type (WT) Myc protein and the mutated Myc proteins were expressed in CV-1 cells as described in the legend to Fig. 2. The Myc-dependent luciferase activity was calculated by subtracting the basal activity (without Myc). Relative luciferase activity was calculated by designating the normalized luciferase activity of wild-type Myc protein as 100%. The data presented were obtained from triplicate samples in a single experiment (mean ± SE) and are representative of results obtained in four separate experiments.

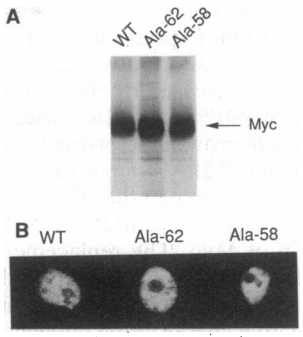

FIG. 6. Expression and cellular localization of the wild-type and mutated, phosphorylation-defective Myc proteins. Transient expression of wild-type (WT) Myc and the mutated [Ala^{58}]Myc (Ala-58) and [Ala^{62}]Myc (Ala-62) was performed. (*A*) Metabolic labeling. The cells were labeled with [^{35}S]methionine and the Myc proteins were isolated by immunoprecipitation and SDS/PAGE. An autoradiograph of a representative gel is presented. (*B*) Indirect immunofluorescence. The cells were fixed and analyzed by indirect immunofluorescence using an anti-Myc antibody and laser scanning confocal microscopy.

Thr-58 and Ser-62 were replaced with Glu. The transactivation by the mutant Myc proteins was similar to that caused by wild-type Myc (Fig. 5*B*). This result is in contrast to the observation of a marked reduction in Myc-stimulated gene expression caused by the replacement of Thr-58 and Ser-62 with Ala (Fig. 5*A*). The lack of a marked effect of replacement with Glu suggests that this acidic residue may partially mimic the effect of Myc phosphorylation. This observation is consistent with the hypothesis that Thr-58 and Ser-62 represent phosphorylation sites that regulate Myc function.

DISCUSSION

Mechanism of Phosphorylation of the Myc Transactivation Domain. One site of phosphorylation of the Myc transactivation domain, Ser-62 (Fig. 3), is a substrate for MAP kinases *in vitro* (22). It is therefore possible that MAP kinases may account for the phosphorylation of Ser-62 *in vivo*. The observation of MAP kinases in the nucleus of serum-stimulated cells (22, 27, 37) is consistent with this hypothesis. However, it is possible that additional kinases may also contribute to the *in vivo* phosphorylation of Myc at Ser-62 [for example, cyclin-dependent protein kinases (22, 23) and glycogen synthase kinase 3 (32)]. Thus, the regulation of Ser-62 phosphorylation may be very complex, involving both growth factor-stimulated and cell cycle-regulated protein kinases.

We obtained evidence that, in addition to the phosphorylation of the Myc transactivation domain at Ser-62, one additional site of phosphorylation can be accounted for by Thr-58 (Fig. 3). The primary sequence surrounding Thr-58 does not conform to the MAP kinase substrate consensus (28), and *in vitro* phosphorylation of Myc at Thr-58 by MAP kinases is not observed (22). Thus, the Thr-58 phosphorylation detected in intact cells must be accounted for by a protein kinase pathway distinct from MAP kinase. The results of mutational analysis suggest that there is an interaction between the phosphorylation sites Thr-58 and Ser-62 (Fig. 4). One possibility that could account for this potential interaction is that Thr-58 is subjected to hierarchical phosphorylation (33) by a protein kinase such as glycogen synthase kinase 3 (34). This hypothesis is supported by the observation of *in vitro* phosphorylation of L-Myc within the conserved proline-rich NH_2-terminal domain by glycogen synthase kinase 3 (32).

Role of the Phosphorylation Sites Thr-58 and Ser-62 During Transactivation of Gene Expression by Myc. Previously we have demonstrated that Myc phosphorylation (and transactivation potential) is increased by the overexpression of the MAP kinase isoform $p41^{mapk}$ (27). Increased phosphorylation of Myc also occurs in growth factor-treated cells and may be cell cycle-dependent (22). To investigate the role of this phosphorylation, we examined the effect of mutations at the phosphorylation sites located within the NH_2-terminal transactivation domain of Myc. The replacement of Thr-58 and Ser-62 with Ala caused a marked inhibition of Myc-stimulated gene expression (Fig. 5*A*). Significantly, the introduction of negative charge at the phosphorylation sites by replacement of Thr-58 and Ser-62 with Glu caused Myc-stimulated gene expression that was similar to that observed for wild-type Myc (Fig. 5*B*). This result indicates that negative charge (Glu) at these phosphorylation sites may partially mimic the effect of phosphorylation. These data indicate a role for Myc phosphorylation at Thr-58 and Ser-62 in the transactivation of gene expression. It is likely that Thr-58 and Ser-62 are phosphorylated by independent mechanisms. Myc activity may therefore be controlled by the coordinate actions of different signaling pathways that lead to the phosphorylation of Myc. A role for both growth factor-stimulated and cell cycle-regulated signal transduction pathways is possible.

Several mechanisms could account for the decreased function of the phosphorylation-defective Myc proteins we described. The defect in Myc-stimulated gene expression could be the result of decreased expression of the mutated protein. However, a direct analysis demonstrates that these mutations did not alter the compartmentalization or expression of the Myc protein (Fig. 6). It is also possible that the intrinsic activity of the mutated Myc proteins was impaired. Myc contains several domains with specific functions—for example, protein–protein interaction (HLH and Zip), DNA binding (basic region), and transactivation. An alteration in the properties of any of these functional domains could account for the data obtained. However, experiments using a GAL4–Myc fusion protein demonstrate that the requirement for phosphorylation is accounted for, in part, by the activity of the Myc transactivation domain (23).

The regulation of the transactivation domain of Myc is similar to that reported for the transcription factors CREB (17) and Jun (18–20). Mutation of Jun at the phosphorylation sites Ser-63 and Ser-73 (19, 20) caused an inhibition of transactivation of gene expression. However, the phosphorylation-defective Myc and Jun proteins are observed to exhibit a low basal transcriptional activity. This is distinct from the effect of CREB phosphorylation within the transactivation domain at Ser-133, which acts as a molecular switch to increase the transactivation function of CREB from an inactive state (17).

Oncogenic Activity of Myc. Increased activity of the Myc protein has previously been associated with oncogenesis (1). It has been shown that the transactivation domain is required for cellular transformation by Myc (35). Tumor formation may result from the activity of Myc as a transcription factor (1). The regulatory phosphorylation sites Thr-58 and Ser-62 may therefore contribute to cellular transformation caused by Myc. However, we have found that the efficiency of focus formation by Rat-1A fibroblasts transfected with Myc is not significantly altered by mutation at the phosphorylation sites Thr-58 and Ser-62 (data not shown). These observations suggest that there may not be a close correlation between cellular transformation and the transactivation potential of c-Myc. A similar lack of correlation between transformation and transactivation has previously been reported for cells transfected with the Jun protooncogene (36).

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