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# The *HOX11* gene encodes a DNA-binding nuclear transcription factor belonging to a distinct family of homeobox genes

**(homeodomain/T-cell leukemia/chromosome translocation)**

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**ABSTRACT** **A translocation involving human chromosome 10, band q24, in a subset of T-cell acute leukemias disrupts a region surrounding the putative oncogene *HOX11*, which encodes a protein with a homeodomain. The HOX11 protein binds to a specific DNA sequence, it localizes to the cell nucleus, and it transactivates transcription of a reporter gene linked to a cis-regulatory element, suggesting that HOX11 functions *in vivo* as a positive transcription activator. PCR analysis shows that the HOX11 homeodomain is a member of a distinct class of homeodomains, representatives of which occur in murine and *Drosophila* genomes. These all contain a threonine residue in place of the more common isoleucine or valine in helix 3 of the homeodomain. HOX11 therefore appears to belong to a family of DNA-binding transactivators of transcription.**

Specific chromosomal translocations are consistently found in human lymphoid and myeloid tumors. Molecular analysis of these translocations has identified numerous presumptive oncogenes that may code for proteins that act as master regulators of groups of genes in T-cell acute lymphoblastic leukemias (T-ALLs) (1). In T-ALLs, three classes of gene encode putative transcription factors involved in different chromosome translocations. These are the genes encoding cysteine-rich LIM proteins designated *RBTN1/Ttg-1* (2, 3) and *RBTN2/Ttg-2* (4, 5) and those encoding basic helix–loop–helix proteins such as *CMYC* (6), *LYL1* (7), *TAL1/SCL* (8, 9), and *TAL2* (10). A third class of gene product implicated in T-ALL is the HOX11 protein encoded by a homeobox gene on human chromosome 10, band q24 (11–14). Other homeobox genes have been implicated in hematopoietic tumors. *Hox 2.4* was found at a site of retroviral insertion in myeloid tumors and was later shown to be capable of transforming 3T3 cells, whereas *Hox 4.1* is deleted in some myeloid leukemias (15–18). A different homeobox gene, designated *PBX1*, located on human chromosome 1q23, was found to be fused to the *E2A* gene in a pre-B-ALL and to give rise to a unique fusion protein, which was also able to transform 3T3 cells (19–21).

A subset of T-ALLs possesses translocations t(10;14) or, more rarely, t(7;10), involving a reciprocal exchange between the 10q24 region encoding *HOX11* and either *TCRD* (14q11) or *TCRB* (7q35). The gene *HOX11*, adjacent to the 10q24 region, is transcriptionally active in T-cell leukemias possessing this translocation (11–14). It encodes a protein with a homeodomain suggesting that, like other such proteins, it regulates the transcription of a specific set of target genes (22). On the other hand, there was no direct evidence that the HOX11 protein is a transcription factor.

We demonstrate that HOX11 does indeed possess the features required of a transcription factor, since it is a nuclear, transactivating DNA-binding protein. We also show that HOX11 belongs to a homeodomain family of which we have identified further members.

## METHODS AND MATERIALS

**Isolation of λ Phage Genomic Clones.** Murine *hox11* and *hox11L2* genomic clones were isolated from libraries prepared from CCE embryonic stem cell or BALB/c liver DNA by screening at low stringency [6× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate), 65°C] with PCR-amplified probes corresponding to exons 1, 2, and 3 of the human *HOX11* gene (nucleotides 770–943, 1043–1213, and 1320–1498, respectively, in ref. 13). λ phage fragments were subcloned into the pBluescript (SK+) vector (Stratagene). DNA sequence was determined with random sonicated clones (23) and sequences automatically assembled (24).

**Immunofluorescence.** A full-length human *HOX11* cDNA with an 11-amino acid MYC tag (EQKLISEEDLN) at the carboxyl-terminal end of the HOX11 protein was constructed by PCR amplification from PER-255 cell line RNA (25) and cloned into the expression vector pEF-BOS (26). COS-1 cells were transfected with 5 μg of DNA using the DEAE-dextran method (27). After 48 hr the cells were seeded overnight on microscope slides. Cells were fixed in 4% (wt/vol) paraformaldehyde for 15 min, washed in phosphate-buffered saline (PBS), and permeabilized in methanol for 2 min. After blocking in 5% (vol/vol) fetal calf serum in PBS for 30 min, an affinity-purified anti-MYC tag antibody, 9E10, was added (5 μg/ml final concentration). After a 30-min incubation at room temperature a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Sigma) was added and incubated for a further 30 min. Fluorescent cells were visualized by epifluorescent microscopy.

**Transactivation Analysis.** The HOX11-expressor plasmid was constructed by cloning the full-length human *HOX11* cDNA into pEF-BOS (26). The expression vectors for Ubx and Abd-B proteins have been described (28). The chloramphenicol acetyltransferase (CAT) reporter plasmid carries a 36-bp fragment (5′-TAATGGTAATGGTAATGGTAATGGTATGGTAATGG-3′) cloned into a CAT plasmid. C3H10T½ cells were transiently cotransfected with 5 μg of CAT reporter plasmid plus test expression plasmid as described (29, 30). Five micrograms of expression vector pRSVL (31), which contains the Rous sarcoma virus long terminal repeat linked to luciferase, was used as an internal normalizing control.

***In Vitro* PCR Binding Site Selection.** A PCR amplification product corresponding to amino acids 200–259 of human HOX11 (13) was subcloned into pGEX-3X (31). A mutant recombinant plasmid that encoded an isoleucine in place of the threonine in the homeodomain was constructed using

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Abbreviations: CAT, chloramphenicol acetyltransferase; ALL, acute lymphoblastic leukemia; FITC, fluorescein isothiocyanate.

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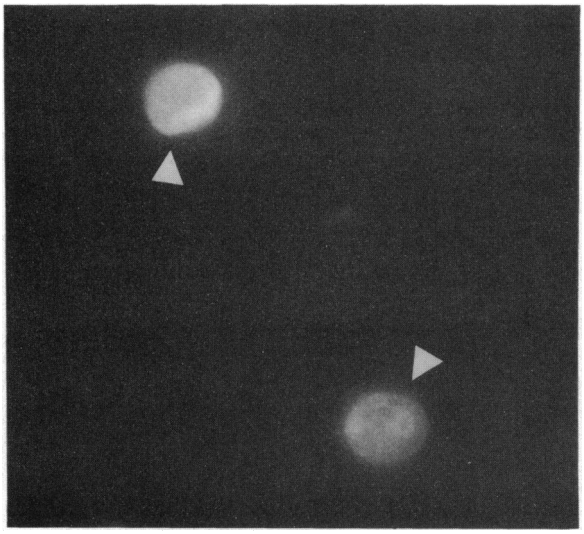

FIG. 1. Subcellular localization of HOX11 protein. COS cells were transfected with a construct that expressed HOX11 fused to a CMYC epitope tag. Cells were incubated with the 9E10 antibody, specific for the CMYC tag, followed by incubation with a FITC-conjugated goat anti-mouse IgG and analysis by immunofluorescence. Fluorescing cells are indicated (arrowheads).

site-directed mutagenesis (32) with the oligomer 5′-GGTCAAAATCTGGTTCC-3′ (mutated base underlined). *In vitro* PCR binding site selection was carried out as described (33–35, 40). After five successive rounds of selection, PCR-amplified DNA was digested with *Bam*HI and *Eco*RI (R76) (33) or *Eco*RI and *Xho* I (R70) (35) and cloned into M13mp18 for sequencing.

**PCR Amplification of *hox11*-Related Homeoboxes.** One microgram of DNA from BALB/c liver or *Drosophila melanogaster* was amplified in a 50-μl reaction with the HOX-

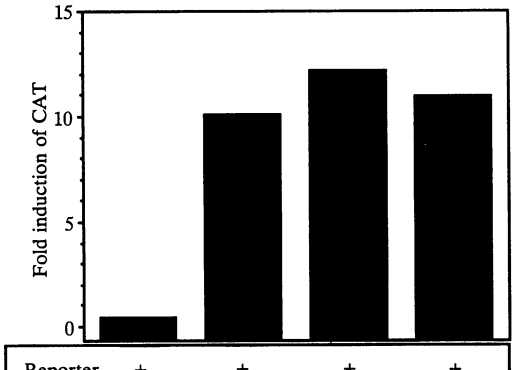


FIG. 2. CAT assays from C3H10T½ cells transfected with HOX11, Ubx, or Abd-B plasmids. A CAT reporter plasmid containing six tandem repeats of the sequence TAATGG [similar to the previously determined optimal DNA motif recognized by the Ubx homeodomain (35)] was cotransfected into C3H10T½ cells with test plasmids from which Ubx, Abd-B, or HOX11 was transcribed. Cells were harvested ≈60 hr later and extracts were assayed for CAT activity. Relative CAT activities were determined by comparing the ratios of acetylated/unacetylated [$^{14}$C]chloramphenicol present in spots cut from the thin-layer chromatographs. Equivalent amounts of protein (15–25 mg as determined with Bio-Rad protein kit) and a reaction time of 1 hr were used in all CAT assays, which kept all values within the linear range. Values (average of four independent experiments) show CAT activities relative to extracts of cells transfected with the CAT reporter alone set to a value of 1.

**A. HOX11 + R76 (n=37)**

TAAC priming site motif (n=23)
1 tcCGTTGAGTGCCTACAGGACCACTTAACg
2 tcATTAGCCGCCCTCGTTCCGCTATAACg
3 tcGACCTAGGGTAATTAGATTATTAACg
4 tcGCGCAATGAGCGATCGGTCGGGTAACg
5 tcCAGACCAATTAAATGATTCTCGTTAACg
6 tcGAGAGCGATAATCGGTACCCTCATAACg
7 tcAACACCCTGCATGACCATTCGCGTAACg
8 tcAACAAAGGTTGCTAAGTACTCATTAACg
9 tcGTTAACCACATCAGCCGTGCAGTTAACg
10 tcCTCGTTAACATAACGACCCCATCTAACg
Consensus: TAAC

TAAC internal motif (n=10)
11 tcATCTGACATAAACAACTACTTAACGTcg
12 tcAGTTAACAACCCCACTTAACACCTTGTcg
13 tcGTTATGCTAACGATGCCTGAGCCTCAcg
14 tcATCAATACGGATCTATAACCATGCcg
15 cgTAGAGTAAGTACCACTTAACGTGACGga
Consensus: TAAC

TAAT internal motif (n=3)
16 tcATTGGCTGCCATTGTAATGTACATTGcg
17 cgGTAATGAGGATGCAAGTAGCCTAATga
18 tcATTAACGCCGGCCAATTATCGTAATGcg
Consensus: TAAT

No obvious motif (n=1)

**B. HOX11 (T->I) + R76 (n=25)**

TAAT motif (n=20)
1 tcGGAGCGGGTATGTGGTAATGGCTATGcg
2 tcGTTGAGCGGCTACTAATGGGGGTCAAATcg
3 tcGACTGGATGGTTAATGGGGCTGTTGAcg
4 tcACCCATTCGTAATCAATTGTGGCCCTAATcg
5 tcCATCTTAGTGTAATTCCCACGCAAGTcg
6 cgGTATGGTCCGGGTCGCTAAGGCTAATga
7 cgGGGTAAGACGTCATAGTGGGGATAATga
8 cgAAGGGGGGTTGGTTAATCACAGCCTTga
Consensus: TAAT

No obvious motif (n=5)

**C. HOX11 + R70 (n=25)**

TAAC motif (n=7)
1 ctTATAACGACCACgg
2 ctGTACTCTAACGCgg
3 ctCTTCGTAACTCAgg
4 ccCTAACTTATTCTag
Consensus: TAAC

TAAT priming site motif (n=6)
5 ctAATCGCGACGTAgg
6 ctAATTGGGGCTGGgg
7 ctAATGCTCGTGGGgg
8 ctAATGATTTAATAgg
9 ctAATGCCCATGTTgg
Consensus: TAAT

TAAT internal motif (n=9)
10 ctGTAATCAGCTGGgg
11 ctGCTAATGCTAGTgg
12 ctGGGGTAATTAGGgg
13 ccGTTAATATGACTag
14 ccAATTAATGTGCAag
Consensus: TAAT

No obvious motif (n=3)

FIG. 3. Selection of sequences of PCR clones selected after HOX11 *in vitro* binding. Uppercase letters denote nucleotides derived from the randomized sequence core of R76 and R70; lowercase letters indicate the invariant flanking sequences. The common motifs (TAAT and TAAC or their complements ATTA and GTTA) shared by the oligonucleotides are underlined. The sequences are aligned relative to the most common motif in each group. The fusion proteins used for binding experiments contained either the normal HOX11 protein (*A* and *C*) or a mutant version in which the threonine residue at position 47 in the homeodomain was replaced by an isoleucine (*B*). The R76 oligonucleotide containing a 26-base random sequence core was used for the experiments in *A* and *B*, whereas the R70 oligonucleotide containing a 12-base random sequence core was used in *C*. For simplicity, not all sequences are shown. The number of actual clones (n) obtained for each category is indicated in parentheses.

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HOX11    MEHLGP--HHL-HPGHAEPISFGIDQILNSPDQGGCMGPASRLQDGEYGLGCLVGGAYTYGGGGSAAATGAGG  70
hox11    .......--.R.-.....................................D.....................GA ...  70
hox11L1  ..PAVLAA...--P-.H..........SGPEP-----------------------------------------  30
hox11L2  ..APAS--AQTP..--H..............DSA---.P.PRRRQLPGRAPR.RPGAAYPSLP.SFA.L.A  67

HOX11    AGAYGTGGPGGPGGPAGGGG-ACSMGPLTGSYNVNMAL-AGGPGPGGG--GGSSGGAGALSAAGVIRVPAHRP  140
hox11    T....A.............G.......P.........-.........GG..GA.....................  143
hox11L1  ---------...GL..GQS.Q-SHGESAAFS.GFHGASGY.PAGSLASL--PRG..VGPG----.........  88
hox11L2  PFEDAGSYSVNLS-------------------------------------------------.AP.........  94

HOX11    LAGAVAHPQPLATGLPTVPSVPAMPGVNNLTGLTFPWMESNRRYTKDRFTv--alspftvtrriGHPYQNRTP  212
hox11    .......................V.....................................--.....................  215
hox11L1  .PVPPPSGAAP.------..GPSGLG.AGG.A.......D.G..FA...L.A--.....sg.............  154
hox11L2  .P...--.P..PSA..AM...---.T.SS.G..N......S..FV.....AAA..T.................  162

                  helix 1          helix 2      helix 3
HOX11    PKKKKPRTSFTRLQICELEKRFHRQKYLASAERAALAKALKMTDAQVKTWFQNRRTKWRRQTAEEREAERQQA  286
hox11    ..............................................................................  289
hox11L1  ..R.......S.S.VL...R..L.................R.............................HR.  227
hox11L2  ..R.......S.V.........................S.................................  235

HOX11    NRILLQLQQEAFQKSLAQPLPADPLCVHNSSLFALQNLQPWSDDSTKITSVTSVASACE                342
hox11    ..........................................................                  345
hox11L1  G.L..H...D.LPRP.RP...P....L.............AE.N-.VA..SGL..VV                  284
hox11L2  S.LM....HD......NDSIQP....L.............EE..S.VPA...LV                    290
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FIG. 4. Comparison of hox11 with related mouse proteins. Alignment of the deduced protein sequences from human HOX11, murine hox11, and two related gene products, hox11L1 and hox11L2. The homeodomain is overlined. Amino acid identity is indicated by dots; dashes correspond to gaps introduced to maximize alignment. Lowercase letters represent 12 amino acids encoded by a segment of DNA immediately upstream of the acceptor splice site of *HOX11* exon 2, which is conserved in the human and murine genes and in the *hox11L1* and *hox11L2* homologues, suggesting that this region may be incorporated into mRNA by alternative splicing and sometimes be translated into protein. Interexon PCR, performed on various mouse tissues utilizing primers from exons 1 and 2 of *hox11*, yielded two DNA products (data not shown) whose sequence confirmed this. The corresponding 12 amino acids may also be alternatively spliced in *hox11L1*. Presumptive helices 1, 2, and 3 of the homeodomain are overbarred and the threonine at position 47 is indicated (arrow).

HOMF12/HOXBACK primers (see Fig. 5*A*) using the touchdown method (36). Initial conditions were 94°C, 1.5 min; 63°C, 1.5 min; 72°C, 2 min. The annealing temperature was reduced by 2°C every two cycles down to 35°C, at which 15 cycles were performed. Amplified products were digested with *Bam*HI and *Eco*RI and cloned into M13mp18 for sequencing.†

## RESULTS

**Nuclear Localization and Transcriptional Transactivation by HOX11.** *HOX* genes encode transcription factors that bind DNA *in vivo* and activate transcription (37). These aspects of the *HOX11* gene were investigated using *in vivo* and *in vitro* methods. The subcellular locus of the HOX11 protein was investigated by transfecting COS cells with an expression construct that produces the human HOX11 protein linked to an amino acid epitope tag. This tag corresponds to part of the human CMYC protein which is specifically recognized by the 9E10 antibody (38), thereby allowing immunofluorescence detection. No immunofluorescence signal was seen in cells transfected with the HOX11-only construct, which lacks the MYC tag (data not shown), whereas those transfected with HOX11-MYC tag produced clear immunofluorescence signal within the nucleus but little cytoplasmic staining (Fig. 1). Thus the HOX11 protein is able to reside in the nucleus, which is, presumably, where it lies in T-ALL with t(10;14) or t(7;10).

We assayed the ability of HOX11 protein to transactivate transcription of a CAT reporter gene linked to a minimal metallothionine promoter that is itself coupled to the specific target sequence TAATGG. C3H10T½ cells were cotransfected with HOX11-expression constructs together with the CAT reporter gene. Control constructs encoding the *Drosophila* homeodomain-containing proteins Ubx and Abd-B were also assayed in this system. All of these homeodomain constructs stimulated production of CAT in the transfected cells when the reporter clone contained the target TAATGG sequence (Fig. 2) but not in its absence (data not shown). These results show that HOX11 is capable of activating transcription *in vivo* through the TAATGG target sequence.

**DNA Sequence Recognition by the HOX11 Homeodomain.** The transcriptional transactivation by HOX11 protein suggests sequence-specific DNA-binding activity. The third helix of homeodomains is important in contacting DNA (39), but this helix of the HOX11 homeodomain has a threonine residue instead of the more usual isoleucine or valine (arrow in Fig. 4), suggesting that the threonine might affect binding specificity. The DNA sequences recognized by the HOX11 homeodomain were analyzed by *in vitro* binding site selection from random sequence oligonucleotides (33, 35, 40). The human HOX11 homeodomain was expressed in the pGEX-3X bacterial expression vector (31) and the fusion protein was bound to glutathione-Sepharose beads followed by incubation with the R76 oligonucleotide, which contained a central 26-nucleotide random sequence core flanked by fixed sequences to serve as PCR priming sites. Specifically bound oligonucleotides were recovered, amplified by PCR, and subjected to a further four rounds of binding site selection. The amplified DNA from the fifth round was sequenced. The DNA sequences of 37 individual oligonucleotides were determined (Fig. 3*A*). Eighty nine percent of these sequences exhibit a motif consisting of TAAC; the C comes from the first residue of the priming site in 70% of cases. Five of the sequences were identical, suggesting a few sequences from the initially large pool have been enriched. Of the clones exhibiting a TAAC motif, 55% also contained a TAAT (or its complement ATTA) sequence, as exemplified by clones 2, 3, 5, 6, and 8 in Fig. 3*A*.

TAAC is a variant of the TAAT sequence recognized by other homeodomains of the antennapedia class (35), which have isoleucine or valine at position 47 rather than the threonine in HOX11. To ascertain whether this replacement in HOX11 changes the binding specificity, a mutant fusion HOX11 protein was made with an isoleucine at position 47 and binding site selection was repeated. Fig. 3*B* shows examples of the sites in 25 independent clones. The core binding site motif is invariable TAAT. The binding site was varied by repeating the site selection using a second oligo-

†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L08614–L08620).

A

Forward

HOXHOMF12

TAGGGATCC GAA CTG GAG AAA CGN TTT
G G A C
E L E K R/S F

Reverse

HOXBNOT (COMPLEMENT SHOWN)

HOXBACK (COMPLEMENT SHOWN)

CN TGG TTT CAG AAT GCN ACG ACN AAA TGG GCN GAATTCCTA
C C C G
T W F Q N R R T K W R

B

| | | |
|---|---|---|
| Group 1 | ↓ | Species |
| M6 | ETQKYLSPPERKRRAKMLQLSERQVKT | mouse |
| M1234 | *TQKYLSPPEKKHRANMSQLRERQVKT | mouse |
| Group 2 | | |
| hox11 | HRQKYLASAERAALAKALKMTDAQVKT | mouse |
| hox11L1 | LRQKYLASAERAALAKALRMTDAQVKT | mouse |
| hox11L2 | HRQKYLASAERAALAKSLKMTDAQVKT | mouse |
| 311 | HKQKYLASAERAALARGLKMTDAQVKT | *Drosophila* |
| BarH1 | ERQKYLSVQERQELAHKLDLSDCQVKT | *Drosophila* |
| Group 3 | | |
| D125 | LYQKYLSPADRDEIAASLGLSNAQVIT | *Drosophila* |
| Group 4 | | |
| 310 | EGQRYLSTPERVELATALGLSETQVKT | *Drosophila* |
| Consensus | --QKYL---ER---A--L-----QVKT | |

FIG. 5. A family of threonine-containing homeodomains in man, mouse, and fly. (*A*) Sequences of the forward and reverse PCR primers used to identify additional HOX11-like homeodomains. Amino acid sequences are shown below the corresponding primer sequences. The primers were based on those that had originally been used to identify homeoboxes of the antennapedia class (42). (*B*) Alignment of predicted homeodomain sequences. Related sequences have been separated into distinct groups. The consensus sequence identifies those amino acid sequences present in at least eight of the nine predicted sequences. *BarH1* and *hox11L1* have been previously reported (13, 42). The asterisk in clone M1234 indicates an in-frame stop codon (TAG) that was present in the only clone sequenced. The threonine in the recognition helix is indicated (arrow).

nucleotide, R70, which has a 12-nucleotide random sequence core lacking a C nucleotide in the priming site sequence (35). Using the unmutated HOX11 homeodomain, binding sites containing either TAAC (28%) or TAAT (60%) were obtained (Fig. 3*C*). These data suggest that the sequence TAAC might indeed be part of an *in vivo* recognition sequence for the HOX11 protein.

**The Murine hox11 and Related Mouse Proteins.** Families of proteins, defined by conservation of their homeodomains, have been well documented (21). The unusual threonine at position 47 in HOX11 suggested that this homeodomain might also constitute a family. Indeed, we had previously isolated a murine homologue of human *HOX11*, designated *hox11L1* (ref. 13; formerly termed *pmur10f*), with marked divergence from human *HOX11* in the amino-terminal half of the predicted protein.

We have now isolated the mouse genomic homologue of *hox11*. The protein predicted by this gene is very similar to its human counterpart (Fig. 4); the only amino acid differences occur within exon 1, which contains 10 amino acid substitutions (4 of which are conservative) and an additional three glycine residues absent from the human sequence. The homeodomain is fully conserved between the two species. In addition to the mouse *hox11* and *hox11L1*, a third gene was isolated from mouse DNA that encodes a homeodomain with

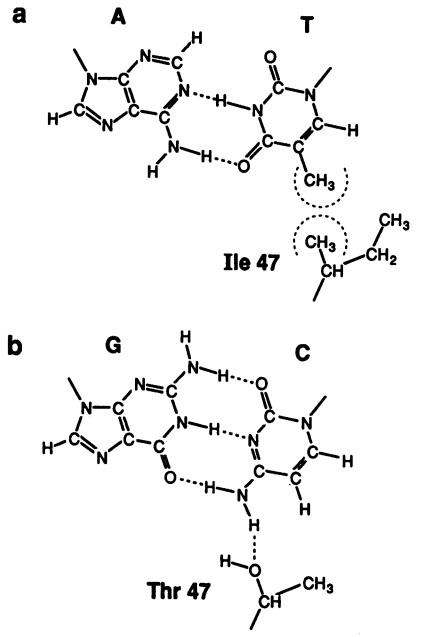


FIG. 6. Sequence-specific interactions by the amino acid at position 47 of the homeodomain. (*a*) Isoleucine in the engrailed homeodomain contacts a thymine by making a hydrophobic methyl–methyl interaction (43). (*b*) Threonine in the HOX11 homeodomain could potentially interact with cytosine by making a hydrogen bond between its hydroxyl group and the amine group of the cytosine.

threonine 47. This gene, designated *hox11L2*, was identified by cross-hybridization with a full-length human *HOX11* cDNA probe at low stringency (6× SSC, 65°C). Fig. 4 compares the predicted protein sequence of this homologue with human and mouse hox11 and mouse hox11L1. The sequence shows a 61.3% homology between hox11 and hox11L1 in a 282-amino acid overlap, 66.3% between hox11 and hox11L2 in a 288-amino acid overlap, and 54.0% between hox11L2 and hox11L1 in a 289-amino acid overlap.

**The HOX11-Related Homeodomains Define a Distinct Subfamily.** The hox11, hox11L1, and hox11L2 homeodomains, along with *Drosophila* BarH1 (41), are the only homeodomains known to possess a threonine at position 47 within helix 3. To determine the extent of this "threonine" family, PCR primers were employed to amplify *HOX11*-related homeobox sequences from the murine and *Drosophila* genomes (see *Methods and Materials* and the legend to Fig. 5). In this way we identified five additional homeoboxes, the murine clones M1234 and M6 and the *Drosophila* clones 310, 311, and D125 (Fig. 5*B*), together with the known murine *hox11* and *hox11L2* homeoboxes. PCR using forward primers specific to the individual homeobox sequences in combination with a reverse primer, HOXBNOT (Fig. 5*A*), confirmed that all of the detected homeoboxes contained a threonine codon at position 47 (Fig. 5*B*).

The protein sequences predicted by these homeoboxes can be classified into four separate categories based on homology (Fig. 5*B*). The only M1234 clone obtained had an in-frame stop codon (TAG) at the position of the 21st amino acid in the homeodomain; this may represent a PCR-incorporated error. Thus, vertebrates and invertebrates possess a subfamily of homeobox genes characterized by a specific threonine residue in helix 3 of the homeodomain.

## DISCUSSION

**The *HOX11* Gene Encodes a Highly Conserved Transcription Factor in Man and Mouse.** The *HOX11* gene is highly expressed in T-ALLs as a result of a t(7:10)(q35;q24) or t(10:14)(q24;q11). The presence of HOX11 protein in the

nucleus, its DNA binding, and its activation of transcription suggest that it contributes to T-cell tumorigenesis by activating a specific subset of tumor-associated target genes. These features embody the master gene model, proposed to explain the consequences of chromosome translocation in T-ALL (1). Incorrect activation by transcription factors constitutes an adverse event capable of disrupting cellular transcription equilibria and ultimately leading to overt leukemia. This mechanism of tumorigenesis may be common to other T-cell leukemia-associated genes such as *RBTN1/Ttg1, RBTN2/Ttg2, LYL1, TAL1/SCL*, and *TAL2*.

***HOX11* Is a Member of a Homeobox Family.** The homeobox of the *HOX11* gene belongs to a distinct subclass of homeodomains possessing a threonine within helix 3. In other homeodomains, isoleucine or valine is most commonly found in this position, but asparagine, leucine, or histidine has also been found there (39). The *hox11* gene family consists of at least three partially homologous members. The only example of a homeodomain protein with threonine was the *BarHI* gene (41). The *in vitro* PCR binding data suggest that the threonine variation in the critical DNA-binding helix may effect binding site recognition. The isoleucine in this position in the engrailed homeodomain–DNA complex (43) contacts the fourth thymine of the TAAT core motif via a methyl–methyl interaction (Fig. 6*a*). The threonine in a similar position in the HOX11 homeodomain could potentially hydrogen bond with the amine side chain of cytosine (Fig. 6*b*), thereby accounting for the binding of this homeodomain to TAAC *in vitro*. The threonine might also contact a thymine residue via a methyl–methyl interaction, in an analogous fashion to isoleucine or valine. This could account for the ability of the HOX11 homeodomain to bind to TAAC and TAAT *in vitro* when the R70 oligonucleotide was used.

The *Drosophila BarHI* and *HOX11* genes exhibit no significant homology outside of the homeobox, suggesting that *HOX11* is not functionally related to this *Drosophila* gene. Two other *Drosophila* genes identified by PCR also show significant homology with HOX11 in the homeodomain (D125 and 311, Fig. 5*B*). Both of these *Drosophila* genes also share sequence homology with the *Drosophila* homeobox genes *NK3* and *NK4/msh-2* (44, 45), suggesting an evolutionary relationship, even though the *NK3* and *NK4/msh-2* genes do not encode the threonine at position 47 of the homeodomain (44, 45). The *Drosophila* 311 sequence is the one most closely related to the HOX11 homeodomain and may be a functional homologue. If the 311 gene is a *HOX11* equivalent, a study of the normal role of 311 in *Drosophila* may give a clue to the function of the *HOX11* gene in man and mouse.

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