DNA-Binding and Transcriptional Properties of Human Transcription Factor TFIID after Mild Proteolysis

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The existence of separable functions within the human class II general transcription factor TFIID was probed for differential sensitivity to mild proteolytic treatment. Independent of whether TFIID was bound to DNA or free in solution, partial digestion with either one of a variety of nonspecific endoproteases generated a protease-resistant protein product that retained specific DNA recognition, as revealed by DNase I footprinting. However, in contrast to native TFIID, which interacts with the adenovirus major late (ML) promoter over a very broad DNA region, partially proteolyzed TFIID interacted with only a small region of the ML promoter immediately surrounding the TATA sequence. This novel footprint was very similar to that observed with the TATA factor purified from yeast cells. Partially proteolyzed human TFIID could form stable complexes that were resistant to challenge by exogenous templates. It could also nucleate the assembly of transcription complexes on the ML promoter with an efficiency comparable to that of native TFIID, yielding similar levels of transcription initiation. These results suggest a model in which the human TFIID protein is composed of at least two different regions or polypeptides: a protease-resistant "core," which by itself is sufficient for promoter recognition and basal transcriptional levels, and a protease-sensitive "tail," which interacts with downstream promoter regions and may be involved in regulatory processes.

The promoter of protein-encoding (class II) genes is composed of multiple DNA elements. The minimum (or basal) promoter often contains a sequence referred to as the TATA box, whereas regulation is conferred by one or several upstream regulatory elements (for a review, see references 13 and 27). Recognition of the promoter by the general transcription factors leads to the formation of stable complexes that signal a gene for transcription by RNA polymerase II. Template challenge analyses have revealed that a particular transcription factor, designated TFIID, is the essential component of these stable complexes and that the TATA box sequence alone is sufficient for TFIID binding (4, 26). Human TFIID is required for transcription of a number of cellular and viral genes in vitro (15). Binding of TFIID to the promoter DNA has been shown to be facilitated by an activity designated TFIIA (4, 6, 17), although an absolute TFIIA requirement for stable complex formation as well as for efficient transcription has not always been found (19, 26). Stable binding of TFIID is also sufficient to maintain promoter function after in vitro nucleosome assembly (29).

DNase I footprinting has revealed interesting features of the TFIID-TATA box interaction. On several promoters, exemplified by that of the human HSP70 gene, the DNase I footprint of human TFIID is restricted to a small region of the promoter around the TATA box sequence (15). In contrast, the same protein protects, on both the adenovirus major late (ML) and human histone H4 promoters, a much larger DNA region extending from 40 base pairs (bp) upstream to 30 or 35 bp downstream of the transcription initiation site (15, 20). This unusual downstream extension of the TFIID footprint, which shows no requirement for specific DNA sequences, has been postulated to reflect wrapping of the DNA around a portion of the TFIID molecule (21). DNase I footprints, covering not only the TATA box region but also the start site of transcription and part of the

leader region, were also observed on several *Drosophila* promoters with a TATA box-binding protein isolated from K_c cells (16).

Recently, a protein was isolated from yeast which can substitute for human TFIID in a heterologous in vitro system reconstituted with the other general transcription factors and RNA polymerase II from HeLa cells (2, 3). This yeast TFIID is a single polypeptide of 27,000 daltons (10), which can form stable preinitiation complexes with various TATA boxes in the absence of any other transcription factor (3). DNase I footprinting has revealed a small (16 bp) region of interaction for yeast TFIID with the ML TATA box (2, 10), and the same nucleotides required for transcriptional activity are also critical for specific binding (2). The unique gene that encodes the yeast TATA factor has been cloned (7, 11, 23) and has revealed potential homologies with the bacterial sigma factor (11). Interestingly, this gene was found to be identical to a known mutation, SPT15, which had been previously isolated as a suppressor of Ty element insertions (5).

The exact relationship between the yeast TATA factor and the TFIID activity isolated from higher eucaryotes remains to be established. Both proteins seem equally capable of nucleating the assembly of the other transcription factors and RNA polymerase II into functional preinitiation complexes (1, 25). However, an extended interaction with the promoter DNA has only been observed with *Drosophila* and human TFIIDs. This single difference could be very significant, since the switch from a limited to an extended TFIID-promoter interaction has been postulated to play a key role in transcription stimulation by several upstream regulatory factors (8, 9). In relation to this, preliminary estimates for the molecular mass of human TFIID (~100 kilodaltons) (15, 17, 18) indicate a much larger protein than the 27-kilodalton polypeptide purified from yeast cells. Thus, it could be that the human TFIID is composed of several polypeptides (or several domains), one of which is equivalent to the smaller yeast TATA factor. To investigate this possibility, we sub-

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jected human TFIID to treatment with various proteases in order to probe the existence of separate domains within this transcription factor.

MATERIALS AND METHODS

Partially purified human transcription factors TFIIB, TFIID, TFIIE, USF, and RNA polymerase II were prepared from HeLa cell nuclear extracts, as previously described (15, 19, 20, 25). Proteases and protease inhibitors were purchased from Boehringer Mannheim Biochemicals, and poly(dG-dC) and radiolabeled nucleotides were purchased from Pharmacia, Inc., and DuPont, NEN Research Products.

Proteolysis of TFIID. Partial proteolysis of TFIID in solution was performed as follows. In a 12.5- μ l final reaction volume, HeLa TFIID (either ω -aminooctyl agarose or DE-52 fractions, 5 μ g of total protein) was incubated with 200 ng of protease in the standard transcription reaction buffer (20 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid] [pH 8.4], 60 mM KCl, 6 mM MgCl₂, 10% glycerol, 5 mM dithiothreitol) for 10 min at 30°C. The reaction was terminated by adding the inhibitor phenylmethylsulfonylfluoride (PMSF) to a 2 mM final concentration. For partial proteolysis of the TFIID-DNA complex, human TFIID and USF (100 fmol; Mono Q fraction [22]) were first incubated with 5.5 fmol of adenovirus ML promoter-containing DNA fragment and 100 ng of poly(dG-dC) carrier DNA in a 23- μ l reaction volume for 60 min at 30°C to affect complete binding. Proteolysis and reaction termination were then performed as above. Proteases used in this investigation included (in units per milligram) chymotrypsin A (90), papain (30), proteinase K (20), subtilisin (5), and trypsin (110).

DNase I footprinting. DNase I footprinting of TFIID-DNA complexes was performed essentially as previously described (15, 20). The footprinting probe consisted of the 690-bp *Xba*I-to-*Nar*I restriction fragment of the plasmid pML(C₂AT)19 Δ -127f (20), labeled upstream of the adenovirus ML promoter on the nontranscribed strand. TFIID-ML promoter complexes were assembled as described above. The addition of USF (100 fmol; Mono Q fraction) was used to facilitate complete TFIID binding to the ML promoter, as previously noted (20, 22), and does not otherwise qualitatively affect the TFIID-DNA interaction (data not shown). DNase I cleavage was initiated by the addition of 3 ng of DNase I and allowed to proceed for 30 s at room temperature. Termination of the DNase cleavage reaction, DNA fragment purification, and gel electrophoresis followed standard protocols (24). Footprinting of other transcription complexes (i.e., preinitiation, energy dependent, and postinitiation) required a second incubation step, as described previously (25). To assemble preinitiation complexes, general transcription factors TFIIB (2.5 U; single-stranded DNA agarose fraction), TFIIE (2.4 U; Bio-Gel A-1.5m fraction), and RNA polymerase II (20 U; phosphocellulose fraction) were added to the preformed TFIID-DNA complex in a final volume of 50 μ l, and the incubation was allowed to proceed for an additional 30 min before footprinting. Energy-dependent complexes were analyzed after 0.4 mM dATP was added, whereas postinitiation complexes were analyzed after a 10-min subsequent incubation in the presence of 1 mM each ATP, CTP, and UTP. The cleavage products of adenosine-specific chemical sequencing reactions were used as markers (12).

In vitro transcription. In vitro transcription assays were essentially performed as described previously (26). Templates derived from plasmid pML(C₂AT)19 Δ -53, containing

either the standard 380-bp or a shortened (~340-bp) G-less cassette (19), were used as indicated. Initially, TFIID (7 U; DE-52 fraction) was preincubated with the DNA in the standard transcription reaction buffer (see above) for 10 min at 30°C to affect template commitment. General transcription factors TFIIB (6.3 U; single-stranded DNA agarose), TFIIE (6.3 U; Bio-Gel A-1.5m fraction), and RNA polymerase II (5 U; phosphocellulose fraction) were then added together with nucleotides (0.6 mM each ATP and UTP, 25 μ M CTP, 13 μ Ci of [α -³²P]CTP at 700 Ci/mmol), and transcription was allowed to ensue. Aliquots corresponding to 1/10 of the total reaction volume were removed periodically, as indicated, and frozen. Further processing of transcription reactions was performed as previously described (28). RNA products were resolved by gel electrophoresis (acrylamide-bisacrylamide, 6.0:0.16%; 8 M urea, 1 $\times$ TBE [Tris-borate EDTA]) and visualized by autoradiography. Quantitation of transcription was performed by excising slices of the dried gel and scintillation counting.

RESULTS

Digestion of human TFIID with subtilisin. The effect of a mild proteolytic digestion of human TFIID by subtilisin on the DNA-binding properties of the transcription factor was first investigated by using DNase I footprinting with a singly end-labeled probe containing the adenovirus ML promoter (Fig. 1). The DNase I footprinting pattern which characterizes native human TFIID bound at the ML promoter contains a region of complete cleavage protection around the TATA box sequence and a second region downstream, extending to position +30, composed of alternated protections and enhanced cleavages (15, 20). When TFIID was first preincubated with the footprinting probe and then submitted to a 10-min digestion with subtilisin, its interaction with the promoter DNA was drastically altered (Fig. 1, lane 2). The novel footprint observed with subtilisin-digested TFIID was considerably smaller than the original TFIID footprint, covering only 21 bp of DNA around the TATA sequence (positions -37 to -17). Interestingly, the exact same cleavage protection pattern was observed when TFIID was submitted to subtilisin digestion before its interaction with the promoter DNA (lane 3). This may indicate the existence of a component within TFIID which is always sensitive to cleavage by subtilisin, whether the transcription factor is free in solution or bound to the DNA. As a control, the simultaneous addition of subtilisin with the specific inhibitor PMSF had no effect on the TFIID footprinting pattern, whether treatment was performed before or after TFIID was allowed to bind to DNA (lanes 1 and 4).

Digestion of TFIID with various proteases. The same small DNase I footprint which characterized the interaction of subtilisin-treated TFIID with the ML promoter was also observed when the transcription factor was submitted to limited digestion with a number of other relatively nonspecific endoproteases, including chymotrypsin, papain, proteinase K, and trypsin (Fig. 2). Again, identical footprinting patterns were observed whether the TFIID was subjected to proteolysis before or after binding to DNA (data not shown). Taken together, these results suggest the existence of a component within TFIID which is resistant to proteolysis and is sufficient for the recognition of specific DNA sequences. We will refer to this protease-resistant component as "core" TFIID and define it as a subset of the native human TFIID which is capable of specific binding to the TATA element. Another portion of TFIID, that which is

FIG. 1. DNase I footprinting of native and partially proteolyzed TFIID on the adenovirus ML promoter. The interaction of TFIID with the ML promoter in the presence of the ML upstream factor USF was analyzed by DNase I footprinting, as described in Materials and Methods. Shown is an autoradiogram of the DNase I cleavage products separated by gel electrophoresis. Lane 1, TFIID (ω -aminooctyl agarose; 0.5 μ g of protein) and USF (Mono Q fraction; 100 fmol) preincubated with DNA [2 fmol of ML promoter and 100 ng of poly(dG-dC)] for 60 min at 30°C to affect complete binding and then treated with 0.1 μ g of subtilisin and 25 pmol of PMSF for 10 min before cleavage by DNase I; lane 2, TFIID and USF preincubated with the DNA and then subjected to treatment with subtilisin for 10 min before the addition of PMSF and cleavage by DNase I; lane 3, TFIID treated with subtilisin for 10 min and incubated with USF and DNA after the addition of PMSF; lane 4, same as lane 3, except that PMSF was present during the incubation of TFIID with subtilisin; lane -, DNase I control cleavage pattern; lane A, markers for adenine-specific chemical sequencing. A schematic representation of the DNA fragment is shown at right, indicating the locations of the USF-binding element (UE), TATA box, initiation site (+1), and transcription cassette (C_2AT).

normally responsible for downstream interaction with the ML promoter, will be referred to as the "tail."

Interaction of the gene-specific transcription factor USF upstream of the ML TATA box has been shown to stabilize the binding of TFIID (20). In agreement with this observation, the presence of USF in our footprinting reactions seemed to facilitate to the complete occupancy of the ML TATA box by TFIID. The interaction of USF with ML DNA was, in most cases, unaffected by proteolytic treatment, indicating that USF, or minimally its DNA-binding domain, is less sensitive to protease attack than is the TATA box factor (Fig. 2). Digestion with trypsin, however, abolished the USF footprint (Fig. 2, lane 6). Since this removal of USF did not affect the footprint of TFIID, it seems that the upstream factor may not be necessary for maintenance of the TFIID-TATA box interaction.

Core TFIID can form stable complexes. Template challenge assays have been used to determine the stability of complexes between transcription factors and the promoter DNA. In the case of class II genes, we and others have shown that binding TFIID to the core promoter element is necessary

FIG. 2. DNase I footprinting of TFIID partially proteolyzed with a variety of nonspecific endoproteases. DNase I cleavage reactions were performed on TFIID-DNA complexes as described for Fig. 1, lane 2, with the substitution of the following proteases: none (lane 1), chymotrypsin (lane 2), papain (lane 3), proteinase K (lane 4), subtilisin (lane 5), and trypsin (lane 6). Also shown is the DNase I control cleavage pattern (lane -) and the adenine-specific cleavage ladder (lane A). A schematic representation of the DNA fragment is shown at right, indicating the locations of the USF-binding element (UE), TATA box, initiation site (+1), and transcription cassette (C_2AT).

and sufficient for commitment of a particular gene to transcription and resistance to challenge by other genes (4, 6, 17, 26). To investigate the role of the TFIID tail in stable complex formation, we used partially proteolyzed TFIID in a template challenge experiment. Core TFIID was preincubated with a first template containing the minimal ML promoter. A second template, containing the identical promoter but encoding a longer transcript, was then added together with the remaining transcription factors and nucleoside triphosphates. Under these conditions, only transcription of the first template was observed throughout the 40-min reaction (Fig. 3). Thus, core TFIID exhibited the same ability as the native transcription factor to form stable complexes that are resistant to challenge by other templates during the course of a standard transcription reaction, indicating that the TFIID tail is not involved in this particular function of the TATA box-binding factor.

Core TFIID is as active in basic transcription as native TFIID. Since both native and core TFIID were capable of forming stable complexes, it was then possible to directly investigate the relative transcriptional efficiencies of these two species through simultaneous transcription reactions. Both forms of TFIID were individually preincubated with two different ML promoter-containing templates to allow stable complex formation. These reactions were then mixed together, and transcription was initiated by the addition of the remaining transcription factors and nucleoside triphosphates. Equivalent amounts of RNA synthesis were observed from both templates throughout the time course of the reaction (Fig. 4). This indicated that native and core TFIIDs were equally capable of reconstituting a basal level

FIG. 3. Transcriptional analysis of stable complexes containing partially proteolyzed TFIID. (A) Graph of RNA synthesis from each template as a function of reaction time and autoradiogram of RNA products (insert). (B) Incubation protocol. Proteinase K-treated TFIID (D^{core} [DE-52 fraction, 4.2 U]) was preincubated with 2 μg of an ML promoter-containing plasmid containing a shortened C_2 AT cassette for a template ($\bullet$). After 20 min, the remaining transcription factors TFIIB (6 U), TFIIE (6 U), and RNA polymerase II (5 U), together with nucleotides (NTPs) and a second ML promoter-containing template having a long C_2 AT cassette ($\circ$), were added to the reaction and transcription was allowed to ensue. Aliquots (1/10 of reaction volume) were removed over the course of 0.5 to 40 min (as indicated in the insert) and processed for analysis by gel electrophoresis.

of transcription from a minimal class II promoter, with regard both to the total amounts of RNA transcripts made and to the rates of their syntheses. Thus, at least in vitro, the tail of TFIID does not seem to play a significant role in the basic transcriptional process.

Comparison of transcription complexes assembled on native and core TFIIDs. Individual steps along the RNA polymerase II transcription initiation pathway are amenable to analysis by DNase I footprinting if saturation of the templates with active transcription complexes can be achieved (25). The fact that core TFIID demonstrated comparable

FIG. 4. Comparison of transcriptional efficiencies between native and partially proteolyzed TFIIDs on minimal ML promoters. (A) Graph of RNA synthesis from each template as a function of reaction time and an autoradiogram of RNA products (insert). (B) Incubation protocol. Reactions were performed essentially as described in the legend to Fig. 3, with the exception that either partially proteolyzed (D^{core}) or native (D) TFIIDs were incubated with the different templates.

DNA-binding and transcriptional activities to native TFIID indicated that it would be possible to quantitatively assemble transcription complexes containing the partially proteolyzed TATA factor. Adding the general transcription factors TFIIB, TFIIE, and RNA polymerase II to a core TFIID-TATA complex resulted in a DNase I footprint identical to that of complete preinitiation complexes containing native TFIID (Fig. 5, lanes 2 and 6). As was the case with native TFIID, no intermediate preinitiation complexes containing only subsets of the other transcription factors were observed (data not shown). Similarly, adding dATP to preinitiation complexes containing core TFIID demonstrated the identical loss of DNase I-enhanced cleavage at the 3' boundary of the footprint to that observed with native TFIID (lanes 3 and 7). Thus the energy-dependent transition, which is now thought to entail the dissociation of a component of TFIIE (1), apparently takes place independent of the presence of

FIG. 5. DNase I footprinting of transcription complexes containing either native or partially proteolyzed TFIID. Transcription complexes containing either intact (lanes 1 to 4) or chymotrypsin-treated (lanes 5 to 8) TFIID were assembled on the ML promoter, as described in Materials and Methods. Lanes 1 and 5, TFIID-DNA complex; lanes 2 and 6, complete preinitiation complex; lanes 3 and 7, energy-dependent transition; lanes 4 and 8, postinitiation complex; lane -, DNase I cleavage control; lane A, adenosine-specific cleavage ladder. A schematic representation of the DNA fragment is shown at right, indicating the locations of the USF-binding element (UE), TATA box, initiation site (+), and transcription cassette (C_2AT).

the TFIID tail. Only after the addition of all nucleoside triphosphates and promoter clearance by the RNA polymerase II were differences observed between the DNase I footprints of complexes containing the two forms of TFIID. As previously reported, native TFIID-containing postinitiation complexes exhibited the identical DNase I footprint to that of the initial TFIID-DNA stable complex (lanes 1 and 4). By contrast, postinitiation complexes containing core TFIID demonstrated a short extension at the 3' boundary of the cleavage protection (down to position -9) which was not present in the initial core TFIID-DNA complex (lanes 5 and 8). This could reflect the continued association of another protein besides TFIID within the postinitiation complex. Whether this additional protein is also normally present with native TFIID remains to be determined, since its detection by DNase I footprinting would normally be obscured by the extended native TFIID-DNA interaction.

DISCUSSION

Separable DNA-binding functions in human TFIID. The DNA-binding properties of the human transcription factor TFIID have remarkable features that set it apart from many other known DNA-binding proteins. Previous studies using a combination of MPE (methidiumpropyl-EDTA) and DNase I footprinting have revealed two distinct regions of interaction between the ML promoter DNA and proteins present in the most purified human TFIID-containing fractions (20). The strongest interaction takes place with the TATA element and

is clearly the result of sequence-specific recognition. A secondary interaction, whose nature is not well understood, takes place with the DNA around the transcription initiation site and transcribed leader region and is apparently sequence independent. Since the human TFIID has not yet been completely purified, it is not possible to completely exclude the possibility that these two footprinting regions reflect the binding of two distinct proteins. If this is the case, interaction of the downstream protein would have to be directed by the prior association of the TATA box-binding protein and our observations would simply indicate a greater sensitivity to proteolysis for this downstream binding protein over the TATA box-binding factor. However, several observations indicate that the two footprinting regions may not result from the independent binding of two different proteins. First, the two activities were found to precisely coelute through multiple chromatographic steps (15). In addition, the two interactions were always observed to take place simultaneously, independent of the concentration of TFIID (unpublished observation). It seems therefore more likely that, despite the complexity of the TFIID footprint on the ML promoter, it only reflects the interaction of a single protein. If this is true, it is quite interesting that the two regions of TFIID interaction within the ML promoter DNA correspond to two distinct domains (or polypeptides) within the TFIID protein, which can be separated by selective protease degradation. It could simply be that the core domain of TFIID, responsible for TATA box recognition, is much more resistant to degradation by proteases than the tail domain, which is normally responsible for 3' promoter interaction. However, another possibility would be that the junction between the TFIID core and tail domains, which could be thought of as a hinge region, is a preferential target for protease attack. Precedent for such a phenomenon has been described in the case of the yeast general transcription factor τ , a protein which serves the equivalent function of TFIID in recognizing the promoters of class III genes and facilitating the assembly of transcription complexes (14).

Analogies between human core TFIID and the yeast TATA factor. The protease-resistant core domain of TFIID appears completely functional in vitro, with respect to sequence-specific DNA binding and stable complex formation. In addition, core TFIID seems as capable as native TFIID of nucleating the assembly of transcription preinitiation complexes and reconstituting basal levels of RNA synthesis from a minimal class II promoter. Each of these properties, together with the restricted interaction with the ML TATA box, also characterizes the TATA factor which has been purified from yeast cells (2, 3, 10). Given the apparent difference in molecular mass between yeast and human factors, one could hypothesize that there would be a second protein in yeast cells which is the analog of the human TFIID tail. There are, however, no indications as to whether the core and tail domains of the human TFIID are encoded by a single large polypeptide or whether the transcription factor is composed of several polypeptides. This particular question will await the complete purification and characterization of this key transcription factor.

Role of the TFIID tail domain. By itself, core TFIID is fully capable of reconstituting basal levels of transcription in vitro. Furthermore, most steps along the transcription reaction pathway are apparently both qualitatively and quantitatively unaffected when core TFIID is substituted for native TFIID. Obviously, these findings raise the question of the role played by the TFIID tail domain. The appearance of periodic sites of DNase I accessibility within the downstream portion of the TFIID-ML promoter interaction has

suggested a model in which the DNA could be wrapped around this region of the TFIID protein (21). Whether or not this is true, the interaction (or lack of interaction) of the TFIID tail with promoter DNA must affect the spatial arrangement of the protein-DNA complex and, therefore, potentially alter its interaction with other transcription components. Since TFIID clearly remains associated with promoter DNA after transcription initiation and promoter clearance by RNA polymerase II, the tail domain of TFIID could possibly play a role in the transcription reinitiation process. For example, the TFIID tail could stabilize the postinitiation complex, thereby facilitating subsequent rounds of initiations. Alternatively, the TFIID tail could play a role in mediating the response of the general transcription machinery to upstream binding by regulatory proteins. Isomerization of the TFIID-adenovirus E4 promoter interaction, from the short to extended modes, has been invoked as a mechanism by which *trans*-acting factors, such as GAL4 and ATF, affect transcription initiation (8, 9). Although our results argue against the hypothesis that the downstream TFIID interaction would be a prerequisite for complete preinitiation complex formation (9), they do not exclude the possibility that the TFIID tail domain could serve as a sensor for upstream factor action. Further studies are currently under way in our laboratories to determine the ability of both native and core human TFIIDs to mediate transcription stimulation *in vitro* for a number of specific transcription factors.

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LITERATURE CITED

- Buratowski, S., S. Hahn, L. Guarente, and P. A. Sharp. 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. *Cell* 56:549-561.
- Buratowski, S., S. Hahn, P. A. Sharp, and L. Guarente. 1988. Function of a yeast TATA element-binding protein in a mammalian transcription system. *Nature (London)* 334:37-42.
- Cavallini, B., J. Huet, J.-L. Plassant, A. Sentenac, J.-M. Egly, and P. Chambon. 1988. A yeast activity can substitute for the HeLa cell TATA box factor. *Nature (London)* 334:77-80.
- Davison, B. L., J.-M. Egly, E. R. Mulvihill, and P. Chambon. 1983. Formation of stable preinitiation complexes between eukaryotic class B transcription factors and promoter sequences. *Nature (London)* 301:680-686.
- Eisenmann, D. M., C. Dollard, and F. Winston. 1989. SPT15, the gene encoding the yeast TATA factor TFIID, is required for normal transcription initiation *in vivo*. *Cell* 58:1183-1191.
- Fire, A., M. Samuels, and P. A. Sharp. 1984. Interactions between RNA polymerase II, factors, and template leading to accurate initiation. *J. Biol. Chem.* 259:2509-2516.
- Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente. 1989. Isolation of the gene encoding the yeast TATA binding protein TFIID: a gene identical to the SPT15 suppressor of Ty element insertions. *Cell* 58:1173-1181.
- Horikoshi, M., M. F. Carey, H. Kakidani, R. G. Roeder. 1988. Mechanism of action of a yeast activator: direct effect of GAL4 derivatives on mammalian TFIID-promoter interactions. *Cell* 54:665-669.
- Horikoshi, M., T. Hai, Y.-S. Lin, M. R. Green, and R. G. Roeder. 1988. Transcription factor ATF interacts with the TATA factor to facilitate establishment of a preinitiation complex. *Cell* 54:1033-1042.
- Horikoshi, M., C. K. Wang, J. A. Cromlish, P. A. Weil, and R. G. Roeder. 1989. Purification of a yeast TATA box-binding protein that exhibits human transcription factor IID activity. *Proc. Natl. Acad. Sci. USA* 86:4843-4847.
- Horikoshi, M., C. K. Wang, H. Fuji, J. A. Cromlish, P. A. Weil, and R. G. Roeder. 1989. Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. *Nature (London)* 341:299-303.
- Iverson, B. L., and P. B. Dervan. 1987. Adenine specific DNA chemical sequencing reaction. *Nucleic Acids Res.* 15:7823-7830.
- Maniatis, T., S. Goodbourn, and J. A. Fischer. 1987. Regulation of inducible and tissue-specific gene expression. *Science* 236:1237-1244.
- Marzouki, N., S. Camier, A. Ruet, A. Moenne, and A. Sentenac. 1986. Selective proteolysis defines two DNA binding domains in yeast transcription factor *tau*. *Nature (London)* 323:176-178.
- Nakajima, N., M. Horikoshi, and R. G. Roeder. 1988. Factors involved in specific transcription by mammalian RNA polymerase II: purification, genetic specificity, and TATA box-promoter interactions of TFIID. *Mol. Cell. Biol.* 8:4028-4040.
- Parker, C. S., and J. Topol. 1984. A Drosophila RNA polymerase II transcription factor contains a promoter-region-specific DNA binding activity. *Cell* 36:357-369.
- Reinberg, D., M. Horikoshi, and R. G. Roeder. 1987. Factors involved in specific transcription by mammalian RNA polymerase II. Functional analysis of initiation factors IIA and IID and identification of a new factor operating at sequences downstream of the initiation site. *J. Biol. Chem.* 262:3322-3330.
- Samuels, M., A. Fire, and P. A. Sharp. 1982. Separation and characterization of factors mediating accurate transcription by RNA polymerase II. *J. Biol. Chem.* 257:14419-14427.
- Sawadogo, M., and R. G. Roeder. 1985. Factors involved in specific transcription by human RNA polymerase II: analysis by a rapid and quantitative *in vitro* assay. *Proc. Natl. Acad. Sci. USA* 82:4394-4398.
- Sawadogo, M., and R. G. Roeder. 1985. Interaction of a gene-specific transcription factor with the major late promoter upstream of the TATA box region. *Cell* 43:165-175.
- Sawadogo, M., and R. G. Roeder. 1986. DNA-binding specificity of USF, a human gene-specific transcription factor required for maximum expression of the major late promoter of adenovirus, p. 147-154. *In* T. Grodzicker, P. Sharp, and M. Botchan (ed.), *Cancer cells/DNA tumor viruses*, vol. 4. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Sawadogo, M., M. W. Van Dyke, P. Gregor, and R. G. Roeder. 1988. Multiple forms of the human gene-specific transcription factor USF. I. Complete purification and identification of USF from HeLa cell nuclei. *J. Biol. Chem.* 263:11985-11993.
- Schmidt, M. C., C. C. Kao, R. Pei, and A. J. Berk. 1989. Yeast TATA-box transcription factor gene. *Proc. Natl. Acad. Sci. USA* 86:7785-7789.
- Van Dyke, M. W., and R. G. Roeder. 1987. Multiple proteins bind to VA RNA genes of adenovirus type 2. *Mol. Cell. Biol.* 7:1021-1031.
- Van Dyke, M. W., R. G. Roeder, and M. Sawadogo. 1988. Physical analysis of transcription preinitiation complex assembly on a class II promoter. *Science* 241:1335-1338.
- Van Dyke, M. W., M. Sawadogo, and R. G. Roeder. 1989. Stability of transcription complexes on class II genes. *Mol. Cell. Biol.* 9:342-344.
- Wasylyk, B. 1988. Transcription elements and factors of RNA polymerase B promoters of higher eukaryotes. *Crit. Rev. Biochem.* 22:77-120.
- Weil, P. A., D. S. Luse, J. Segall, and R. G. Roeder. 1979. Selective and accurate initiation of transcription at the Ad2 major late promoter in a soluble system dependent on purified RNA polymerase II and DNA. *Cell* 18:469-484.
- Workman, J. L., and R. G. Roeder. 1987. Binding of transcription factor TFIID to the major late promoter during *in vitro* nucleosome assembly potentiates subsequent initiation by RNA polymerase II. *Cell* 51:613-622.