Two Consecutive Zinc Fingers in Sp1 and in MAZ Are Essential for Interactions with cis-Elements*

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The zinc finger proteins Sp1 and Myc-associated zinc finger protein (MAZ) are transcription factors that control the expression of various genes. Regulation of transcription by these factors is based on interactions between GC-rich DNA-binding sites (GGGGCGG for Sp1 and GGGAGGG for MAZ) and the carboxyl-terminal zinc finger motifs of the two proteins. Sp1 and MAZ have three and six zinc fingers, respectively, and the details of their interactions with cis-elements remain to be clarified. We demonstrate here that Sp1 and MAZ interact with the same GC-rich DNA-binding sites, apparently sharing DNA-binding sites with each other. We found that the DNA binding activities of Sp1 and MAZ depended mainly on consecutive zinc fingers, namely the second and third zinc fingers in Sp1 and the third and fourth zinc fingers in MAZ. Furthermore, the interactions of the zinc finger proteins with the same cis-elements appear to play a critical role in the regulation of gene expression. It seems plausible that two consecutive zinc finger motifs in a zinc finger protein might be essential for interaction of the protein with DNA.

The regulation of gene expression is mediated by the binding of transcription factors to cis-elements in promoter regions. The promoter regions of many eukaryotic genes contain GC-rich sequences (1), and some of the most widely distributed promoter elements are GC boxes and related motifs. The zinc finger proteins Sp1 and Myc-associated zinc finger protein (MAZ) are transcription factors that bind to GC-rich sequences, namely GGGCGG and GGGAGGG, respectively, to regulate the expression of various target genes.

Sp1 was originally characterized as a ubiquitous transcription factor of 778 amino acids that recognized GC-rich sequences in the early promoter of simian virus 40 (2, 3). The DNA-binding domain of Sp1 consists of three contiguous C2H2-type zinc fingers (4). The amino-terminal region contains two serine- and threonine-rich domains and two glutamine-rich domains, which are essential for the transcriptional activity of Sp1 (5). The carboxyl-terminal domain of Sp1 is involved in synergistic activation and interactions with other transcription factors. Sp1 is considered to be a constitutively expressed transcription factor and has been implicated in the regulation of a wide variety of housekeeping genes, tissue-specific genes, and genes involved in the regulation of growth (6). It interacts with many factors, such as YY1 (7), E2F (8), and p300 (9). Moreover, Sp1-null mouse embryos exhibit severely retarded growth and die within 10 days after displaying a wide range of abnormalities (10).

MAZ was first identified as a transcription factor that bound to a GA box (GGGGAGGG) at the ME1a1 site of the c-myc promoter and to the CT-element of the c-myc gene (11). It is a zinc finger protein with six C2H2-type zinc fingers at the carboxyl terminus, a proline-rich region, and three alanine repeats. It is expressed ubiquitously, albeit at different levels in different tissues (12). It can regulate the expression of numerous genes, such as c-myc (11, 13), genes for insulin I and II (14), the gene for CD4 (15), the gene for the serotonin receptor (16), and the gene for nitric oxide synthase (17).

The sequences of the binding sites for Sp1 and MAZ are very similar, and they are often present in the same gene. However, the details of the interactions of Sp1 and MAZ with GC-rich cis-elements remain unknown. We report here that Sp1 and MAZ share DNA-binding sites with each other and that the specific binding of Sp1 and of MAZ to the DNA-binding sites depends on pairs of consecutive zinc fingers.

MATERIALS AND METHODS

Plasmids—A DNA fragment (from nucleotide nt −383 to minus70) from the MAZ promoter was obtained by digestion with appropriate restriction enzymes, filled in, and inserted into the HindIII site of pSV000CAT (18), via a pHindIII linker, to generate pMAZCAT. pCMV-Sp1 and pGEX-Sp1 were kindly provided by Dr. R. Chiu (UCSC School of Medicine, Los Angeles, CA). We constructed pGEX-MAZ-ΔF12, pGEX-MAZ-ΔF3, pGEX-MAZ-ΔF4, pGEX-MAZ-ΔF34, pGEX-MAZ-ΔF56, pGEX-Sp1-ΔF1, pGEX-Sp1-ΔF2, pGEX-Sp1-ΔF3, and pGEX-Sp1-ΔF23 by subcloning the appropriate DNA fragments that had been amplified by the PCR into pGEX-2T (Amersham Pharmacia Biotech). pGEX-MAZ-X and pGEX-Sp1-X were constructed by amplification of inserted DNA by PCR with exchange of the respective zinc finger domains of Sp1 and/or MAZ and subcloning into pGEX-2T. The inserts of pGEX-MAZ-ΔF34, pGEX-Sp1-ΔF23, pGEX-MAZ-X, and pGEX-Sp1-X were subcloned into pDNA3 (Invitrogen, San Diego, CA) to generate pCMV-MAZ-ΔF34, pCMV-Sp1-ΔF23, pCMV-MAZ-X, and pCMV-Sp1-X.

Cell Culture, Transfection, and Assay of Chloramphenicol O-acetyltransferase (CAT) Activity—HeLa cells, 293 cells, and NIH3T3 cells were grown in Dulbecco’s modified Eagle’s medium, and NCI-H460 cells were grown in RPMI 1640 medium that contained 10% fetal bovine serum.

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1 The abbreviations used are: MAZ, Myc-associated zinc finger protein; nt, nucleotide; CAT, chloramphenicol acetyl transferase; GST, glutathione S-transferase; PCR, polymerase chain reaction.
serum (Life Technologies, Inc.). Cells were transfected with plasmid DNA using the FuGENETM 6 transfection reagent (Roche Molecular Biochemicals) according to the protocol provided by the manufacturer. The assay of CAT activity was performed as described elsewhere (12, 19).

**DNase I Footprinting and Gel Shift Assay**—The DNA probe (nt −383 to −70) were radiolabeled by the Klenow large fragment of DNA polymerase (New England BioLabs, Inc., Beverly, MA) using [α-32P]dCTP. The reaction was performed in 50 μl of buffer that contained 25 mM Tris-HCl (pH 8.0), 6 mM MgCl2, 0.5 mM EDTA, 10% glycerol, 0.5 mM dithiothreitol, 50 mM KCl, and a radiolabeled DNA probe with an extract of HeLa cells, GST-Sp1, GST-MAZ, and GST (glutathione S-transferase), respectively. The reaction mixture was first chilled on ice for 30 min and then 0.1 unit of DNase I (TAKARA, Kyoto, Japan) was added. The incubation was continued on ice for 4 min. The reaction was stopped by the addition of 25 μl of buffer that contained 20 mM EDTA, 0.5% SDS, and 250 μg/ml tRNA as a carrier. The reaction products were purified by phenol-chloroform extraction and ethanol precipitation and were then subjected to electrophoresis on a DNA sequencing gel. Gel shift assays were performed as described elsewhere (6, 20).

**RESULTS**

**Sp1 and MAZ Share DNA-binding Sites**—The sequences of the DNA-binding sites of Sp1 and MAZ are very similar. To investigate whether Sp1 and MAZ might bind to the same cis-elements, we prepared DNA probes from the GC-rich promoter of the human gene for MAZ (from nt −383 to −70), which contains multiple putative binding sites for both Sp1 and MAZ, for gel shift assays using the nuclear extract of HeLa cells (12). We prepared three probes: the SM probe, nt −313 to −284, which contained one putative Sp1-binding site and one putative MAZ-binding site that partially overlapped (Fig. 1A); the M probe, nt −232 to −216, which contained one putative MAZ-binding site (Fig. 1B); and the S probe, nt −153 to −137, which contained one putative Sp1-binding site (Fig. 1C). We detected one major and one minor DNA-protein complex (B1 and B2) with the SM probe that included the overlapping binding sites for Sp1 and MAZ (Fig. 1A). Addition of unlabeled oligodeoxynucleotides to reaction mixtures revealed competition by the unlabeled wild-type SM probe (lanes 5–7), but not by the mutant SM probe, for binding to MAZ and Sp1 (lanes 2–4). As shown in Fig. 1A, the retarded bands corresponding to B1 and B2 were shifted still further upon addition of the antibodies against Sp1 and MAZ (lanes 9 and 11). Control antibodies did not affect the mobilities of the complexes (lanes 12 and 13). These results indicated that Sp1 and MAZ specifically recognized overlapping sites in the same cis-element. Similarly, we detected supershifted bands when antibodies against MAZ or Sp1 were added to reaction mixtures with the M probe or S probe (Figs. 1, B and C, lanes 2 and 4). The results indicated that both Sp1 and MAZ interacted with the putative MAZ-binding sites and the putative Sp1-binding sites in all three probes. We also examined other GC-rich cis-elements in the MAZ promoter and found that the DNA-binding sites were recognized similarly by Sp1 or by MAZ or by both (data not shown).

We next performed DNase I footprinting assays to determine whether the factors did, in fact, bind to these putative cis-elements in cells. We used a nuclear extract of HeLa cells for the assays. All of the putative binding sites for Sp1 and MAZ were protected from nuclease digestion (Fig. 2A). In an attempt to identify whether Sp1 and MAZ could bind to these sites, we used purified GST-Sp1 and GST-MAZ fusion proteins in DNase I footprinting assays. As shown in Fig. 2B, all of the sixteen putative binding sites for Sp1 and MAZ were protected from nuclease digestion by GST-MAZ (lanes 8–10), and 13 of the 16 putative binding sites for Sp1 and MAZ were protected by GST-Sp1 (lanes 3–5). No cis-elements were protected when we used GST alone as a negative control (Fig. 2C). The patterns of protection obtained with purified GST-Sp1 were almost the same as those obtained with purified GST-MAZ. Thus, both fusion proteins bound to almost the same cis-elements as the respective binding proteins in a nuclear extract of HeLa cells. These results indicated that Sp1 and MAZ could bind to the same cis-elements and suggested, moreover, that Sp1 and MAZ might share DNA-binding sites.

**Pairs of Consecutive Zinc Finger Motifs in Sp1 and MAZ Are Essential for DNA Binding Activity**—We found that the second and third zinc fingers of Sp1 are strongly homologous to the fourth and third zinc fingers of MAZ, respectively (Fig. 3A). To identify whether these zinc fingers are necessary for interaction with GC-rich sequences, we generated a mutant GST-Sp1 and GST-MAZ fusion proteins with deletions of the zinc finger motifs (Figs. 3, B and C), and we examined the DNA binding abilities of these fusion proteins in gel shift assays. The DNA binding activity of the GST-Sp1 fusion protein decreased significantly when either the second or the third zinc finger motif had been deleted (GST-Sp1-ΔF2 and GST-Sp1-ΔF3; Fig. 3B).
FIG. 2. Sp1 and MAZ share DNA-binding sites. A, all putative binding sites for Sp1 and MAZ were protected in the DNase I footprinting assay. Putative binding sites for Sp1 and MAZ are indicated by rectangles. The protected sites are indicated by arrowheads. Lane 1, no nuclear extract of HeLa cells; lanes 2, 3, and 4, 2, 4, and 8 μg of nuclear extract (as protein), respectively; lane 5, G+A sequencing reaction. B, Sp1 and MAZ share the same DNA-binding sites. Putative binding sites for Sp1 and MAZ are indicated by rectangles. Protected sites are indicated by arrowheads. Lane 1, No protein; lanes 2, 3, and 4, 2, 4, and 8 μg of GST fusion protein, respectively; lane 5, G+A sequencing reaction. C, GST protein was used as the control.

FIG. 3. Two of the multiple zinc fingers of both Sp1 and MAZ are required for binding to DNA. A, homology among pairs of zinc fingers in Sp1 and MAZ. The amino acid sequences of two C2H2-type zinc fingers of Sp1 and MAZ are aligned. Conserved amino acids and consensus amino acids are indicated by red letters. P refers to a specific zinc finger. B, two of these zinc fingers of Sp1 are required for binding. A construct designated GST-Sp1, and a series of deletion variants were constructed as indicated schematically and used in gel shift assays with the S and M probes. C, two of the six zinc finger motifs of MAZ are required for binding. The GST-MAZ construct and a series of deletion variants were used in gel shift assays with the S and M probes. D, the similar DNA binding abilities of pairs of zinc fingers from Sp1 and MAZ. Chimeric constructs GST-Sp1-X and GST-MAZ-X were used for gel shift assays. 0.1 ng (10,000 cpm) of labeled probe and 1 μg of GST fusion protein were used in each assay in B, C and D.
assays were performed using HeLa cells. MAZ repressed the promoter activity of MAZ. Transfections and CAT assays were used to transfect the cells before assays of CAT activity. Promoter activities of MAZ-CAT fusion genes are expressed relative to the activity of pMAZCAT, which was taken arbitrarily as 1.0.

Because two consecutive zinc fingers in both Sp1 and MAZ were required for binding to GC-rich DNA sequences, we exchanged the two pairs of consecutive zinc fingers between GST-Sp1 and GST-MAZ (Fig. 3D). The chimeric fusion proteins bound both to Sp1- and to MAZ-binding sites after the pairs of consecutive zinc fingers had been exchanged with one another (GST-Sp1-X and GST-MAZ-X, Fig. 3D). This result confirmed the involvement of the pairs of consecutive zinc fingers in Sp1 and MAZ in the binding to GC-rich sequences in vitro.

FIG. 4. Sp1 and MAZ repress the activity of a MAZ-CAT reporter gene. HeLa cells were cultured in 35-mm dishes. A, Sp1 and MAZ repressed the promoter activity of MAZ. Transfections and CAT assays were performed using HeLa cells ± pCMV-Sp1 and/or pCMV-MAZ. One μg of reporter plasmid and 1 μg of ectopic expression plasmid were used to transfect the cells before assays of CAT activity. Promoter activities of MAZ-CAT fusion genes are expressed relative to the activity of pMAZCAT in the absence of pCMV-Sp1, which was taken arbitrarily as 1.0. B, repression by Sp1 and MAZ in HeLa cells, 293 cells, NCI-H460 cells, and NIH3T3 cells. Promoter activities of MAZ-CAT fusion genes in each line of cells are expressed relative to the activity of pMAZCAT, which was taken arbitrarily as 1.0. C and D, repression by Sp1 and MAZ of the promoter activity of the MAZ-CAT reporter gene was dose-dependent. The concentrations of pCMV-Sp1 and pCMV-MAZ are indicated. pRSVCAT was used as the control. E, the two consecutive zinc fingers are essential for the repression. Transfections and CAT assays were performed using HeLa cells in the presence of pCMV-Sp1, pCMV-Sp1-ΔF23, pCMV-Sp1-X, pCMV-MAZ, pCMV-MAZ-ΔF34, or pCMV-MAZ-X individually. Promoter activities of MAZ-CAT fusion genes are expressed relative to the activity of pMAZCAT, which was taken arbitrarily as 1.0. F, similar effects of the consecutive zinc fingers of Sp1 and MAZ on repression of the MAZ-CAT reporter. The concentrations of pCMV-Sp1 and pCMV-MAZ are indicated.

Using the minimum effective dose of ectopically expressed Sp1, we examined the effect of increasing amounts of the MAZ expression vector and found that MAZ can replace Sp1 to repress the reporter activity of MAZ-CAT (Fig. 4D). This repression was also dose-dependent. Similar repression was detected when we examined the ability of Sp1 to replace MAZ in transcriptional repression of the MAZ-CAT reporter gene (data not shown). These results indicate that repression of transcription by Sp1 and by MAZ does not involve cooperation between the two proteins. The binding of Sp1 and MAZ to the GC-rich elements seemed to be independent since we failed to detect any cooperation between Sp1 and MAZ in the repression of gene expression. These observations might reflect the competition for DNA-binding sites.

We next studied the effects of the two consecutive zinc fingers of Sp1 and MAZ on the expression of MAZ. Plasmids expressing the wild-type protein, the deletion mutant that lacked the two consecutive zinc fingers, and a chimera with zinc fingers in Sp1 and MAZ were required for binding to GC-rich DNA sequences, we exchanged the two pairs of consecutive zinc fingers between GST-Sp1 and GST-MAZ (Fig. 3D). The chimeric fusion proteins bound both to Sp1- and to MAZ-binding sites after the pairs of consecutive zinc fingers had been exchanged with one another (GST-Sp1-X and GST-MAZ-X, Fig. 3D). This result confirmed the involvement of the pairs of consecutive zinc fingers in Sp1 and MAZ in the binding to GC-rich sequences in vitro.

Sp1 and MAZ Repress Expression of the Gene for MAZ—We transfected HeLa cells with MAZ-CAT reporter constructs in the presence or absence of an Sp1- or a MAZ-expression vector. The CAT reporter activity was inhibited significantly in the presence of Sp1 and of MAZ (Fig. 4A), whereas the ectopic expression of Sp1 and MAZ had no effect on the transcription of pRSVCAT, the control plasmid. These results indicated that both Sp1 and MAZ repressed transcription of the MAZ gene. We also examined the inhibitory effects of Sp1 and MAZ on the MAZ promoter in other cell lines, namely 293 cells, NCI-H460 cells, and NIH3T3 cells. Both Sp1 and MAZ repressed the activity of the MAZ promoter in these cell lines, albeit to different extents in different cell lines (Fig. 4B). The effects of repression by Sp1 and MAZ were clearly in a dose-dependent fashion (Fig. 4C).

No DNA binding activity of the GST-Sp1 fusion protein was detected when both zinc fingers had been deleted (GST-Sp1-ΔF23; Fig. 3B). These results clearly indicated that the second and third zinc fingers of Sp1 were essential for its DNA binding ability. Similarly, the DNA binding activity of the GST-MAZ fusion protein decreased when the third and/or the fourth zinc finger of MAZ had been deleted (GST-MAZ-ΔF3, GST-MAZ-ΔF4, and GST-MAZ-ΔF34; Fig. 3C). The DNA binding ability of MAZ was not affected when the first two zinc fingers or the last two zinc fingers had been deleted (GST-MAZ-ΔF12 and GST-MAZ-ΔF56, Fig. 3C). These results indicated that the third and fourth zinc fingers of MAZ are essential for its DNA binding activity.

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consecutive zinc fingers from MAZ or Sp1 were constructed and used to transfect HeLa cells. Repression of the MAZ promoter was reversed in the absence of the two consecutive zinc fingers used to transfect HeLa cells. Repression of the MAZ promoter consecutive zinc fingers from MAZ or Sp1 were constructed and  

The numbers of zinc finger motifs in zinc finger proteins vary from only two to more than a dozen (24–27). It is possible that some but not all of the multiple zinc fingers are required for the binding to cis-elements. In view of the physical space available at cis-elements and the size of a zinc finger motif, it seems reasonable to postulate that two consecutive zinc fingers might be essential for direct interaction with a cis-element while additional zinc fingers might play an auxiliary role in the interaction or might be involved in cooperation with other factors. For example, the Ikaros protein contains six zinc fingers; the first four zinc fingers are involved in interactions with a cis-element, while the two carboxyl-terminal zinc fingers are involved in homodimerization of the protein itself (24, 28). We observed similar results for transcription factors Sas3 (29) and RIP60 (30).

There are three and six C2H2-type zinc finger motifs in the carboxyl-terminal regions of Sp1 and MAZ, respectively. Despite the difference in the number of zinc finger motifs between Sp1 and MAZ, the DNA binding abilities of the two proteins are very similar. The second and third zinc fingers of Sp1 are strongly homologous to the fourth and third zinc fingers of

DISCUSSION

Many transcription factors, including Sp1 and MAZ, have zinc finger motifs and can bind to the GC-rich cis-elements that are widely distributed in the promoters, enhancers, and locus-control regions of housekeeping genes as well as tissue-specific genes (21). The consensus sequence of Sp1-binding sites is very similar to that of MAZ-binding sites, and both types of sites are often present in the same gene. We examined the DNA binding activities of the two factors in gel shift assays (Fig. 1) and found that Sp1 bound to consensus Sp1-binding sites as well as to consensus MAZ-binding sites. Similarly, MAZ bound to the consensus binding sites for both MAZ and Sp1. The results of the gel shift assays indicated that both Sp1 and MAZ recognized the same cis-elements. We also performed DNase I footprinting assays to determine whether Sp1 and MAZ might share DNA-binding sites. We used a nuclear extract of HeLa cells, purified GST-Sp1, and purified GST-MAZ for these assays. All of the sixteen putative binding sites for Sp1 and MAZ in the probe were protected from nucleolytic digestion by the GST-MAZ fusion protein, and 13 of the 16 putative binding sites for Sp1 and MAZ were protected by the GST-Sp1 fusion protein (Fig. 2B). The patterns of protection obtained with purified GST-Sp1 and GST-MAZ were almost the same as that

obtained with a nuclear extract of HeLa cells (Fig. 2A), indicating that Sp1 and MAZ shared the same DNA-binding sites.

It has been reported that a number of transcription factors can bind to the same GC-rich DNA-binding sites, and functional interference by Sp1 with NF-κB at the same DNA-binding site has been reported (22). It has also been reported that Sp1 binds to a variety of GC-rich nucleotide sequences as well as to the consensus Sp1-binding site (23). Our observations that MAZ and Sp1 share binding sites indicate that the regulatory activity associated with some GC-rich elements is consistent with cooperative interactions by multiple transcription factors, such as zinc finger proteins, at the same or overlapping DNA motifs.

The numbers of zinc finger motifs in zinc finger proteins vary from only two to more than a dozen (24–27). It is possible that some but not all of the multiple zinc fingers are required for the binding to cis-elements. In view of the physical space available at cis-elements and the size of a zinc finger motif, it seems reasonable to postulate that two consecutive zinc fingers might be essential for direct interaction with a cis-element while additional zinc fingers might play an auxiliary role in the interaction or might be involved in cooperation with other factors. For example, the Ikaros protein contains six zinc fingers; the first four zinc fingers are involved in interactions with a cis-element, while the two carboxyl-terminal zinc fingers are involved in homodimerization of the protein itself (24, 28). We observed similar results for transcription factors Sas3 (29) and RIP60 (30).

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**Fig. 5. Schematic representation of the binding to DNA of MAZ, Sp1, and variants of the two proteins.** A, models of the Sp1 complex and the Sp1-X complex with DNA were superimposed by aligning the α carbons of fingers and the phosphorus and C1’ atoms of the corresponding binding subsites. The structure of Sp1 is shown in violet and the targeted DNA subsite is shown in blue. For Sp1-X, the color code is yellow and green. Panels B and C show that Sp1 makes contact with the target DNA via hydrogen bonds. The targeted DNA in B contains a MAZ-binding site, and that in C contains an Sp1-binding site. D, models of the MAZ complex and the MAZ-X complex with DNA are superimposed. The structures of the MAZ and MAZ-X are shown in *violet* and *yellow*, respectively. The corresponding targeted DNA subsites are shown in blue and green, respectively. Panels E and F show that MAZ makes contact with the target DNA via hydrogen bonds. The target DNA in E contains a MAZ-binding site, and that in F contains an Sp1-binding site. Hydrogen bonds are indicated by *yellow bars* in B, C, E, and F. The models were generated with the Molecular Modeling Software SYBYL (TRIPOS Associates, Inc., St. Louis, MO).
MAZ, respectively (Fig. 3A). In our analysis by gel shift assays of mutant GST-Sp1 and GST-MAZ fusion proteins with deletions of zinc fingers, we found that GST-Sp1 no longer bound to the Sp1-binding site or to the MAZ-binding site when the second and third zinc fingers had been deleted (Fig. 3D). Similarly, the DNA binding activity of the GST-MAZ fusion protein decreased when the third and/or the fourth zinc finger of MAZ had been deleted (Fig. 3C). These results indicated that the second and third zinc fingers of Sp1 and the third and fourth zinc fingers of MAZ were necessary for the DNA binding activity of the respective proteins. Because two adjacent zinc fingers in both Sp1 and MAZ were required for binding to GC-rich DNA sequences, we exchanged the pairs of consecutive zinc fingers between GST-Sp1 and GST-MAZ (Figs. 3D, 4, E and F). The binding of the chimera GST-Sp1/MAZ to both Sp1- and MAZ-binding sites was unchanged after the pairs of zinc fingers had been exchanged with one another, indicating that the pairs of consecutive zinc finger motifs could substitute for one another. Moreover, the extent of the repression of the MAZ-CAT reporter was similar with the reciprocal combination of another. Moreover, the extent of the repression of the MAZ-binding sites was unchanged after the pairs of zinc fingers had been exchanged (Fig. 4). These results indicated that the pairs of zinc fingers of Sp1 and MAZ can be interchanged without loss of the ability to repress transcription (Figs. 4, E and F). We do not know the exact roles in vivo of Sp1 and MAZ in the control of transcription of the target genes with GC-rich elements. Our transcription experiments indicated that the extent of regulation of expression of a target gene with GC-rich elements by Sp1 and MAZ is dose-dependent. We cannot rule out the possibility that each factor might function at different stages of the cell cycle and might recruit different cofactors to the same cis-elements. Further studies are required for a full understanding of the molecular mechanism of action of Sp1 and MAZ in vivo, but it is clear that two consecutive zinc finger motifs in Sp1 and in MAZ are essential for the binding of these proteins to DNA and for the coordinated repression of transcription.

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