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Sp1 activation of a TATA-less promoter requires a species-specific interaction involving transcription factor IID

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ABSTRACT

Sp1 is a ubiquitous activator of numerous TATA-containing and TATA-less promoters within the human genome. This transcription factor is distinct from several other mammalian activators because it cannot stimulate transcription of reporter genes when ectopically expressed in *Saccharomyces cerevisiae*. Here we report that in cultured cells from *Drosophila melanogaster* human Sp1 efficiently activates transcription from synthetic promoters containing TATA boxes, but not from promoters that contain an initiator instead of a TATA box. The inability of Sp1 to activate initiator-mediated transcription did not result from inactivity of the consensus initiator element used for the experiments, as other initiator functions were conserved in *Drosophila* cells. Interestingly, a difference between the *Drosophila* and human TFIID complexes was found to be responsible for the selective inability of Sp1 to activate initiator-mediated transcription in *Drosophila*; in a complementation assay with a TFIID-depleted HeLa cell extract both the *Drosophila* and human TFIID complexes supported TATA-mediated transcription, but only the human complex supported initiator-mediated transcription. These results suggest that a species-specific interaction is required for activation of TATA-less promoters by Sp1, revealing a difference in transcriptional activation mechanisms between vertebrates and invertebrates.

INTRODUCTION

Sp1 was isolated from HeLa cell extracts over 12 years ago as a sequence-specific DNA binding protein capable of activating transcription from the simian virus 40 (SV40) early promoter in a cell-free assay (1,2). Subsequent studies demonstrated that Sp1 contributes to activation of numerous viral and cellular genes (3–5). Because it was the first sequence-specific activator of pol II genes identified and because of its widespread role in regulating transcription the functional properties and biochemical activities of Sp1 have been analyzed in considerable detail.

The DNA binding activity of Sp1 is mediated by a zinc finger region near the C-terminus of the protein and transcriptional activation involves four distinct domains (6–8). The A and B activation domains near the N-terminus each contain a glutamine-rich region and an adjacent region rich in serines and threonines. When targeted to a promoter by a heterologous DNA binding domain these domains strongly activate transcription (see for example 9,10). The C and D activation domains, which flank the zinc finger region in the C-terminal half of the protein, activate transcription poorly by themselves, but augment the activities of the A and B domains (8). The D domain also acts in concert with the A and B domains to direct formation of Sp1 multimers, which are essential for synergistic activation of transcription (11–14).

During the past few years progress has been made towards an understanding of the mechanism by which Sp1 stimulates transcription. Like many activators Sp1 requires the transcription factor IID (TFIID) complex for efficient stimulation of transcription *in vitro* (15–18). Furthermore, Sp1 can directly stabilize binding of TFIID to core promoter elements (19). Physical interactions have been detected between Sp1 and three components of human TFIID: TBP, $hTAF_{II}130$ and $hTAF_{II}55$ (9,20–23). TBP and $hTAF_{II}130$ interact with the glutamine-rich domains of Sp1, whereas $hTAF_{II}55$ binds to the C-terminus (9,20–23). The functional relevance of the Sp1–$hTAF_{II}130$ interaction has been supported by *in vitro* transcription experiments with reconstituted *Drosophila* TFIID subcomplexes, in which the *Drosophila* homolog of $hTAF_{II}130$, $dTAF_{II}110$, was found to play an essential role during Sp1 activation (24,25). Sp1 may also interact with other components of the pol II preinitiation complex (26).

Further insight into the functions of the Sp1 activation domains was recently provided by an analysis of their abilities to activate transcription through core promoters with different structures (10). This analysis revealed that the isolated glutamine-rich domains, when fused to a heterologous GAL4 DNA binding domain, possess a strong preference for a core promoter containing an Inr element; activation was not detected if the core promoter contained only a TATA box. In contrast, full-length Sp1 activated transcription with equal efficiency through core promoters

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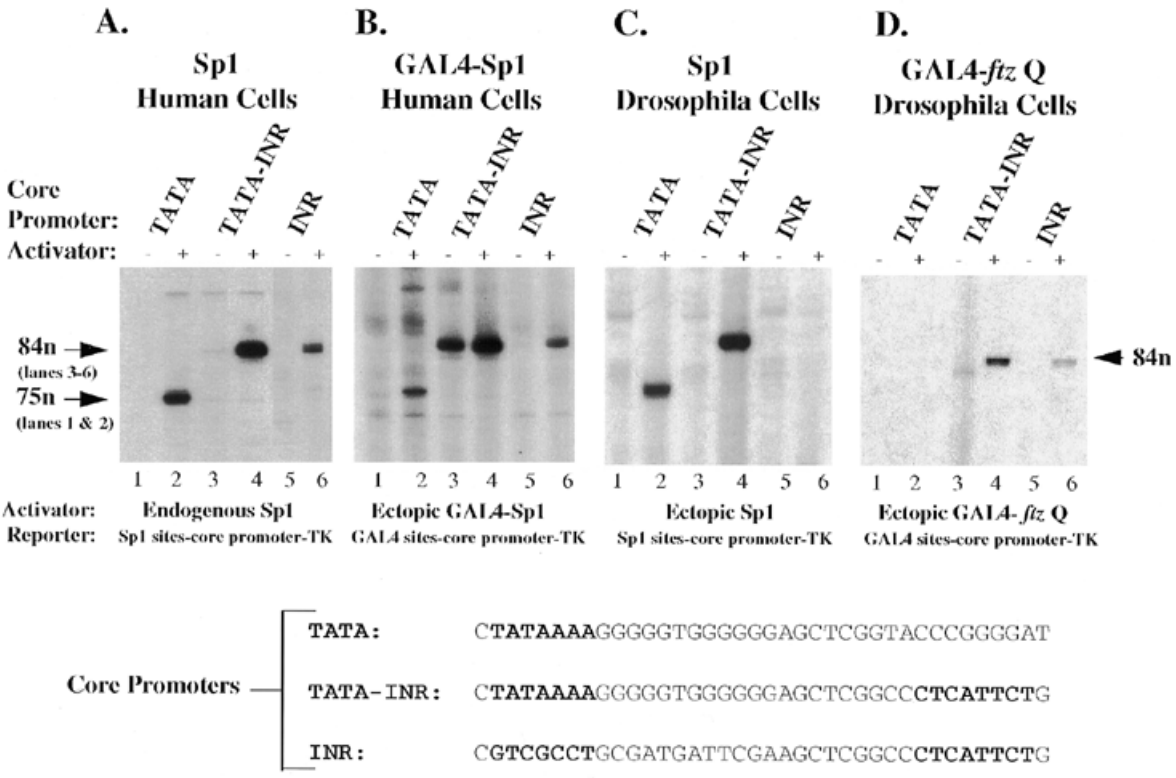

Figure 1. Species-specific activation of a TATA-less promoter by Sp1. Transient transfection experiments were performed with reporter plasmids containing multiple Sp1 or GAL4 binding sites upstream of the three core promoters shown at the bottom and a HSV-TK reporter gene. Specific RNA transcripts were analyzed by primer extension, yielding a 75 nt cDNA product for the TATA promoter and an 84 nt product for the TATA–Inr and Inr promoters. (**A**) Reporter plasmids containing (lanes 2, 4 and 6) or lacking (lanes 1, 3 and 5) Sp1 binding sites were analyzed by transient transfection and primer extension. (**B**) 293 cells were co-transfected with the three reporter plasmids containing GAL4 binding sites and with (lanes 2, 4 and 6) or without (lanes 1, 3 and 5) a GAL4–Sp1 (full-length) expression plasmid. (**C**) *Drosophila* SL2 cells were co-transfected with the three reporter plasmids containing Sp1 binding sites and with (lanes 2, 4 and 6) or without (lanes 1, 3 and 5) an Sp1 expression plasmid (pPacSp1). (**D**) *Drosophila* SL2 cells were co-transfected with the three reporter plasmids containing GAL4 bindings sites and with (lanes 2, 4 and 6) or without (lanes 1, 3 and 5) a GAL4–ftzQ expression plasmid.

containing either a TATA box or an Inr. Activation of a promoter containing only a TATA box apparently requires the combined functions of the N-terminal and C-terminal activation domains, revealing clear functional differences between the domains.

To investigate the mechanisms of Sp1 activation from different core promoters in greater detail we wished to ectopically express wild-type and mutant Sp1 proteins in cells that do not express an endogenous Sp1 protein. Since all mammalian cells express Sp1, we employed the *Drosophila melanogaster* SL2 cell line, which does not express the tissue-restricted *Drosophila* homolog of Sp1 (27). The SL2 cell line has been used successfully for several other studies of Sp1 (8,9,11,13), including the original analysis of the Sp1 activation domains. The unexpected results obtained from our analysis provide further insight into the mechanisms of transcriptional activation by Sp1, into the differences between TATA- and Inr-mediated transcription and into the evolution of the pol II transcription apparatus.

MATERIALS AND METHODS

Plasmids

The basic reporter plasmids used for the transient transfection assays have been described previously (10). These plasmids contain multiple binding sites for GAL4 or Sp1 upstream of three different core promoters (Fig. 1) and a herpes simplex virus thymidine kinase (HSV-TK) gene. The plasmids used for *in vitro* transcription experiments are the same as plasmids II (TATA; 15), IV (TATA/Inr; 15), I (weak TATA/Inr; 15) and VI-c (Inr; 28), except that the Sp1 sites were deleted from VI-c. The expression plasmids for the full-length and mutant Sp1 proteins have been described previously (8,13). For simplicity we renamed some of the deletion mutants, with the name used here corresponding to the deleted domain: $pPac_0$ is the expression vector lacking Sp1 sequences; ΔB, ΔA1, ΔA2, ΔBc, ΔBn, ΔD and ΔDc are ΔA0, C2-2E, pPack, D23, D9, N619 and N636 respectively (8,13). The M21 and M37 substitution mutants described previously (9) were inserted into the context of the ΔA2 protein. The GAL4–Sp1 expression plasmid (10), the GAL4–ftzQ expression plasmid (29,30), the plasmids with variable spacing between TATA and Inr (31), the reverse TATA–Inr plasmid (31) and the plasmids with different sequences 25 bp upstream of the TdT Inr element (28) have been described previously. All plasmids were purified by column chromatography (Qiagen Inc.).

Transient transfections

The human embryonic kidney cell line 293 and the *Drosophila* cell line SL2 were grown and transfected by the calcium phosphate method as described previously (8,10). The transfected human and

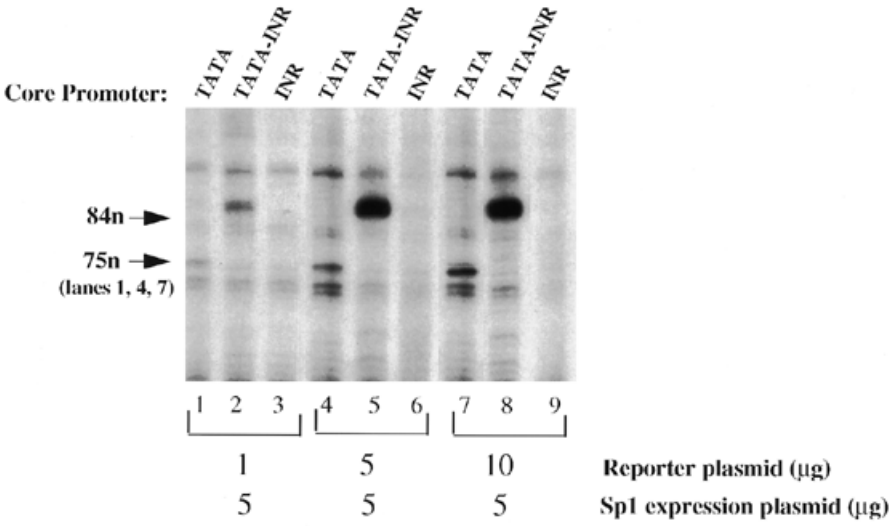

Figure 2. Titration of reporter plasmids in transfected *Drosophila* SL2 cells. SL2 cells were co-transfected with 5 μg pPacSp1 expression vector and with 1 (lanes 1–3), 5 (lanes 4–6) or 10 ug (lanes 7–9) of the three reporter plasmids containing Sp1 binding sites. Cytoplasmic RNA was isolated and analyzed by primer extension, yielding a 75 nt product with the TATA plasmid and an 84 nt product with the TATA–Inr and Inr plasmids.

Drosophila cells were incubated for 48 h at 37 and 25°C respectively and then total cytoplasmic RNAs were isolated by a Nonidet P-40 lysis method (28). Promoter strengths were analyzed by primer extension, using 30 μg RNA and a 20 nt primer complementary to a HSV-TK sequence (28).

In vitro transcription

In vitro transcription reactions with nuclear extracts derived from HeLa cells or *Drosophila* embryos (a kind gift from Dr Al Courey, UCLA) were performed as described previously (28,32) except the reactions with *Drosophila* extracts were incubated at 21°C. Each reaction contained 300 or 500 ng template and 100 μg extract. RNA transcripts were analyzed by primer extension as described (32). TFIID-depleted extracts were prepared by heat treatment of HeLa extracts at 47°C for 20 min (33). The epitope-tagged human and *Drosophila* TFIIDs were isolated by immunoaffinity chromatography as described (18,34).

RESULTS

Sp1 cannot efficiently activate transcription in *Drosophila* cells from an Inr-containing promoter

The human Sp1 protein efficiently activates transcription in mammalian cells from synthetic promoters containing multiple Sp1 binding sites and either a TATA box, an Inr or both TATA and Inr elements (10,15,28,32). This result is reproduced in Figure 1A. In addition, a protein containing the GAL4 DNA binding domain fused to full-length Sp1 stimulates transcription of reporter plasmids containing multiple GAL4 binding sites upstream of the TATA, Inr or TATA–Inr core promoters (10). This result is reproduced in Figure 1B. The reporter plasmids used for these experiments contain multiple upstream binding sites for Sp1 (Fig. 1A) or GAL4 (Fig. 1B) upstream of the three different core promoters (Fig. 1, bottom) and a HSV-TK reporter gene. Forty eight hours after transfection of human 293 cells with the reporter plasmids alone (Fig. 1A) or with the reporter plasmids and a GAL4–Sp1 expression plasmid (Fig. 1B) total RNAs were isolated and analyzed by primer extension. (It should be noted that Figure 1B was derived from a much longer exposure than Figure 1A, resulting in the appearance of unactivated transcription signals.)

Interestingly, when the same reporter plasmids were tranfected into the *Drosophila* SL2 cell line in the absence and presence of a well-characterized Sp1 expression plasmid (pPacSp1; 8) the relative promoter strengths observed were strikingly different from those observed in the human cells. Although the transcripts directed by the TATA and TATA–Inr promoters were easily detectable (Fig. 1C, lanes 2 and 4), the transcripts directed by the Inr promoter were undetectable (lane 6), even with long exposures (data not shown). The selective inability of Sp1 to activate Inr-mediated transcription was confirmed by transfection of SL2 cells with increasing concentrations of the reporter plasmid (Fig. 2) or with increasing concentrations of the Sp1 expression plasmid (data not shown). In more than 20 experiments a specific primer extension signal has never been detected with the Inr reporter plasmid, despite consistently strong signals with this reporter in human cell lines. Similar results were obtained following transfection of SL2 cells with an expression plasmid for Sp3, a human protein that is closely related to Sp1 and that binds to the same DNA sequence element (35; data not shown).

The inability of Sp1 to activate transcription from the Inr promoter could result from a defect in the Inr element itself or from a defect in the ability of Sp1 to activate Inr-mediated transcription. To distinguish between these possibilities a fusion protein containing the GAL4 DNA binding domain and a transcriptional activation domain from the *Drosophila ftz* gene was expressed in *Drosophila* SL2 cells (29,30). This protein efficiently stimulated transcription from the Inr promoter and the TATA–Inr promoter, but less efficiently from the TATA promoter (Fig. 1D). Preferential activation of Inr-mediated transcription has been observed previously in human cells with the isolated glutamine-rich domains of Sp1 (10) and with the Elf-1 transcription factor (36), but the mechanism underlying this preferential activation remains unknown. Nevertheless, efficient activation of

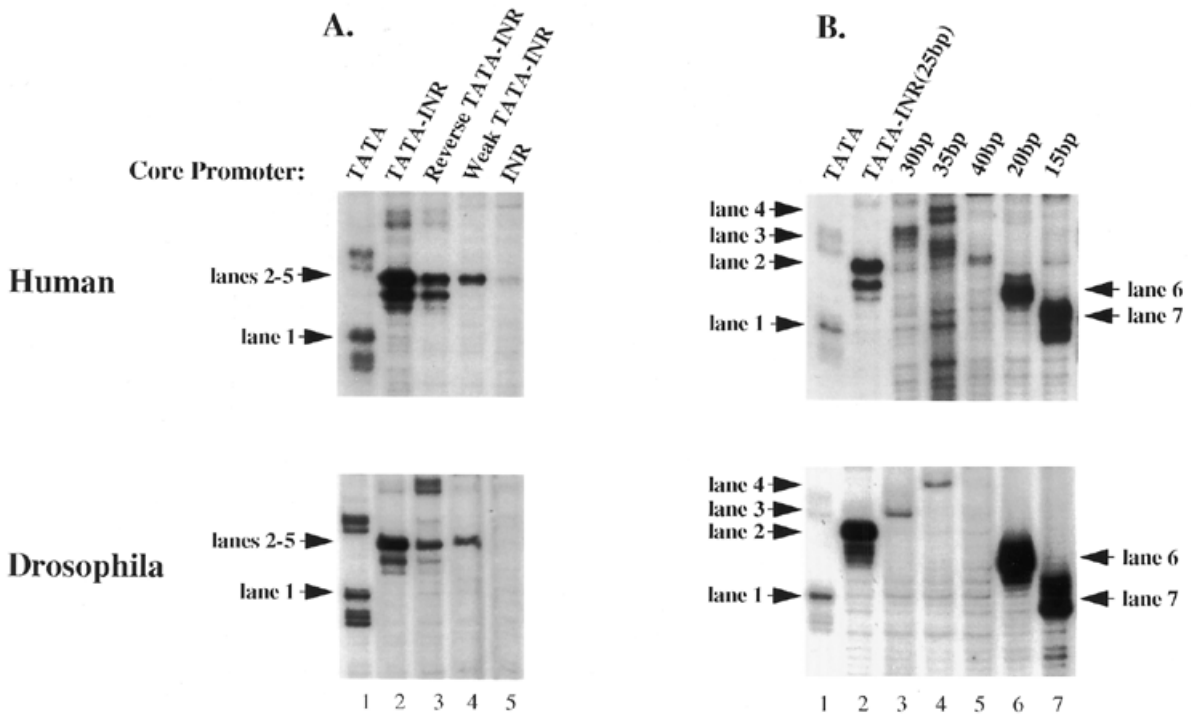

Figure 3. Similar properties of TATA and Inr elements in *Drosophila* and HeLa nuclear extracts. (**A**) *In vitro* transcription reactions were performed in nuclear extracts from HeLa cells (top panel) or *Drosophila* embryos (bottom panel). RNA products were analyzed by primer extension. Core promoters tested included a TATA box alone (lane 1), TATA–Inr (lane 2), a reverse TATA sequence, TTTATA, upstream of an Inr (lane 3), a weak TATA sequence, CATATG, upstream of an Inr (lane 4) and a GC-rich sequence (see Fig. 1) upstream of the Inr (lane 5). cDNA products were 70 (lane 1) or 79 nt (lanes 2–5). (**B**) *In vitro* reactions with promoters containing variable spacing between TATA and Inr were performed in HeLa (top panel) or *Drosophila* (bottom panel) extracts. Plasmids tested included a TATA box alone (lane 1) or TATA and Inr elements separated by 25, 30, 35, 40, 20 or 15 bp (lanes 2–7 respectively).

Inr-mediated transcription by the GAL4–ftz activator suggests that the Inr element functions perfectly well in *Drosophila* cells. Taken together, these results suggest that a species-specific defect in the Sp1 activation mechanism is responsible for the inability of Sp1 to activate the Inr promoter in SL2 cells.

The TdT Inr functions in *Drosophila* cells

The results in Figure 1D suggest that the TdT Inr functions properly in *Drosophila* cells. To confirm this hypothesis other properties of the consensus TdT Inr element were compared in human and *Drosophila* cells. To rule out potential contributions from specific activator proteins *in vitro* transcription experiments in nuclear extracts from HeLa cells and *Drosophila* embryos were employed. Unlike transfection experiments, the *in vitro* transcription experiments are sufficiently sensitive for detection of basal transcription directed by the isolated core promoter elements.

Figure 3A reveals that the basic properties of TATA and Inr elements are comparable in nuclear extracts from *Drosophila* and man. In both extracts the promoter containing the isolated TATA box led to the expected 70 nt cDNA product (Fig. 3A, lane 1; see also Fig. 3B, lane 1). [The bands above and below the specific TATA signal were observed in previous experiments (31) and are believed to be non-specific background bands.] The TATA–Inr promoter yielded the expected 79 nt product in both extracts (which is 9 nt longer than the 70 nt cDNA product because of the 9 bp Inr insertion). The TATA–Inr promoter was stronger than the TATA promoter in both extracts, although the degree of synergy between the TATA and Inr elements varied from experiment to experiment (Fig. 3A, lane 2; see also Fig. 3B, lanes 1 and 2). Specific transcription in both extracts was also directed by promoters containing the TdT Inr element downstream of weaker TATA sequences, one containing a reverse consensus TATA box, TTTATA (Fig. 3A, lane 3), and the other containing the sequence CATATG (Fig. 3A, lane 4). Very little transcription was directed by the promoter containing the TdT Inr element downstream of the G/C-rich sequence used for the experiments in Figure 1 (Fig. 3A, lane 5).

Another established property of TATA and Inr elements is that the synergy between them is dependent on spacing (31). As previously reported (31), strong synergy was observed in HeLa extracts when the two elements were separated by 25 bp (Fig. 3B, top panel, lane 2; the 25 bp refers to the distance from the TATAAA sequence to the transcription start site), but synergy was not observed when the elements were separated by 30, 35 or 40 bp (Fig. 3B, top panel, lanes 3–5). Furthermore, as previously reported (31), strong synergy was retained in HeLa extracts when the TATA and Inr elements were separated by 20 or 15 bp (Fig. 3B, top panel, lanes 6 and 7). These same properties were observed in *Drosophila* extracts (Fig. 3B, bottom panel), confirming that the synergistic properties of TATA and Inr elements are similar in *Drosophila* and man.

The evidence that the consensus TdT Inr functions in *Drosophila* cells is consistent with previous studies, which revealed that the *Drosophila* Inr consensus sequence is very similar to the mammalian consensus. The *Drosophila* Inr consensus was

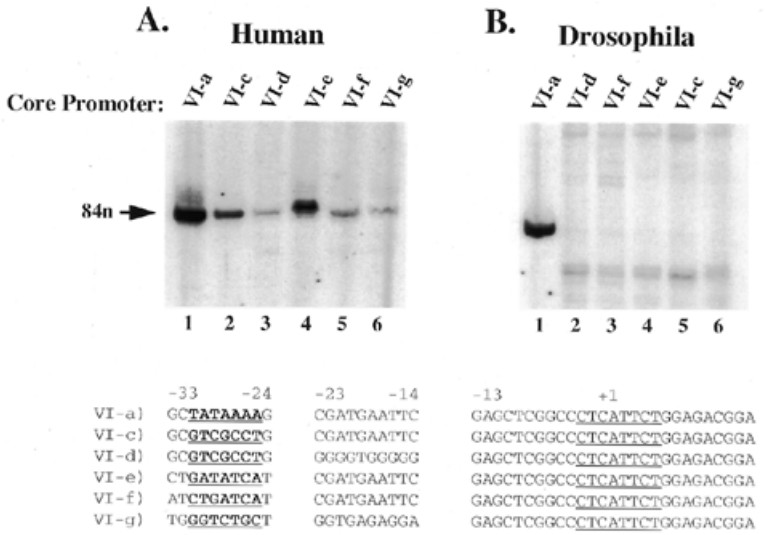

Figure 4. Sp1 activation in *Drosophila* cells requires a strong TATA box. (**A**) Reporter plasmids containing Sp1 binding sites upstream of the core promoters shown at the bottom were transfected into human 293 cells. RNA products were analyzed by primer extension. (**B**) The reporter plasmids containing Sp1 binding sites, an Inr and variable upstream sequences were co-transfected into *Drosophila* SL2 cells with the pPacSp1 expression plasmid. RNA products were analyzed by primer extension.

originally proposed by sequence comparisons of the start site regions from numerous *Drosophila* promoters (37) and by a demonstration that *Drosophila* TFIID selectively binds to this consensus sequence (38). More recently we showed that the functional Inr consensus sequences in *Drosophila* and man are indistinguishable, using an *in vitro* transcription assay in *Drosophila* embryo extracts with core promoters that contain a TATA box and numerous Inr mutants (39). Taken together, these results suggest that the inability of Sp1 to activate Inr-mediated transcription in *Drosophila* cells does not result from a fundamental difference in human and *Drosophila* Inr elements.

Transcriptional activation by Sp1 in *Drosophila* cells requires a strong TATA box

The results in Figure 1 reveal that Sp1 efficiently activates transcription in *Drosophila* cells from a promoter containing a consensus Inr combined with a strong TATA box, but not a consensus Inr lacking a TATA box (Fig. 1C). These results suggest that a strong TATA box is required for Sp1 activation. An alternative explanation, however, is that the G/C-rich sequence which replaces the TATA box in the –25 region of the Inr promoter strongly inhibits function of the *Drosophila* transcription machinery during Sp1-activated transcription. To distinguish between these possibilities promoters containing different sequences between –24 and –33 were analyzed in human and *Drosophila* cells (Fig. 4). As previously reported (28), all of the promoters direct efficient activated transcription following transfection into human 293 cells (Fig. 4A). The strengths of these promoters roughly correlate with the affinities of their upstream sequences for TBP (28). In striking contrast, *Drosophila* cells supported Sp1-activated transcription only from the promoter containing the strong consensus TATA box (Fig. 4B). These results demonstrate that the inability of Sp1 to activate Inr-mediated transcription in *Drosophila* is not restricted to promoters containing upstream regions with a high G/C content.

Functional domains of Sp1 required for activation of TATA and TATA–Inr promoters

Our original goal in studying Sp1 in *Drosophila* cells was to develop an assay for defining the Sp1 domains required for activation of TATA- and Inr-mediated transcription. The results described above demonstrate that we cannot use *Drosophila* cells for this purpose. However, since the Inr enhances Sp1-activated transcription when the core promoter includes a TATA box, the Sp1 domains required for activation of the TATA promoter can be compared with the domains that allow the Inr to enhance TATA-mediated transcription.

Figure 5 shows the relative strengths of the TATA and TATA–Inr promoters when activated by several Sp1 mutants (8,13). In general, the effects of these mutations with the Sp1–TATA–Inr and Sp1–TATA promoters were similar to the effects observed previously with reporter plasmids containing multiple Sp1 sites upstream of natural core promoters with TATA boxes (8,13). Interestingly, with each of the mutants the ability of the Inr to enhance promoter strength was not strongly affected. For example, relative to wild-type Sp1 the ΔB mutation resulted in enhanced transcription from both promoters, with strong Inr activity maintained (Fig. 5, compare lanes 3 and 4 with 5 and 6). Strong Inr activity was also maintained with mutations that had little effect on promoter strength (e.g. ΔA1 and ΔBn), as well as with mutations that reduced promoter strength (ΔA2, M21, ΔDc and ΔD). [As expected, none of the Sp1 mutants stimulated transcription from the Inr promoter (data not shown).] These results suggest that the Sp1 domains required for TATA-mediated transcription cannot be distinguished from the domains required for Inr activity in a promoter that also contains a TATA box.

TFIID contributes to the species specificity of Sp1 activation

The results presented above demonstrate that in *Drosophila* cells Sp1 cannot activate transcription of a TATA-less promoter, even though the Inr element within this promoter is functional. These

results could reflect an inability of a domain of Sp1 to be properly modified when expressed in *Drosophila* cells. Alternatively, it could reflect the absence of an essential event during Sp1 activation that involves the *Drosophila* general transcription machinery. The TFIID complex is an attractive candidate for the 'defective' component of the *Drosophila* general transcription machinery because of its important role in mediating transcriptional activation (40).

To determine if TFIID is involved in the species specificity observed, we employed an *in vitro* complementation assay. HeLa nuclear extracts were depleted of TFIID activity by a standard procedure, which involves heat treatment at 47°C (33). As expected, the heat treatment abolishes the extract's ability to support transcription (Fig. 6, lanes 1–4). When highly purified human TFIID (18,19) was added to the extract strong transcription from the four promoters containing Sp1 binding sites was restored (lanes 5–8). The relative promoter strengths obtained were similar to those routinely observed with crude nuclear extracts (10,15). In contrast, when purified *Drosophila* TFIID was added to the extracts strong transcription was detected with the Sp1–TATA and Sp1–TATA–Inr promoters, but transcription was not detected with the Sp1–Inr promoter (lanes 9–12).

These results reveal an interesting species-specific difference between the human and *Drosophila* TFIID complexes. The most likely explanation for this difference is that *Drosophila* TFIID is unable to support Sp1 activation of the Inr promoter. Because we are unable to detect significant basal transcription from the Inr promoter in HeLa cell extracts, however, we cannot completely rule out the possibility that *Drosophila* TFIID possesses a more general defect in supporting Inr activity. Nevertheless, based on the *in vivo* evidence that the *Drosophila* defect is specific for Inr-mediated transcription stimulated by Sp1, the *in vitro* defect is also likely to be specific for Sp1 stimulation.

DISCUSSION

The data presented in this manuscript demonstrate that a vertebrate-specific interaction involving TFIID is essential for Sp1 activation of a TATA-less promoter. Although several models could explain

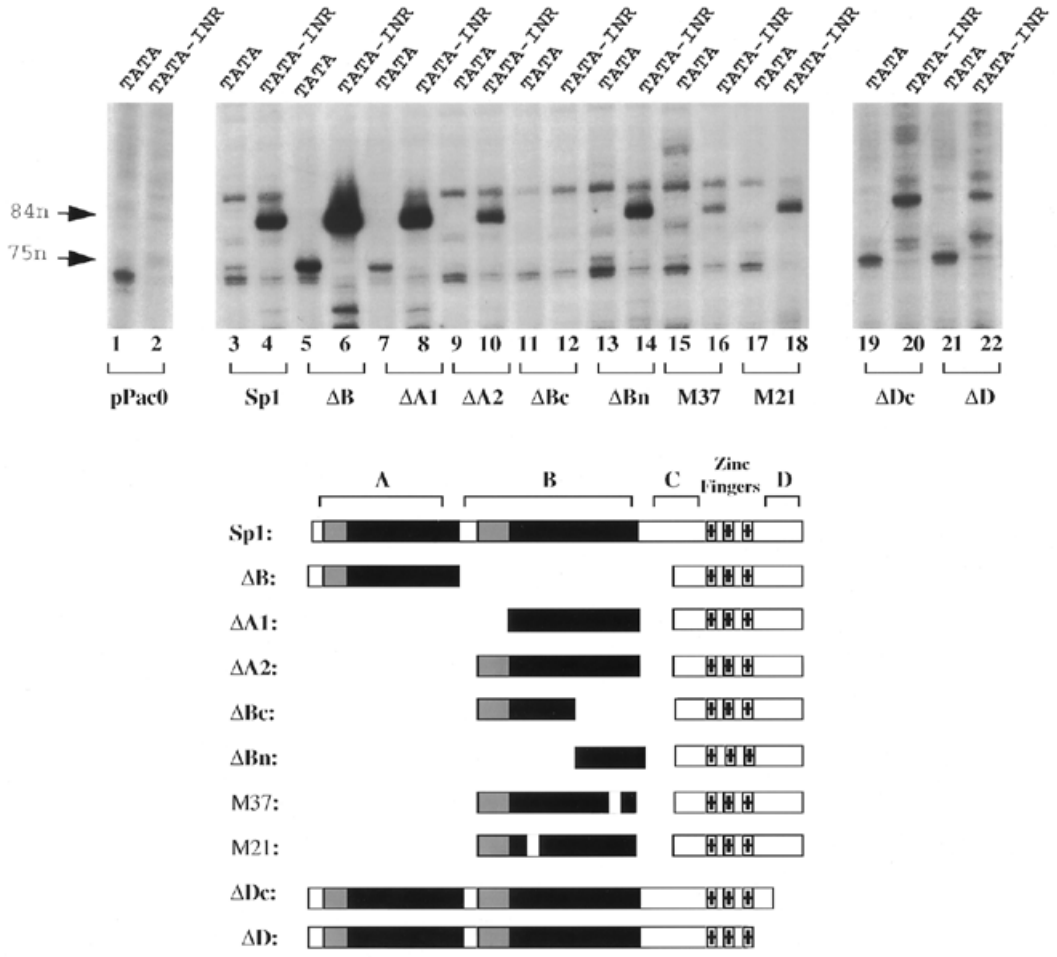

Figure 5. Relative abilities of Sp1 mutant proteins to activate transcription from the TATA and TATA–Inr promoters. Transient transfection experiments were performed in *Drosophila* SL2 cells with reporter plasmids containing multiple Sp1 binding sites upstream of TATA (odd numbered lanes) and TATA–Inr (even numbered lanes) core promoters. An expression vector lacking an insert (pPac0, lanes 1 and 2) or containing wild-type or mutant Sp1 sequences (lanes 3–33, plasmid indicated below each lane) were introduced by co-transfection. Background bands were detected on these gels below the 75 nt TATA cDNA product and above the 84 nt TATA–Inr cDNA product. At the bottom the wild-type and mutant Sp1 proteins are depicted schematically, with activation domains A–D indicated.

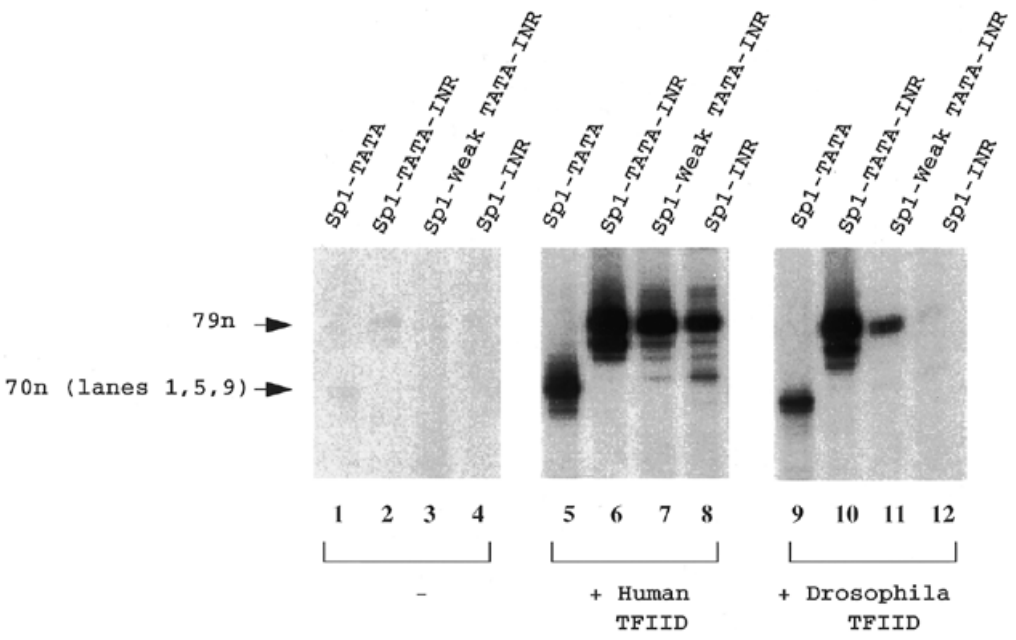

Figure 6. Species-specific activation of TATA-less promoters by Sp1 involves TFIID. *In vitro* transcription assays were performed with HeLa cell extracts that had been depleted of TFIID activity by mild heat treatment (33). Plasmids containing Sp1 binding sites upstream of four core promoters were tested in the TFIID-depleted extract alone (lanes 1–4) or supplemented with purified epitope-tagged human TFIID (lanes 5–8) or *Drosophila* TFIID (lanes 9–12).

this finding, an attractive hypothesis is that a specific interaction between Sp1 and human TFIID is non-functional with *Drosophila* TFIID. Since three different interactions between Sp1 and TFIID have been described (9,20–23), a defect in any one of the interactions could explain the results. An alternative hypothesis is that the *Drosophila* TFIID complex lacks a domain or subunit which carries out an essential interaction with another general transcription factor or which stabilizes the TFIID–DNA interaction during Sp1 activation from TATA-less promoters. According to the data the missing TFIID domain would be critical only during stimulation of TATA-less transcription by a specific class of activation domains. Furthermore, the interaction would not be essential for basal Inr recognition or function or for Inr function during Sp1 activation of a TATA-containing promoter.

We favor the latter of these two hypotheses because the Sp1 mutant analysis adds an additional level of complexity to the former hypothesis. The mutant analysis revealed that the Sp1 Bc and A domains are necessary for transcriptional activation in *Drosophila*. We previously showed that either of these domains is sufficient for activation of Inr-mediated transcription in human cells, when tested as GAL4 fusion proteins (10). Since these domains appear to function in *Drosophila* cells, they are unlikely to contribute to the species-specific defect. It is possible that these domains carry out two critical interactions, one of which cannot take place when Sp1 is expressed in *Drosophila*. However, because of the small size of the Bc domain it seems unlikely to carry out two essential interactions, leading us to favor the second hypothesis proposed above.

The component of human TFIID that contributes to the vertebrate-specific activity remains to been established. A small number of human TAFs have not been found in *Drosophila* (40) and must therefore be considered as candidates. However, any of the TFIID subunits may lack a critical domain or possess an evolutionarily diverged domain that cannot impart the essential activity. We also have not determined whether other components of the transcription machinery are involved in the essential species-specific function. To gain insight into this question *in vitro* transcription experiments with human TFIID added to *Drosophila* extracts must be performed. Unfortunately, *Drosophila* extracts do not support efficient activation by Sp1 or other activators, but rather support anti-repression (data not shown).

The results obtained in *Drosophila* cells with co-transfected Sp1 are interesting to compare with the results obtained in mammalian cells with GAL4–Sp1 fusion proteins (10). In the mammalian experiments the N-terminal glutamine-rich activation domains were found to selectively activate transcription from promoters containing an Inr element. Activation of promoters containing only a TATA box required fusion proteins with both the N-terminal and the C-terminal domains of Sp1 (10). Consistent with the mammalian studies, all of the Sp1 proteins which activated transcription from the TATA-only promoter in *Drosophila* cells contain both N-terminal and C-terminal domains. Interestingly, GAL4 fusion proteins containing only the glutamine-rich activation domains of Sp1 were inactive with any of the core promoters when expressed in *Drosophila* cells (data not shown). Although the reason for the inactivity was not explored in depth, the results are consistent with our expectations; the glutamine-rich domains appear to require an Inr to function but in *Drosophila* cells Sp1 activation through an Inr is defective.

The selective inability of Sp1 to activate TATA-less transcription is consistent with a previous study by Pugh and Tjian (16), who found that purified *Drosophila* TFIID could not support Sp1-activated transcription from a TATA-less promoter when added to a human *in vitro* transcription assay. That study compared partially purified human TFIID to highly purified *Drosophila* TFIID in an *in vitro* assay. Since only the human TFIID supported Inr-mediated transcription, the proposed explanation was that an essential protein present in partially purified human TFIID was lost during extensive purification of the *Drosophila* TFIID. The *in vivo* experiments

shown here, combined with the *in vitro* experiments performed with highly purified human TFIID, strongly suggest that the deficiency observed in the previous study resulted from species specificity.

Although the results shown here are consistent with those described above (16), they are inconsistent with a recent study by Colgan and Manley (29), who reported that in *Drosophila* cells Sp1 activates an Inr-containing promoter even more strongly than a TATA–Inr promoter. In that study the transcription start sites were not determined because promoter activity was measured by an indirect CAT assay, rather than by direct RNA analysis. Since numerous AT-rich sequences are capable of functioning as TATA boxes (28,41,42), it remains possible that the promoter activity detected was mediated by AT-rich sequences within the vector rather than by the Inr element. Consistent with this hypothesis, we have had considerable difficulty studying Inr activity using indirect reporter assays and have found that the reporter activities routinely differ from the specific reporter mRNA levels (data not shown). Another finding which might explain the CAT assay results (29) is that the Sp1–Inr–CAT plasmids used in the previous study contain a sequence downstream of the Inr that exhibits homology to the DPE reported by Burke and Kadonaga (34). The presence of a DPE might facilitate Sp1 stimulation through an Inr in *Drosophila* cells and might explain why the Inr promoter was stronger than the TATA–Inr promoter in those studies (29).

When ectopically expressed in *Saccharomyces cerevisiae* Sp1 possesses no transcriptional activation function (43), whereas in mammalian cells Sp1 activates all relevant promoters. It therefore might not be surprising that in *Drosophila* some but not all of the Sp1 activation properties are functional. Clearly, this result does not suggest that the *Drosophila* genome lacks TATA-less promoters, as numerous examples have been reported. Moreover, Figure 1D shows that a specific activation domain from the *Drosophila ftz* gene is capable of activating transcription from TATA-less promoters in *Drosophila*. Instead, the results suggest that the types of domains capable of activating transcription in *Drosophila* cells from promoters that lack a TATA box are more limited than the domains used for TATA-less transcription in mammalian cells. An understanding of the mechanistic basis of this vertebrate-specific activity will provide insight into the mechanism of transcription activation in general and the mechanism of activation from TATA-less promoters in particular.

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