Communication

Homeodomain Interaction with the β Subunit of the General Transcription Factor TFIIE*

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Homeodomain-containing proteins play a crucial role as transcriptional regulators in the process of cell differentiation. The homeodomain performs a dual function in this regard, acting as a DNA binding domain and participating in protein-protein interactions that enhance DNA binding specificity or regulatory activity. Here we describe a homeodomain-class-specific interaction with the β subunit of the general transcription factor TFIIE. We show that the Antennapedia and Abdominal-B homeodomains bind to TFIIEβ, but the even-skipped homeodomain does not. Using a two-hybrid assay performed in cultured cells, we demonstrate that the homeodomain-TFIIEβ interaction occurs in vivo. The Abdominal-B homeodomain is shown to activate transcription in vitro, and this activation can be blocked with anti-TFIIEβ antibody without affecting basal transcription levels. Together with published data demonstrating an interaction between proteins containing even-skipped class homeodomains and the TATA-binding protein (Um, M., Li, C., and Manley, J. L. (1995) Mol. Cell. Biol. 15, 5007-5016; Zhang, H., Catron, K. M., and Abate-Shen, C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1764-1769), these results suggest various homeodomain containing proteins interact with different general transcription factors, an observation that may have important implications for transcriptional regulation.

The homeodomain is a structural motif originally defined by homologous sequences found in the Drosophila homeotic selector proteins, a family of proteins responsible for specifying positional identity along the anterior-posterior axis of a developing embryo (1, 2). It is now well established that the homeodomain functions as a DNA binding domain and that homeotic proteins function as transcription factors that regulate the expression of genes necessary for segment identity (3). The homeodomain motif was subsequently found in numerous developmentally regulated proteins from species as diverse as sponges to mammals implying a functionally conserved mechanism of regulating gene expression during development (4). The extraordinary degree of conservation found among homeodomain sequences suggests that they evolved from a common ancestor through gene duplication. Presumably, sequence variation in the homeodomain and the surrounding protein accumulated as the duplicated genes acquired new developmental roles; however, sequence divergence must have also been restricted by molecular interactions necessary for function.

The mammalian Hox genes contain homeodomains that are more similar to the homeodomains found in Drosophila homeotic genes than any other homeodomain sequence. When expressed in fly embryos, some Hox genes can partially substitute for the function of their Drosophila orthologs (5-8). This is an intriguing observation given that, with few exceptions, the only conserved sequence between orthologous pairs is the homeodomain itself and a short stretch of 6-9 amino acids just amino-terminal to the homeodomain. One explanation for this functional substitution is that the homeodomain, aside from recognizing specific binding sites in target promoters, also participates in interactions with other conserved proteins required for transcriptional regulation of effector genes (9-12).

One group of highly conserved proteins required for basal levels of transcription in vitro are the general transcription factors (GTFs), which form the transcription preinitiation complex. To establish high levels of expression, assembly of the GTFs is thought to be facilitated through protein-protein interactions with sequence specific DNA binding transcription factors (13-15). Interaction with the GTFs is usually mediated by a regulatory domain in the sequence specific transcription factor; however, these interactions may also involve the DNA binding domain (16-18). Of particular interest to this study is the recent demonstration that proteins containing either the even-skipped (eve) or Msx-1 homeodomains interact with an essential GTF, the TATA-binding protein (19, 20). Although the exact role of the eve homeodomain remains undefined, it is required to mediate the interaction between TBP and the eve repression domain. The binding of the TBP to Msx-1 protein, however, occurs primarily through an interaction with the Msx-1 homeodomain.

Here we extend these reports of homeodomain-GTF interactions by demonstrating that the Abdominal-B (Abd-B) homeodomain can bind to the β subunit of TFIIE (TFIIEβ), but does not interact with TBP. Moreover, we show that while the diverged homeodomains Antennapedia and Abd-B interact with TFIIEβ, the even-skipped homeodomain does not. Our data indicate that the Abd-B homeodomain interaction with TFIIEβ occurs in vivo and that it can facilitate transcriptional activation in vitro. These data suggest that homeodomains mediate interactions with various basal transcription factors in a class-specific manner, which may have important implications for transcriptional regulation during development.

EXPERIMENTAL PROCEDURES

DNA Constructs—All transfection effector plasmids are based on pH60-1 (41), which contains sequences encoding the Gal4 DNA binding domain (amino acids 1-147) cloned 3’ of the Rous sarcoma virus long terminal repeat (RSV LTR). To make RSV-TFIIEβ, a Xbal-HindIII fragment from plasmid His6TFIIEβ, encoding a histidine-tagged TFIIEβ (a gift from Dr. R. Roeder) was subcloned into pH60-1 replacing the existing Gal4 DNA binding domain. RSV-NHD was made by replacing the Gal4 DNA binding domain of pH60-1 with a Dral-XbaI fragment

* The abbreviations used are: GTF, general transcription factor; TBP, TATA-binding protein; TAF, TBP-associated factor; CAT, chloramphenicol acetyltransferase; DTT, dithiothreitol; CTD, carboxyl-terminal domain; GST, glutathione S-transferase; RSV LTR, Rous sarcoma virus long terminal repeat; PCR, polymerase chain reaction.

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from plasmid pΔC18 encoding the entire DfdΔAC/Abd-B protein as well as some flanking Dfd cDNA sequences (25). The plasmid RSV-GN was made by PCR amplification of a DNA fragment encoding the N domain. The primers used for this reaction were GCTCTAGATACAATGGA-

C18 encoding the entire Dfd from plasmid p

GB5CAT has been described previously in Yang and Evans (41).

Protein Binding Assay—DNA fragments encoding the three homeodomains and the N domain were amplified by PCR and cloned into pGEX-2T (43) using restriction sites introduced by the PCR primers. The sequence of each set of primers is available upon request. The induction and purification of the GST fusion proteins was performed as described in Ausubel et al. (44). The use of equal amounts of protein in the glutathione beads pull down assay was confirmed by equivalent staining intensities on Coomassie Blue-stained SDS-polyacrylamide gels. The TNT-coupled reticulocyte lysate system (Promega) was used to transcribe and translate the GTFs and TAFs in vitro under conditions recommended by the manufacturer. Plasmids encoding the Drosophila GTFs and TAFs were obtained from the laboratories of Drs. R. Tjian or J. T. Kadonaga, while the human TFIIE subunit clones were obtained from Dr. R. Roeder. Two micrograms of a preformed GST fusion protein-glutathione bead complex was incubated with the indicated amount of 35S-labeled protein at room temperature (25°C) for 30 min in a 60-μl reaction containing 1 × binding buffer (1 × BB). The 1 × BB contained 25 mM Hepes (pH 7.8), 80 mM KCl, 0.1% Nonidet P-40, 10% glycerol, 1.0 mM DTT, 0.1 mM EDTA, 2.5 μg/ml leupeptin, 1 mg/ml bovine serum albumin, and 5.0 mM MgCl2. The beads were pelleted, washed four times with 100 μl of 1 × BB, then boiled in SDS sample buffer (44). Bound proteins were resolved by 12% SDS-polyacrylamide gel electrophoresis for autoradiography. Band quantitation was performed using a Fuji PhosphoImaging system.

RESULTS

Transcription initiation requires a number of multisubunit GTFs, including TFIIID, TFIIA, TFIIH, TFIIIE, TFIIIF, and TFIIH. In Drosophila, TFIIID is composed of TBP and at least eight TBP-associated factors or TAFs (21, 22). To investigate if homeodomains interact with GTFs or TAFs in vitro, a series of binding assays were performed with a GST-Abd-B homeodomain fusion protein and 35S-labeled Drosophila TAFs including TAF30a, TAF30b, TAF40, TAF60, TAF80, TAF110 and TAF150. We also tested the Drosophila GTFs, TBP, and TFIIID and human TFIIIE and TFIIIEβ. Of all GTFs and TAFs tested, binding at levels above that observed with GST alone was only observed with TFIIIEβ. Approximately 50% of the input TFIIIEβ bound to the GST-Abd-B homeodomain fusion protein (Fig. 1, lanes 1–4). The addition of 20 pmol of a double-stranded oligonucleotide encoding a high affinity Abd-B homeodomain binding site did not detectably alter the binding interaction with TFIIIEβ or affect the negative interaction with the other GTFs and TAFs.2

Homeodomains can be divided into classes based on sequence homology within and outside the homeodomain region (23, 24). Since the Abdominal-B homeodomain is representative of a relatively small class, we asked if sequence differences found in homeodomains of various classes can affect binding to TFIIIEβ in vitro. A representative of the Antennapedia class, the Drosophila Antennapedia homeodomain (AntpHD), binds to approximately 60% of input labeled TFIIIEβ (Fig. 1, lane 5). In contrast, a representative of a more diverged class of homeodomains, the Drosophila even-skipped homeodomain (eveHD), fails to bind TFIIIEβ at levels above that retained by GST alone under identical conditions (Fig. 1, lane 6). The eveHD binds a labeled DNA fragment containing known eve binding sites, suggesting that the fusion protein retained biological activity.2 These results indicate that sequence differences among homeodomain classes can affect TFIIIEβ binding affinity in vitro.

We next asked if the interaction observed in vitro between the Abd-B homeodomain and TFIIIEβ could occur in vivo. We reasoned that in cultured cells, artificially high concentrations of 2 A. Zhu and M. A. Kuziora, unpublished results.
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of TFIIEβ might interfere with the ability of proteins containing the Abd-B homeodomain to regulate transcription by blocking interaction with the basal transcription complex. We have shown previously that the Abd-B homeodomain can weakly stimulate transcription, but at levels that would be insufficient for accurately measuring changes in response to high TFIIEβ activation (25). To obtain higher levels of transcriptional activation, we used the DfdΔAC/Abd-B protein, which essentially consists of the Abd-B homeodomain fused to an activation domain found in the Deformed homeotic protein called the N domain (25). Sequences encoding this protein were cloned downstream of the RSV LTR promoter to create RSV-NHD (Fig. 2A). Transfection of RSV-NHD activates transcription approximately 20-fold above levels observed upon transfection of the −33AdhCATHB reporter plasmid alone (data not shown). To obtain a high cellular concentration of TFIIEβ, we constructed the expression plasmid RSV-TFIIEβ in which coding sequences from a TFIIEβ cDNA are placed downstream of a RSV LTR promoter (Fig. 2A). As shown in Fig. 2B, cotransfection of RSV-NHD with increasing amounts of RSV-TFIIEβ results in up to approximately 60% reduction in CAT activity without significantly affecting the level of transcription in the absence of RSV-NHD. We conclude that exogenous TFIIEβ interferes with the ability of the DfdΔAC/Abd-B protein to interact with the transcription complex.

Several lines of evidence suggest that the inhibition described above is due to an interaction between TFIIEβ and the Abd-B homeodomain rather than with other regions of the DfdΔAC/Abd-B protein or other transcription factors. First, a GST-N domain fusion protein does not bind to TFIIEβ (Fig. 1, lane 7). To confirm this observation in vivo, we cotransfected a construct encoding a Gal4 DNA binding domain-N domain fusion protein (RSV-GN; Fig. 2A) and RSV-TFIIEβ with a CAT reporter plasmid bearing 5 tandem Gal4 DNA binding sites (GB5CAT; Fig. 2A). As shown in Fig. 2B, the level of CAT reporter activation by RSV-GN is only reduced by approximately 10% in the presence of high concentrations of TFIIEβ, indicating that TFIIEβ does not significantly interact in vivo with the Gal4-N domain fusion protein. As a third control, we made use of the observation that the VP16 acidic activation domain interacts with TFIIEβ (26). As shown in Fig. 2B, cotransfection with RSV-TFIIEβ does not significantly affect the activity of a fusion protein consisting of the VP16 acidic activation domain and the Gal4 DNA binding domain. These experiments show that only a construct containing an Abd-B homeodomain is affected by overexpression of TFIIEβ, suggesting a specific interaction is possible in vivo between the homeodomain and the TFIIEβ subunit.

We next asked if an interaction between the Abd-B homeodomain and TFIIEβ could enhance transcriptional regulation. We tested the ability of the GST-Abd-B homeodomain fusion protein to stimulate transcription in vitro using a HeLa cell nuclear extract. The GST-Abd-B homeodomain fusion protein stimulates transcription approximately 2.5-fold using the −33AdhCATHB reporter construct as a template (Fig. 3, columns 1–3). Activated transcription was not observed from the −33AdhCAT reporter construct which lacks Abd-B binding sites (data not shown). The modest activation mediated by the Abd-B homeodomain can be blocked by preincubating the nuclear extract with increasing amounts of anti-TFIIEβ antibody, without affecting basal transcription levels (Fig. 3, columns 4–11). These results support the hypothesis that the Abd-B homeodomain can contribute to transcriptional regulation through interaction with TFIIEβ.
The three-dimensional structures of several homeodomain-DNA complexes reveal that homeodomains with diverged primary sequences are folded into remarkably similar globular structures (27-30). Mutational analysis of protein-protein interactions involving the homeodomain have identified several amino acids that are involved in determining the specificity of the interaction (10, 31, 32). In general, these amino acids are considered to be solvent exposed as they are positioned on the opposite side of the homeodomain that contacts DNA. Residues in positions 11, 14, 15, and 22 of helix one, positions 32 and 36 of helix two, and position 24 in the intervening loop have been implicated in homeodomain-protein interactions and are identical or similar in the Antp and Abd-B homeodomains. One or more of these amino acids are likely to play a role in determining the specificity of the TFIIIE β interaction with the homeodomain.

How can an interaction between TFIIIE β and the homeodomain contribute to transcriptional regulation? At a biochemical level, TFIIIE promotes the phosphorylation of the carboxyterminal domain (CTD) of RNA polymerase II by TFIIH (33, 34), but also inhibits a helicase activity shown by TFIIH that may be required to unwind the DNA prior to transcription initiation (35). Since the phosphorylated RNA polymerase II is the form of the enzyme that actively elongates transcripts (36, 37), the ability of a homeodomain to attract TFIIIE to the initiation complex would serve to stimulate transcription by enhancing the kinase activity of TFIIH, resulting in a completely phosphorylated CTD. Alternatively, since TFIIIE inhibits the helicase activity of TFIIH, it has been proposed that TFIIIE might be removed from the complex following CTD phosphorylation (38). This is consistent with the observation that TFIIIE binds unphosphorylated RNA polymerase II, but not to the phosphorylated form (39). Together with conformational changes that occur in the transcription complex during initiation that may alter access to the GTFs (40), the removal of TFIIIE from the complex could be facilitated by interaction with a homeodomain. This scheme might have the added advantage of retaining TFIIIE in the vicinity of the target promoter, thereby aiding subsequent rounds of initiation.

Unlike the results obtained with the Abd-B and Antp homeodomains, we failed to detect and interaction between the eve homeodomain and TFIIIE β. In contrast, an interaction between the eve protein and TBP has been reported (19). The eve homeodomain does not bind TBP in vitro, but it is required for the interaction between TBP and the eve repression domain and functions in some undetermined manner to stabilize the interaction. The mouse Msx-1 protein, which contains a homeodomain of the eve class, also interacts with TBP in vitro (20). In this case, a direct interaction between the Msx-1 homeodomain and TBP can be demonstrated. Of particular interest to this study was the finding that the Msx-1 protein does not interact with TFIIIE β (20). Thus homeodomains differ in their ability to interact with GTFs. Given the degree of sequence similarity within a homeodomain class, the interactions with specific GTFs may also be conserved. It follows that homeodomain-mediated interactions with other highly conserved proteins such as extradenticle/Pbx (10, 11) or SRF (12) might be retained among members of a homeodomain class. These interactions may be sufficient for orthologous vertebrate Hox proteins to partially mimic the transcriptional regulation of appropriate target promoters in fly embryos despite the lack of significant sequence homology outside of the homeodomain region.

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