TAFs and TFIIA Mediate Differential Utilization of the Tandem *Adh* Promoters

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Summary

The D. melanogaster alcohol dehydrogenase (*Adh*) gene is transcribed from two tandem promoters that are differentially utilized at various stages during development. To determine the mechanism of promoter selectivity, we have analyzed the activity of the *Adh* promoters both in vitro and in transfected cells. We found that selective promoter utilization is controlled by distinct initiator elements. Reconstitution of *Adh* transcription with purified components requires a specific TBP–TAF complex that, in concert with TFIIA, directs differential *Adh* promoter transcription. Fractionation of this TBP–TAF complex reveals that TAF$_{II}$150 is required for discrimination between the proximal and distal promoters. We propose a mechanism for regulating differential promoter utilization during Drosophila development that involves the recognition of specific initiator elements by TAFs in the TFIID complex.

Introduction

Embryonic development and cellular differentiation are complex processes regulated by the precise temporal and spatial expression of specific genes. Studies of gene expression during Drosophila development have revealed a complex array of regulatory proteins, many of which are transcription factors (reviewed by Jäckle and Sauer, 1993; St Johnson and Nüsslein-Volhard, 1992). Several of these transcription factors recognize and interact with gene-specific DNA elements (enhancers or silencers) located upstream or downstream of protein-coding genes. Through these specific protein–DNA interactions, unique combinations of enhancer/silencer elements regulate transcription initiation at core promoters (Tjian and Maniatis, 1994). This interplay between enhancer factors and the basal transcription apparatus results in a tightly regulated cascade of gene expression during development. While the importance of enhancer/silencer elements and their corresponding binding factors in governing gene transcription is well established, less is known about the potential regulatory properties of core promoter elements.

Transcription initiation of protein-coding genes occurs at core promoters, which typically consist of a TATA box, initiator, and downstream elements. The ordered assembly of the basal transcription apparatus (Zawel and Reinberg, 1993) at core promoters is thought to begin with the binding of TFIID, an essential transcription factor composed of the TATA box–binding protein (TBP) and eight or

more TBP-associated factors (TAFs) (Goodrich and Tjian, 1994). It is commonly accepted that an important step in promoter recognition is mediated by the binding of TBP to the TATA box. However, the existence of TATA-less promoters and the discovery of TAFs suggest that promoter recognition might also be mediated by TAF–DNA interactions (Kaufmann and Smale, 1994; Martinez et al., 1994; Purnell et al., 1994), an idea supported by the ability of TFIID and other TBP–TAF complexes, such as SL1 and TFIIIB (or SNAP$_c$), to discriminate among promoters transcribed by RNA polymerase I, II, and III (pol I, II, and III), respectively (Goodrich and Tjian, 1994; Hernandez, 1993). Among the subunits of TFIID, TAF$_{II}$150 displays sequence-specific DNA-binding activity at select core promoters (Verrijzer et al., 1994). Likewise, TAF$_I$63, a subunit of SL1, has been cross-linked to DNA elements in the ribosomal RNA promoter (Rudloff et al., 1994). These findings are consistent with the notion that TAFs may contribute significantly to the promoter recognition process.

In this study, we analyze the role of TAFs in directing core promoter selectivity during transcription of the alcohol dehydrogenase gene (*Adh*) in Drosophila cells. The tissue- and stage-specific transcription of the *Adh* gene is controlled by tandem promoters (termed distal and proximal) in combination with upstream regulatory elements (Ayer and Benyajati, 1992; Corbin and Maniatis, 1989a, 1990; Falb and Maniatis, 1992). Interestingly, the distal and proximal *Adh* promoters are differentially transcribed during fly development (Benyajati et al., 1983; Savakis and Ashburner, 1986): the distal promoter is primarily utilized in early–mid stage embryos and in adult flies, whereas the proximal promoter is active during late embryonic and early–mid stage larval development. Regulation of the tandem *Adh* promoters has been extensively analyzed by transgenic fly experiments, revealing that *Adh* gene expression is at least in part controlled by the upstream *Adh* larval and adult enhancers (Corbin and Maniatis, 1989a). Utilization of the proximal promoter in larvae is dependent on the *Adh* larval enhancer. By contrast, while the *Adh* adult enhancer strongly stimulates distal promoter activity, transcription from the distal promoter in adults is still maintained, although at a reduced level, after removal of the adult enhancer. Thus, the selective utilization of the distal promoter can occur even in the absence of the upstream enhancer (Corbin and Maniatis, 1989a, 1989b). These results suggest that distal promoter utilization may be regulated by an additional mechanism possibly mediated by core promoter elements. Therefore, a detailed analysis of *Adh* transcription held the promise of novel insights regarding the role of core promoter elements in regulating transcription during development.

Here, we identify core elements that direct promoter-selective *Adh* gene transcription and purify the factors necessary for promoter recognition. First, using a combination of in vitro transcription and transfection experiments, we establish the role of core elements in differential promoter

utilization. Second, we identify specific transcription factors that control promoter switching by reconstituting promoter-selective *Adh* transcription using purified Drosophila factors. Our data reveal novel properties of TFIID and TFIIA that allow Drosophila to discriminate between tandem promoters governing a regulatory switch during development.

Results

Role of Initiator Elements in the *Adh* Promoter Switch

The Drosophila *Adh* gene is differentially transcribed from two promoters during embryonic development (Heberlein and Tjian, 1988; Savakis and Ashburner, 1986). The distal promoter is active in a brief burst lasting from 8 to 20 hr after egg laying and is subsequently shut off. Transcription from the proximal promoter is activated 12–16 hr after egg laying and increases during late embryogenesis (Figure 1A). Thus, in early- to mid-stage embryos the switch is "on" for the distal promoter and "off" for the proximal promoter, whereas the situation reverses a few hours later. This temporal pattern of *Adh* transcription can be reproduced in vitro using nuclear extracts derived from embryos at different developmental stages (Heberlein and Tjian, 1988).

We have used nuclear extracts derived from embryos to study the regulatory mechanisms governing *Adh* distal versus proximal promoter utilization. Nuclear extracts from 0- to 12-hr-old embryos can direct accurate initiation from a 3.2 kb wild-type template (3.2wt) containing *Adh* sequences from position −662 upstream of the distal promoter to position +2509, which also encompasses the proximal promoter (Figures 1B and 1C). In vitro transcripts were detected by primer extension analysis and revealed that the distal promoter was preferentially transcribed relative to the proximal promoter (Figure 1C, lane 2), consistent with the pattern of transcription in early–mid stage embryos. A truncated version of the distal promoter (D-46) containing only sequences between positions −46 and +100 was also active, but showed decreased levels of transcription relative to the longer template (compare lanes 1 and 2 in Figure 1C). This was not surprising, since the upstream region contains the *Adh* enhancer and binding sites for *Adh* distal factor-1, which has been shown to stimulate the distal promoter in vitro and in vivo (England et al., 1990). However, despite the lack of upstream regulatory sequences, the truncated promoter has considerable activity, consistent with transgenic fly experiments in which distal transcription was observed even in the absence of sequences upstream of position −128 (Corbin and Maniatis, 1989a, 1989b). These observations suggest that distal promoter activity is not strictly dependent on the *Adh* enhancers. Instead, activity of the distal promoter appears to be partly mediated by downstream or core promoter elements.

To identify sequence elements responsible for select distal promoter activity during early embryogenesis, we constructed a chimeric promoter, in which the distal initiator element (DIE) was substituted for the corresponding
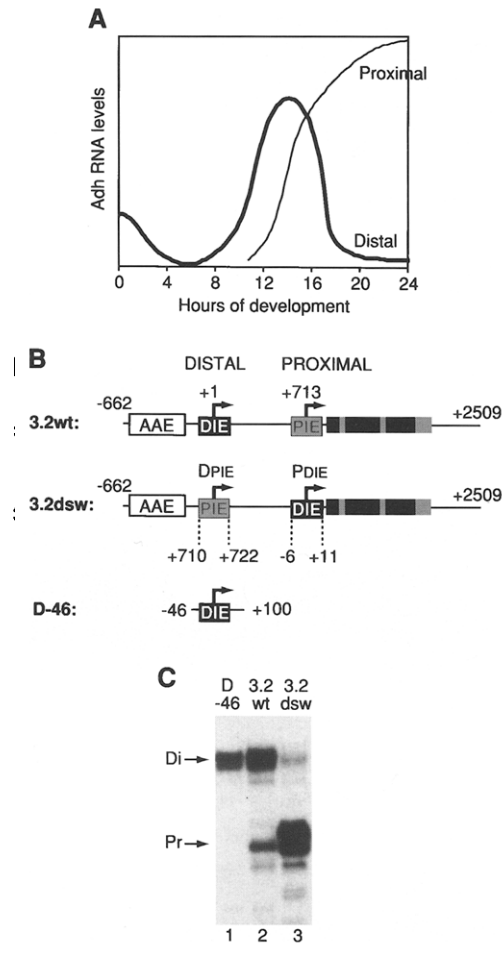

Figure 1. Differential *Adh* Promoter Transcription In Vivo and In Vitro

(A) Diagram showing the accumulation of *Adh* RNA transcribed from the distal and proximal promoters during Drosophila embryonic development. RNA levels were detected by primer extension. Data were taken from Heberlein and Tjian (1988).

(B) Genomic *Adh* fragments used for in vitro transcription and transfection experiments. Template 3.2wt contains a 3.2 kb fragment of the wild-type *AdhF* allele from position −662 to position +2509 relative to the distal transcription start site. *Adh* protein-coding sequences are shown in black (exons), whereas introns and 3' untranslated sequences are shown in gray. Template 3.2dsw is identical to 3.2wt, except that the DIE and the PIE have been swapped: distal initiator sequences from position −3 to position +10 were substituted with the PIE (position +710 to position +722). Similarly, proximal sequences from position +707 to position +723 (equal to positions −6 and +11, respectively, relative to the proximal transcription start site) were substituted with the DIE (position −6 to position +11). The numbers in the figure are relative to the distal transcription start sites. The original distance between the TATA box and the initiator element was maintained in the initiator swaps (see Figure 4A for sequences). The template D-46 contains distal promoter sequences from −46 to +100. AAE, *Adh* adult enhancer.

(C) In vitro transcription using embryo nuclear extracts fractionated by heparin–agarose chromatography. Distal and proximal promoter transcripts were detected by primer extension analysis using two ^{32}P-labeled primers complementary to distal and proximal transcripts, respectively. The relative migration of distal (Di) and proximal (Pr) extension products is indicated.
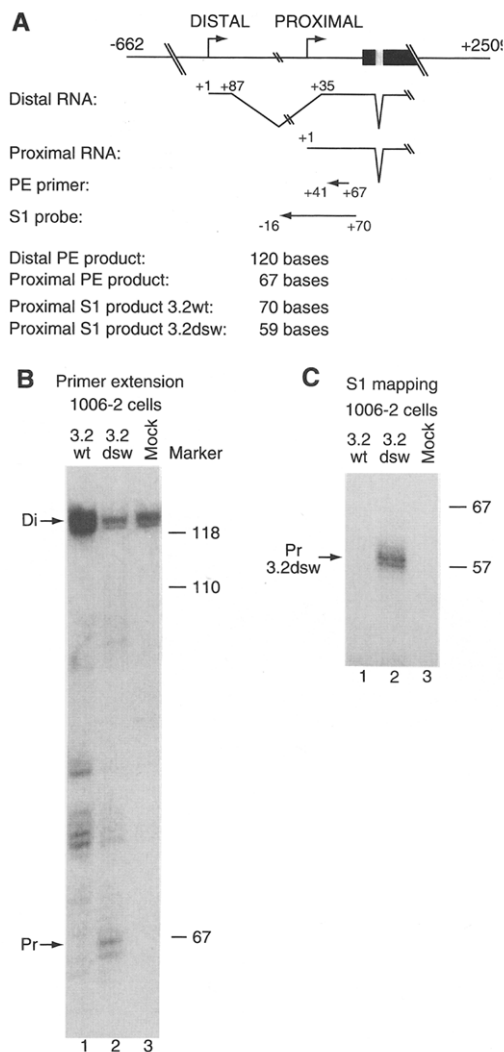
A

DISTAL   PROXIMAL

-662   +250!

Distal RNA: +1 +87   +35

Proximal RNA: +1

PE primer: +41 +67

S1 probe: -16   +70

Distal PE product:	120 bases
Proximal PE product:	67 bases
Proximal S1 product 3.2wt:	70 bases
Proximal S1 product 3.2dsw:	59 bases

B Primer extension
1006-2 cells

3.2 3.2 Mock Marker
wt   dsw

Di→   — 118

                       — 110

Pr→   — 67

1   2   3

C S1 mapping
1006-2 cells

3.2 3.2 Mock
wt   dsw

                       — 67
Pr
3.2dsw →   — 57

1   2   3

Figure 2. The Initiator Element Directs Differential *Adh* Promoter Transcription In Vivo

(A) Diagram illustrating RNA mapping technique using primer extension analysis and S1 nuclease protection. Since most of the distal transcript upstream of the proximal promoter is spliced, the proximal and distal mRNAs are of similar size, but have distinct 5' termini. After splicing, distal RNA from position +87, relative to the distal initiation site, is fused to position +35, relative to the proximal start site, resulting in an RNA that is 53 nt longer than the proximal RNA. The primer for primer extension analysis is complementary to distal and proximal RNA sequences between positions +41 and +67 relative to the proximal start site. The S1 probe, used only for detection of the proximal transcript, is complementary to coding sequences from position -16 to position +70 relative to the proximal start site.

(B) Primer extension analysis of total RNA (15 µg) extracted from Drosophila 1006-2 cells transfected with the *Adh* 3.2wt (lane 1) or 3.2dsw (lane 2) plasmids, along with a luciferase internal standard plasmid. Both distal and proximal promoter transcripts were detected using the same primer complementary to the 5' untranslated region of the *Adh* gene (see [A]). The transcript detected in mock transfected cells (lane 3) is from the endogenous *Adh* gene and originates from the distal promoter. The positions of distal (Di) and proximal (Pr) primer extension products are indicated. The size in nucleotides of single-stranded DNA standards is indicated to the right. Transfection efficiency was determined by luciferase assay (348 U and 514 U for 3.2wt and 3.2dsw, respectively). The decrease in endogenous distal promoter transcription (lane 2) is probably due to competition from the transfected tem-

region from the proximal promoter within the 3.2 kb *Adh* genomic fragment. At the same time, the initiator of the proximal promoter (PIE) was replaced by distal initiator sequences, thereby generating the 3.2 kb double-swap template (3.2dsw) (Figure 1B). The presence of the PIE severely reduced distal promoter activity (Figure 1C). Indeed, exchange of the initiator region was more devastating for distal promoter activity than removal of upstream regulatory sequences. Interestingly, the DIE was not only required for robust transcription of the wild-type distal promoter, but its presence was also sufficient to direct high levels of transcription from the proximal promoter (P$_{DIE}$) in the context of the double-swap template (Figure 1C, lane 3). A comparison of the wild-type and double-swap templates revealed that whichever promoter contained the DIE became preferentially transcribed in 0- to 12-hr embryonic extracts, suggesting that promoter utilization in vitro is highly dependent on this initiator element. Thus, the differential activity observed between the distal and proximal promoters in early- to mid-stage embryos may also be dictated, at least in part, by these different initiator elements.

To confirm the regulatory properties of the DIE in the context of intact Drosophila cells, we transfected 3.2wt and 3.2dsw templates into 1006-2 embryonic cells (derived from 3- to 14-hr-old embryos (Simcox et al., 1985)). This Drosophila cell line expresses *Adh* transcripts only from the distal promoter and thus mimics the situation observed in early embryos (Benyajati et al., 1987). Distal and proximal promoter transcription was assayed by primer extension and S1 nuclease mapping of total RNA (S1 mapping was performed only to confirm the presence of the proximal transcript). As expected, transfection of 1006-2 cells with the wild-type template, 3.2wt, revealed efficient distal promoter transcription (Figure 2B, lane 1), but no detectable proximal promoter transcription (lane 1). By contrast, distal promoter transcription above the endogenous *Adh* background transcription was not detected from the transfected 3.2dsw plasmid (Figure 2B, lanes 2 and 3), whereas transcription from the proximal promoter was greatly enhanced (compare lanes 1 and 2 in Figure 2B, as well as lanes 1 and 2 in Figure 2C; S1 mappping was performed to substantiate the level of proximal promoter transcription detected by primer extension analysis). These results confirm the observation that the presence of the DIE is linked to high levels of transcription. Moreover, our results suggest that the PIE and the DIE play a pivotal role in directing differential transcription of the two *Adh* promoters both in vitro and in cultured embryonic cells.

plate. RNA quality was determined by primer extension using an actin-specific primer, 5C-P1 (Heberlein and Tjian, 1988), which only revealed minor differences between samples (data not shown). Transfection experiments were repeated three or four times with similar results.

(C) S1 nuclease protection showing the proximal transcript from the same set of transfections as in (B). S1 mapping was performed to verify the presence of a proximal transcript. The distal S1 product is not shown.

Analysis of Differential Promoter Utilization
in Reconstituted Transcription Reactions

To dissect the mechanism governing the *Adh* promoter switch, we sought to identify different components of the transcriptional machinery required to direct promoter selectivity. By fractionation of 0- to 12-hr embryo nuclear extracts, we have separated the basal transcription factors (Figures 3A and 3B) and, wherever possible, substituted for them with purified recombinant factors, e.g., *Drosophila* TBP (dTBP), dTFIIB, human TFIIE34 (hTFIIE34), hTFIIE56, dTFIIF large and small subunits, and dTFIIA large and small subunits (Figure 3C). For these studies, we constructed a template that contained distal promoter sequences from position −46 to position +12 fused to a 250 bp G-less cassette. The proximal promoter template included sequences from position −40 to position +10 fused to a 377 bp G-less cassette (Figure 4A).

Since dTAF$_{II}$150 has previously been shown to bind to promoter sequences (Verrijzer et al., 1994), we determined whether a partially purified TFIID fraction could direct differential *Adh* promoter recognition. A TFIID fraction from an S300 sizing column (see Figure 3A) was tested in an in vitro transcription reaction reconstituted with the purified transcription factors dTFIIB, hTFIIE, dTFIIF, dTFIIH, and dRNA pol II. As shown in Figure 4B (lanes 1 and 3), TFIID directed preferential transcription of the distal rather than the proximal G-less template. In contrast, differential promoter activity was not observed with TBP, suggesting that one or more activities in the TFIID fraction are required to discriminate between the distal and proximal core promoters (Figure 4B). To identify these activities further, we purified the S300 TFIID fraction by Mono Q chromatography, thereby separating the bulk of TFIID from TFIIA (see Figure 3A). Transcription from the distal promoter (D$_{DIE}$) with the Mono Q–purified TFIID fraction, which does not contain TFIIA (data not shown), was significantly reduced, but maximum activity could be restored upon addition of the TFIIA fraction (Figure 4C, lanes 1–3). Substitution of the distal initiator by the proximal initiator (template D$_{PIE}$) resulted in decreased distal promoter activity (Figure 4C, lanes 3 and 4), whereas insertion of the distal initiator into the proximal promoter to form P$_{DIE}$ increased proximal promoter transcription (Figure 4C, lanes 7 and 8). These results confirm, using a purified transcription system, that the distal and proximal initiators can direct selective *Adh* promoter utilization. By contrast, differential transcription from the various distal and proximal promoter templates was not observed with TBP (Figure 4D), suggesting that differential distal and proximal initiator function requires TFIID.

Comparison of the nucleotide sequences within the regions used for the initiator swaps revealed distinct distal and proximal initiator motifs (Figure 4A). To map further the nucleotide sequence critical for distal promoter function, we substituted the distal initiator sequence (ATTATT) with the proximal motif (AACAAC) to form a mutant template (D$_{MIE}$) (Figure 4A). Transcription from the D$_{MIE}$ template was strongly reduced relative to the wild-type distal promoter, D$_{DIE}$, and comparable with the level observed with the wild-type proximal (P$_{PIE}$) promoter (Figure 4C).
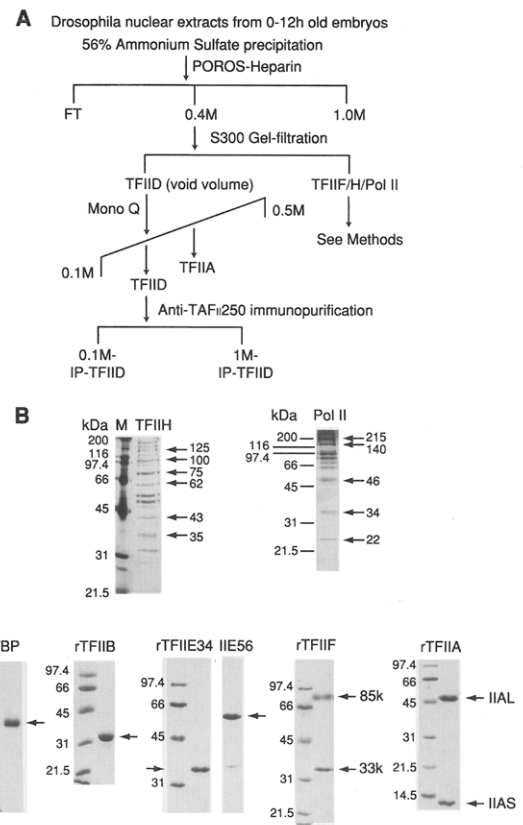
Figure 3. Purified Basal Transcription Factors Used in Reconstituted Transcription Reactions

(A) Purification scheme for separation of basal factor activities from *Drosophila* embryo nuclear extracts. The TFIID fraction was selected based on its ability to direct differential *Adh* promoter transcription and was further purified using a monoclonal antibody directed against the dTAF$_{II}$250 subunit of the TFIID complex. The immunopurification included several washes with buffer containing either 0.1 M NaCl (0.1M-IP-TFIID) or 1.0 M NaCl (1M-IP-TFIID). For information on the purification of TFIIF, TFIIH, and RNA pol II, see Experimental Procedures.

(B) Silver-stained SDS–polyacrylamide gels showing the subunit composition of TFIIH (10 µl; equivalent to 100 transcription reactions) and RNA pol II (5 µl; equivalent to 20 transcription reactions) purified from 0- to 12-hr *Drosophila* embryos. Size markers are indicated. Protein bands with sizes corresponding to subunits of hTFIIH are indicated: 125 kDa, XPC; 100 kDa, ERCC3; 75 kDa, ERCC2; 62 kDa, p62; 43 kDa, p44; 35 kDa, p34 (Drapkin et al., 1994; Humbert et al., 1994). The sizes of previously described RNA pol II subunits are indicated to the right (Weeks et al., 1982).

(C) Coomassie-stained SDS–polyacrylamide gel showing the recombinant purified basal factors used for in vitro transcription. The molecular mass standards are given in kilodaltons. dTBP and dTFIIB, the two subunits of hTFIIE (34k and 56k), the small subunit of dTFIIF (33k), and both subunits of dTFIIA (IIAL, 48k and IIAS, 14k) were produced in bacteria, whereas the large subunit of dTFIIF (85k) was expressed in baculovirus-infected SF9 cells. The subunits of dTFIIF and dTFIIA were subject to combined renaturation and further purified as protein complexes.

This effect was only observed with TFIID and TFIIA, but not with TBP (Figure 4D). To test whether the distal initiator, in the context of its own promoter, was sufficient to direct transcription initiation, we mutated the TATA box motif, TATTT, into the sequence CCTTG. As expected, these
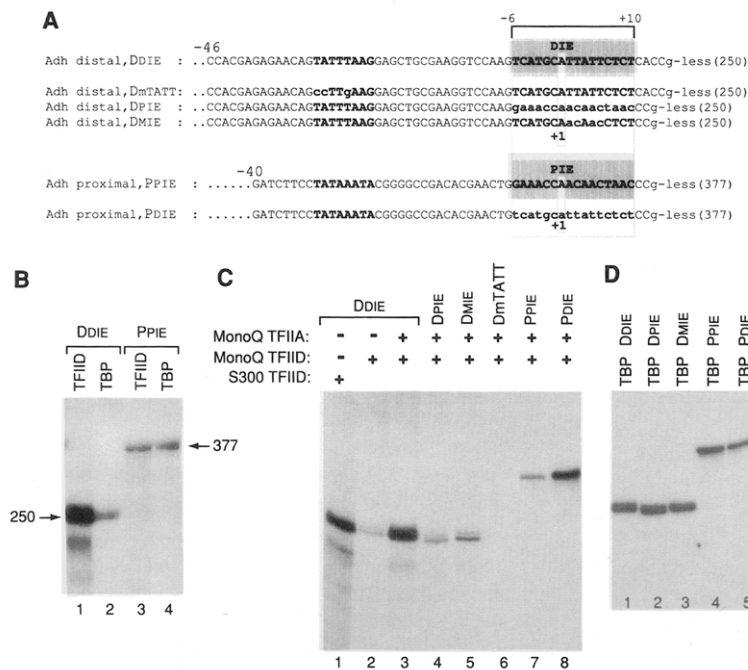
A

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                                                        -6            +10
                            -46                                  DIE
Adh distal,DDIE   : ..CCACGAGAGAACAGTATTTAAGGAGCTGCGAAGGTCCAAGTCATGCATTATTCTCTCACCg-less(250)

Adh distal,DmTATT : ..CCACGAGAGAACAGccTTgAAGGAGCTGCGAAGGTCCAAGTCATGCATTATTCTCTCACCg-less(250)
Adh distal,DPIE   : ..CCACGAGAGAACAGTATTTAAGGAGCTGCGAAGGTCCAAGgaaaccaacaactaacCCg-less(250)
Adh distal,DMIE   : ..CCACGAGAGAACAGTATTTAAGGAGCTGCGAAGGTCCAAGTCATGCAacAacCTCTCCg-less(250)
                                                                      +1
                            -40                                 PIE
Adh proximal,PPIE : ......GATCTTCCTATAAATACGGGGCCGACACGAACTGGAAACCAACAACTAACCCg-less(377)

Adh proximal,PDIE : ......GATCTTCCTATAAATACGGGGCCGACACGAACTGtcatgcattattctctCCg-less(377)
                                                                      +1
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Figure 4. Differential *Adh* Promoter Transcription Reconstituted with Purified Components

(A) In vitro transcription templates contain sequences from positions −46 to +12 (D_{DIE}) and from positions −40 to +10 (P_{PIE}) of the *Adh* distal and proximal promoters, respectively. Proximal promoter constructs direct the expression of a 377 nt G-less cassette, whereas distal promoter constructs direct the expression of a 250 nt G-less cassette (the first 250 nt within the two G-less cassettes are identical). The construct D_{PIE} was generated by exchanging the DIE with the PIE (in the abbreviation D_{PIE}, D refers to the distal promoter, and the subscript PIE refers to the initiator element). Likewise, P_{DIE} is the proximal promoter with the DIE. D_{MIE} has four point mutations at positions +2, +3, +5, and +6 that change the distal initiator motif ATTATT into the proximal motif AACAAC. The distal promoter construct DmTATT contains point mutations destroying the distal TATT box motif. For each construct, the +1 position is indicated. The TATA motifs and initiator elements are shown in bold. Sequence alterations are shown in lower case.

(B) Differential distal and proximal promoter transcription, in vitro, using the D_{DIE} and P_{PIE} G-less cassette templates described in (A). D_{DIE} and P_{PIE} were transcribed in a purified transcription system (see Figure 3 and Experimental Procedures) using either TFIID (S300 sizing column fraction) or dTBP. The distal and proximal promoter transcripts are 250 and 377 nt in length, respectively.

(C) Functional role of distal core promoter elements in reconstituted in vitro transcription. The transcription templates are indicated above each lane and described further in (A). S300 TFIID (lane 1) was further fractionated on a Mono Q column, thereby separating TFIID (Mono Q–TFIID) from TFIIA (Mono Q–TFIIA) (also see Figure 3A). The Mono Q–TFIID fraction used in this study represents a subpopulation of the entire TFIID pool; see Experimental procedures for details.

(D) In vitro transcription of various distal and proximal promoter templates using 2 ng of dTBP in the reconstituted transcription system.

mutations abolished distal promoter activity (Figure 4C, lane 6). These studies suggest that maximum distal promoter activity requires the core initiator element and TATA box. Moreover, reconstitution of differential *Adh* promoter transcripiton in the purified transcription system indicates that *Adh* promoter selectivity is dependent on TFIID and perhaps TFIIA.

Role of TFIIA in Promoter Selectivity

The role of TFIIA in both activated and basal transcription in vitro has recently been confirmed using the recombinant large and small TFIIA subunits (Yokomori et al., 1994). In Figure 4C, we demonstrated that a TFIIA fraction was required for maximum *Adh* distal promoter activity. To determine whether the TFIIA component of this fraction indeed contributes to the observed promoter-selective function, we tested distal promoter transcription with purified bacterially expressed dTFIIA. Transcription reactions reconstituted with TFIID confirmed that purified recombinant TFIIA subunits (rTFIIA) could fully substitute for the partially purified embryonic TFIIA fraction (eTFIIA) to stimulate distal promoter activity (Figure 5A, lanes 1–3). However, if TBP instead of TFIID was used in these transcription reactions, dTFIIA was found to have no effect on transcription from the distal *Adh* promoter (Figure 5A, lanes 4 and 5). To assess further the role of TFIIA in promoter selectivity, we have carried out in vitro transcription reactions in the presence of both the distal and proximal templates in the same reaction. Transcription with TBP or TFIID in the

absence of recombinant TFIIA revealed similar levels of transcription from both the distal and proximal promoters (Figure 5B, lanes 1 and 2). However, upon addition of recombinant TFIIA, TFIID preferentially directed transcription from the distal promoter relative to the proximal promoter (Figure 5B, lane 3), whereas TFIIA had no effect on transcription with TBP (data not shown). When the same transcription reactions were performed with templates containing the initiator swaps, recombinant TFIIA preferentially stimulated transcription from the promoter containing the distal initiator P_{DIE} (Figure 5C). These experiments establish that differential distal and proximal promoter transcription is mediated through the initiator elements by components in the TFIID fraction and requires TFIIA.

Regulation of Promoter Selectivity by Components Associated with TFIID

Our results thus far strongly suggest that components associated with TFIID may be essential for directing selectivity between the *Adh* proximal and distal promoters. To determine whether the TFIID complex itself might be important for directing promoter selectivity, we have carried out in vitro transcription reconstituted with immunopurified TFIID. TFIID was immunopurified using a monoclonal antibody recognizing the $TAF_{II}250$ subunit of TFIID (see Figure 3A), and transcription was performed on the beads. This highly purified TFIID complex displayed both stimulation by TFIIA and the ability to discriminate between the initia-
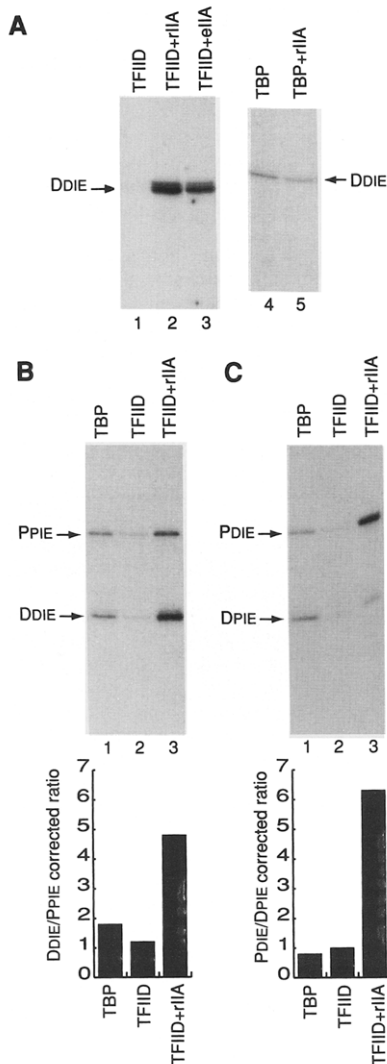
Figure 5. TFIIA Is Required for Differential *Adh* Promoter Transcription

(A) Reconstituted in vitro transcription using the D_{DIE} template containing the wild-type distal core promoter fused to a G-less cassette (also see Figure 4A). Transcription with the Mono Q–TFIID fraction was carried out in the absence (lane 1) or in the presence of either recombinant dTFIIA (rIIa, 25 ng, lane 2) or the Mono Q–TFIIA embryonic fraction (eIIA, lane 3). Transcription with recombinant dTBP (0.1 ng) in the absence (lane 4) or presence (lane 5) of 25 ng of recombinant dTFIIA (rIIA) is shown.

(B) Cotranscription of G-less distal and proximal promoter templates (in purified transcription system) using dTBP (lane 1), Mono Q–TFIID (lane 2), or Mono Q–TFIID with recombinant dTFIIA (lane 3). The relative transcriptional efficiencies cannot be directly derived from the autoradiogram, since the proximal transcript labels with a specific acitivity that is approximately 1.5-fold higher than that of the distal transcript owing to the different sizes of the two transcripts. The corrected ratios are shown in the histogram below the autoradiogram.

(C) As in (B), except that the distal and proximal initiators were swapped. The histogram shows the corrected ratios of P_{DIE^-} and D_{PIE^-} derived transcription.

tors of distal and proximal *Adh* promoters (Figure 6A). To identify which components associated with TFIID, i.e., TAFs, were required for transcriptional selectivity, we subjected the immunopurified complex to treatment with 1

M salt (1M-IP-TFIID). Interestingly, TFIID that had been washed with 1 M salt lost the ability to discriminate between the distal and proximal *Adh* promoters (Figure 6B, lane 2). This result suggested the presence of a promoter-selective activity that was loosely associated with the TBP–TAF complex. Indeed, when the flowthrough fraction (the unbound material) from the immunopurification was added to the 1M-IP-TFIID beads, differential *Adh* promoter transcription was restored (Figure 6B, lanes 2 and 3). The flowthrough fraction alone had no detectable TFIID activity (Figure 6B, lane 4) and when added to TBP failed to stimulate promoter selectivity (data not shown). Thus, one or more components that form a metastable complex with TFIID appear to be required for differential *Adh* distal and proximal promoter recognition.

To identify the activity required for promoter selectivity, we tested the 1M-IP-TFIID complex for the presence of TAFs by Western blot analysis and found $TAF_{II}250$, $TAF_{II}110$, $TAF_{II}80$, $TAF_{II}60$, $TAF_{II}40$, $TAF_{II}30\alpha$, and TBP (data not shown). However, no $TAF_{II}150$ was detected by Western blot analysis; instead, a protein with an apparent molecular mass of 125 kDa was detected, possibly a $TAF_{II}150$-related or proteolytic product (marked with an X in Figure 6C, lane 3). In contrast, $TAF_{II}150$ was found in the active TFIID fraction and in the flowthrough fraction from the immunopurification (Figure 6C, lanes 1 and 2), as well as in 0.1M-IP-TFIID (data not shown). This observation suggested that $TAF_{II}150$, or a closely related protein, might be loosely associated with this TBP–TAF complex and could be dislodged by a high salt wash. To test whether $TAF_{II}150$ contributes to differential promoter utilization in an initiator-dependent manner, we incubated the promoter-selective TFIID fraction with antibodies directed against $TAF_{II}150$. Transcription reactions supplemented with this antibody-treated TFIID were no longer able to discriminate between templates containing the distal and proximal initiator (Figure 6D, lanes 3–6). To further substantiate the role of $TAF_{II}150$ in promoter selectivity, we incubated purified recombinant $TAF_{II}150$ with the 1M-IP-TFIID complex that had been depleted of $TAF_{II}150$. Silver-stained SDS–polyacrylamide gels of the 1M-IP-TFIID before and after incubation with $TAF_{II}150$ revealed that $TAF_{II}150$ could be incorporated into this TBP–TAF complex (Figure 6E). Furthermore, this incorporation of $TAF_{II}150$ strongly stimulated transcription from the promoter bearing the distal initiator, but had no effect on the promoter with the proximal initiator (Figure 6D, lanes 7–10). These data suggest that, in addition to TFIIA, differential promoter recognition is mediated by TAFs in the TFIID complex and that $TAF_{II}150$ plays a critical role in this process.

Stage-Specific *Adh* Promoter Transcription In Vitro

In late-stage embryos, the proximal promoter is more active than the distal promoter (see Figure 1A). To test whether this decrease in distal promoter activity is due solely to developmental regulation of initiator selectivity or to more complex mechanisms, we performed in vitro transcription reactions using nuclear extracts derived from 16- to 20-hr-old embryos. These late-embryo extracts were fractionated by heparin–agarose chromatography and
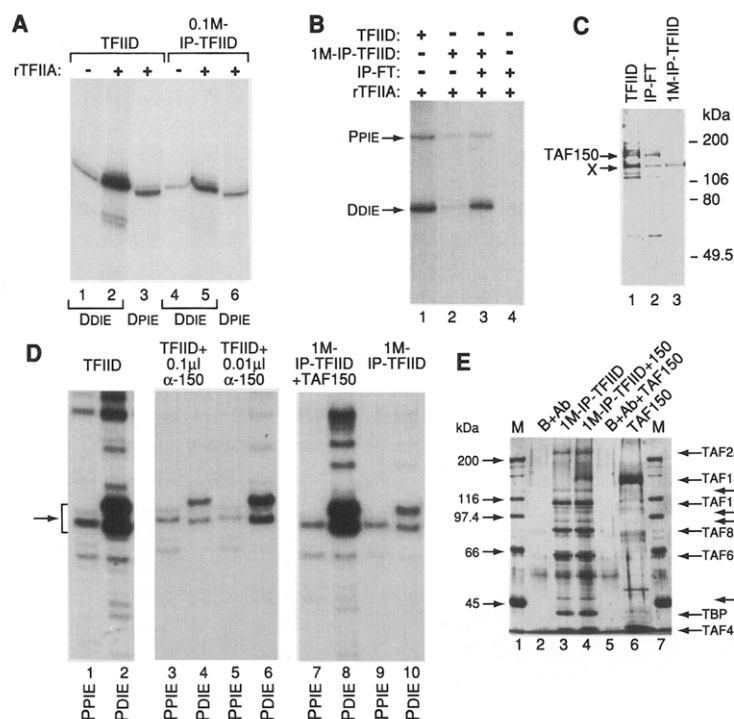
A

B

C

D

E

Figure 6. dTAF$_{II}$150 Is Required for Differential Adh Promoter Transcription

(A) Reconstituted in vitro transcription with Mono Q–TFIID fraction (lanes 1–3) and immunopurified Mono Q–TFIID (0.1M-IP-TFIID, lanes 4–6; see Figure 3A). Transcription with the immunopurifed complex was performed on the beads. G-less transcription templates, D$_{DIE}$ and D$_{PIE}$, are indicated below the autoradiogram.

(B) Cotranscription of G-less D$_{DIE}$ and P$_{PIE}$ templates in the reconstituted in vitro transcription system. Differential distal and proximal promoter transcription with Mono Q–TFIID (TFIID) in the presence of recombinant dTFIIA (rTFIIA) is shown (lane 1). Transcription with 1M-IP-TFIID was performed on the beads (lanes 2 and 3). We preincubated 2 μl of the flowthrough fraction from the immunopurification (IP-FT) with 1M-IP-TFIID and TFIIA or TFIIA only prior to transcription (lanes 3 and 4).

(C) Western blot analysis of Mono Q–TFIID (lane 1), flowthrough fraction from TFIID immunopurification (IP-FT, lane 2), and 1M-IP-TFIID (lane 3). The blot was probed with anti-TAF$_{II}$150 antibodies. The positions of full-length TAF$_{II}$150 and a 125 kDa protein (marked X) immunoreactive with anti-TAF$_{II}$150 antibodies are indicated with arrows.

(D) Reconstituted in vitro transcription using templates 3.2wt and 3.2dsw. Initiator-dependent transcription from P$_{PIE}$ and P$_{DIE}$ was detected using the proximal primer for primer extension analysis. The arrow indicates the position of the correct primer extension product. All reactions were performed in the presence of recombinant dTFIIA. Lanes 1 and 2, Mono Q–TFIID; lanes 3–6, Mono Q–TFIID preincubated with 0.1 μl or 0.01 μl of anti-TAF$_{II}$150 antibodies; lanes 7 and 8, 1M-IP-TFIID incubated with recombinant purified TAF$_{II}$150 (unbound TAF$_{II}$150 was removed prior to transcription); lanes 9 and 10, 1M-IP-TFIID (depleted of TAF$_{II}$150).

(E) Silver-stained SDS–polyacrylamide gel showing the protein pattern of the immunopurifed TFIID complexes used in (C) and (D). Lanes 1 and 7, molecular mass markers; lane 2, beads with cross-linked anti-TAF$_{II}$250 monoclonal antibodies; lane 3, 1M-IP-TFIID; lane 4, 1M-IP-TFIID after incubation with recombinant purified TAF$_{II}$150; lane 5, beads with cross-linked anti-TAF$_{II}$250 monoclonal antibodies incubated with recombinant TAF$_{II}$150; lane 6, recombinant purified TAF$_{II}$150.

then used to direct transcription from the 3.2wt and 3.2dsw templates. In stark contrast to the early-embryo extracts, these late-embryo extracts transcribed the proximal promoter much more efficiently than the distal promoter (Figure 7, lane 3), consistent with the in vivo pattern of Adh promoter utilization. Interestingly, when the initiator elements were swapped, transcription from the proximal promoter was increased, whereas transcription from the distal promoter remained repressed (Figure 7, lane 4). Addition of recombinant TFIIA did not rescue distal promoter transcription, but rather stimulated overall transcription, suggesting that down-regulation of distal promoter activity is not due to lack of TFIIA. Western blot analysis revealed that TAF$_{II}$150 was present in late-embryo extracts at a level comparable with that detected in early embryos (data not shown). Thus, all the components necessary for preferential distal versus proximal initiator activity appear to be present and active in extracts from late-stage embryos. Therefore, down-regulation of distal promoter transcription is most likely not mediated by developmental regulation of TFIIA or TAF$_{II}$150.

Discussion

Regulation of Adh gene expression during Drosophila development is governed by a complex array of transcrip-

tional control elements that have been characterized both in vivo and in vitro (Abel et al., 1992, 1993; Ayer and Benyajati, 1990, 1992; Benyajati et al., 1987, 1992; Corbin and Maniatis, 1989a, 1990; England et al., 1990; Heberlein et al., 1985). These studies have revealed a likely mechanism for proximal promoter selection during larval stages, but how selective distal promoter utilization is achieved in early embryos and later in adults is not understood. Here we present evidence suggesting that the tandem Adh promoters are differentially transcribed in the embryo owing to critical differences in core promoter elements. We have reconstituted differential Adh promoter transcription in vitro using purified components and provide evidence that selective Adh promoter utilization is mediated by a specific TBP–TAF complex in combination with TFIIA. Our data show that TAFs in the TFIID complex, in particular TAF$_{II}$150, are required for discrimination between the Adh distal and proximal initiator elements. These findings suggest that TAF$_{II}$150, in the context of the TFIID complex and in concert with TFIIA, mediates the recognition of the distal core promoter and hence effects high distal promoter activity. This transcriptional selectivity is critically dependent on the DIE and fails to occur on the proximal promoter. This is consistent with footprint experiments using immunopuriifed TFIID showing that efficient binding to the distal promoter is dependent on TFIIA and that se-
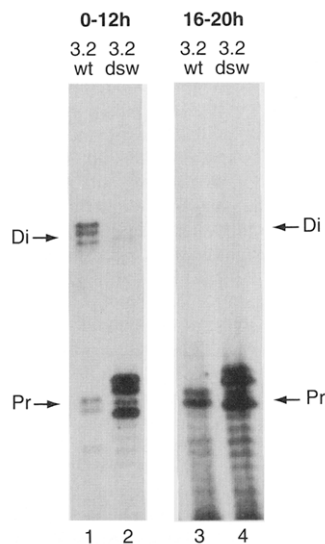
Figure 7. The Distal Promoter Is Inactive in Extracts from Late-Stage Embryos

In vitro transcription experiment using heparin–agarose fractionated (H.4) nuclear extracts from 0- to 12-hr and 16- to 20-hr-old embryos. The 3.2wt and 3.2dsw templates are described in Figure 1 and do not contain the larval enhancer. We used 14 µg of H.4 in all reactions. Transcription reactions with extracts from 16- to 20-hr embryos (lanes 3 and 4) were supplemented with 25 ng of recombinant TFIIA.

quences downstream of position +1 are protected from DNase I digestion, which is characteristic of TAF$_{\mathrm{II}}$150 binding (data not shown; Verrijzer et al., 1994).

While our data suggest that TFIIA and TAF$_{\mathrm{II}}$150 are necessary for efficient distal promoter function, preliminary results indicate that these transcription factors may not be sufficient because we have been unable to reconstitute differential Adh promoter transcription with a TBP–TAF$_{\mathrm{II}}$250–TAF$_{\mathrm{II}}$150 complex in the presence of TFIIA. The failure of this minimal triple complex to restore Adh distal promoter activity suggests that additional TAFs or factors associated with TFIID may be required. In particular, differential Adh promoter utilization may be dependent on a TFIIA-mediated function that cannot be supported by TBP, TAF$_{\mathrm{II}}$250, and TAF$_{\mathrm{II}}$150 alone. Possibly, functional cooperation between TFIIA and TAF$_{\mathrm{II}}$150 may require a novel subunit of TFIID in addition to the identified TAFIIs. Indeed, the TFIID complex that is competent to direct differential Adh promoter selectivity contains several uncharacterized proteins, possibly TAFs, (molecular masses of 125 kDa, 100 kDa, 90 kDa, and 46 kDa in Figure 6E) in addition to the previously described subunits of TFIID. Importantly, these putative novel TAFs appear to be in substoichiometric amounts consistent with a role in promoter-specific rather than general transcription functions. Future analysis of the role played by TFIIA in distal initiator selectivity may provide more detailed insight into the molecular mechanism governing differential promoter utilization.

Developmental Regulation of Promoter Utilization

Our findings suggest that differential Adh promoter utilization in Drosophila melanogaster is regulated in part by

initiator selectivity. We postulate that the discrimination between the different initiators by the TFIID–TFIIA complex serves as a transcriptional switch that selectively turns on distal rather than proximal promoter transcription. Taken together, the in vivo and in vitro data indicate that this mechanism is likely to be operative in directing differential Adh promoter utilization during Drosophila development. Although our experimental system has focused on early–mid embryonic stages, we speculate that this mechanism may also direct selection of distal promoter utilization in adult flies.

Our data also show that the distal promoter is inactive while the proximal promoter is active in extracts from late-stage embryos, consistent with the in vivo pattern of Adh promoter utilization. However, differences in the initiator elements of the distal and proximal core promoters are not sufficient to account for the switch in promoter utilization during late embryogenesis, because a proximal promoter containing the distal initiator retains high levels of transcriptional activity, while a distal promoter containing the proximal initiator remains repressed. Thus, differential initiator function that is dependent on TAF$_{\mathrm{II}}$150 and TFIIA is not sufficient to down-regulate the distal promoter in late-stage embryos. Instead, the distal promoter may be targeted by a stage-specific repressor that prevents the formation of an initiation complex. In support of this model, it has been reported that a putative repressor activity binds to sequence elements downstream of the DIE (Benyajati et al., 1992). Occupation of this particular site, located between positions +8 and +17, may sterically hinder the interaction between TFIID and the distal promoter. Moreover, repression of distal promoter function could account for the inability of the larval enhancer to activate the distal promoter during early- to mid-stage larval development (Corbin and Maniatis, 1989a). These obsevations suggest that both positive and negative regulatory functions mediated by specific core promoter elements contribute to differential utilization of the tandem Adh promoters. Our data, taken in combination with previous studies, suggest that the developmentally regulated Adh promoter switch is controlled by two distinct mechanisms. The first involves an enhancer-mediated event that selectively targets the proximal Adh promoter, as described by Corbin and Maniatis (1989a) (Figure 8B). This proximal promoter selectivity may, in part, be due to repression of the distal promoter. The second mechanism involves differential core promoter utilization directed by the recognition of DIE versus PIE by a TBP–TAF complex in concert with TFIIA (Figure 8A).

Why has D. melanogaster evolved tandem promoters to direct expression of Adh? Utilization of tandem promoters to direct developmentally regulated gene expression is not unique to the Adh gene of D. melanogaster. A dual Adh promoter organization found in a distantly related Drosophila species also shows patterns of temporal- and tissue-specific expression similar to those in D. melanogaster (Moses et al., 1990). Tandem promoter structures are also not restricted to Adh genes. For example, both the Drosophila antennapedia gene (Jorgensen and Garber, 1987) and the mouse α-amylase gene (Amy-1^a) (Schibler et

A

Early embryos
and adults

TBP/TAFs+TFIIA

B

Late embryos and
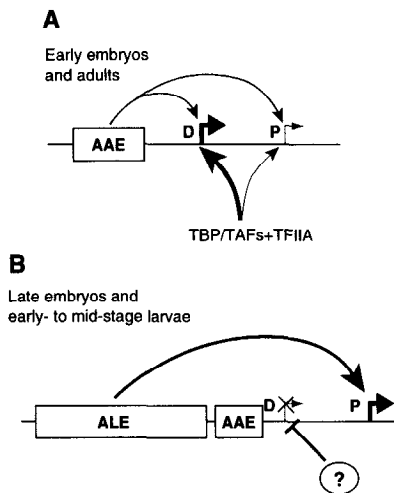early- to mid-stage larvae

Figure 8. Model for Differential *Adh* Promoter Utilization during Development

(A) The *Adh* distal promoter is switched on during early-embryonic and adult developmental stages. It has previously been shown that the *Adh* adult enhancer (AAE) has the potential to stimulate both promoters and is thus not promoter selective (Corbin and Maniatis, 1989a). However, the inherent strength of the distal promoter, owing to the ability of TBP–TAFs and TFIIA to differentiate between the DIE and PIE, results in distal rather than proximal promoter utilization.

(B) The *Adh* distal promoter is switched off during late-embryonic and most of larval development. In contrast, the proximal promoter is active and specifically stimulated by the *Adh* larval enhancer (ALE). The distal promoter is inactivated owing to repression, which may explain its inability to respond to the ALE.

al., 1983) utilize tandem promoters to effect tissue-specific and developmental regulation. In light of the differential *Adh* core promoter functions we have described here, it is plausible that tandem promoters have evolved as a mechanism necessary to regulate complex patterns of gene expression that neither promoter can accommodate alone. Therefore, Drosophila may have taken advantage of tandem promoters that, in concert with distinct enhancers and repressors, provide the flexibility required for proper *Adh* gene expression during embryonic, larval, and adult developmental stages.

Experimental Procedures

DNA Templates

The plasmid 3.2wt contains a 3.2 kb XbaI fragment of the wild-type *AdhF* allele and is identical to the plasmid pXba3.2, which has been described previously (Ayer and Benyajati, 1990). The plasmid 3.2dsw was constructed by PCR as follows: proximal initiator sequences from positions +710 to +722 were fused to the distal promoter at position +11 (fusion between +722 and +11), subcloned into pBluescript, confirmed by sequencing, and excised by partial digestion with RsaI (introduced by PCR and overlapping position +710) and StuI (distal position +285). The plasmid 3.2wt was cut with StuI and NsiI (distal position −4), and the 3' overhang was removed by T4 DNA polymerase and blunt end ligated to the RsaI–StuI fragment. Plasmids containing NsiI–RsaI fusions (distal −4 fused to proximal +710) and StuI–StuI fusions were selected. Similarly, distal initiator sequences from position −6 to position +11 were inserted into the proximal promoter between positions +706 and +724 (fusion between −6 and +706 and between +11 and +724), subcloned into pBluescript, confirmed by sequencing, excised with Eco47III (position +575) and BanII (position +724), and inserted into the 3.2 kb *Adh* fragment (containing the proximal initiator

swap) cut with Eco47III and BanII. Numbers are given relative to the distal transcription start site.

The 250 nt G-less cassette was constructed by PCR using the previously described 377 bp G-less cassette, pC2AT, as a template (Sawadogo and Roeder, 1985). The G-less distal and proximal promoter constructs were generated by PCR using the plasmids pDΔ5'-46 and pPΔ5'-40 (England et al., 1990; Heberlein et al., 1985). A SmaI site was introduced downstream of the transcription start site (at position +10 or +12) and used for fusing the promoters to the Ecl136II site in the beginning of the G-less cassette. Similarly, the initiator swaps and point mutations were introduced by PCR and are shown in Figure 4A.

Transfection Experiments

The Drosophila cell line 1006-2 was grown in M3 medium supplemented with 10% fetal bovine serum, glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 µg/ml). Cells (2 × 10⁷) were plated per 10 cm dish the day before transfection. Cells were transfected with the calcium phosphate coprecipitation method (Di Nocera and Dawid, 1983). Each plate received 15 µg of 3.2wt or 3.2dsw plasmid along with 30 ng of luciferase expression plasmid (pGL2; Promega) and 15 µg of salmon sperm carrier DNA.

Cells were harvested 48 hr posttransfection, and 5% of the cells from each plate was removed and assayed for luciferase activity (as described by Promega). Total RNA was isolated from the remaining cells.

RNA Procedures

Transfected 1006-2 cells were lysed in 0.7 ml of denaturing solution (4 M guanidinium thiocyanate, 25 mM sodium citrate [pH 7], 0.5% N-lauroylsarcosine, and 0.1 M β-mercaptoethanol) per 10⁷ cells. Total RNA was prepared from the lysates essentially as described previously (Chomczynski and Sacchi, 1987), solubilized in 50 µl of double-distilled water, and treated with 10 µg of proteinase K in the presence of 100 µl of buffer containing 20 mM EDTA, 1% SDS, 0.2 M NaCl. RNA was extracted with phenol-chloroform-isoamylalcohol, ethanol precipitated, and resuspended in 50 µl of double-distilled water. We used 15 µg of RNA for primer extension analysis and S1 nuclease mapping. Primers and RNA were combined in a total volume of 15 µl in 1× hybridization buffer (2 mM Tris [pH 7.9], 0.2 mM EDTA, 250 mM NaCl) and heated to 90°C for 3 min, slowly cooled to 55°C, and allowed to anneal for an additional 90 min. Primer extension analysis was performed as described for in vitro transcription products. Samples for S1 mapping were supplemented with 200 µl of S1 mix (350 U of S1 nuclease [GIBCO BRL], 30 mM sodium acetate [pH 4.6], 1 mM zinc acetate, 5% v/v glycerol, and 250 mM NaCl) and incubated at 22–24°C for 30 min. Samples were readjusted to pH 7.9 with 15 µl of 2 M Tris (pH 7.9), extracted with phenol/chloroform, and ethanol precipitated. Samples were resolved on 8% sequencing gels.

In Vitro Transcription

In vitro transcription and primer extension analysis were performed essentially as described elsewhere (Heberlein et al., 1985), except that 200 ng of template was used in a total volume of 20 µl (supplemented with 0.005% NP-40), and protein and DNA were preincubated at 20°C for 30 min (in a total volume of 15 µl) prior to addition of nucleotides. The distal primer was complementary to distal sequences between positions +56 and +83, whereas the proximal primer was complementary to proximal sequences between positions +41 and +67 relative to the proximal transcription start site.

G-less transcription reactions were performed as described above, except that the nucleotide mix contained 650 µM ATP and CTP, 25 µM UTP, 12.5 µM 3'-O-meGTP, 5 µCi of [α-³²P]UTP (3000 µCi/mmole), and 12 U of RNase T1.

In vitro transcription reactions reconstituted with purified components were always supplemented with 12.5 ng of dTFIIB, 1.5 ng of hTFIIE56, 7.5 ng of hTFIIE34, 30 ng of dTFIIF (both subunits) or with 0.4 µl of purified dTFIIF fraction (from Mono S), 0.1 µl of purified dTFIIH fraction (from POROS-ether), and 25 ng of purified dRNA pol II fraction (Mono Q or POROS-heparin). Transcription reactions supplemented with dTFIIA contained either 25 ng of rdTFIIA (both subunits) or 0.5 µl of eTFIIA fraction from Mono Q chromatography (Figure 3A). Transcription reactions supplemented with dTBP contained 0.1–2 ng of recombinant dTBP. Transcription reactions supplemented with TFIID

contained 0.5 μl of S300–TFIID or 0.5 μl of Mono Q–TFIID. Transcription with immunopurified TFIID was performed on the beads. We used 2 μl of beads equilibrated with 0.1 M HEMG buffer (25 mM HEPES [pH 7.6], 0.1 mM EDTA, 12.5 mM MgCl₂, 10% v/v glycerol, 100 mM KCl, and 0.01% NP-40) per transcription reaction.

Protein Expression and Purification

dTBP was expressed in bacteria as described previously (Hoey et al., 1990); cells were disrupted by sonication, and the recombinant TBP was purified to homogeneity by polyethylenimine precipitation, phosphate–cellulose, and POROS–heparin chromatography. Bacterially expressed dTFIIB was purified by ammonium sulfate precipitation, phosphate–cellulose, and Mono S chromatography, essentially as described previously (Ha et al., 1991). The 56 kDa and 34 kDa subunits of hTFIIE were expressed and purified essentially as described elsewhere (Peterson et al., 1991). The large subunit of dTFIIF (Kephart et al., 1994) was expressed in SF9 cells infected with recombinant bacculovirus (Pharmingen) and purified on POROS–HQ in the presence of 5 M urea. The small subunit of dTFIIF (Frank et al., 1995) was expressed in bacteria and purified on a Mono S column. The large (1.5 mg) and small (2.1 mg) subunits were combined in the presence of 3 M urea, renatured by dialysis, and purified as a complex on a Mono Q column. The two subunits of dTFIIA were expressed in bacteria, solubilized in the presence of 7 M urea, and renatured together by dialysis. The soluble protein complex was further purified by POROS–HQ chromatography.

Full-length TAF₁₁150 (Verrijzer et al., 1994) was expressed in bacculovirus-infected SF9 cells and purified by POROS–heparin chromatography.

The heparin–agarose H.4 fractions used for in vitro transcription (Figures 1C and 7) were prepared as previously described (Heberlein and Tjian, 1988). We used 4–6 μl of H.4 with a protein concentration of 2.5–3 mg/ml per transcription reaction.

TFIID, TFIIF, TFIIH, and pol II were purified from nuclear extracts prepared from 0- to 12-hr-old embryos (Heberlein and Tjian, 1988). Nuclear extracts derived from approximately 250 g of embryos were fractionated on a POROS–heparin column. TFIID was subsequently separated from TFIIF, TFIIH, and pol II by Sephacryl S300 sizing-column chromatography. TFIIF, TFIIH, and pol II were separated by Mono Q chromatography. TFIIF was further purified on a Mono S column. TFIIH was further purified on Mono S and POROS–ether columns. The polymerase used in some of the transcription reactions was purified by POROS–ether and POROS–heparin chromatography. All transcription factors were either diluted or dialyzed to 0.1 M HEMG prior to in vitro transcription.

TFIID was further purified on a Mono Q column, which separated the bulk of TFIID from TFIIA. A subpopulation of the TFIID pool (termed Mono Q–TFIID) was selected based on its ability to direct differential promoter transcription and used in all subsequent experiments. TFIID from the Mono Q–TFIID fraction was further purified using a monoclonal antibody recognizing dTAF₁₁250 (30H9 or 2B2; Weinzierl et al., 1993), which was covalently attached to protein G beads: 100 μl of Mono Q–TFIID was incubated for 12–14 hr with 50 μl of protein G–dTAF₁₁250 beads in 0.1 M HEMG. The beads were collected, and the flowthrough fraction, IP-FT, containing the unbound material, was saved. The beads were either washed with 0.1 M HEMG, generating 0.1M-IP-TFIID, or with 1.0 M HEMG, generating 1M-IP-TFIID, which was subsequently washed with 0.1 M HEMG. IP-TFIID beads (1 M) that were incubated with dTAF₁₁150 were supplemented with 25 ng of dTAF₁₁150 in a total volume of 2.5 μl of 0.15 M HEMG per 1 μl bead. After a 4–6 hr incubation, unbound dTAF₁₁150 was removed by washing with 0.1 M HEMG.

Acknowledgments

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