Activation of the Adenovirus Major Late Promoter by Transcription Factors MAZ and Sp1

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Multiple binding sites for the transcription factors MAZ and Sp1 within the adenovirus type 5 major late promoter have been identified by DNase I protection studies. In the proximal region of the promoter, both MAZ and Sp1 interact with GC-rich sequences flanking the TATA box. Two MAZ binding sites are centered at −18 and −36 relative to the transcriptional initiation site. Sp1 bound only to the −18 GC-rich sequence. Several sites of interaction were also evident in the distal region of the promoter. Both MAZ and Sp1 interacted with a sequence centered at −166, and MAZ bound weakly to an additional site centered at −130. Overexpression of MAZ or Sp1 activated expression from the major late promoter in transient expression assays. Mutational analysis of the GC-rich sequences in the major late promoter suggested that a primary target of MAZ activation is the GC-rich sequences flanking the TATA sequence, whereas Sp1 requires the distal GC-rich sequence elements to stimulate gene expression. This activation is enhanced by the adenovirus E1A protein, and evidence for interaction between E1A and both transcription factors was obtained by using an immunoprecipitation assay. Activation by MAZ and Sp1 also was observed in transfection studies using the complete adenovirus type 5 genome as the target. Increased levels of late mRNA from both the L1 and L5 regions were observed when MAZ or Sp1 expression plasmids were transfected with viral DNA. Unexpectedly, activation of the major late promoter by MAZ and Sp1 was detected irrespective of whether the viral DNA could replicate.

The adenovirus major late promoter (MLP) controls expression of the major late transcription unit that encodes most of the viral structural proteins and several nonstructural proteins (reviewed in reference 22). The MLP is active during both early and late periods of infection but reaches maximal activity after the onset of DNA replication. Genetic and biochemical studies have identified a number of transcription factor binding sites and corresponding DNA-binding proteins that regulate expression from the MLP. These include the TATA box-binding protein (TBP) and the TFIID complex that bind the TATA element, the USF/MLTF binding site at −50, a CAAT box near −70, an initiator site at +1, and downstream elements that bind to a protein complex that includes cellular factors and the viral IVa2 protein (reviewed in reference 22). Most of these factor binding sites are conserved in the MLPs of divergent adenovirus serotypes, supporting the conclusion that these sites are important for appropriate transcriptional regulation (Fig. 1 and reference 25).

An interesting architectural feature of the MLP is the presence of GC-rich sequences surrounding the TATA box (Fig. 1). These sequences are well conserved in human adenoviruses as well as some other adenoviruses (Fig. 1 and reference 25), which implies a functional importance of the sequences to the MLP. Although the GC-rich elements can be extensively substituted with AT base pairs without inhibiting activity of the major late promoter in a whole-cell extract (29), mutations in the upstream TATA-proximal GC-rich element reduced the activity of the MLP in virus-infected cells (3). Further, Yu and Manley (30) found that the TATA-proximal GC-rich sequences formed nuclease-sensitive structures when the MLP was present in supercoiled plasmid DNA, but the physiological significance of this observation is not clear.

We have been interested in the transcription regulation of GC-rich promoters by the zinc finger proteins MAZ and Sp1 (20). Since the GC-rich sequences in the MLP are potential binding sites for MAZ and Sp1, we have examined the ability of these factors to interact with the promoter and regulate its activity. Our results suggest that both factors can interact with the GC-rich sequences in the MLP, stimulate MLP activity, and respond to the E1A protein.

MATERIALS AND METHODS

Plasmids, viruses, and cells. Expression plasmids that produce influenza virus epitope-tagged MAZ and Sp1 were previously described (20). The 289-amino-acid residue E1A protein cDNA (13S E1A) was expressed from the cytomegalovirus (CMV) promoter (25). An epitope-tagged YY1 expression plasmid was prepared by generating a cDNA clone that included the first leader and part of the major late start site cloned into pGL2-basic. These constructs were prepared by PCR using pBl DNA polymerase (Stratagene). The promoter fragment was cloned into the luciferase reporter plasmid pGL2-basic (Promega). Minimal MLP constructs contain sequences from −48 to +11 relative to the major late start site cloned into pGL2-basic. These constructs were prepared by cloning double-stranded oligonucleotides into the luciferase vector. A plasmid that supplied hybridization probes for detection of major late L1 RNA 5′ ends was prepared by generating a cDNA that included the first leader and part of the second leader. This cDNA was fused to promoter sequences from −260 to +1 and cloned into vector pSP72 (Promega). A genomic DNA clone containing part of the L5 region was prepared by cloning the adenovirus type 5 (Ad5) DNA sequence from 89 to 92 map units into pGem3 (Promega).

The Ad5 E1A-minus mutant, d512 (11), was propagated in 293 cells which express the E1A protein (6), and viral DNA was prepared from purified virus as described earlier (19). Infections were performed at a multiplicity of 20 PFU/cell.

HeLa cells were maintained in Dulbecco's minimal essential medium supplemented with 10% Fetal Clone II serum (HyClone Laboratories). 293 cells were grown in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum (HyClone Laboratories).

Expression assays. HeLa and 293 cells were transfected by the calcium phosphate precipitation method, harvested, and processed for luciferase assays as described earlier (20). A modified protocol was used when viral DNA was transfected into 293 cells (24). Viral DNA and expression plasmid were combined, and the solution was adjusted to a final concentration of 0.3 M CaCl₂ in a total volume of 1 ml. To form the precipitate, 1 ml of HEPES-buffered saline

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MAZ and Sp1 cooperate with E1A to activate the MLP.

After establishing that the GC-rich sequences in the MLP interacted with MAZ and Sp1, we tested whether the transcription factors affected the activity of the promoter. To do this experiment, we used transient expression assays to examine the effect of overexpression of MAZ or Sp1 on the activity of an MLP reporter plasmid. Sequences from −260 to +11 relative to the major late start site were cloned into a luciferase reporter plasmid, and this construct was cotransfected with expression plasmids that encoded epitope-tagged MAZ or Sp1. We also examined the effect of overexpression of the 286-amino-acid residue E1A activator protein encoded by the Ad5 135 mRNA (reviewed in reference 22). MAZ increased luciferase activity by a factor of 40 to 50, whereas E1A or Sp1 provided a more modest increase of 4- to 10-fold (Fig. 3A).

Interestingly, when MAZ and E1A were cotransfected, the effect of the two proteins was multiplicative, yielding a 200-fold increase over the value observed with vector alone. Similarly, the combination of E1A and Sp1 produced very large increases that approached 200-fold in some experiments.

To confirm that MAZ and Sp1 were being produced from the transfected plasmids, we analyzed cell extracts for the presence of the epitope-tagged proteins by Western blot assay. Both proteins were expressed (Fig. 3B, lanes 2 and 5), and we also noted that there was a reproducible strong enhancement of Sp1 expression in cells that also received E1A (Fig. 3B, lane 6). This increase in the level of Sp1 may contribute to the reporter activation detected in cells transfected with E1A plus Sp1. Expression of E1A had negligible effects on the level of MAZ protein expression (Fig. 3B, lane 4).

We measured the steady-state level of luciferase RNA (Fig. 3C) to be certain that the activation by MAZ or Sp1 and the combined effect with E1A were due to increased RNA accumulation from the MLP. Quantification of total RNA from transfected cells by hybridization and nuclear S1 digestion produced results that were in good agreement with the results from the transient expression assays. Luciferase RNA levels were undetectable in cells transfected with the reporter gene and the empty expression vector (Fig. 3C, lane 2). Similarly, cotransfection with E1A alone or Sp1 alone did not provide the level of stimulation necessary to detect luciferase RNA (Fig. 3C, lanes 4 and 6). This result was consistent with those of the transient assays that indicated that E1A or Sp1 alone activated the reporter to a relatively modest extent (Fig. 3A).

The stimulation by MAZ was greater in the luciferase assay, and this was also true for detection of the mRNA (Fig. 3C, lane 3). A band of about 75 nucleotides is clearly evident, and this was also true for detection of the mRNA (Fig. 3C, lane 3). A band of about 75 nucleotides is clearly evident, and the size is consistent with correctly initiated mRNA derived from the MLP-luciferase expression plasmid. Furthermore, just as predicted from the luciferase assays, the combined effects of MAZ plus E1A or Sp1 plus E1A produced the largest increase...
FIG. 2. Analysis of DNA-protein interactions in the MLP by DNase I protection. (A) Increasing amounts of MAZ protein were incubated with an MLP fragment spanning nucleotides +47 to -260 relative to the start site that was end labeled with 32P at nucleotide +47. After limited digestion with DNase I, the footprint reaction products were processed and electrophoresed in a sequence gel next to a GA sequencing ladder. Bars at the sides of the autoradiograms highlight the regions of protection. Black and grey bars represent strong and weaker MAZ binding sites, respectively. Nucleotide positions relative to the start site are indicated at the left. (B) Experiment performed as described above, but also including footprint reactions containing Sp1 protein (hatched bars). (C) Summary of footprinting experiments that show the binding sites for MAZ and Sp1. Data were scanned and cropped by using Ofoto software, and figures were prepared by using Canvas 3.5 software.
in RNA levels (Fig. 3C, lanes 5 and 7), generating about three- to fourfold more reporter RNA than when only MAZ was expressed with the reporter gene.

The combined effect of E1A and MAZ or E1A and Sp1 suggested that E1A might interact with these zinc-finger proteins, and an earlier study has shown that Sp1 and E1A can form a complex in vitro (16). To confirm the earlier result with Sp1 and test for the possible interaction of E1A with MAZ, we performed immunoprecipitation experiments. Vectors expressing influenza virus epitope-tagged MAZ or Sp1 expression vectors were transfected into HeLa cells in the absence or presence of E1A. Protein from extracts of transfected cells was immunoprecipitated with anti-influenza virus epitope tag antibody, subjected to electrophoresis in a sodium dodecyl sulfate-polyacrylamide gel, and blotted to nitrocellulose. Duplicate Western blots were then probed with either an anti-E1A or anti-epitope tag antibody. The antibody to the epitope tag demonstrated that MAZ and Sp1 were immunoprecipitated from the transfected cells (Fig. 3D, left panel). In agreement with earlier in vitro results, the antibody to E1A showed that E1A was coprecipitated with Sp1 (Fig. 3D, right panel, lane 1).

In extracts of cells transfected with MAZ and E1A, it is evident that some E1A coprecipitates with MAZ (Fig. 3D, right panel, lane 3), although substantially less E1A is coprecipitated with MAZ than MAZ and E1A (Fig. 3D, right panel, lane 2).
MAZ than with Sp1. This result might indicate that the MAZ-E1A interaction is less stable to the immunoprecipitation conditions than the interaction between E1A and Sp1. However, it is likely that the reduced level of E1A coprecipitated with antibody to epitope-tagged MAZ reflects at least in part the substantially lower level of MAZ expression than of Sp1 expression in the transfected cells that received plasmids expressing the transcription factor plus E1A.

MAZ activates transcription through GC sequences flanking the TATA motif. The most intriguing DNA-protein interaction between the MLP and the GC-rich binding factors occurs at the −18 and −36 GC sequences immediately flanking the TATA box (Fig. 1). The footprints generated by MAZ or Sp1 in this region of the promoter actually span the TATA sequence (Fig. 2). We performed a mutational analysis to determine whether these GC-rich sequences participate in the activation of the MLP. A minimal MLP (−45 to +11) that included only the −36 GC sequence, the TATA element, the −18 GC sequence, and the initiator region was constructed, and mutant derivatives were produced (Fig. 4A) with multiple base-pair substitutions disrupting the −18 GC motif (M1), the −36 GC motif (M2), both GC motifs (M3), or both GC motifs as well as the TATA and initiator elements (M4). The effect of the mutations on DNA-protein interactions was examined by footprint analysis (Fig. 4B and C). On the wild-type minimal promoter, the pattern of interaction at the −18 and −36 GC sequences was identical to that observed for the full-length promoter; two MAZ binding sites and one Sp1 site were evident. Mutation of the −18 GC sequence (M1) reduced the size of the MAZ footprint consistent with disruption of one MAZ binding site, and the M1 mutation completely blocked interaction by Sp1. Thus, the −18 GC mutation confirms that MAZ interacts with two separate sites in the minimal promoter region and that a single Sp1 binding site is present. The −36 GC mutation (M2) reduced the size of the region protected by MAZ, confirming that the −36 GC sequence is also a MAZ binding site, but did not alter the Sp1 footprint. The dou-

FIG. 4. Effects of mutations in the GC-rich sequences flanking the TATA motif on MAZ and Sp1 binding. (A) Sequence of the wild-type (WT) minimal MLP and its mutant derivatives. (B) DNase I footprint analysis was performed to assay MAZ (B) and Sp1 (C) binding to wild-type and mutant MLPs. The probe DNA was 5’-end labeled in the luciferase coding region. The strong (black bars) and weak (white bars) MAZ footprints and the Sp1 footprint on wild-type DNAs are designated at the left. Sequence positions relative to the start site are shown next to the GA sequence reaction.

FIG. 5. Effects of MLP mutations on the activity of the minimal MLP. Luciferase reporter plasmids were prepared with the minimal promoter fragments shown Fig. 4A. (A) The in vitro transcription activity of wild-type (WT) and mutant MLPs was assayed in a whole-cell extract. Reaction products were analyzed by primer extension and denaturing polyacrylamide gel electrophoresis. The template DNAs used in the transcription reactions are indicated above the lanes. Migration of the 75-base marker is indicated at the left, and the MLP-specific band is marked by an arrow. (C) Transfection experiments using wild-type and mutant MLP luciferase plasmids. Plasmids (0.2 μg) were transfected into 293 cells with effector plasmids (1 μg) expressing MAZ (grey bars) or Sp1 (hatched bars). Activation was calculated from seven independent experiments.
ble-GC sequence mutation (M3) substantially blocked the ability of both MAZ and Sp1 to interact with the promoter.

To test the effects of these mutations on promoter activity, supercoiled template DNAs carrying the promoter variants were used to direct in vitro transcription in a whole-cell extract, and reaction products were assayed by primer extension. Mutations in the GC sequences reduced the efficiency of transcription (Fig. 5A, lanes 2 to 5). Mutation of the −18 sequence (M1) reduced transcription by a factor of about 2 relative to the wild-type promoter, and mutation of the −36 GC sequence (M2) reduced transcription about threefold. Mutation of both GC sequences (M3) produced a more significant reduction of fivefold. Transcription reactions programmed with a promoter carrying mutations in both GC sequences, the TATA box, and initiator (M4), with the vector without a promoter sequence or with no template DNA, did not produce detectable product (Fig. 5A, lanes 6 to 8).

We also examined the ability of overexpressed MAZ and Sp1 to activate the minimal promoter and its mutant derivatives in transfected cells. 293 cells were used in this assay since they contain the adenovirus E1A protein and both MAZ and Sp1 very strongly activate the MLP in the presence of the viral transcriptional activator (Fig. 3A). Cells were transfected with each MLP construct together with an effector plasmid expressing influenza virus epitope-tagged MAZ or Sp1. The GC mutations affected activation by MAZ but had relatively little effect on the modest activation by Sp1 (Fig. 5B). Either single GC mutation (M1 or M2) had little effect on activation by MAZ, but when both GC mutations were present (M3), activation by MAZ was reduced to a factor of about 10 to 15, compared to 30- to 50-fold for the wild-type minimal promoter. The MLP with mutations in all of its motifs (M4) and the promoterless luciferase plasmid exhibited a fivefold activation by MAZ. This activation, as well as the consistent two- to threefold activation of all constructs by Sp1, is probably due to GC-rich sequences in the luciferase vector residing outside the MLP.

The failure of Sp1 to activate the minimal promoter through the GC sequences flanking the TATA motif (Fig. 5B) suggests that Sp1 acts through its upstream binding site centered at −166 (Fig. 2) to influence transcription of the MLP. Consistent with this proposal, Sp1 cooperated with E1A to strongly activate a reporter that contained this upstream GC element (Fig. 3A).

**Activation of the MLP residing in the viral genome by MAZ and Sp1.** To further test the capability of MAZ and Sp1 to activate the MLP, we examined activation of the MLP from within the viral genome. In this case, additional upstream or downstream sequences not present in plasmid constructs might influence activity of the promoter, other viral gene products might affect its regulation, and viral DNA replication could influence its activity. Transfection of the viral DNA molecule, rather than infection with virus, was used so that the effects of added MAZ and Sp1 could be effectively monitored by cotransfection with expression vectors. 293 cells were transfected with adenovirus DNA under conditions that allowed DNA replication to occur or in the presence of hydroxyurea, which blocked DNA replication. RNA was harvested at 48 h after infection, and total RNA was isolated. The RNA was hybridized to a 32P-end-labeled probe designed to detect the 5’ end of L1 RNA (A) or RNA from the L5 region (B). The presence (+) or absence (−) of hydroxyurea (HU) during the 48-h transfection period is indicated. The sizes (in nucleotides) of marker DNAs are indicated on the left. Negative control RNA was prepared from mock-transfected cells, and positive control RNA was isolated from cells infected with adenovirus at a multiplicity of 20 PFU/cell. (C) Replication of transfected adenovirus DNA. Viral DNA was harvested at 72 h after transfection by the Hirt procedure and analyzed by Southern blotting. A 32P-labeled riboprobe specific for the Ad5 HindIII E fragment was used as the hybridization probe.

As predicted by the experiments using reporter plasmids (Fig. 3A and 5B), cotransfection of genomic viral DNA with plasmids expressing MAZ or Sp1 stimulated expression from the MLP. The level of L1 RNA was increased two- to fivefold by both MAZ and Sp1 (Fig. 6A, lanes 1, 3, and 5). The addition of hydroxyurea markedly inhibited the accumulation of viral DNA (Fig. 6C) as well as L1 RNA (Fig. 6A; compare lanes 1 and 2), consistent with the reduced activity of the major late promoter in infected cells before the onset of DNA replication (reviewed in reference 20). In the presence of the drug, MAZ or Sp1 stimulated the accumulation of L1 RNA by as much as
a factor of 17 (Fig. 6A, lanes 4 and 6). MAZ and Sp1 produced similar effects, consistent with results of the transient assays using luciferase reporters containing the more complete (−260 to +11) MLP (Fig. 3A). The level of L1 RNA in cells cotransfected with genomic DNA plus MAZ or Sp1 was very high, comparable to the amount that accumulated in 293 cells infected with Ad5 at a multiplicity of 20 PFU/cell (Fig. 6A, lane 8).

The transcription factors also stimulated transcription through the L5 region of the major late transcription unit. L5 RNA accumulation was substantially blocked by hydroxyurea in cells receiving the viral genome without the MAZ or Sp1 expression plasmid (Fig. 6B, lane 2). Hydroxyurea treatment also blocked L5 RNA accumulation in infected cells (data not shown). This block is consistent with earlier work showing that only the 5′ proximal domain of the major late transcription unit (L1 and L2) is transcribed in the absence of viral DNA replication (reviewed in references 17, 22, and 27).

When MAZ was cotransfected with viral DNA, there was a moderate increase in L5 RNA accumulation in the absence of hydroxyurea and a strong stimulation of L5 RNA accumulation when DNA synthesis was blocked with the drug (Fig. 6B, lanes 3 and 4). Sp1 did not stimulate L5 RNA accumulation as effectively as MAZ in the absence of DNA replication (Fig. 6B, lane 6), and L5 RNA levels from transfected DNA, even the presence of MAZ, were substantially less than the levels achieved after infection (Fig. 6B, lane 8). Finally, as a control, we tested for activation of the MLP by an expression plasmid that encoded YY1, another zinc finger protein (23). There is no known role of DNA replication in the activation of this promoter is unclear (reviewed in reference 22). Conceivably, MAZ and possible that Sp1 family members influence MLP activity through these sequences.

Earlier work had demonstrated that Sp1 and E1A interact in vitro (16). Activation of the MLP residing in the viral genome by MAZ or Sp1 was most pronounced when DNA replication was blocked by hydroxyurea (Fig. 6). This may mean that overexpression of MAZ or Sp1 can substitute for the MLP activation function normally mediated by DNA replication. So far, the role of DNA replication in the activation of this promoter is unclear (reviewed in reference 22). Conceivably, MAZ and Sp1 function as a normal part of the transcriptional activation mechanism that depends on DNA replication. Replication might generate genomic templates that are more accessible to MAZ and Sp1, and the increased recruitment of these factors in turn could help to attract the other components of a transcription initiation complex. A higher concentration of MAZ or Sp1, coupled with the delivery of naked DNA to the cell by transfection, might eliminate the need for a more easily accessible template and compensate for the inhibition of DNA replication by hydroxyurea. We were surprised that overexpression of MAZ, and to a more limited extent Sp1, enhanced the accumulation of L5 RNA synthesis in the absence of DNA replication (Fig. 6). Normally, DNA replication is a prerequisite for transcription of the distal portion of the major late transcription unit that includes the L5 region, but the mechanism controlling the extent to which the unit is transcribed remains obscure (reviewed in reference 22). The observation that activation of the MLP in the absence of DNA replication leads to the accumulation of L5 RNA suggests that full-length transcription might simply be a mass action effect; i.e., as the promoter becomes more active and more molecules of RNA polymerase begin to transcribe the unit, then more molecules succeed in traveling to the end of the unit, producing L5 RNA.

Yu and Manley (29) examined the transcriptional activity in HeLa whole-cell extracts of an extensive set of MLP derivatives containing base-pair substitutions in the GC-rich elements flanking the TATA motif. Several of their variants with multiple G-to-A transitions in the GC-rich sequences exhibited wild-type activity in the cell-free assay. In contrast, our substitution mutants, which prevented MAZ and Sp1 binding to the GC-rich elements (Fig. 4), were somewhat (as much as 2.5-fold) less active than the wild-type minimal MLP. There are several possible explanations for these apparently conflicting results. Different mutations were assayed in the two studies, and it is not known whether the mutations analyzed in the earlier experiments blocked binding of MAZ and Sp1. The different results might also result from the use of different MLP segments in the in vitro transcription assays: the earlier study used a sequence from −66 to +193, and our experiments used the sequence from −45 to +11. Factors that bind within the larger segment of the MLP, but do not have access to the minimal MLP, could obscure the effect of mutations in the GC-rich sequences that flank the TATA motif.

Brunet et al. (3) studied the effects of mutations within the GC-rich elements flanking the TATA motif on the adenovirus chromosome within infected cells. Although multiple G-to-A transitions in the GC-rich sequence downstream of the TATA element had no observable effect, substitutions in the upstream GC-rich region reduced the activity of the MLP by a factor of 2 to 6. Thus, our results with a minimal MLP (Fig. 5), as well as results of a mutational analysis of the MLP on the viral genome (3), argue that GC-rich sequences adjacent to the TATA motif contribute to the full activity of the MLP.

Do these GC elements contribute to MLP activity by serving as binding sites for MAZ and Sp1? Overexpressed Sp1 does not activate a minimal MLP, but it is possible that Sp1 is not limiting in 293 cells, and for this reason added Sp1 does not influence activity of a minimal MLP reporter. Also, other members of the Sp1 family (7, 13) might play a role in the activation. MAZ clearly activates the minimal MLP (Fig. 5B), and so it is likely that MAZ and possible that Sp1 family members influence MLP activity through these sequences.

When MAZ is bound to the GC-rich sequences centered at −18 and −36, its DNase I footprint overlaps the TATA motif (Fig. 2 and 4B). Further, when the TFIID-TFIIB-TFIIB complex interacts with the promoter during the formation of an initiation complex, TFIIB interacts with the promoter DNA both upstream and downstream of the TATA sequence (5, 18, 26). In the case of the MLP, these contacts would occur
within the GC-rich sequences at which MAZ resides. It is possible that MAZ, TFIIA, and TFIIIB are able to contact these domains of the MLP simultaneously. Our attempts to demonstrate a simultaneous interaction of these factors with the MLP have so far produced equivocal results. It is also conceivable that when MAZ interacts with the GC-rich sequences flanking the TATA motif, TBP might be excluded from binding directly to the promoter DNA. In this case, TFIIID could be brought to the promoter through protein-protein interactions. It is noteworthy that two single base-pair changes in the TATA motif reduced but did not fully block the expression of properly initiated transcripts from the MLP in infected cells (21). Perhaps TFIIID is brought to the promoter exclusively through its interaction with MAZ and Sp1 in this mutant virus. We have previously postulated that MAZ might bring TFIIID to promoter sequences in the absence of identifiable TATA motifs in the serotonin 1A receptor, where MAZ and Sp1 sites are found near a series of transcriptional start sites that do not appear to have corresponding TATA elements (20). The potential for MAZ, and perhaps Sp1 family members, to direct TFIIID to the major late promoter in the absence of a direct TBP-DNA interaction raises the intriguing possibility that two alternative mechanisms of initiation operate at the MLP. One mode of initiation would involve direct binding of TFIIID to the TATA motif, and the other would depend on protein-protein interactions to bring TFIIID to a promoter containing bound MAZ or Sp1.

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