Transcriptional Repression by the Human Homeobox Protein EVX1 in Transfected Mammalian Cells* 

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The human homeobox protein EVX1 (EVX1) is thought to play an important role during embryogenesis. In this study, the effect of EVX1 on gene transcription has been investigated in transfected mammalian cells. EVX1 expression represses transcription of a reporter gene directed by either cell-specific or viral promoter/enhancer sequences in a variety of mammalian cell lines and in a concentration-dependent manner. Transcriptional repression is independent of the presence of DNA-binding sites for EVX1 in all the promoters we tested. Furthermore, repression by EVX1 is evident also using a TATA-less minimal promoter in the reporter construct. A carboxy-terminal proline/alanine-rich region of EVX1 seems to be responsible for the transcriptional repression activity, as suggested by transfection of EVX1 mutants. We speculate that the repressor function of EVX1 contributes to its proposed role in embryogenesis.

Homeobox genes code for transcription factors containing a trihelical DNA-binding motif, termed homeodomain (HD) (Refs. 1–3, and references cited therein). The HD is highly conserved with respect to structure and function throughout evolution and is a common component of numerous proteins that regulate transcription during development (for reviews, see Refs. 4–6). The functions of HD proteins range from activities in directing pattern formation to more restricted roles in regulating specific cell fate (3, 7). The products of vertebrate homeobox-containing genes have been shown to bind DNA in vitro (8, 9) and to regulate gene transcription through specific target sequences in cell culture (10, 11). It is unlikely that HD-containing proteins exert their different selective functions in vivo only through protein-DNA interactions, since they share similar DNA-binding specificities in vitro (4, 5). Thus, it is presumed that although a single HD has the potential to bind to many DNA-target sites it may be specifically recruited into a functional complex at only a subset of those sites by selective protein-protein interactions (12–15). Furthermore, in some cases the HD has been demonstrated to be dispensable for activity in vivo (16, 17).

Recently, the human homeobox genes EVX1 and EVX2 have been isolated and sequenced (18, 19). These genes encode proteins containing a HD closely related to that encoded by the Drosophila even-skipped, which belongs to the pair-rule class of segmentation genes and is required for the proper development of the metameric body plan of the fruit fly (20–22). Also the murine Evx1 and Evx2 genes have been cloned, and Evx1 expression pattern during mouse embryogenesis has been studied (23). During early embryogenesis, Evx1 is expressed in a biphasic manner. From day 7 to 9 of development its expression emerges at the posterior end of the embryo within the primitive ectoderm and later in the mesoderm and neuroectoderm. From day 10 to 12.5, Evx1 transcripts are restricted to specific cells within the neural tube and hindbrain, while no expression is detectable in a variety of adult tissues. Spyropoulos et al. (24) have recently shown that the targeted disruption of the Evx1 gene in mice causes early postimplantation lethality of the conceptus.

Several authors have reported that the even-skipped gene product acts as a transcriptional repressor both in in vitro assays (25–27) and in cell transfection experiments (28, 29). On the contrary, J ones et al. (30) showed that the mouse Evx1 protein induces the expression of a reporter gene driven by the chicken Tenascin-C promoter. The same authors restricted the sequences that contributed to the activation to a segment containing a TRE known to bind transcription factors belonging to the AP-1 family. Currently, the role of the human EVX1 and EVX2 proteins in gene transcription is unknown.

The aim of our study was to investigate the transcriptional activity of EVX1 in transfected mammalian cells. Our data indicate that EVX1 expression strongly reduces, in a concentration-dependent manner, the basal and activated transcription of the reporter gene CAT directed by a variety of cell-specific and viral promoters in several different mammalian cell lines. EVX1 transcriptional repressor function is evident using both TATA-containing and TATA-less promoters. We also show that the repressor function of EVX1 is contained within a C-terminal region rich in alanine and proline residues and is independent of the presence of DNA-binding sites for EVX1.

EXPERIMENTAL PROCEDURES

Expression and Purification of EVX1 Recombinant Protein—EVX1 protein was produced in the baculovirus system according to the method previously described in detail (9) with minor modifications. Briefly, pVL941/EVX1 was obtained by inserting the filled in Sau3AI–Sau3AI (1453 bp) EVX1 DNA fragment (18) in the filled in BamHI cloning site of pVL941 (Pharmingen, San Diego, CA). Recombinant baculovirus particles (Baculogold/EVX1) were obtained by transfecting Sf9 cells with the wild type Baculogold™ (Pharmingen) viral DNA together with pVL941/EVX1 and purified by limiting dilution and dot hybridization. For the production of EVX1 protein, 500 × 10⁶ Sf9 cells
were infected with five plaque-forming units/cell of the recombinant virus, and cells were recovered and lysed as described previously (9). Both the cytoplasmic and the nuclear lysates were analyzed by SDS-polyacrylamide gel electrophoresis and stained with silver. A band that was absent in lysates from cells infected with a control virus was present in the profile of the nuclear lysates, accounting for approximately 20% of HeLa cells. The reconstituted protein was stored in buffer D supplemented with 20% glycerol at −80 °C.

General handling techniques for baculovirus expression system were performed essentially as described in Ref. 31.

Cells, Antibodies, and Western Blotting—HeLa human cervix carcinoma cells, F9 teratocarcinoma cells, and InR1-G9 hamster glucagonoma cells (32) were electrophoresed in 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes according to standard procedures. The filters were incubated with the supernatant of hybridoma cells, followed by an alkaline phosphatase-conjugated goat anti-mouse antibody from Sigma. Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-1-phosphate were used as the substrates (Promega, Madison, WI) with a positive reaction resulting in purple color.

Immunochemistry and Immunohistochemistry—For Immunofluorescence, cytoplasts were prepared and fixed in acetone at −20 °C for 5 min. Cells were incubated for 2–16 h at room temperature with supernatant of hybridoma cells, followed by extensive washes and by incubation with rhodaminated goat anti-mouse IgG antibody from Sigma. Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-1-phosphate were used as the substrates (Promega, Madison, WI) with a positive reaction resulting in purple color.

DNA Transfections and CAT Assays—InR1-G9 and β-TC1 cells transient transfection was performed essentially as described by Fehmann and Habener (44) with the modification reported in Ref. 38. HeLa and F9 cells were transiently transfected by the calcium phosphate technique, performed exactly as reported in Ref. 45. Transient transfection of NIH-3T3 by the electroporation technique was performed using a Bio-Rad apparatus as follows: 4 × 10⁶ NIH-3T3 cells in complete medium were electroporated by applying a voltage of 0.25 kV and a capacitance of 960 microfarads. In each transfection experiment the cell number of each well was controlled by both protein concentration assays and cell counting, and variation never exceeded 10%.

Results

Expression of the EVX1 Protein and Production of Monoclonal Antibodies—EVX1 was expressed in the baculovirus system and purified to near homogeneity (Fig. 1A), as described under “Experimental Procedures.” When analyzed by SDS-polyacrylamide gel electrophoresis, the recombinant protein showed an apparent molecular mass higher (62 kDa) than predicted by its amino acid sequence (42.5 kDa). Mouse monoclonal antibodies against EVX1 were raised and characterized (36–38) and were a gift from Drosophila melanogaster (Drosophila melanogaster) (36) and Rhodnius prolixus (37). Mouse monoclonal antibodies against EVX1 were produced and characterized (34, 35). Determination of Ig classes was carried out by standard procedures using the Mouse Typer Sub-Isotyping kit (Bio-Rad).

For immunoblot analysis, nuclear lysates of infected SF9 cells were electrophoresed in 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes according to standard procedures. The filters were incubated with the supernatant of hybridoma cells, followed by extensive washes and by incubation with rhodaminated goat anti-mouse IgG antibody from Sigma. Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-1-phosphate were used as the substrates (Promega, Madison, WI) with a positive reaction resulting in purple color.

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recombinant EVX1). On the contrary, p[-0.35]GLU-CAT does not contain this consensus sequence. Homeobox proteins have been reported to play important roles in cell-specific gene transcription in pancreatic islet cells (48–50). Thus, it was not surprising to find a putative EVX1 DNA-binding site in the proglucagon gene promoter. As previously reported, p[-1.1]GLU-CAT and p[-0.35]GLU-CAT direct high levels of CAT transcription in InR1-G9 cells, p[-0.35]GLU-CAT being less effective than p[-1.1]GLU-CAT (38). As shown in Fig. 2A and in Fig. 4, the basal transcriptional activity of either p[-1.1]GLU-CAT or p[-0.35]GLU-CAT was approximately 90% reduced when each of the reporter plasmids was cotransfected with the expression plasmid pCMV-EVX1 in comparison with pRC-CMV alone. Similar results were obtained using β-TC1 insulinoma cells (not shown).

To investigate whether the transcriptional repression is restricted to the specific promoter we used in the above described experiments, other reporter plasmids containing mammalian and viral promoter/enhancer regions were tested in different mammalian cell lines. The cotransfection of pCMV-EVX1 with pRSV-CAT, pCAT-promoter, and p7B2-I-CAT, containing 524 bp of the 3' LTR of the Rous sarcoma virus (39), 202 bp of the SV40 promoter (51), and 1.5 kilobases of the 5' flanking region plus 1.5 kilobases of the first intron of the human 7B2 gene (which encodes a neuroendocrine molecular chaperone expressed in insulinoma and glucagonoma cells) (52), respectively, decreased the transcriptional activity of the reporter by 50–90% in different cell lines (Fig. 4, and data not shown). These results indicate that the transfected EVX1 exerts its transcriptional repressor activity in cells either expressing or not expressing the endogenous EVX1 and that this effect is not promoter-specific.

The observed repression of CAT activity by EVX1 corresponds to a decrease in CAT mRNA, as measured either by RNase protection analysis or by RT-PCR experiments (Fig. 5). It is noteworthy that the CAT transcript is strongly reduced in EVX1-transfected cells 24 h after transfection and that the reduction persists after 46 h (Fig. 5A). This finding suggests that the observed EVX1 effect on CAT activity predominantly depends on the transcription rate of CAT mRNA. This finding

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is supported by the RT-PCR experiments depicted in Fig. 5B. In each transfection experiment, both the expression and the correct transport to the nucleus of the EVX1 protein were checked by immunofluorescence using mAb B6–41 (Fig. 1D, and data not shown).

EVX1 Represses Transcription from a TATA-less Minimal Promoter—Although the results presented above suggest that the effect of EVX1 does not require its binding to a specific DNA motif, we investigated whether EVX1 represses gene transcription from a reporter plasmid containing only a single promoter element, the 17-bp mammalian terminal deoxynucleotidyl transferase initiator element (pInr-CAT) (41) belonging to a class of promoters that have no apparent TATA element. We used a minimal TATA-less promoter to eliminate any possible interference from sequences that could mimic the core sequence ATTA known to interact with homeobox proteins. As shown in Fig. 6, in InR1-G9 cells, the weak transcriptional activity of pInr-CAT was 90% inhibited by the cotransfection of pCMV-EVX1. In order to increase the transfection efficiency, we transfected NIH-3T3 cells by electroporation and obtained similar results. Cells cotransfected with pCMV-EVX1 showed a transcriptional activity of pInr-CAT approximately 75% lower than cells cotransfected either with pRC-CMV alone or with pCMV-HOXD4 (Fig. 6).

The Deletion Mutant ΔEVX1, Which Lacks the C Terminus, Loses the Ability to Inhibit Transcription—No dear amino acid sequence similarities between the repressing domains of different transcriptional repressors have been described. These domains are, however, usually rich in alanine, glutamine, and/or proline residues (29, 53, 54). The EVX1 protein does contain such a region rich in alanine and proline residues carboxy-terminal to the HD. Thus, we constructed an expression vector (pCMV-ΔEVX1) cloning a deletion mutant lacking the 3' 157 nucleotides of the EVX1 coding sequence in the pRC-CMV (Fig.
Our findings indicate that a human HD protein, EVX1, is a potent transcriptional repressor and that this effect is independent of the presence of its specific DNA-binding sites in the promoter. Here we show that EVX1 represses basal and activa-

ted transcription directed by TATA-containing and TATA-

less promoters. Furthermore, the carboxyl-terminal region of the protein seems to contain the "repressor domain(s)." It has been shown in Drosophila that Eve binds DNA (55) and functions as a sequence-specific transcriptional repressor in transfected cells (28, 29) and in transcriptionally competent extracts (25). However, Han and Manley (29), using Eve deletion mutants, observed a significant correlation between strong repression and weak binding to DNA. Moreover, TenHarmsel et al. (27), in addition to a repressor mechanism based on Eve binding to its DNA sites, described also a second mechanism that does not require binding to DNA. These authors suggested that Eve might interact with the general transcription factors to inhibit their function. Thus, the finding that EVX1, which is homologous to Eve, represses reporter gene transcription directed by promoters lacking any obvious binding site for the protein is not totally unexpected. Furthermore, other HD proteins seem to behave similarly. Msx-1, a murine HD protein, inhibits transcription with a mechanism very similar to that we describe for EVX1 (54). Also SCIP, a member of the POU class of HD proteins, actively represses transcription of myelin-specific genes independently of the presence of its specific DNA binding sites on the promoter (56). Much evidence indicates that the function of HD proteins in transcriptional regulation may not be limited to their interaction with specific DNA-binding sites. Although HD proteins share similar DNA-binding properties in vitro, they present different functional specificities in vivo, and this is likely due to protein-protein

Fig. 5. RNase protection and RT-PCR analysis of the CAT transcript in transfected InR1-G9 cells. A, subconfluent cultures of InR1-G9 cells were transiently transfected using the calcium phosphate technique with p[−1.1]GLU-CAT together with either pRC-CMV or pCMV-EVX1. Total RNA was prepared at different times, and 10 μg RNA were subjected to RNase protection analysis as indicated under "Experimental Procedures." Lane 1, RNA CAT probe; lane 2, negative control; lanes 3 and 4, RNA from pCMV-transfected cells; lane 5, RNA from pCMV-EVX1-transfected cells. Lane 6, RNA from pCMV-EVX1-transfected cells, 24 and 46 h after transfection, respectively; lanes 7 and 8, RNA from pCMV-EVX1-transfected cells, 24 and 46 h after transfection, respectively. Expression of β-actin is shown in the lower part of the panel as an internal control. The numbers to the left indicate the length of the fragments in nucleotides. B, cell transfection and RNA isolation were performed as in panel A. 5 μg of RNA were subjected to RT-PCR as described under "Experimental Procedures." RT-PCR products were electrophoresed, and agarose gels were stained with ethidium bromide and photographed. The bands corresponding to the CAT transcript were densitometrically scanned, and the relative absorbances (expressed in arbitrary units) of two independent experiments were 23.1 (lane 1, pRC-CMV transfected cells) and 2.5 (lane 2, pCMV-EVX1 transfected cells).

DISCUSSION

Our findings indicate that a human HD protein, EVX1, is a potent transcriptional repressor and that this effect is independent of the presence of its specific DNA-binding sites in the promoter. Here we show that EVX1 represses basal and activa-

Fig. 6. EVX1 represses the transcriptional activity of a minimal TATA-less promoter in transiently transfected InR1-G9 and NIH-3T3 cells. Cells were transiently transfected in suspension using the DEAE-dextran technique with 10 μg of the reporter construct and 10 μg of effector plasmids, as indicated. 46 h after the transfection, cells were harvested, and the CAT activity present in aliquots of cell lysates was assayed, as detailed under "Experimental Procedures." Results represent the average (± S.E.) of three independent experiments (performed in duplicate). In the lower part of the figure, a representative autoradiogram of one experiment (performed in duplicate) for each cell line is shown.
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Fig. 7. The deletion mutant ΔEVX1 lacking the C terminus loses the ability to repress transcription in transfected cells. A, a schematic representation of EVX1, the deletion mutants, HOXC6, and the chimeric construct HOXC6/EVX1. Details on the construction of the mutant expression vectors are reported under "Experimental Procedures." B, immunohistochemical analysis of transiently transfected INR1-G9 cells with pcMV-ΔEVX1 utilizing mAb B6–41. Magnification is × 250. C, INR1-G9 was transiently transfected with 10 μg of the reporter plasmid p-1.1KLU-CAT and 10 μg of the effector plasmids indicated. 46 h after transfection, cells were harvested, and the CAT activity present in aliquots of cell lysates was assayed as detailed under "Experimental Procedures." Bars represent the average (± S.E.) of three independent experiments performed in duplicate.

interactions (4, 5, 14, 57, 58). Furthermore, several authors have shown that proteins lacking all or part of their HD may retain some aspects of function (16, 17, 54, 59). Therefore, all of this experimental evidence indicates that the simple interaction between the HD and its DNA-binding site can be dispensable for some transcriptional properties of homeobox-containing proteins.

A comparative analysis of the amino acid sequences of EVX1 and Eve revealed that, apart from the highly conserved HD, the two proteins show an alanine/proline-rich region present at the C-terminal end of both proteins in a position very close to the HD (data not shown). Although glutamine/alanine/proline richness is not a universal feature of active repressors, these domains have been found not only in Eve, but also in other well characterized transcriptional repressors such as engrailed, Krüppel, Wilms tumor gene product, and Msx-1 (53, 54). Furthermore, the Dr1 protein, which also contains a glutamine/alanine-rich region, has been shown to interact with TFIID and to inhibit transcription (60). Indeed, we have shown that EVX1 protein, while still able to bind DNA with high affinity, loses its ability to repress transcription when the alanine- and proline-rich carboxy-terminal portion of the protein is removed. Furthermore, this region confers transcriptional inhibitory potential to the HD protein HOXC6 which is per se unable to affect transcription of the reporter plasmids used.

In recent years there has been an explosion of information regarding the role of sequence-specific DNA binding proteins in the selective activation of eukaryotic promoters. On the contrary, less is known about how DNA binding proteins repress transcription (for reviews, see Refs. 53 and 61–63). However, it is reasonable to believe that selective repression is an important mechanism of transcriptional control and that it could be widely used during development. Our results seem to rule out most of the repression mechanisms proposed for the eukaryotic gene transcriptional regulation (53, 61–63). First, EVX1 does not compete with an activator protein for binding to the same DNA sequence, as it can inhibit promoters lacking its binding site. Second, a mechanism by which EVX1 would interact with a specific activator protein, preventing its contact with the transcription machinery, is made unlikely by the generality of the phenomenon we observed. A mechanism that must be taken into account when repression is observed, particularly in transient transfection assays, is termed "squelching" (64). In this case, transcriptional repression paradoxically results from the overexpression of an activating protein. Such repression requires neither specific DNA binding sites nor an intact DNA binding domain in the overexpressed protein and appears to result from the sequestration of other transcription factors with which the activator naturally interacts. Our data are not consistent with this explanation since (i) transcriptional activation was never observed under any circumstances we used, regardless of the promoter, the cell type or the concentration of the transfected EVX1 expression vector; (ii) the amount of the expression plasmid required for repression is within the range that is normally used to measure transcriptional activity in transient transfection assays (54); and (iii) preliminary results obtained in pools of HeLa cells stably transfected with pcMV-EVX1, which express relatively low levels of the protein in comparison to the transient transfectants, indicate that EVX1 represses transcription of transiently transfected reporter constructs.6 On the basis of our data, we can speculate that EVX1 functionally interferes with a general transcription factor. However, this interference cannot be due to a displacement of the factor caused by the binding of EVX1 to the TATA sequence, which could in principle mimic homeobox protein binding sites, because of its action on TATA-less promoters. The results seem then to indicate a mechanism by which EVX1, as other repressor proteins (60, 65–67), interferes with a general transcription factor at an early stage of the assembly of the transcription initiation complex, thereby preventing its further assembly.

The potential for EVX1 to repress transcription through protein-protein rather than protein-DNA interactions may be an important feature of its proposed role as a regulator of embryogenesis.

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REFERENCES

1. Levine, M., and Hoey, T. (1988) Cell 55, 537–540
2. Hayashi, S., and Scott, M. P. (1990) Cell 63, 883–894
3. McGinnis, W., and Krumlauf, R. (1992) Cell 68, 283–302
4. Scott, M. P., Tamkun, J. W., and Hartzell, G. W. (1989) Biochim. Biophys. Acta

4. P. Briata and G. Corte, unpublished results.
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