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Chromatin Is Permissive to TATA-binding Protein (TBP)-mediated Transcription Initiation*

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Preinitiation complex assembly is nucleated by the binding of TFIID to the promoters of protein coding genes transcribed by RNA polymerase II. TFIID is comprised of the TATA-binding protein (TBP) and TBP-associated factors (TAF_{II}s). We investigated the transcription properties of TBP and TFIID on chromatin templates. On naked templates both TBP and purified TFIID are able to initiate basal transcription. However, on chromatin templates only TBP mediates transcription initiation in a heat-treated extract, whereas TFIID does not. Moreover, TBP-mediated chromatin transcription is blocked in a nontreated extract. These observations suggest that a chromatin-targeted repressor is present in crude extracts and that chromatin *per se* is not refractory to transcription mediated by TBP. As TBP can function through TAF_{II}-independent and TAF_{II}-dependent pathways, the repression of TBP-mediated basal transcription may be an additional level to the control of Pol II transcription initiation on chromatin.

Transcription initiation of protein-encoding genes requires the assembly of a productive preinitiation complex (PIC)¹ comprising RNA polymerase II (Pol II) and the general transcription factors (1). Binding of TFIID is followed by the subsequent ordered interactions of the other general transcription factors (TFIIA, -IIB, -IIE, -IIF, -IIH) and Pol II to yield a productive PIC (2). TFIID is a protein complex composed of TBP and a number of TBP associated factors (TAF_{II}s) (3). The discovery that TBP is not only a component of the TFIID complex but is also present in transcription complexes functioning in Pol I and III transcription, placed TBP as a central player in

transcription (4).

In vitro recombinant TBP can substitute for TFIID in reconstituted basal transcription systems but does not support transcription from TATA-less promoters (5). Moreover, in *Drosophila* and human cell-free systems TFIID but not TBP could support activator-dependent transcription. These findings suggested that TAF_{II}s are essential both for transcription initiation and for the response to transcriptional activators *in vitro* (3). Evidence showing that TAF_{II}s may function as coactivators *in vivo* has come from either mammalian cell transfection experiments (6) or from genetic studies in *Drosophila* (7). However, in contrast to the above cell-free systems, activation of *in vitro* transcription in a TBP-dependent manner was reported in a TAF_{II}-depleted nuclear extract (8).

Two active forms of TBP have been recently reported in yeast, a TAF-dependent and a TAF-independent form, that can mediate Pol II transcription *in vivo* (9, 10). Novel complexes consisting of TAF_{II}s and a plethora of other proteins have also been identified from yeast and human cells, *i.e.* TFTC, PCAF/GCN5, STAGA, and SAGA (11). These complexes do not contain TBP or TBP paralogues, although they may participate in promoter recognition, transcription activation, and chromatin modifications. In addition, a TBP-TFIIA-containing complex (TAC) has recently been described in embryonic carcinoma cells that does not contain TAF_{II}s (12).

Despite their ability to mediate basal transcription on naked templates, the minimal set of Pol II transcription factors, including TFIID, are not sufficient for Pol II transcription on DNA templates packaged into chromatin (13, 14). Although the function of TBP and TFIID during basal transcription from naked DNA templates has been widely studied, their precise role during transcription on chromatin templates remains elusive. Here, we analyzed the transcriptional properties of TBP and TFIID on chromatin.

EXPERIMENTAL PROCEDURES

Chromatin Assembly—*Drosophila* embryo extracts were prepared, and chromatin was assembled essentially as described (15). Typically, chromatin templates were assembled with 3 mg of *Drosophila* S-190 extract, 1 μg of calf thymus core histones, 1 μg of supercoiled plasmid (see below), and an ATP regenerating system (30 mM creatine phosphate, 3 mM ATP, 4.2 mM MgCl₂ and 6 μg of creatine kinase) in a 100–μl volume. After a 5-h incubation period, chromatin was either analyzed by micrococcal nuclease digestion or processed for transcriptional analysis.

Transcription *in Vitro*—HeLa nuclear extract (NE) preparation and Gal4-VP16 purification have been described (16). The heat inactivation of the NE (usually 300 μl) was carried out at 45 °C for 15 min as described in (17). Transcription reactions were performed using 25 ng of 17M5/pAL7 (AdMPL+1) and 25 ng of pG1 (Glob+1) templates (16). Purified recombinant TBP (16) or immunopurified TFIID (18) were incubated with the templates for 10 min at 4 °C. Where indicated Gal-VP16 was preincubated with the templates for 10 min at 4 °C. Transcription reactions (25 μl) were performed with either normal or

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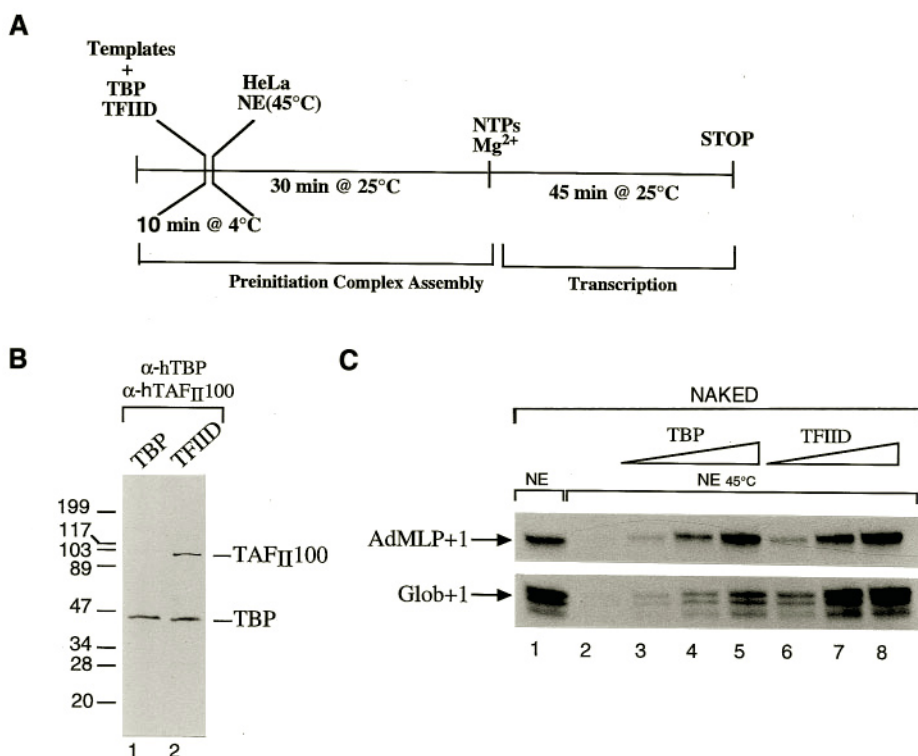
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¹ The abbreviations used are: PIC, preinitiation complex; Pol, RNA polymerase; TBP, TATA-binding protein; TAF_{II}s, TBP associated factors; TAC, TBP-TFIIA-containing complex; NE, nuclear extract; mAb, monoclonal antibody, AdMPL, adenovirus major late promoter; Glob, rabbit β-globin promoter.

FIG. 1. Promoter-specific requirements for TBP and TFIID on naked transcription.

A, outline of the transcription assay. *NE(45 °C)*, inactivation of HeLa nuclear extract at 45 °C for 15 min. **B**, normalization of recombinant TBP and immunopurified TFIID by immunoblotting using mAbs raised against TBP (3G3) and hTAF_{II}100 (1TA). The sizes of the protein markers in kDa are indicated. **C**, TBP- and TFIID-dependent transcription from AdMLP (*AdMLP+1*) and the rabbit β -globin (*Glob+1*) promoters. Transcription reactions were assembled on naked DNA templates as depicted in panel A. When used, TBP and TFIID were prebound to the template for 10 min at 4 °C before nuclear extracts (NE) were added. Lanes 3 and 6, 4 and 7, and 5 and 8 were normalized to contain the same amounts of TBP.



heat-inactivated NEs and incubated for 30 min at 25 °C. Transcription was initiated by the addition of NTPs (0.5 mM) and MgCl₂ (5 mM) and carried out for 45 min at 25 °C. Specific transcripts were analyzed by S1 mapping (16).

Immunoprecipitation and Immunoblotting—Immunoprecipitation was performed as described in (18). Immunoblotting was performed using anti-hTBP, anti-hTAF_{II}30, anti-hTAF_{II}100, and anti-hTAF_{II}135 mAbs by standard methods (19).

RESULTS

To analyze the role of TBP and TFIID during transcription initiation from naked or chromatin-assembled templates, first we normalized the amounts of TBP to be used in the reactions, either as recombinant TBP or immunopurified TFIID complex, by immunoblotting using a mAb raised against the recombinant human TBP (16) (Fig. 1B). Then using equivalent units of TBP and TFIID on naked templates from two different promoters (Fig. 1, A and C). As a source of remaining general transcription factors and other accessory factors we used a heat-treated HeLa cell NE in which the transcriptional activity of TBP and TFIID could be monitored (17). Transcription in the heated NE was completely dependent upon the addition of TBP or TFIID (Fig. 1C). Equivalent units of TBP and TFIID resulted in comparable levels of basal transcription initiation from the adenovirus major late promoter (AdMLP) (Fig. 1C). However, TFIID-dependent basal transcription was more efficient from the rabbit β -globin promoter (Glob) than with TBP (in Fig. 1C, compare lanes 3–5 to 6–8 and in Fig. 2E, lane 8 to 9). Note, that transcription from the two templates have been carried out in the same reaction. Thus, our results show that on certain promoters TAF_{II}s can have a positive effect on transcription initiation from naked templates.

Next, the AdMLP and the Glob templates were assembled into chromatin with an average nucleosome repeat length of 176 base pairs (± 3 base pairs; data not shown), and the efficiency of chromatin assembly was monitored by micrococcal nuclease digestion (Fig. 2B). To analyze the transcriptional properties of TBP and TFIID on chromatin templates, the chromatin-assembled AdMLP and the Glob templates had been

preincubated with TBP or TFIID for 10 min prior to the addition of the heat-treated NE (Fig. 2A). Surprisingly, recombinant TBP alone can mediate transcription initiation in a dose-dependent manner from the chromatin-assembled AdMLP and, to a lesser extent, from the β -globin promoter (Fig. 2C). In contrast, the same amount of TFIID that mediated efficient transcription initiation from naked DNA templates exhibited only a very minor enhancement of transcription from the chromatin-assembled AdMLP and globin promoters (lanes 7–10). However, on the chromatin-assembled AdMLP template that contains five GAL4 binding sites, the chimeric activator, Gal4-VP16, strongly stimulated TFIID-mediated transcription (Fig. 2D, lanes 5–8). These results together suggest that TAF_{II}s can impair initiation of basal, but not activated, transcription when nucleated by TFIID on chromatin templates.

To test whether efficient TBP-dependent transcription on chromatin was solely due to the heat treatment, we used either normal or heat-treated NEs (Fig. 2E). DNA templates were assembled with chromatin or not and preincubated with TBP or TFIID for 10 min, and then the nontreated or the heat-treated NEs were added (Fig. 2E). Using the chromatin-assembled templates neither TBP or TFIID was able to recover or enhance transcription in the normal nuclear extract (Fig. 2E, lanes 4–6). In contrast, in the heat-inactivated NE TBP mediated transcription from the chromatin templates (Fig. 2E). Note that TBP-mediated transcription was much more efficient from the chromatin-assembled AdMLP template than from the Glob template (Fig. 2C, lanes 3–6 and Fig. 2E, lane 11), suggesting that the observed effect may be dependent on the TATA box of the promoter used. However, TFIID had only a weak effect on chromatin transcription when added to the heat-treated NE (Fig. 2E). These results suggest that on certain promoters chromatin is permissive to transcription initiation mediated by TBP but not by TFIID and that the heat treatment may destabilize a chromatin-targeted repressor in the nuclear extract (see also "Discussion").

The action of TBP-mediated transcription on chromatin templates was further analyzed at the level of transcription initi-

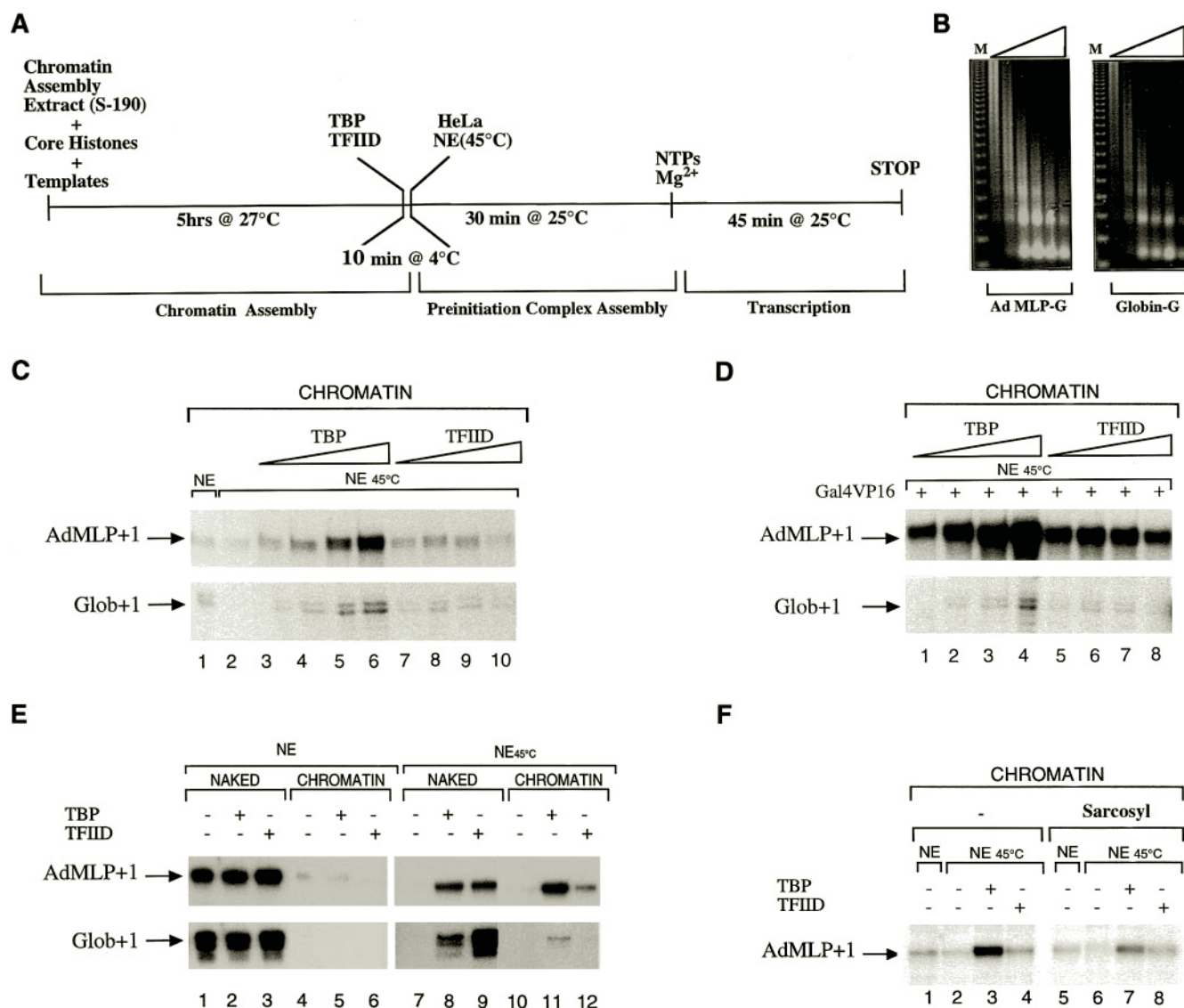


FIG. 2. TBP-mediated basal transcription is not blocked on chromatin templates. *A*, outline of chromatin assembly and transcription assays. *B*, chromatin templates were digested with micrococcal nuclease and the DNA fragments were analyzed by agarose gel electrophoresis and ethidium bromide staining. *M*, 123-base pair DNA ladder. *C*, TBP- and TFIID-dependent chromatin transcription. AdMLP (*AdMLP+1*) and β -globin (*Glob+1*) promoter-containing templates were assembled in chromatin, equivalent units of TBP or TFIID were prebound to these templates, and then normal (*NE*, lane 1) or heat-inactivated nuclear extracts (*NE45°C*, lanes 2–10) were added as indicated. *D*, TBP- and TFIID-dependent activated chromatin transcription. The AdMLP template contains 5 GAL4 binding sites upstream of the adenovirus major late minimal promoter. GAL4-VP16 was preincubated with the AdMLP and β -globin promoter-containing chromatin templates for 10 min before TBP or TFIID were added. The samples shown in panels *C* and *D* were analyzed on the same gel and thus exposed for the same time. *E*, TBP- and TFIID-dependent transcription in normal and heat-inactivated nuclear extracts on naked and chromatin templates. *F*, TBP-dependent chromatin transcription is blocked by sarcosyl at the level of reinitiation. Preinitiation assembly reactions were assembled as indicated in panel *A*. In lanes 5–8 sarcosyl was added 1.5 min after the addition of NTPs and MgCl₂.

ation. To eliminate ongoing initiation in the transcription reaction, we performed single round transcription experiments on the chromatin-assembled AdMLP template. Transcription was initiated for 90 s by addition of NTPs, then sarcosyl was added to a final concentration of 0.08% to prevent *de novo* PIC formation as well as initiation at preformed PICs (20). Using nontreated NE no differences were observed on chromatin with or without sarcosyl in the absence of TBP and TFIID (Fig. 2*F*). Moreover, in the heat-treated extract the low TFIID-dependent transcription levels were not influenced by sarcosyl (Fig. 2*F*). However, a strong decrease was observed in the TBP-mediated transcription initiation on the chromatin template when using heat-treated NE (Fig. 2*F*). Thus, on chromatin templates TBP and TFIID have little or no effect on single round basal transcription, but TBP seems to be involved in the maintenance of high levels of activator-independent reinitiation.

Next we examined the effect of heat treatment of the NE on endogenous TFIID. Nuclear extracts were either kept on ice or heat inactivated at 45 °C before immunoprecipitation was performed with an anti-TBP mAb, and TBP-associated proteins were analyzed by Western blot (Fig. 3). In normal NE, several TAF_{II}s were coimmunoprecipitated together with TBP (Fig. 3). In heat-treated nuclear extract TBP dissociated from TFIID because none of the tested TAF_{II}s (*i.e.* TAF_{II}250, TAF_{II}135, TAF_{II}100, TAF_{II}55, and TAF_{II}30) were coimmunoprecipitated with TBP (Fig. 3 and data not shown). Thus, we hypothesize that after the dissociation from the TFIID complex, TBP loses its transcriptional activity because it undergoes a conformational change and/or a posttranslational modification. Moreover, it seems that all the other activities that have been reported to be able to mediate Pol II transcriptional initiation

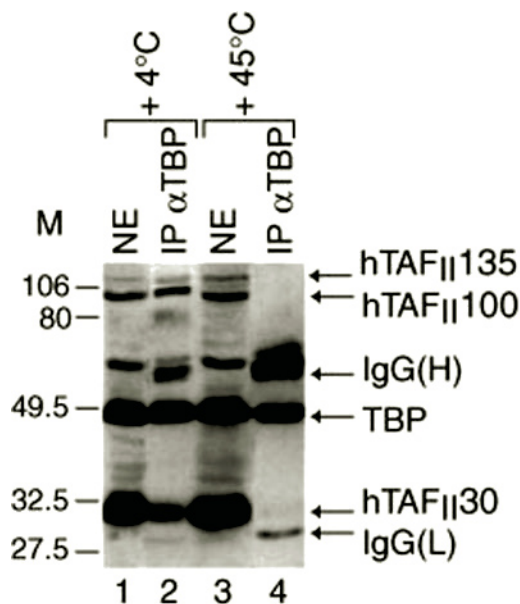


FIG. 3. Heat-treatment dissociates TBP from the TFIID complex. Nuclear extracts (NE) were heat-treated at 45 °C or kept at 4 °C. TBP-containing complexes immunoprecipitated (IP) with an anti-TBP mAb (α TBP) and associated proteins were tested by immunoblotting using anti-hTBP, anti-hTAF_{II}135, anti-hTAF_{II}100, and anti-hTAF_{II}30 mAbs. Cross-reacting immunoglobulin light and heavy chains are indicated as IgG(L) and IgG(H), respectively. M, molecular mass marker in kDa.

(TFTC, TAC, and other TBP-like factors (21) are also heat inactivated in this extract.

DISCUSSION

Our results demonstrate that chromatin *per se* is not refractory to activator-independent PIC formation and that subsequent transcription initiation on every promoter, such as TBP-dependent basal transcription, is almost as efficient on chromatin-assembled AdMLP template as it is on naked DNA in the heat-treated nuclear extract.

The recent discovery of TAF_{II}-independent and TAF_{II}-dependent forms of TBP (9, 10) and the TAC complex free of TAF_{II}s (12), give TBP function a new turn. Thus it is important to identify nuclear activities that regulate TBP-dependent (TAF_{II}-independent) basal transcription. Binding of TBP to the TATA sequence seemed to be inhibited by nucleosomes, and this inhibition could be overcome by ATP-dependent alterations in nucleosomal DNA structure mediated by hSWI/SNF (22). Here we show that TBP-mediated basal transcription on chromatin is blocked in a "normal" NE and that heat treatment can relieve this inhibition on certain promoters. Thus, it seems that a chromatin-targeted inhibitory factor exists in NE that blocks transcription initiation by the TAF_{II}-independent forms of TBP only on chromatin. However, activators may relieve the repression by the inhibitory factor as TBP-dependent transcription can be stimulated in a normal NE (data not shown). FACT, a heat labile elongation factor that can relieve chromatin-mediated blocks and that blocks TBP-mediated transcription on naked DNA in the absence of TFIIE and TFIIF (23, 24), may be a candidate for this inhibitory factor. Whether FACT and/or other yet unknown factors are required for this chromatin-targeted inhibitory activity remain to be determined. Alternatively, it is also conceivable that heat treatment causes dissociation of a nuclear multisubunit transcription complex, which subsequently releases a nonheat labile factor that facilitates the observed TBP-dependent basal transcription on chromatin.

When TBP was co-incubated with heat-treated NE under

chromatin transcription conditions, we were unable to co-purify TAF_{II}s together with TBP (data not shown). This observation together with the clear difference between the TBP- and TFIID-mediated transcription on chromatin and with the immunoprecipitation results suggest that TFIID does not reform under our assay conditions. Thus, the observed differences between TBP and TFIID are due to TAF_{II}-independent *versus* TAF_{II}-dependent transcription initiation on chromatin. In contrast to the TBP-mediated transcription initiation, the inability of TFIID to efficiently initiate basal transcription on chromatin may indicate that TAF_{II}s are able to exert negative effects on TBP-dependent basal transcription on chromatin or that TFIID is simply sterically excluded from chromatin. On naked templates TAF_{II}s have been reported to have negative effects on basal transcription (25, 26). Surprisingly, under our assay conditions and on naked templates we have not observed negative effects by TAF_{II}s on basal transcription as TFIID was as active as free TBP. However, on chromatin templates TAF_{II}s seem to inhibit basal but not activated transcription as activators strongly stimulated TFIID-mediated transcription (Fig. 2D). Thus, our data suggest that on chromatin TAF_{II}s may impair only TFIID-dependent basal transcription initiation.

Emerging evidence suggest that reprogramming of chromatin and of TBP- and TBP-like factor-mediated transcription initiation during distinct developmental stages of embryogenesis, where fundamentally different chromatin states exist, are precisely regulated and coupled events (27, 28). Our study thus identifies a potential contributor(s) to this regulation and provides mechanistic explanations toward the understanding of chromatin-regulated transcription initiation events *in vivo*.

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