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An alternatively spliced form of the transcription factor Sp1 containing only a single glutamine-rich transactivation domain

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ABSTRACT **Protein–protein interactions involving specific transactivation domains play a central role in gene transcription and its regulation. The promoter-specific transcription factor Sp1 contains two glutamine-rich transcriptional activation domains (A and B) that mediate direct interactions with the transcription factor TFIID complex associated with RNA polymerase II and synergistic effects involving multiple Sp1 molecules. In the present study, we report the complementary DNA sequence for an alternatively spliced form of mouse Sp1 (mSp1-S) that lacks one of the two glutamine-rich activation regions present in the full-length protein. Corresponding transcripts were identified in mouse tissues and cell lines, and an Sp1-related protein identical in size to that predicted for mSp1-S was detected in mouse nuclear extracts. Cotransfection analysis revealed that mSp1-S lacks appreciable activity at promoters containing a single Sp1 response element but is active when multiple Sp1 sites are present, suggesting synergistic interactions between multiple mSp1-S molecules. The absence of a single glutamine-rich domain does not fully explain the properties of the smaller protein and indicates that additional structural features account for its unique transcriptional activity. The functional implications of this alternatively spliced form of Sp1 are discussed.**

Sp1 is a ubiquitously expressed transcription factor that plays a primary role in the regulation of a large number of gene promoters (1). It consists of multiple functional domains, including a zinc-finger DNA binding region and four transcriptional activation domains: A, B, C, and D. Domains A and B both contain serine/threonine-rich and glutamine-rich sequences. Glutamine-rich domains can be found in several other transcription factors and have been implicated in protein–protein interactions (2, 3). In the case of Sp1, domains A and B have been shown to mediate specific binding to transcription factor TFIID via the TATA-box-binding protein (TBP)-associated factor (TAF) designated $TAF_{II}110$ (4), and synergistic interactions involving multimeric complexes of Sp1 (5). Domain D has been implicated in binding interactions between Sp1 and the transcription regulator YY1 (6) as well as in Sp1 synergism (5), and the zinc-finger DNA-binding domain was recently implicated in Sp1 interactions with the TAF designated $TAF_{II}55$ (7).

Regulation of gene transcription requires the complex interplay of a diverse array of trans-acting proteins, and this diversity is created in part by the synthesis of alternative transcription factor isoforms that contain distinct DNA-binding and/or transactivation domains (8–11). Such isoforms are generated by differential promoter usage and alternative RNA splicing or translational initiation and can exhibit transcriptional properties that are highly divergent or even opposing. While two distinct forms of Sp1 have been identified (apparent masses 95 and 105 kDa) that differ in their state of phosphorylation (12), it generally has been assumed that a single primary sequence exists for this protein, since only one Sp1 gene is found in the mouse and human (13, 14). Here we report the identification of an alternatively spliced, 48-kDa form of mouse Sp1 termed mSp1-S that lacks the entire glutamine-rich A transactivation domain as well as the serine/threonine-rich region of the B domain. It is further shown that mSp1-S possesses transcriptional properties distinct from the full-length protein. The implications of these results in terms of structure–function relationships and the possible role of mSp1-S are discussed.

MATERIALS AND METHODS

cDNA Characterization. A 3.2-kb mouse Sp1 cDNA previously isolated from F9 cells (13) was sequenced in its entirety in the plasmid pBluescript SK(+) (Stratagene) by the dideoxy chain-termination method using a series of external and internal primers. Sequence analyses and comparisons were performed with software from the Genetics Computer Group (Madison, WI).

RNA Analysis. RNA extraction, RNA blot-hybridization (Northern) analysis, and RNase protection assays were performed as described (15). The plasmid pmSp1-0.6 was prepared by subcloning a 560-bp *Eco*RI–*Pst* I fragment derived from pMusSp1-11 (13) into pBluescript SK(−). Antisense riboprobes were generated after *Eco*RI digestion. For Northern blotting, 10 μg of poly(A)$^+$ RNA were run for each sample, and membranes were hybridized after transfer to GeneScreen-*Plus* membranes (DuPont) with the pmSp1-0.6 cDNA probe. PCR amplification of RNA was performed as described (16) with mouse mammary leukemia virus reverse transcriptase (GIBCO/BRL) and Vent DNA polymerase (New England Biolabs). Sequences of primers used were as follows: (*i*) 5′-GGACAGGTCAGTTGGCAG-3′ (nucleotides 524–541 in mSp1-S); (*ii*) 5′-CCTCCAGCTTCAGGCTGT-3′ (nucleotides 457–474); and (*iii*) 5′-GGCGGTGCCGCCTTTTCT-3′ (nucleotides 185–203).

Protein Analyses. Nuclear extracts were prepared from mouse tissues and cells using previously described protocols (17). Immunoblotting (Western blotting) was performed by enhanced chemiluminescence (DuPont) as recommended by the manufacturer. The primary antibody (Sp1 PEP2, Santa Cruz Biotechnology) was generated against a peptide sequence that is conserved in mouse, rat, and human Sp1.

Cell Transfection and Chloramphenicol Acetyltransferase (CAT) Assays. The reporter plasmids BCAT-1, BCAT-2, −115tkCAT, tkCAT15, and −115tkCAT15 and the expression vector pPacSp1 containing the human Sp1 cDNA inserted downstream of the actin 5C gene promoter have been previously described (1, 5, 18). pPacSp1S was generated by subcloning a *Sac* I–*Nde* I fragment containing the entire mSp1-S

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Abbreviations: CAT, chloramphenicol acetyltransferase; TBP, TATA box-binding factor; TAF, TBP-associated factor.

coding sequence into the *Xho* I site of pPacU with *Xho* I linkers.

SL2 *Drosophila* cells were transfected by the calcium phosphate method and assayed for activity as described (1). Briefly, cells were plated on 100-mm plates at a density of $1–2 \times 10^7$ cells per plate. Each plate received 2 μg of reporter plasmid, 5–200 ng of expression vector, and pBluescript carrier DNA to bring the total amount to 10 μg. CAT assays were performed by using the organic-phase extraction method of Seed and Sheen (19), and data are presented as the averages of duplicate samples from at least two or three independent experiments.

RESULTS

cDNA Sequencing and RNA Analysis. A 3.2-kb cDNA for mouse Sp1 was previously isolated from F9 cells (13), which represented the longest Sp1-related cDNA identified. Sequencing of this cDNA revealed an encoded protein (mSp1-S; ≈48 kDa) that is essentially identical to full-length rat Sp1 (20) over its N-terminal and C-terminal regions but is missing a contiguous 319 amino acid sequence located between residues 55 and 373 of the rat protein (Fig. 1). The region absent from mSp1-S corresponds to the entirety of the glutamine-rich and serine/threonine-rich regions of the A domain as well as the serine/threonine-rich region of domain B (Fig. 2). The resulting product thus contains an intact DNA-binding zinc-finger region, both C and D domains important for transcriptional activity, but only one glutamine-rich transactivation domain, lacking any serine/threonine-rich region.

RNase protection analysis was used to detect the presence of authentic mSp1-S transcripts in mouse tissues and cell lines. An RNA probe was generated from the 5′ end of the cDNA, which spanned the region of divergence between mSp1-S and full-length rat Sp1 (pmSp1-0.6; Fig. 3*A*). Specific bands representing the fully protected probe (556 nucleotides) were present in mouse kidney and liver and in mouse F9, P19, and NG-108 cell lines (Fig. 3 *A* and *B*). Additional bands of 300–310 and 250 nucleotides were also detected, which correspond well to the expected sizes of protected fragments derived from alternatively spliced, full-length Sp1 transcripts. Consistent with this, protection analysis using a 445-nucleotide RNA probe shortened at the 5′ end yielded a set of smaller, 147- to 143-nucleotide 5′-end fragments that agreed with the predicted RNA splice site, in addition to the fully protected 445-nucleotide fragment and the doublet at nucleotides 300–310 observed with the longer probe (data not shown).

Further evidence for multiple, alternatively spliced Sp1 mRNAs in the mouse was obtained by Northern and reverse transcription–PCR analyses. In addition to 8.2-kb Sp1 transcripts corresponding to full-length Sp1 (21), two additional mRNAs ≈3.8 and ≈3.2 kb long also were detected in lower abundance on RNA blots of poly(A)$^+$ RNA from mouse cell lines and tissues (Fig. 3*C*). The size of the smaller mRNA is identical to that for the mSp1-S cDNA; however, whether one or both of the shorter transcripts encode mSp1-S is unknown.

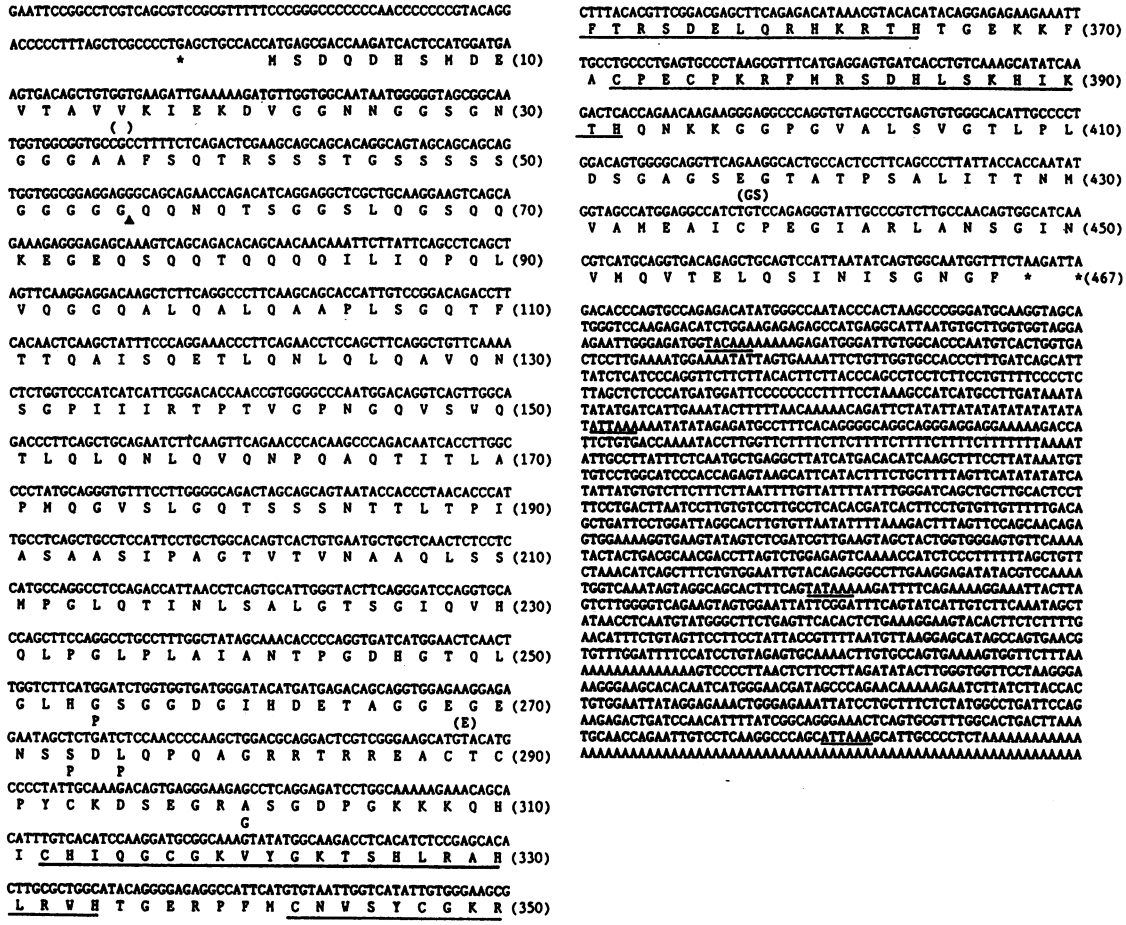

FIG. 1. cDNA sequence of mSp1-S. The coding region (467 amino acid residues) is numbered and is given below the nucleotide sequence. Differences between the rat (20) and mouse Sp1 protein sequences within common regions are shown below the mouse sequence (additions and deletions are indicated by parentheses with or without residue symbols, respectively). The site of sequence divergence in which a 319-amino acid region in rat Sp1 is absent from the mSp1-S sequence is indicated by a triangle. Underlined sequences in the coding region refer to the three zinc fingers in the Sp1 DNA-binding domain, while potential polyadenylylation signals are underlined in the 3′ untranslated region. The position of in-frame stop codons upstream and downstream of the mSp1-S coding region is indicated by asterisks.

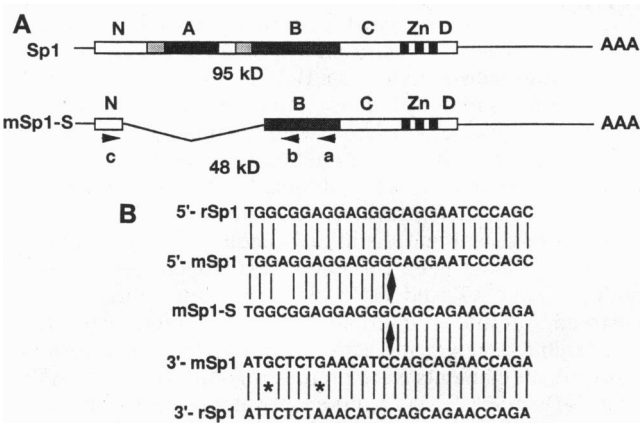

FIG. 2. (*A*) Schematic comparison between the protein deduced from the mSp1-S cDNA sequence and full-length Sp1. The Sp1 structure is based on the complete coding sequence of the rat protein (20) (788-amino acid residues). The positions of domains A, B, C, D, and the three zinc fingers are based on previous studies of human Sp1 (5), and domain N represents additional N-terminal sequences within rat Sp1 that were not present in the partial human cDNA (the rat and human proteins exhibit >97% sequence homology at the protein level; ref. 20). The gap in the mSp1-S structure represents a contiguous 319-residue region that is absent in the smaller mouse protein. The positions of primers used for reverse transcription and PCR amplification (a, b, and c) are indicated below the mSp1-S diagram. (*B*) Sequence comparison of mouse Sp1 and mSp1-S based on reverse transcription–PCR analysis. Total RNA from mouse kidney was reverse-transcribed with primer a and subsequently amplified by using primers b and c. The sequence spanning the putative splice site region for mouse Sp1-S (nucleotides 244–269) is shown along with corresponding sequences for the mouse Sp1 PCR product (mSp1) and for rat Sp1 (rSp1). Comparison with Sp1 sequences located 5′ of the presumptive splice site is shown above the mSp1-S sequence, while comparison of sequences downstream of this site is shown below. Asterisks indicate nucleotide differences between rat and mouse Sp1, and the position of the putative splice site is indicated by the vertical double arrowheads. The sequence of the mouse Sp1 PCR product exhibited >90% homology to rat and human sequences at the nucleotide level, while the 300-bp product was identical in sequence to that for mSp1-S (data not shown). A conservative base mutation was found in the mSp1 sequence 5′ of the splice site, which may represent a PCR-generated artifact or diversity among individual mice.

It should be noted that a smaller Sp1 mRNA (5.2 kb) is also detected in rat tissues (20). To further characterize variant Sp1 mRNAs in mouse tissues, reverse transcription-PCR was used to amplify sequences spanning the putative splice site in mSp1-S, where its sequence diverges from the full-length protein (Fig. 2*A*). Products similar in size to those predicted for mSp1-S (300 bp) and full-length Sp1 (>1 kb) were identified by Southern analysis with the pmSp1-0.6 cDNA probe (data not shown). Analysis of the larger product revealed the presence of sequences highly homologous (>90%) to the A domain of full-length rat and human Sp1 that is missing in mSp1-S and confirmed the location of the putative splice site suggested by RNase protection and the mSp1-S cDNA sequence (Fig. 2*B*).

Protein Studies. We next searched for proteins corresponding to mSp1-S in mouse nuclear extracts. An Sp1-related protein having an apparent mass of 49 kDa was identified by Western analysis along with larger forms of 80 and 95–105 kDa in mouse kidney, liver, P19, and F9 cells, the larger bands corresponding to the full-length protein (Fig. 4). The 49-kDa band was consistently observed in multiple preparations, and incubation of extracts at room temperature for various lengths of time did not alter the observed protein pattern (data not shown), suggesting that it was not simply a product of proteolytic digestion. Further, a similar 49-kDa band was not observed in extracts from mouse spermatogenic cells or HeLa

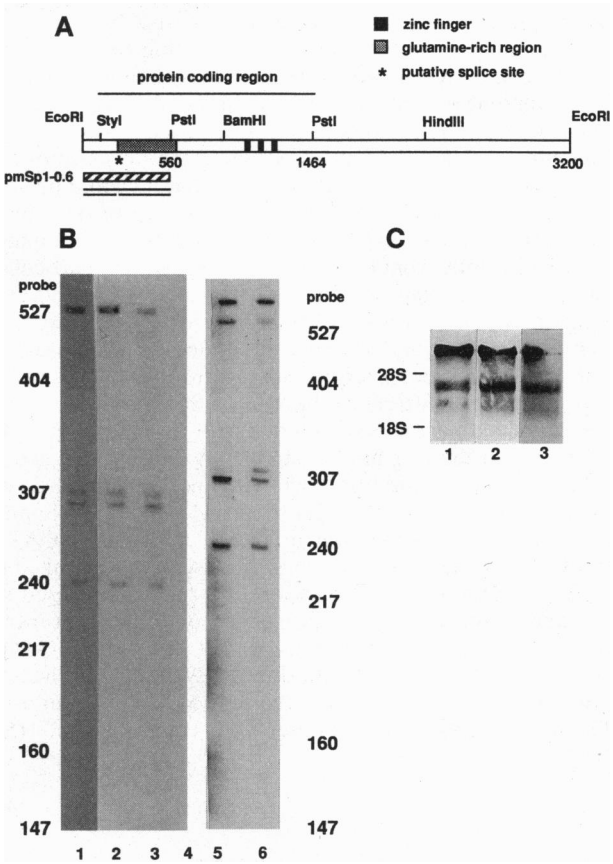

FIG. 3. RNase protection and Northern analysis of mSp1-S transcripts. (*A*) Diagram of the mSp1-S cDNA showing the positions of various restriction sites, including those used to generate the pmSp1-0.6 riboprobe for protection analysis. The asterisk indicates the location of the gap in the mSp1-S cDNA sequence relative to that for rat Sp1. The origins of protection fragments resulting from the riboprobe are indicated by solid lines below the pmSp1-0.6 riboprobe (hatched box). (*B*) Protection products derived from the pmSp1-0.6 riboprobe when using 30 μg of total RNA are shown for NG-108 cells (lane 1), P19 cells (lane 2), F9 cells (lane 3), yeast RNA (lane 4), adult mouse kidney (lane 5), and liver (lane 6). Sizes are shown in nucleotides. Weaker 450-nucleotide bands also were detected in F9 and P19 cell samples after long exposure of autoradiograms (unpublished observations). The overall length of the undigested pmSp1-0.6 probe was 628 nucleotides (556 of the mSp1-S sequence and 72 of plasmid sequence). (*C*) Northern blot analysis of mouse F9 cells (lane 1), day-14 embryos (lane 2), and NG-108 neuroblastoma–glioma cell hybrid (lane 3).

cells (Fig. 4). The lower amounts of the 49-kDa band relative to the 95- and 105-kDa species in mouse kidney and liver are consistent with the lower levels of fully protected fragments in these tissues (Fig. 3*B*). The nature and origin of the 80-kDa protein is uncertain, although possibly it arises from one of the smaller transcripts (perhaps the 3.8-kb form).

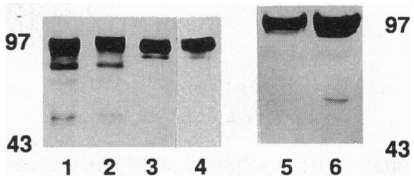

FIG. 4. Detection of Sp1-related proteins in mouse nuclear extracts. Western blot of total nuclear extract proteins (60 μg) from mouse kidney (lane 1), mouse liver (lane 2), mouse spermatogenic cells (lane 3), HeLa cells (lane 4), P19 cells (lane 5), and F9 cells (lane 6). Size is shown in kDa.

Attempts were also made to detect mSp1-S by using UV photocrosslinking and protein–DNA blotting analysis. This proved inconclusive because of the presence of more abundant GC box-binding proteins apparently unrelated to Sp1 in mouse nuclear extracts (data not shown).

Functional Analysis of mSp1-S. While the presence of the zinc-finger domain in mSp1-S indicated that it should possess appropriate DNA-binding activity, the absence of domain A sequences suggested that its transcriptional properties might differ significantly from those of full-length Sp1. In particular, previous domain mapping studies of human Sp1 found that both glutamine-rich transactivation domains were required for synergistic interactions involving multiple Sp1 response elements and for a process termed superactivation (5). As noted above, additional differences between mSp1-S and the previously examined human Sp1 include the N-terminal sequences not present in the original human cDNA and the absence in mSp1-S of serine/threonine-rich sequences. Therefore, we compared the transactivation properties of mSp1-S and human Sp1 in SL2 cells using various promoter constructs. BCAT-1 and BCAT-2 contain one or two Sp1 consensus sites linked to the E1b TATA box, respectively, and have been used to characterize the synergistic properties of Sp1 and various deletion mutants derived from it (5). Sp1 activated transcription from BCAT-1 8-fold and exhibited synergistic activation of BCAT-2, as previously reported [although this synergistic effect was not as great as that observed in the earlier study (5)] (Fig. 5*A*). In contrast, mSp1-S showed negligible transactivation of the single Sp1-site promoter BCAT-1 but did stimulate transcription substantially from BCAT-2, although to a smaller extent (7-fold) than Sp1. This stimulation represents a rather substantial synergistic effect when compared with the extremely low activity seen for mSp1-S at the single-site promoter. Similar results were obtained with a different set of promoter constructs previously used to examine interactions between proximal and distal Sp1-binding sites adjoining the thymidine kinase gene promoter (18). Again, Sp1 activated both −105tkCAT and tkCAT15 containing either a single upstream Sp1 site or multiple downstream sites, respectively, and exhibited synergistic activation when both upstream and downstream elements were present together (−105tkCAT15) (Fig. 5*A*). mSp1-S was a poor activator when upstream or downstream Sp1 sites were present alone but showed increased (synergistic) activity when both elements were present (Fig. 5*A*).

These results indicated that mSp1-S was a relatively weak activator of transcription from Sp1 response elements and suggested the possibility that it may function as an antagonist of Sp1-induced transactivation. To test this, Sp1 and mSp1-S were coexpressed along with either the BCAT-1 and BCAT-2 promoters. Instead of reducing activation, the combination of mSp1-S and Sp1 was synergistic with respect to promoters containing either one or two Sp1 response elements (Fig. 5 *B* and *C*). While mSp1-S was a relatively poor coactivator of the

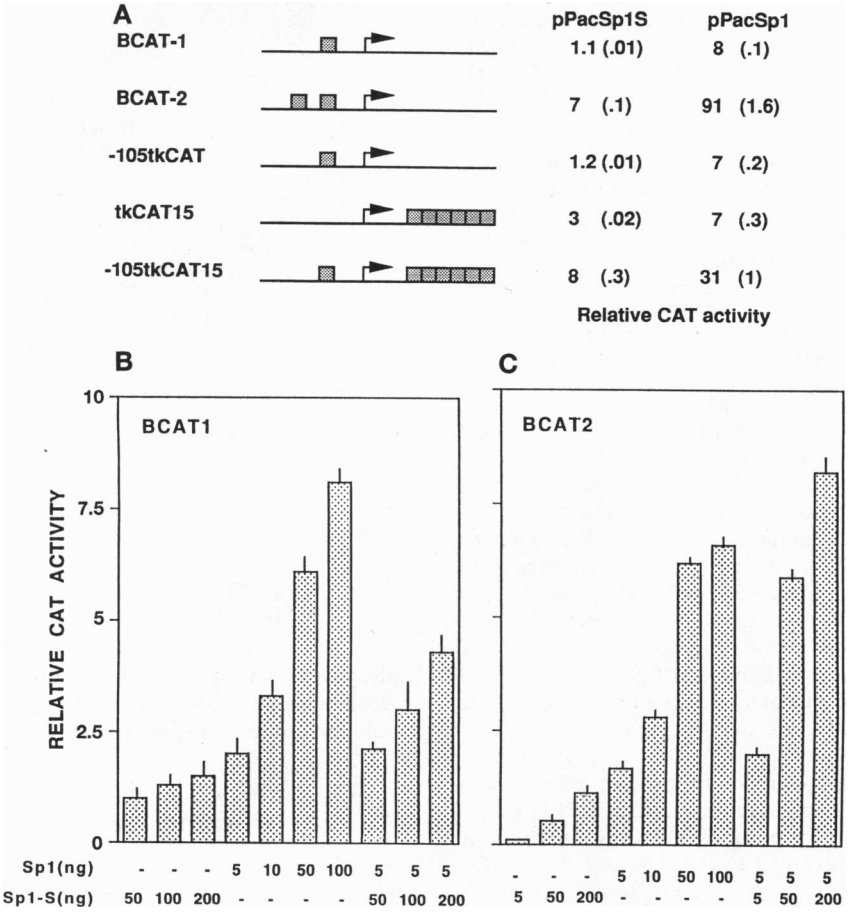

FIG. 5. Cotransfection analysis of mSp1-S in SL2 cells. (*A*) The expression vectors pPacSp1S and pPacSp1 were coexpressed individually with each of the reporter vectors shown containing one or more Sp1 response elements linked to the CAT reporter gene. Data for CAT activity are given under each expression vector, with basal activity in each case being defined as 1 as in previous studies (5). Standard errors are shown in parentheses. Quantitatively similar results were obtained in three separate experiments. (*B*) Interactions between mSp1-S and Sp1 at a promoter containing a single Sp1 response site. Differing amounts of Sp1 and mSp1-S were tested either alone or in combination with the reporter plasmid BCAT-1 in SL2 cells. (*C*) mSp1-S/Sp1 interactions with the two-site promoter, BCAT-2. Basal activity of reporter plasmids was set at 1 in both *B* and *C*.

BCAT-1 promoter containing a single Sp1 site, it exhibited marked synergistic interaction with Sp1 when we used the multiple-site promoter BCAT-2. This is analogous to the results obtained above for mSp1-S alone.

DISCUSSION

The present findings demonstrate the existence of a novel, alternatively spliced form of Sp1 in which all of domain A and the serine/threonine-rich region of domain B have been deleted. Together domains A and B are necessary for maximal synergistic activity of Sp1 with promoters containing multiple Sp1 sites, and this appears to reflect a requirement for distinct glutamine-rich regions to interact with TFIID and for Sp1 complex formation (5). Sp1 superactivation and $TAF_{II}110$ binding activities reside within the C-terminal third of domain B (4, 5), which is conserved in mSp1-S. Based on these considerations, one might predict that this smaller protein would retain the ability to interact with Sp1 and $TAF_{II}110$ but would lack significant synergistic activity, since it contains only one glutamine-rich domain. While mSp1-S is a weaker activator of promoters containing multiple Sp1 sites, it does exhibit a substantial synergistic effect under these conditions. This is due to its negligible activity at promoters containing a single Sp1 site and the apparent ability to compensate for this deficit when multiple sites are present. These observations are not consistent with the deletion of the A domain alone, since the mutant form of human Sp1 lacking this domain was found to retain substantial activity at single-site promoters but exhibited weak synergism (5). This suggests that the specific structure of mSp1-S reduces its ability to activate promoters containing a single Sp1 site by inhibiting Sp1 tetramer formation and/or interactions with TBP-associated factors. mSp1-S differs structurally from the human Sp1 A-domain mutant, ΔA, by the lack of serine/threonine-rich sequences associated with domain B and by the presence of a complete N-terminal region (5). These results may directly indicate a primary role for the serine/threonine-rich regions of Sp1 in the transactivation process. While glycosylation and phosphorylation within these regions have been implicated in the regulation of Sp1 activity (22, 23), a specific role for these domains has not been elucidated to date (1). Serine/threonine-rich domains have been implicated in transactivation by other transcription factors, including growth hormone factor-1 (GHF-1) (7). The possibility cannot be ruled out, however, that other structural alterations, including the juxtapositioning of the N-terminal and B domains, also influence the transactivation properties of mSp1-S.

The ability of multiple mSp1-S molecules, when bound to promoters containing more than one Sp1 response element, to compensate for their individual deficiencies presumably reflects mutual interactions that enhance recruitment of TAFs to the promoter site (5). The precise mechanisms by which such interactions occur are not clear, but multiple Sp1 domains appear to be involved, including domains A, B, and D, and the zinc-finger DNA-binding region (4–6, 24). Those domains mediating the synergistic effects of mSp1 remain to be determined, although domains B and D and the zinc-finger region would seem likely candidates on the basis of the existing data.

The abundance of mSp1-S in mouse tissues and cell lines so far examined appears to be relatively low in comparison with Sp1. However, conditions may exist in which expression of mSp1-S is selectively induced, such as during development. Developmental regulation of alternative RNA splicing has been observed for numerous transcription factor isoforms (11, 25–27). It is also worth noting in this context that two *Drosophila* homologues of mSp1-S were recently identified, both of which contain a single glutamine-rich domain and perform pivotal roles during development (28, 29). Although mSp1-S is less active than Sp1 with model promoters, it cannot be ruled out that mSp1-S activates (or represses) a select subset of native promoters because of novel interactions with other transcription factors. Specifically, mSp1-S may exhibit altered interactions with YY1, TBP, $TAF_{II}110$ or the inhibitory molecule Sp1-I (30, 31). Another possibility worth investigating is that it serves a nontranscriptional role. For example, recent studies indicate the involvement of transcription factors in replication and DNA repair (32, 33).

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1. Courey, A. J. & Tjian, R. (1988) *Cell* **55,** 887–898.
2. Foulkes, N. S., Melstrom, B., Benusiglio, E. & Sassone-Corsi, P. (1992) *Nature (London)* **355,** 80–84.
3. Hoey, T., Weinzierl, R. O. J., Gill, G., Chen, J.-L., Dynlacht, B. D. & Tjian, R. (1993) *Cell* **72,** 247–260.
4. Gill, G., Pascal, E., Tseng, Z. H. & Tjian, R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 192–196.
5. Pascal, E. & Tjian, R. (1991) *Genes Dev.* **5,** 1646–1656.
6. Lee, J.-S., Galvin, K. M. & Shi, Y. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6145–6149.
7. Theill, L. E., Castrillo, J.-L., Wu, D. & Karin, M. (1989) *Nature (London)* **342,** 945–948.
8. Foulkes, N. S., Borrelli, E. & Sassone-Corsi, P. (1991) *Cell* **64,** 739–749.
9. Molina, C. A., Foulkes, N. S., Lalli, E. & Sassone-Corsi, P. (1993) *Cell* **75,** 875–886.
10. Delmas, V., Laoide, B. M., Masquilier, D., de Groot, R. P., Foulkes, N. S. & Sassone-Corsi, P. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4226–4230.
11. Bach, I. & Yaniv, M. (1993) *EMBO J.* **12,** 4229–4242.
12. Jackson, S. P., MacDonald, J. J., Lees-Miller, S. & Tjian, R. (1990) *Cell* **63,** 155–165.
13. Saffer, J. D., Thurston, S. J., Annarella, M. D. & Compton, J. G. (1990) *Genomics* **8,** 571–574.
14. Szpirer, J., Szpirer, C., Riviere, M., Levan, G., Marynen, P., Cassiman, J. J., Wiese, R. & DeLuca, H. F. (1991) *Genomics* **11,** 168–173.
15. Kilpatrick, D. L., Zinn, S. A., Fitzgerald, M., Higuchi, H., Sabol, S. L. & Meyerhardt, (1990) *Mol. Cell. Biol.* **10,** 3717–3726.
16. Kawasaki, E. S. (1991) in *PCR Protocols: A Guide to Methods and Applications*, eds. Innis, M. A., Gelfand, D. H., Shinsky, J. J. & White, T. J. (Academic, San Diego), pp. 21–26.
17. Galcheva-Gargova, Z., Tokeson, J. P., Karagyosov, L. K., Ebert, K. M. & Kilpatrick, D. L. (1993) *Mol. Endocrinol.* **7,** 979–991.
18. Courey, A. J., Holtzman, D. A., Jackson, S. P. & Tjian, R. (1989) *Cell* **59,** 827–836.
19. Seed, B. & Sheen, J. Y. (1988) *Gene* **67,** 271–277.
20. Imataka, H., Sogawa, K., Yasumoto, K.-I., Kikuchi, Y., Sasano, K., Kobayashi, A., Hayami, M. & Fujii-Kuriyama, Y. (1992) *EMBO J.* **11,** 3663–3671.
21. Kadonaga, J. T., Curner, K. R., Masiarz, F. R. & Tjian, R. (1987) *Cell* **51,** 1079–1090.
22. Jackson, S. P., MacDonald, J. J., Lees-Miller, S. & Tjian, R. (1990) *Cell* **63,** 155–165.
23. Jackson, S. P. & Tjian, R. (1988) *Cell* **55,** 125–133.
24. Chiang, C.-M. & Roeder, R. G. (1995) *Science* **267,** 531–536.
25. Kozmik, Z., Kurzbauer, R., Dorfler, P. & Busslinger, M. (1993) *Mol. Cell. Biol.* **13,** 6028–6035.
26. Takeda, H., Matsuzaki, T., Oki, T., Miyagawa, T. & Amanuma, H. (1994) *Genes Dev.* **8,** 45–59.
27. Gogos, J. A., Hsu, T., Bolton, J. & Kafatos, F. C. (1992) *Science* **257,** 1951–1955.
28. Wimmer, E. A., Jackle, H., Pfeifle, C. & Cohen, S. M. (1993) *Nature (London)* **366,** 690–694.
29. Bronner, G., Chu-LaGraff, Q., Doe, C. Q., Cohen, B., Weigel, D., Taubert, H. & Jackle, H. (1994) *Nature (London)* **369,** 664–668.
30. Kim, S.-J., Onwuta, U. S., Lee, Y. I., Li, R., Botchan, M. R. & Robbins, P. D. (1992) *Mol. Cell. Biol.* **12,** 2455–2463.
31. Chen, L. I., Nishinaka, T., Kwan, K., Kitabayashi, I., Yokoyama, K., Fu, Y.-H. F., Grunwald, S. & Chiu, R. (1994) *Mol. Cell. Biol.* **14,** 4380–4389.
32. Rivier, D. H. & Rine, J. (1992) *Science* **256,** 659–664.
33. Drapkin, R., Reardon, J. T., Ansari, A., Huang, J.-C., Zawel, L., Ahn, K., Sancar, A. & Reinberg, D. (1994) *Nature (London)* **386,** 769–772.