THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 11, Issue of March 15, pp. 9598–9605, 2002

Printed in U.S.A.

Sp1 Transcriptional Activity Is Up-regulated by Phosphatase 2A in Dividing T Lymphocytes*

Received for publication, November 30, 2001
Published, JBC Papers in Press, January 4, 2002, DOI 10.1074/jbc.M111444200

Isabelle Lacroix, Carol Lipcey, Jean Imbert, and Brigitte Kahn-Perlès‡

From the Unité de Cancérologie Expérimentale, U119 INSERM, 27 boulevard Leï Roure, 13009 Marseille, France

We have followed Sp1 expression in primary human T lymphocytes induced, via CD2 plus CD28 costimulation, to sustained proliferation and subsequent return to quiescence. Binding of Sp1 to wheat germ agglutinin lectin was not modified following activation, indicating that the overall glycosylation of the protein was unchanged. Sp1 underwent, instead, a major dephosphorylation that correlated with cyclin A expression and, thus, with cell cycle progression. A similar change was observed in T cells that re-entered cell cycle following secondary interleukin-2 stimulation, as well as in serum-induced proliferating NIH/3T3 fibroblasts. Phosphatase 2A (PP2A) appears involved because 1) treatment of dividing cells with okadaic acid or cantharidin inhibited Sp1 dephosphorylation and 2) PP2A dephosphorylated Sp1 *in vitro* and strongly interacted with Sp1 *in vivo*. Sp1 dephosphorylation is likely to increase its transcriptional activity because PP2A overexpression potentiated Sp1 site-driven chloramphenicol acetyltransferase expression in dividing Kit225 T cells and okadaic acid reversed this effect. This increase might be mediated by a stronger affinity of dephosphorylated Sp1 for DNA, as illustrated by the reduced DNA occupancy by hyperphosphorylated Sp factors from cantharidin- or nocodazole-treated cells. Finally, Sp1 dephosphorylation appears to occur throughout cell cycle except for mitosis, a likely common feature to all cycling cells.

Sp1 is the founding member of a multigene family of transcription factors including Sp1, Sp2, Sp3, Sp4 (see Refs. 1–3 for reviews), and Sp5 proteins (4, 5). Both Sp1 and Sp3 are abundant and ubiquitous, whereas Sp4 is mainly expressed in neuronal tissues and Sp5 exhibits a dynamic and highly restricted expression pattern during embryogenesis. This protein family shares three highly conserved zinc finger DNA binding motifs that recognize GC or GT/CACC boxes present in many promoters. Sp1 was first viewed as a constitutive transcriptional activator regulating basal expression of many cellular and viral genes. However, it is now established that Sp1 activity is modulated in response to numerous signals and that this factor plays a critical role in cell growth and differentiation. Sp1 mediates the induction of dihydrofolate reductase (6) and thymidine kinase (7) genes associated with DNA synthesis and is therefore intricately linked to growth/cell cycle progression. In line with this, the ectopic expression of truncated Sp1 prolongs the S phase and reduces the growth rate (8). Conversely, Sp1 mediates cell division arrest by up-regulating the expression of genes coding for negative regulators of the cell cycle such as $p21^{Waf1/Cip1}$, in p53-dependent growth control (9) or p53-independent pathway of terminal differentiation (10–12).

Sp1's manifold roles can be reconciled if one integrates the multiple time-ordered regulations to which this factor is itself submitted. First, Sp1 is a direct target of numerous cell cycle regulators, which activate or repress its activity. For instance, cyclin A (13, 14) and cyclin D1 (15, 16), as well as their respective targets E2F (17, 18) and the retinoblastoma protein (19–21), physically interact with Sp1. It is of interest that cyclin D1 and retinoblastoma protein modulate Sp1 cross-talk with the basal transcriptional machinery through their direct association with the TATA-box-associated protein $TAF_{II}250$ (15, 22). Second, Sp1 exerts its transcriptional activation with the help of a growing list of inducible specific transcription factors such as nuclear factor-κB (23, 24) and Stat (25, 26), as well as with co-modulators (see Ref. 27 for review) such as Smad family factors (28), CBP/p300 (29, 30), and the cofactor required for Sp1 activation multiprotein complex (31). Third, Sp1 undergoes two types of inducible post-translational modifications that influence its activity. Sp1 contains a number of potential *O*-linked glycosylation sites (32) and changes in glycosylation influence the stability of the factor (33) as well as its interactions with other proteins (34). Sp1 also has multiple serine and threonine phosphorylation sites, the steady-state phosphorylation of which relies on several kinases and phosphatases. Many examples of growth/cell cycle-related increases in phosphorylated Sp1 activity have been reported. Thus, serum stimulation of human fibroblasts up-regulates Sp1 in mid-G1 by inducing its association to a kinase activity (6). Moreover, cyclin A-cyclin-dependent kinase complex enhances Sp1 activity (14). In addition, cell infection by SV40 or human immunodeficiency virus type 1 (HIV-1)[1] enhances Sp1 activity by means of a DNA-dependent protein kinase (DNA-PK) (35, 36). Conversely, decreased activity of phosphorylated Sp1 can also occur, as in the case of the casein kinase II (CKII)-mediated inhibition observed upon terminal differentiation of the liver (37, 38). It is noteworthy that the serine/threonine phosphatase I (PP1) counteracts Sp1 phosphorylation in a positive manner (39), but the phosphatase inhibitor okadaic acid has a negative effect (39, 40).

Despite accumulating data on modulations of Sp1 expression

* This work was supported in part by INSERM and by grants from the Association pour la Recherche sur le Cancer, Ligue Nationale contre le Cancer, and Comité des Bouches-du-Rhône de la Ligue Nationale contre le Cancer. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: INSERM U119, 27 boulevard Leï Roure, 13009 Marseille, France. Tel.: 33-4-91-75-82-74; Fax: 33-4-91-26-03-64; E-mail: kahn@marseille.inserm.fr.

[1] The abbreviations used are: HIV, human immunodeficiency virus; FCS, fetal calf serum; EMSA, electrophoretic mobility shift assay; mAb, monoclonal antibody; WGA, wheat germ agglutinin; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase; CKII, casein kinase II; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PP2B, phosphatase 2B; IL-2, interleukin-2; IL-2R, interleukin-2 receptor; DMEM, Dulbecco's modified Eagle's medium.

and function in growing or differentiating cells, no study has yet described what occurs in normal fully dividing cells. In this report, we intended to fill this gap by following Sp1 behavior in human primary T lymphocytes triggered via CD2 plus CD28 costimulation to sustained division and reversal to quiescent survival (41). Our results strongly suggest that Sp1 is dephosphorylated and up-regulated by phosphatase 2A (PP2A) concurrently to active cell division. Furthermore, we show that Sp1 remains dephosphorylated throughout cell cycle except for mitosis.

EXPERIMENTAL PROCEDURES

T Cell Purification, Activation, and Treatments—T cell purification from peripheral blood and activation were performed as previously described (42). Primary T cells were maintained in RPMI, 10% fetal calf serum (FCS). Stimulations were performed with a combination of the following mAbs used at saturating concentrations. Anti-CD2 mAb 39Cl.5 (rat IgG2a) and 6F10.3 (mouse IgG1) were used as purified mAbs at 10 μg/ml each. Anti-CD28 248 (mouse IgM), and anti-CD3 289 (mouse IgG2a) were obtained from Dr. A. Moretta (Cancer Institute, Genova, Italy) and were used either as ascitic fluid (1/400 dilution) or as purified mAb (10 μg/ml). T cell activation was controlled by proliferation assays and CD25/IL-2Rα expression. Re-stimulation of primary T cells was performed, between day 12 and 15 after initial CD2+CD28 triggering, with recombinant IL-2 (360 IU/ml, Chiron). *In vivo* treatment of cells with phosphatase inhibitors or cell synchronizing agents was performed overnight on dividing populations, as indicated in figure legends.

The IL-2-dependent human T cell line Kit225 (43) was maintained in RPMI 1650, 10% FCS, 2 mM L-glutamine (complete RPMI) plus 360 IU/ml recombinant IL-2. The cells were arrested in a quiescent state by washing them four times in medium alone and culturing them in complete RPMI without IL-2 for 24 h (for transient transfection assays) or for 2 days (for biochemical analysis). NIH/3T3 cells were grown in DMEM, 10% FCS, 2 mM L-glutamine (complete DMEM) and arrested in a quiescent state by washing them 4 times in medium alone and culturing them in DMEM, 0.5% FCS for 48 h.

Cell Cycle Analysis and Sorting—Cell cycling was assessed following propidium iodide staining of the cells as follows. Briefly, cells were fixed with ice-cold 70% ethanol, treated (30 min, 37 °C) with RNase A (250 μg/ml) and propidium iodide (15 μM), and analyzed for G_1, S, and G_2/M phases with a Becton Dickinson FACSCan and CellQuest software. Sorting according to cycle progression was performed following *in vivo* labeling (l h, 37 °C) of viable cells with Hoechst 33342 (10 μM) and propidium iodide (15 μM) with a Becton Dickinson Vantage cell sorter.

T Cell Extracts, Immunoprecipitations, and Immunoblotting—Cytosolic and nuclear extracts were prepared as previously described (42), except for additional protease and phosphatase inhibitors. Briefly, pelleted nuclei were resuspended in 50 μl (per 10^7 cells) of buffer C (50 mM Hepes, pH 7.8, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol) supplemented with inhibitor mix M (10 mM sodium fluoride, 1 mM sodium molybdic acid, 1 μM pepstatin, 74 μM antipain, 50 μM calpain inhibitor 1, 100 μM L-1-tosylamido-2-phenylethyl chloromethyl ketone, 25 μM E64D, 10 μM leupeptin, and 4 mM Pefabloc® SC). Protein concentration was measured with a commercial kit (Bio-Rad) by the method of Bradford (44).

Nuclear extracts were used, as such, for Western blots and EMSA analyses or diluted 1/5 with buffer C′ (50 mM Hepes, pH 7.8, 0.1 mM EDTA, and 0.6% Nonidet P-40) supplemented with inhibitor mix M plus 1.2 mM sodium orthovanadate, pH 7.0, and 12 mM sodium pyrophosphate, for immunoprecipitations. Nuclear extracts, first pre-cleared with protein A-Sepharose (50 μl of sedimented beads) and normal rabbit serum (1/500 dilution) for 30 min at 4 °C, were treated with immunoprecipitating antibodies (2 h, 4 °C) and next by protein A-Sepharose (50 μl, 30 min, 4 °C). Immunoprecipitates were washed four times in buffer C″ (50 mM Hepes, pH 7.8, 150 mM NaCl, 0.1 mM EDTA, 0.5% Nonidet P-40) supplemented with inhibitor mix M plus 1 mM sodium orthovanadate and 10 mM pyrophosphate. Proteins bound to washed protein A-Sepharose beads were eluted and reduced by warming the samples at 95 °C for 3 min in sample buffer and then fractionated by SDS-PAGE. Pre-stained protein markers (Biolabs) were run in parallel. Proteins extracted from whole cells by direct boiling of dry cell pellets in sample buffer were similarly fractionated. Immunoblotting was performed as previously described (41). For quantification, films were analyzed by densitometry using a Bio Image whole band analyzer (Millipore Co.).

Binding of Sp1 to Wheat Germ Agglutinin—Whole cell extracts (40 mM Tris, pH 6.8, 1% SDS, 2.5% β-mercaptoethanol) were diluted 10-fold in 50 mM Hepes, pH 7.4, buffer, supplemented with 150 mM NaCl, 10 mM NaF, 4 mM Pefabloc® SC, 10 μM leupeptin, and, when indicated, 50 mM *N*-acetyl-D-glucosamine, in a total volume of 500 μl. Wheat germ agglutinin (WGA) agarose beads (Amersham Biosciences, Inc.) were then added (50 μl sedimented), and the mixture was shaken for 45 min at 4 °C. After four washes in the previous buffer, sedimented beads were boiled and bound proteins were fractionated by SDS-PAGE as described above.

Antisera—Anti-Sp1 (sc-59X), Sp3 (sc-644X), and Sp4 (sc-645X) polyclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) or kindly provided by Dr. Suske (Institut für Molekularbiologie und Tumorforschung, Marburg, Germany) for anti-Sp1 and anti-Sp3 or from our own facilities for anti-Sp4. Anti-cyclin A monoclonal antibody was from Sigma. Anti-p85 phosphatidylinositol 3-kinase catalytic subunit was from Upstate Biotechnology, Inc. Anti-PP2A catalytic α subunit was from Transduction Laboratories. Anti-Stat5b and anti-phospho-StatA/B polyclonal antibodies were from R&D Systems and Upstate Biotechnology, Inc., respectively. Anti-CKII α and β subunit polysera were generous gifts from Dr. Litchfield (Manitoba Institute, Winnipeg, Canada).

Electrophoretic Mobility Shift Assay—Oligonucleotides were end-labeled, annealed and purified as previously described (41). Reactions with indicated amounts of nuclear proteins were performed in a 20-μl final volume of binding buffer (20 mM Tris, pH 7.5, 60 mM KCl, 2 mM EDTA, 5 mM dithiothreitol, 4% Ficoll, and 50 μg/ml poly(dI·dC)) containing 50,000 cpm probe, for 30 min at 4 °C. Unlabeled double-stranded competitor or specific antiserum was pre-incubated with cell extracts for 20 min at 4 °C prior to addition of the probe. Binding reaction mixtures were separated on a 5% nondenaturing polyacrylamide gel, and, following electrophoresis, dried gels were exposed as described previously (41).

Plasmids—The minimal (−40/+80) and 2κB/3Sp (−119/+80) HIV-1 LTR-chloramphenicol acetyltransferase (CAT) vectors were generous gifts from Dr. E. Schaeffer (Unité 338, INSERM, Strasbourg, France) (45). The minimal −37tkCAT and SV6-tkCAT vectors were kindly provided by Dr. R. Tjian (University of California, Berkeley, CA) (46), and pCMV PP1 and pCMV PP2A expression vectors by Dr. P. Lefebvre (Faculté de Médecine, Lille, France) (47).

Transient Transfections and CAT Assays—IL-2 starved Kit225 cells were electrotransfected as described elsewhere (48) with CAT reporter genes and expression vectors, as indicated in figure legends. Following transfection, cells were maintained in culture for 20 h either in presence (360 IU/ml) or in absence of recombinant IL-2. Cell lysis and CAT assays were performed with the CAT enzyme-linked immunosorbent assay kit (Roche Molecular Biochemicals) according to the manufacturer's instructions with 50 μg of cell extracts.

RESULTS

Sp1 Expression Is Modified in Dividing T Lymphocytes—Stimulation of human primary T lymphocytes with CD2+CD28 mAbs induces sustained cell proliferation, IL-2 secretion, and high IL-2 receptor α chain (IL-2Rα) expression (42, 49, 50). Moreover, actively dividing cells ultimately reverse to quiescence about 2 weeks after initial triggering and then survive for several days. In this respect, costimulation of T lymphocyte via CD2+CD28 provides a valuable model to uncover the evolution of Sp1 expression and activity from a quiescent state to cell growth, active proliferation, and proliferation arrest.

We first analyzed the time-course modulation of Sp1 expression in parallel to that of the cell cycle marker cyclin A. This analysis was done several times on T lymphocyte populations from different healthy donors. Immunoblots performed with nuclear cell extracts with polyclonal anti-Sp1 and monoclonal anti-cyclin A antibodies (Fig. 1*A* for representative analysis) showed that Sp1 electrophoretic migration underwent a major change (compare *lanes 1* and *5*), concomitant with the up-regulation of cyclin A expression and thus with cell division. Thus, novel fast-migrating forms were detected that were also present in T lymphocytes triggered to division via CD3/T cell receptor or CD3/T cell receptor+CD28 (data not shown). This change was transient because Sp1 from T cells reverted to quiescence migrated similarly to Sp1 from freshly purified resting cells (*lane 7*). In addition, quantification of Sp1 signal from

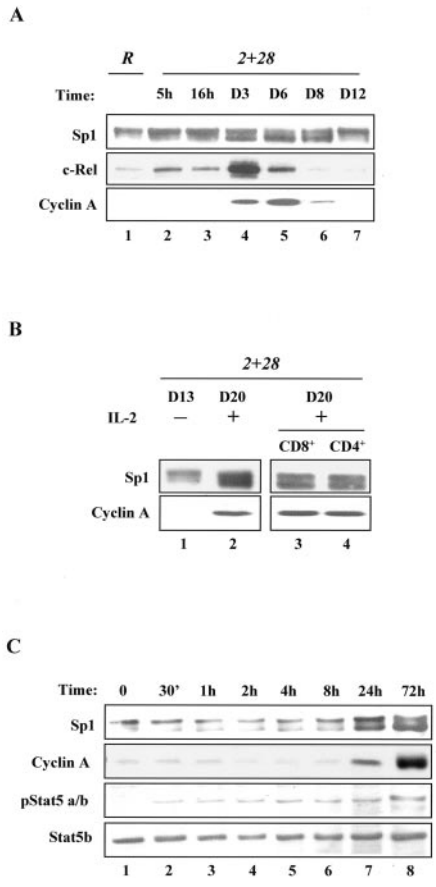

FIG. 1. **Sp1 expression is modified in dividing T lymphocytes.** Purified resting primary human T lymphocytes were stimulated with anti-CD2 (39C1.5+6F10.3; 10 μg/ml each) plus anti-CD28 (248 ascites; 1/400 dilution) for the times indicated *above* the *lanes*. Cells were either boiled directly in SDS reducing buffer for all cell extracts (*W.C.E.*) or separated into nuclear and cytosolic fractions, as described under "Experimental Procedures." After transfer to an Immobilon-P membrane, the extracts (5×10^5 cell eq/cell extracts or 15 μg of nuclear proteins) were probed with various polyclonal or monoclonal antisera. *A*, time-course expression of Sp1, c-Rel, and cyclin A in nuclear extracts from resting (*R*) and CD2+CD28-stimulated T cells. *B*, purified resting primary human T lymphocytes were triggered with anti-CD2 plus anti-CD28 and re-stimulated at day 13 with recombinant IL-2 (360 IU/ml). Sp1 and cyclin A expression in whole cell extracts from day 13 (*D13*) quiescent cells (*left panel*, *lane 1*) or re-dividing populations stimulated at day 13 for 7 days (*D20*) with recombinant IL-2 (*left panel*, *lane 2*) and sorted for $CD8^+$ (*right panel*, *lane 3*) or $CD4^+$ (*right panel*, *lane 4*) cells. *C*, time-course expression of Sp1, cyclin A, phospho-Stat5 A/B, and Stat5b in whole cell extracts from T cells re-stimulated with IL-2 at day 13.

whole cell extracts, normalized to that of the loading charge control phosphatidylinositol 3-kinase p85, indicated that this protein was overexpressed to a mean of 5-fold from resting to actively dividing cells (data not shown). We reported in a previous study of the modulation of Rel/nuclear factor-κB proteins that the nuclear expression of c-Rel subunit is dramatically increased in dividing T lymphocytes (41). We therefore investigated whether the changes in expression of nuclear Sp1 and c-Rel were time-related. We found that c-Rel maximal expression preceded Sp1 modifications and cyclin A maximal expression (compare *lanes 4* and *5* in Fig. 1*A*).

Changes in Sp1 Migration Require Cell Cycle Entry—Given that CD2+CD28-induced T lymphocyte proliferation is mostly sustained by T cell autocrine/paracrine secretion of IL-2 (51), Sp1 modifications are likely to result from the activation of the IL-2/IL-2R pathway. We thus tested whether T lymphocytes that had reversed to quiescence on day 13 could re-divide following secondary IL-2 triggering and, if so, whether Sp1 expression resembled that of fully dividing CD2+CD28-triggered populations. As illustrated by cyclin A expression, IL-2-stimulated T lymphocytes could re-enter cell cycle and Sp1 protein recovered a migration pattern similar to that observed in the first division round (Fig. 1*B, left panel*). Because Sp1 conversion to fast-migrating forms is only partial, we investigated whether it might be restricted either to CD4- or to CD8-positive T cell populations (52) that both grow and divide following primary anti-CD2+CD28 or secondary IL-2 triggering (data not shown). IL-2-stimulated dividing $CD4^+$ and $CD8^+$ cells (38 and 57%, respectively, for the represented donor) were thus sorted to above 95% purity and whole cell extracts analyzed as previously. As shown in Fig. 1*B* (*right panel*), Sp1 had a similar expression in both populations. Given this homogeneous response, we used our IL-2-synchronized re-stimulation protocol to define when Sp1 modifications proceeded along the IL-2/IL-2R transduction pathway. The early activation steps were assessed by the appearance of Stat5 tyrosine-phosphorylated forms (53) detected with an anti-pStat A/B polyclonal antibody, and cell division by the expression of cyclin A. As illustrated in Fig. 1*C*, Sp1 modifications were most delayed compared with pStat induction (30 min) and concomitant with cyclin A maximal up-regulation (72 h).

Sp1 changes observed in dividing T lymphocytes might either be T-specific or else apply to any proliferating cells. NIH/3T3 fibroblasts can be arrested in a quiescent state or synchronously re-induced to enter cell cycle, according to FCS concentration in culture medium. We thus analyzed the time-course expression of Sp1 in re-stimulated NIH/3T3 cells. As shown in Fig. 2*A*, representative of one of several individual experiments, and quantified in Fig. 2*B*, the proportion of Sp1 slower migrating forms started to decrease from 20 h after serum addition. At this time a great proportion of the cells had entered cell cycle, as assessed by the high cyclin A expression and by the increased proportion of cells in S or G_2/M phases (*lane 3*). Notably, change in Sp1 migration persisted after 34 h when a majority of cells had reversed to G_0/G_1 phase (*lane 4*) after division, suggesting that it was independent of cell cycle progression.

Sp1 Modifications Occur throughout Cell Cycle except for Mitosis—To further analyze Sp1 expression all through the cell cycle, we undertook to sort G_0/G_1, S and G_2/M lymphocytes from anti-CD2+CD28-stimulated dividing populations or treat these populations with cell cycle blocking agents. As shown in Fig. 3*A*, extracts from G_0/G_1 and G_2/M (sorting 1, *left upper panel*) or G_0/G_1 and S (sorting 2, *right upper panel*) enriched populations displayed similar Sp1 migration profiles, despite the expected variations in cyclin A expression (*lower panel*). No change was found either (Fig. 3*B, upper* and *lower panels*) following synchronization of cells in late G_1 with mimosine (54). Thus, as for NIH/3T3 fibroblasts, Sp1 modifications appear concurrent to overall cell cycle progression and not restricted to certain phases. However, because mitotic cells only represent a very small population of growing cultures, biochemical changes occurring in mitosis cannot be appreciated without prior enrichment. We therefore used the metaphase synchronizing agent nocodazole (54) to recover an enriched M phase population (Fig. 3*C*, *upper panel*). Interestingly, extracts from nocodazole-treated populations showed a profound modification in Sp1 migration profile with diminished faster migrating forms and strongly increased slower migrating ones (Fig. 3*C*, *lower panel*). Thus, Sp1 appears to undergo particular posttranslational changes during mitosis compared with G_0/G_1, S, and G_2 phases.

Sp1 Appears to Be Dephosphorylated by PP2A in Dividing T Lymphocytes—Lower molecular weight Sp1 forms might arise

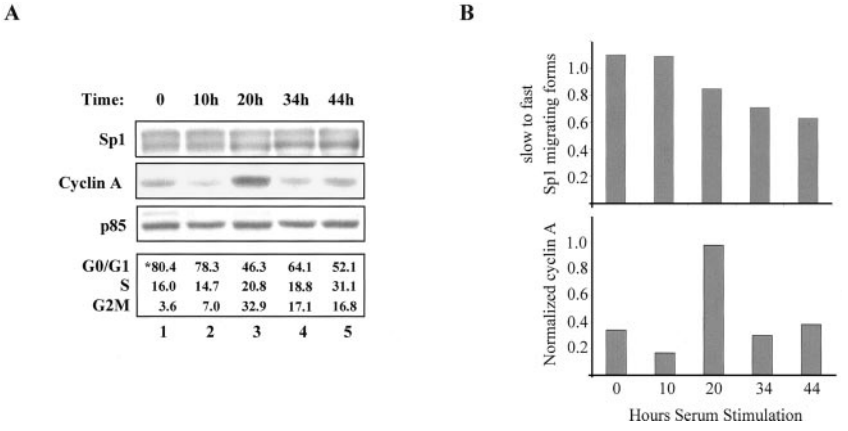

FIG. 2. **Sp1 expression in serum-stimulated NIH/3T3 fibroblasts.** Mouse NIH/3T3 fibroblasts were arrested in quiescence or re-induced to enter cell cycle as described under "Experimental Procedures." Whole cell extracts were prepared and analyzed as for Fig. 1. *A*, time-course expression of Sp1, cyclin A, and p85 in whole cell extracts from serum-stimulated cells. *, percentage of cells gated in G_0/G_1, S, and G_2/M phases by fluorescence-activated cell sorting analysis of propidium iodide-stained cells. *B*, quantifications of protein levels by densitometric analysis of Fig. 1. *A*, Western blot. *Upper panel*, ratio of Sp1 slower to faster migration form value; *lower panel*, ratio of cyclin A to p85 value.

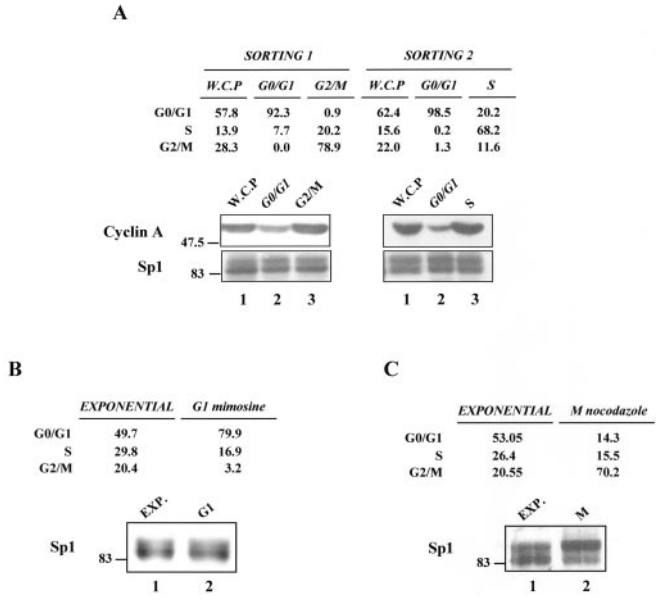

FIG. 3. **Sp1 electrophoretic mobility along T cell cycle.** Purified T lymphocytes were costimulated with anti-CD2 plus anti-CD28 antibodies as for Fig. 1. *A*, day 6 activated whole cell population (*W.C.P.*) was labeled *in vivo* with Hoechst 33342 and G_0/G_1 and G_2/M (sorting 1) or G_0/G_1 and S (sorting 2) populations were sorted and re-analyzed for enrichment (percentage of gated cells, *upper left* and *right panels*) as described under "Experimental Procedures." Whole cell extracts from sorted populations were immunoblotted for Sp1 and cyclin A expression (*lower left* and *right panels*), as described for Fig. 1. *B*, day 6 activated T cells were cultured overnight in the presence of mimosine (100 μM). Control cells were left untreated (exponential). Cells were then recovered and either fixed and propidium-stained for cycle analysis (percentage of cells gated in G_0/G_1, S, and G_2/M phases, *upper panel*) or lysed for immunoblotting (*lower panel*), as described under "Experimental Procedures." *C*, day 6 activated T cells were cultured overnight in the presence of nocodazole (0.33 μM). Control cells were left untreated (exponential). Cells were then analyzed as for *B* (*upper panel,* cell cycle; *lower panel*, immunoblot).

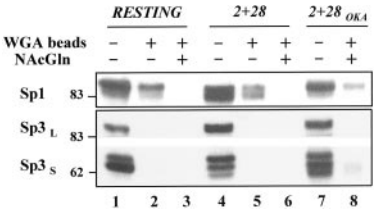

FIG. 4. **Sp1 *O*-glycosylation in resting and dividing T lymphocytes.** Purified T lymphocytes were costimulated with anti-CD2 plus anti-CD28 as described in Fig. 1. Whole cell extracts (*W.C.E.*), from resting or day 6 dividing cells treated, or not, with okadaic acid (*OKA*) were incubated with WGA-agarose beads in presence or absence of *N*-acetyl-D-glucosamine inhibitor. Total cell proteins (*lanes 1*, *4*, and *7*) or proteins bound to WGA beads in the absence (*lanes 2*, *5*, and *8*) or in the presence (*lanes 3* and *6*) of *N*-acetyl-D-glucosamine were fractionated by SDS-PAGE and immunoblotted with anti-Sp1 and anti-Sp3 polysera. The latter reagent is directed against an Sp3 C-terminal peptide and thus recognizes both Sp3 long ($Sp3_L$) and Sp3 short ($Sp3_S$) isoforms (69).

from deglycosylation and/or dephosphorylation. We first followed Sp1 glycosylation by its capacity to bind to WGA lectin (32). Binding specificity was controlled in the presence of the competitive sugar *N*-acetyl-D-glucosamine. As shown in Fig. 4, Sp1 slow and fast migrating forms, detected in resting and dividing cells respectively (*lanes 1* and *4*), bound equally well to WGA beads (*lanes 2* and *5*). Thus, no global deglycosylation was detected. Notice in comparison that Sp1-related factor Sp3 did not bind to WGA beads and therefore did not appear to be glycosylated.

Inducible dephosphorylation of Sp1 was suggested by its reversion to a resting-like migration profile in extracts from dividing cells cultured in the presence of okadaic acid phosphatase inhibitor (Fig. 4, *lanes 7* and *8*). Because okadaic acid binds to the catalytic subunits of PP1 and PP2A family proteins (although with a 200-fold higher affinity for PP2A) but not to that of calcineurin (PP2B) (55), we also looked at the *in vivo* effect of cantharidin, a second PP1/PP2A inhibitor (with a 10-fold higher affinity for PP2A) (56, 57), as well as cyclosporin A calcineurin inhibitor (58). As shown in Fig. 5*A*, treatment of dividing lymphocytes with cantharidin, but not cyclosporin A, induced a reversion of Sp1 migration profile similar to that with okadaic acid. This again suggested the involvement of PP1 or, more likely, PP2A. Necessarily, we checked by fluorescence-activated cell sorting analysis of cell DNA content that neither okadaic acid nor cantharidin affected cell cycling at their effective doses (data not shown). These results led us test whether Sp1 was an *in vitro* substrate for either PP1 or PP2A. Nuclear cell extracts from resting T lymphocytes were first treated with both enzymes in the absence or presence of okadaic acid as specificity control. As shown in Fig. 5*B*, both treatments led to the appearance of Sp1 faster migrating forms. Because Sp1 dephosphorylation was likely to operate in dividing T lymphocytes, we also looked at the *in vitro* dephosphorylation of cycling cells that had been cultured with okadaic acid to inhibit the *in vivo* dephosphorylation. As illustrated and quantified in Fig. 5*C*, the ratio of Sp1 higher to lower molecular forms diminished after treatment with both phosphatases, indicating that these can indeed increase Sp1 *in vitro* dephosphorylation.

PP1 or PP2A might down-regulate Sp1 phosphorylation either by inhibition of a kinase activity or by direct dephosphorylation of the factor. As a first insight into these alternative mechanisms, we tested whether either PP1 or PP2A physically

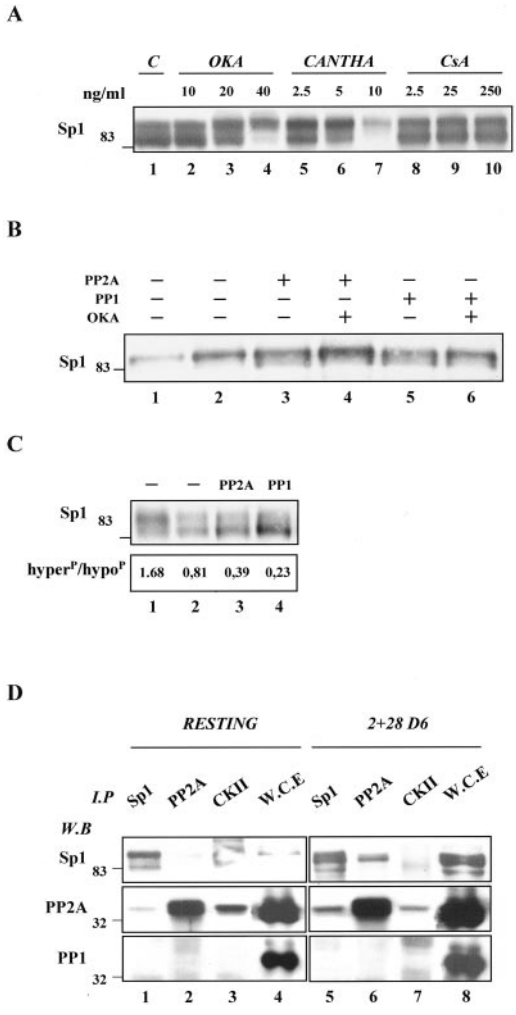

FIG. 5. **PP2A candidate for Sp1 dephosphorylation in dividing T lymphocytes.** Purified T lymphocytes either were left untreated or costimulated by anti-CD2 plus anti-CD28 antibodies as described for Fig. 1. *A*, day 6 activated cells were cultured overnight in the absence (*C*) or in the presence of increasing doses of okadaic acid (*OKA*), cantharidin (*CANTHA*), or cyclosporin A (*CsA*). Whole cell extracts (*W.C.E.*) were immunoblotted as for Fig. 1. *B*, nuclear extract from resting T lymphocytes was either boiled directly in SDS sample buffer (*lane 1*) or incubated 30 min at 30 °C without (*lane 2*), or with PP2A heterotrimeric (ABC) complex (Upstate Biotechnology, Inc., ≈1 milliunit) or PP1 catalytic subunit (Sigma, 5–15 units) in absence (*lanes 3* and *5*) or in presence (*lanes 4* and *6*) of okadaic acid. *C*, nuclear extract from day 6 activated cells cultured overnight with okadaic acid (30 ng/ml) was either boiled directly (*lane 1*) or incubated with PP2A (*lane 3*) or PP1 (*lane 4*) as for Fig. 1*B*. Immunoblots were scanned by densitometry (*lower panel*). *D*, nuclear extracts from day 6 activated cells were purified, immunoprecipitated (*I.P.*) and immunoblotted (*W.B.*) as described under "Experimental Procedures."

interacted with Sp1. Nuclear cell extracts from resting or dividing T cells were immunoprecipitated with anti-Sp1 or anti-PP2A (catalytic α subunit) antibodies and analyzed by immunoblotting with PP1, PP2A, and Sp1 antibodies (Fig. 5*D*). Immunoprecipitation with an anti-CKIIα serum was performed as a positive control, as this catalytic subunit was reported to interact with PP2A in quiescent cells (59). As expected, CKIIα strongly associated with PP2A, and not with PP1, in nuclei from resting T lymphocytes and much more weakly in nuclei from dividing cells (*lane 3 versus lane 7*). Conversely, an increased interaction of PP2A, but not of PP1, with Sp1 was detected in nuclei from dividing lymphocytes compared with those from resting cells (*lanes 5* and *6 versus lanes 1* and *2*). This interaction was also detected in cytosolic extracts suggesting that it preceded Sp1 transport to the nucleus (data not shown). Taken together with the above data, these results reinforced the possibility that PP2A mediates Sp1 dephosphorylation in dividing T lymphocytes. Interestingly, PP2A appeared to interact neither with Sp1, nor with CKIIα, during mitosis (data not shown).

Phosphatase 2A Increases Sp1 Transactivation Activity in Dividing T Lymphocytes—The occurrence of dephosphorylated Sp1 forms in dividing T cells and the likely involvement of PP2A led us examine the role of this enzyme on the transcriptional activity of Sp1 sites. The T lymphoma cell line Kit225 was chosen as a substitution model as it is easily transfectable and can be arrested in quiescence or grown exponentially in the absence of presence of IL-2, respectively. Moreover, Sp1 undergoes the expected modifications when Kit225 cells enter division (Fig. 6*A*). We assayed the transient expression of the CAT reporter gene under the control of the six tandem Sp1 binding sites from SV40 early promoter (SV6-tkCAT) (60) or the three adjacent Sp1 sites from HIV-1 LTR promoter. The latter were used combined to the two adjacent κB sites (LTR −119 CAT) because they have a weak promoter activity by themselves (61). As illustrated in Fig. 6*B*, IL-2 mitogenic stimulation of Kit225 cells enhanced the activity of both reporters, suggesting that it increases Sp1 transcriptional activity in dividing cells. Such increase might result either from a greater number of Sp1 proteins and/or from a greater transcriptional activity of Sp1 dephosphorylated forms. In this case, overexpressed PP2A should potentiate the IL-2 induction of Sp sites promoting activity. As shown in Fig. 6*C*, cotransfection of Kit225 cells with PP2A expression vector enhanced significantly the IL-2 effect ($p = 0.036$), whereas cotreatment of cells with okadaic acid abolished this enhancement ($p = 0.04$). In contrast, cotransfection with PP1 had no significant effect on the IL-2 induction, nor was this induction diminished by okadaic acid (data not shown).

Hypophosphorylated Sp1 Forms Expressed in Dividing T Lymphocytes Display Increased DNA Binding Capacity—Dephosphorylation of Sp1 might modulate its transcriptional activity in multiple ways. To assess whether this change modified Sp1 DNA Binding activity and parallel our functional assays, we analyzed the *in vitro* occupancy of the HIV-1 LTR 3Sp motif by nuclear proteins from dividing CD2+CD28-stimulated T lymphocytes. Because cantharidin or nocodazole alters Sp1 phosphorylation status, we compared the EMSA profiles obtained from untreated cells, or cells cultured in the presence of either drug. As shown in Fig. 7*A*, 10 μM cantharidin treatment led to a diminished occupancy of the 3Sp probe compared with the untreated control and to a simplified pattern of slow migrating complexes (*lanes 4–6 versus lanes 1–3, upper* and *lower panels*). This occupancy was even more reduced after nocodazole treatment (*lanes 7–9*, *upper* and *lower panels*). The complex retardation pattern of control EMSA likely reflects the presence of the three adjacent Sp1 binding sites that are progressively occupied and the multiplicity of Sp family members that can bind to an Sp1 motif. Both Sp1 and Sp3, but also Sp4, are expressed by T lymphocytes (our own data), and all these proteins are expected to bind to the 3Sp probe in a noncooperative manner (62). We thus asked whether the reduced occupancy, observed following cantharidin treatment, reflected a qualitative change in Sp protein recruitment. DNA-binding specificity of the protein complexes was verified by competition with a 100-fold excess of unlabeled wild type or mutated probe (Fig. 7*B*, *lane 2* and *lane 3,* respectively) and characterization of protein complexes by including anti-Sp1, -Sp3, or -Sp4 polyclonal antibodies in the reaction mixtures, as indicated *above* the *lanes*. Although we could not determine the protein composition of all the multiply occupied retarded complexes from

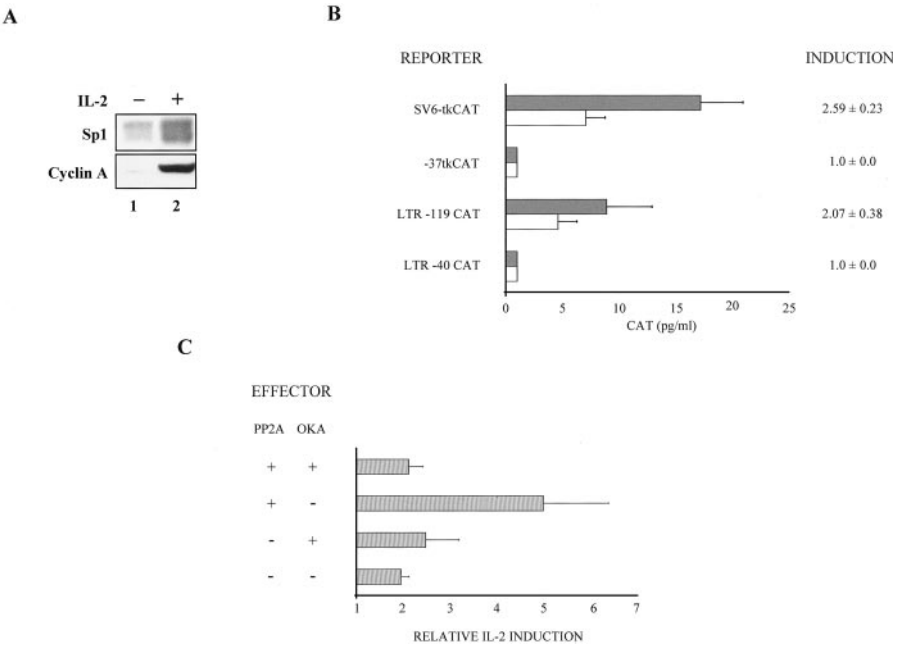

FIG. 6. **Transient expression assays of Sp1 site-driven reporter genes in human Kit225 T cells.** *A*, whole cell extracts (*W.C.E.*) from IL-2-starved (−) or restimulated (+) Kit225 cells were immunoblotted with anti-Sp1 or anti-cyclin A antibodies as for Fig. 1. *B*, starved Kit225 cells were electrotransfected with HIV-1 LTR minimal (−40 CAT, 20 μg) and 2κB3Sp (−119 CAT, 15 μg) or recombined SV40 promoter minimal (−37tkCAT, 5 μg) and 6Sp (SV6-tkCAT, 5 μg) reporter constructs, as described under "Experimental Procedures." Transfected cells were then grown for 20 h either in absence (*open boxes*) or in presence (*hatched boxes*) of IL-2, before CAT assay. IL-2 induction relative to unstimulated level is indicated at the *right* of the histogram. *C*, starved Kit225 cells were transfected with the 6Sp reporter construct in the presence of either PP2A expression vector or corresponding empty vector (1 μg of each) and grown, as for *B*, in presence or absence of IL-2. Okadaic acid (30 ng/ml) was added, when indicated, for the last 12 h of culture. Values presented in *B* and *C* are means of at least four independent determinations, and *error bars* represent standard deviations of the mean. Statistical differences were estimated by Student's *t* test.

control samples, predominant binding of Sp1 and Sp3 factors was detected from both untreated and cantharidin-treated samples (see supershifts of bands B and C in presence of anti-Sp1 antibody and of bands A and C in the presence of anti-Sp3), together with a weak binding of Sp4 (compare remaining band B in presence of anti-Sp1 plus anti-Sp3 antibodies to abrogation of band B in presence of anti-Sp1, anti-Sp3, and anti-Sp4 antibodies). Thus, inhibition of phosphatase activity by cantharidin appeared to cause quantitative rather than qualitative alteration in the DNA-binding capacity of Sp1 and its pairs.

DISCUSSION

Sp1 DNA binding and transcriptional activities are modulated in part by modifications in phosphorylation of serine and/or threonine residues located along its various functional domains. The complexity of such modulation is illustrated by the many target residues and protein kinases that have been implicated (6, 36, 37, 63). Furthermore, increased phosphorylation either enhances (6, 64) or inhibits Sp1 DNA binding and transactivation (65). Conversely, dephosphorylation can increase both activities (38, 40). These discrepancies most likely illustrate the fact that Sp1 regulation has been analyzed at different stages of cell growth and differentiation and that multiple triggering signals and cell types have been used. In this context, we have chosen to follow Sp1 behavior from quiescence to active proliferation and subsequent growth arrest, in a unique cell activation model. Costimulation of highly purified peripheral T lymphocytes by anti-CD2 plus anti-CD28 antibodies offers this possibility as it triggers a long term cell proliferation of both CD4 and CD8 positive subpopulations followed by their reversal to quiescent survival. Strikingly, we detected major quantitative and qualitative modifications of Sp1 protein expression concurrent with active cell division, *i.e.* from day 4 to 8 following initial triggering. Hence, the amount of Sp1 increased, and new faster migrating forms appeared. These changes were transient as the number of the latter forms diminished when cell populations reentered quiescence. The autocrine/paracrine secretion of IL-2 essentially drives CD2+CD28-induced T cell proliferation (42). Therefore, Sp1 changes in expression are likely to be late consequences of the engagement of the IL-2/IL-2R transduction module. In favor of this, we observed similar changes after re-stimulation of quiescent T cells with purified IL-2. Although the IL-2/IL-2R module is T-specific, our findings strongly suggest that Sp1 changes occur upon cell multiplication independently of the initial mitogenic signal. First, they were detected long after activation of the Janus tyrosine kinase/signal transducers and activators of transcription (JAK/STAT) pathway in recycling IL-2-stimulated lymphocytes; and, second, an increased amount of faster migration forms was also detected in serum-induced dividing NIH/3T3 fibroblasts.

Faster migration forms suggested either a deglycosylation or a dephosphorylation event. Binding of Sp1 to WGA, taken as read-out for glycosylation (32), appeared unchanged ruling out a major deglycosylation. A decrease in phosphorylation and the involvement of either PP1, or more likely PP2A, phosphatase was suggested instead by the absence of Sp1 faster migrating forms in extracts from dividing T cell populations treated with okadaic acid or cantharidin. Moreover, purified PP1 and PP2A could dephosphorylate Sp1 from nuclear extracts of the latter cells, as well as of resting T cells. Of interest, we found that PP2A, but not PP1, strongly associated with nuclear Sp1 in dividing T cells. Moreover, PP2A, but not PP1, enhanced the IL-2-driven up-regulation of Sp1 site-mediated transcriptional activity. In contrast to our data, PP1 has been implicated in glucose-induced dephosphorylation of Sp1 (39, 40). The latter studies, however, did not determine whether PP1 physically interacted with Sp1, and it is conceivable that the enzyme acts

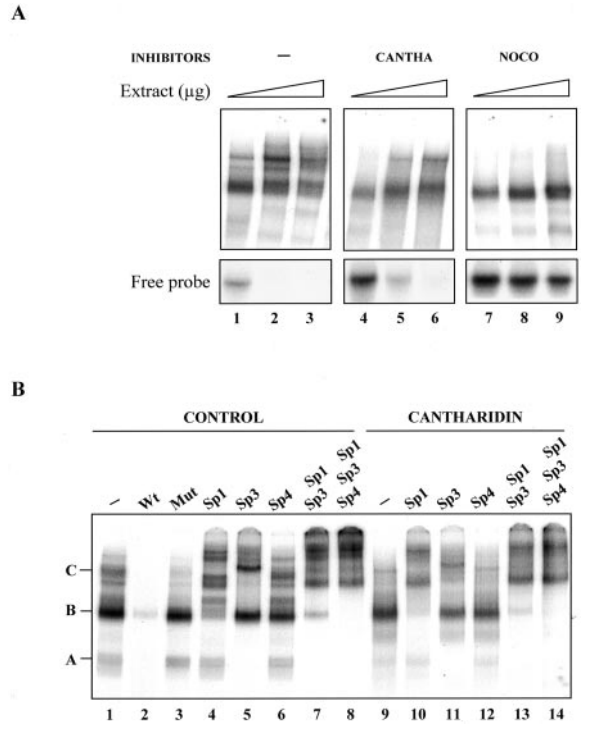

FIG. 7. ***In vitro* occupancy of the HIV-1 LTR 3Sp DNA motif by nuclear proteins from dividing T lymphocytes.** Purified primary T lymphocytes were stimulated by anti-CD2+CD28 antibodies, as described in Fig. 1. *A*, day 6 activated T cells were either left untreated or cultured overnight with cantharidin (7.5 μM) or nocodazole (0.33 μM) as indicated *above* the *lanes*. Nuclear or M phase extracts were prepared and subjected to EMSA using increasing amounts of proteins (*lanes 1*, *4*, and *7*, 1 μg; *lanes 2*, *5*, and *8*, 2 μg; *lanes* 3, *6*, and *9*, 4 μg) and labeled HIV-1 3Sp probe, as described under "Experimental Procedures." Only the *upper parts* of the gels containing the retarded complexes and the *lower parts* containing the free probes are represented. *B*, serological characterization of HIV-1 3Sp-binding proteins detected with 2 μg of nuclear extracts from control (*lanes 1–8*) or cantharidin-treated cells (*lanes 9–14*). DNA-binding specificity of control protein complexes was analyzed by competition with 100-fold excess wild-type (*lane 2*) or mutated (*lane 3*) cold oligonucleotide added 30 min at 4 °C prior to labeled probe. Protein complexes (*lanes 4–14*) were characterized with anti-Sp polyclonal antisera (indicated *above* the *lanes*) added 20 min at 4 °C prior to the probe. Only the *upper part* of the gel containing the retarded complexes is represented. *A–C*, identified protein-DNA complexes from control and cantharidin-treated T lymphocytes, respectively. *A*, Sp3 short forms; *B*, Sp1, Sp3 long forms, and Sp4; *C*, Sp1 and Sp3 short or long forms.

upstream of a PP2A/Sp1 signaling complex. We are currently testing whether Sp1 is a direct target of PP2A or whether this phosphatase interacts with and inhibits an Sp1 kinase also present in a multiprotein complex. This would decrease Sp1 phosphorylation, too. It is noteworthy that PP2A associates with various kinases such as p70 S6 kinase (66), the Ca^{2+}-calmodulin-dependent protein kinase IV (67), and CKIIα catalytic subunit (37). As CKII phosphorylates Sp1 and decreases its DNA binding activity upon liver terminal differentiation (37, 38), it is tempting to speculate that PP2A counteracts this kinase in fully dividing cells. Our T cell model should allow the testing of this hypothesis.

As for members of the AP1 transcription complex (68), Sp1 phosphorylation appears to vary in a cell cycle-dependent manner. As already mentioned, it increases in mid-G_1 following serum stimulation of normal human fibroblasts. Given that this phosphorylation does not affect the ratio of slower to faster migrating forms in the Sp1 doublet, it is likely to target only one or a small subset of Sp1 phosphorylation sites (6). We cannot exclude a similar variation in growing T lymphocytes because our analysis did not allow detection of minor phosphorylation changes. What our results clearly suggest, however, is that, once cells have entered division, Sp1 undergoes a major dephosphorylation which is sustained throughout the cell cycle, except for mitosis. This overall dephosphorylation does not preclude a concomitant fine-tuning of phosphorylation, as suggested by the modulation of Sp1 activity by cyclin A-cyclin-dependent kinase complex in S and G_2 phases (14). In this respect, it is beyond the scope of our report, but of major interest, to map the Sp1 residues that are targets of (de)phosphorylation. This will allow to progress in the understanding of the complex steady-state phosphorylation that governs Sp1 activity.

Acknowledgments—We thank P. Lécine and A. Vicart for critical reading of the manuscript.

REFERENCES

1. Cook, T., Gebelein, B., and Urrutia, R. (1999) *Ann. N. Y. Acad. Sci.* **880,** 94–102., 94–102
2. Lania, L., Majello, B., and De Luca, P. (1997) *Int. J. Biochem. Cell Biol.* **29,** 1313–1323
3. Suske, G. (1999) *Gene* (*Amst.*) **238,** 291–300
4. Harrison, S. M., Houzelstein, D., Dunwoodie, S. L., and Beddington, R. S. (2000) *Dev. Biol.* **227,** 358–372
5. Treichel, D., Becker, M., and Gruss, P. (2001) *Mech. Dev.* **101,** 175–179
6. Black, A. R., Jensen, D., Lin, S. Y., and Azizkhan, J. C. (1999) *J. Biol. Chem.* **274,** 1207–1215
7. Rotheneder, H., Geymayer, S., and Haidweger, E. (1999) *J. Mol. Biol.* **293,** 1005–1015
8. Chen, F., Zhang, F., Rao, J., and Studzinski, G. P. (2000) *Anticancer Res.* **20,** 661–667
9. Torgeman, A., Mor-Vaknin, N., Zelin, E., Ben-Aroya, Z., Lochelt, M., Flugel, R. M., and Aboud, M. (2001) *Virology* **281,** 10–20
10. Biggs, J. R., Kudlow, J. E., and Kraft, A. S. (1996) *J. Biol. Chem.* **271,** 901–906
11. Gartel, A. L., Goufman, E., Najmabadi, F., and Tyner, A. L. (2000) *Oncogene* **19,** 5182–5188
12. Pagliuca, A., Gallo, P., and Lania, L. (2000) *J. Cell. Biochem.* **76,** 360–367
13. Borja, P. F., Collins, N. K., Du, P., Azizkhan-Clifford, J., and Mudryj, M. (2001) *EMBO J.* **20,** 5737–5747
14. Haidweger, E., Novy, M., and Rotheneder, H. (2001) *J. Mol. Biol.* **306,** 201–212
15. Adnane, J., Shao, Z., and Robbins, P. D. (1999) *Oncogene* **18,** 239–247
16. Watanabe, G., Albanese, C., Lee, R. J., Reutens, A., Vairo, G., Henglein, B., and Pestell, R. G. (1998) *Mol. Cell. Biol.* **18,** 3212–3222
17. Karlseder, J., Rotheneder, H., and Wintersberger, E. (1996) *Mol. Cell. Biol.* **16,** 1659–1667
18. Lin, S. Y., Black, A. R., Kostic, D., Pajovic, S., Hoover, C. N., and Azizkhan, J. C. (1996) *Mol. Cell. Biol.* **16,** 1668–1675
19. Chen, L. I., Nishinaka, T., Kwan, K., Kitabayashi, I., Yokoyama, K., Fu, Y. H., Grunwald, S., and Chiu, R. (1994) *Mol. Cell. Biol.* **14,** 4380–4389
20. Noe, V., Alemany, C., Chasin, L. A., and Ciudad, C. J. (1998) *Oncogene* **16,** 1931–1938
21. Udvadia, A. J., Templeton, D. J., and Horowitz, J. M. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 3953–3957
22. Shao, Z., Ruppert, S., and Robbins, P. D. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 3115–3119
23. Krehan, A., Ansuini, H., Bocher, O., Grein, S., Wirkner, U., and Pyerin, W. (2000) *J. Biol. Chem.* **275,** 18327–18336
24. Perkins, N. D., Agranoff, A. B., Pascal, E., and Nabel, G. J. (1994) *Mol. Cell. Biol.* **14,** 6570–6583
25. Cantwell, C. A., Sterneck, E., and Johnson, P. F. (1998) *Mol. Cell. Biol.* **18,** 2108–2117
26. Look, D. C., Pelletier, M. R., Tidwell, R. M., Roswit, W. T., and Holtzman, M. J. (1995) *J. Biol. Chem.* **270,** 30264–30267
27. Naar, A. M., Ryu, S., and Tjian, R. (1998) *Cold Spring Harbor Symp. Quant. Biol.* **63,** 189–199
28. Moustakas, A., and Kardassis, D. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 6733–6738
29. Billon, N., Carlisi, D., Datto, M. B., van Grunsven, L. A., Watt, A., Wang, X. F., and Rudkin, B. B. (1999) *Oncogene* **18,** 2872–2882
30. Xiao, H., Hasegawa, T., and Isobe, K. (2000) *J. Biol. Chem.* **275,** 1371–1376
31. Ryu, S., Zhou, S., Ladurner, A. G., and Tjian, R. (1999) *Nature* **397,** 446–450
32. Jackson, S. P., and Tjian, R. (1988) *Cell* **55,** 125–133
33. Han, I., and Kudlow, J. E. (1997) *Mol. Cell. Biol.* **17,** 2550–2558
34. Roos, M. D., Su, K., Baker, J. R., and Kudlow, J. E. (1997) *Mol. Cell. Biol.* **17,** 6472–6480
35. Chun, R. F., Semmes, O. J., Neuveut, C., and Jeang, K. T. (1998) *J. Virol.* **72,** 2615–2629
36. Jackson, S. P., MacDonald, J. J., Lees-Miller, S., and Tjian, R. (1990) *Cell* **63,** 155–165
37. Armstrong, S. A., Barry, D. A., Leggett, R. W., and Mueller, C. R. (1997) *J. Biol. Chem.* **272,** 13489–13495
38. Zhang, S., and Kim, K. H. (1997) *Arch. Biochem. Biophys.* **338,** 227–232
39. Daniel, S., Zhang, S., DePaoli-Roach, A. A., and Kim, K. H. (1996) *J. Biol. Chem.* **271,** 14692–14697

40. Schafer, D., Hamm-Kunzelmann, B., and Brand, K. (1997) *FEBS Lett.* **417,** 325–328
41. Kahn-Perles, B., Lipcey, C., Lecine, P., Olive, D., and Imbert, J. (1997) *J. Biol. Chem.* **272,** 21774–21783
42. Costello, R., Lipcey, C., Algarte, M., Cerdan, C., Baeuerle, P. A., Olive, D., and Imbert, J. (1993) *Cell Growth Differ.* **4,** 329–339
43. Hori, T., Uchiyama, T., Tsudo, M., Umadome, H., Ohno, H., Fukuhara, S., Kita, K., and Uchino, H. (1987) *Blood* **70,** 1069–1072
44. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254
45. Rohr, O., Aunis, D., and Schaeffer, E. (1997) *J. Biol. Chem.* **272,** 31149–31155
46. Courey, A. J., and Tjian, R. (1988) *Cell* **55,** 887–898
47. Lefebvre, P., Gaub, M. P., Tahayato, A., Rochette-Egly, C., and Formstecher, P. (1995) *J. Biol. Chem.* **270,** 10806–10816
48. Rameil, P., Lecine, P., Ghysdael, J., Gouilleux, F., Kahn-Perles, B., and Imbert, J. (2000) *Oncogene* **19,** 2086–2097
49. Cerdan, C., Martin, Y., Courcoul, M., Brailly, H., Mawas, C., Birg, F., and Olive, D. (1992) *J. Immunol.* **149,** 2255–2261
50. Pierres, A., Lopez, M., Cerdan, C., Nunes, J., Olive, D., and Mawas, C. (1988) *Eur. J. Immunol.* **18,** 685–690
51. Costello, R., Cerdan, C., Pavon, C., Brailly, H., Hurpin, C., Mawas, C., and Olive, D. (1993) *Eur. J. Immunol.* **23,** 608–613
52. Whitmire, J. K., and Ahmed, R. (2000) *Curr. Opin. Immunol.* **12,** 448–455
53. Leonard, W. J., and O'Shea, J. J. (1998) *Annu. Rev. Immunol.* **16,** 293–322
54. Krek, W., and DeCaprio, J. A. (1995) *Methods Enzymol.* **254,** 114–124
55. Schonthal, A. H. (1998) *Front. Biosci.* **3,** D1262–D1273
56. Honkanen, R. E. (1993) *FEBS Lett.* **330,** 283–286
57. Li, Y. M., and Casida, J. E. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 11867–11870
58. Matsuda, S., and Koyasu, S. (2000) *Immunopharmacology* **47,** 119–125
59. Heriche, J. K., Lebrin, F., Rabilloud, T., Leroy, D., Chambaz, E. M., and Goldberg, Y. (1997) *Science* **276,** 952–955
60. Jones, K. A., Kadonaga, J. T., Luciw, P. A., and Tjian, R. (1986) *Science* **232,** 755–759
61. Perkins, N. D., Edwards, N. L., Duckett, C. S., Agranoff, A. B., Schmid, R. M., and Nabel, G. J. (1993) *EMBO J.* **12,** 3551–3558
62. Pascal, E., and Tjian, R. (1991) *Genes Dev.* **5,** 1646–1656
63. Rohlff, C., Ahmad, S., Borellini, F., Lei, J., and Glazer, R. I. (1997) *J. Biol. Chem.* **272,** 21137–21141
64. Kumar, A. P., and Butler, A. P. (1998) *Biochem. Biophys. Res. Commun.* **252,** 517–523
65. Leggett, R. W., Armstrong, S. A., Barry, D., and Mueller, C. R. (1995) *J. Biol. Chem.* **270,** 25879–25884
66. Westphal, R. S., Coffee, R. L., Marotta, A., Pelech, S. L., and Wadzinski, B. E. (1999) *J. Biol. Chem.* **274,** 687–692
67. Westphal, R. S., Anderson, K. A., Means, A. R., and Wadzinski, B. E. (1998) *Science* **280,** 1258–1261
68. Bakiri, L., Lallemand, D., Bossy-Wetzel, E., and Yaniv, M. (2000) *EMBO J.* **19,** 2056–2068
69. Kennett, S. B., Udvadia, A. J., and Horowitz, J. M. (1997) *Nucleic Acids Res.* **25,** 3110–3117

Sp1 Transcriptional Activity Is Up-regulated by Phosphatase 2A in Dividing T Lymphocytes

Isabelle Lacroix, Carol Lipcey, Jean Imbert and Brigitte Kahn-Perlès

J. Biol. Chem. 2002, 277:9598-9605.
doi: 10.1074/jbc.M111444200 originally published online January 4, 2002
