cell cycle progression.

Peptide hormones other than PRL may also function within the nucleus. The mitogenic activity of fibroblast growth factor depends on its nuclear translocation sequence (*15*). Other peptide ligands that translocate into the nucleus such as IL-1 (*16*), platelet-derived growth factor (*17*), and insulin (*18*) may function in a similar manner. Further study of the internalization and intracellular interactions of PRL may define the mechanisms through which this peptide hormone influences cell cycle progression and immune responsiveness.

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Deregulation of a Homeobox Gene, HOX11, by the t(10;14) in T Cell Leukemia

MASAHIKO HATANO, CHARLES W. M. ROBERTS, MARK MINDEN, WILLIAM M. CRIST, STANLEY J. KORSMEYER*

Molecular cloning of the t(10;14)(q24;q11) recurrent breakpoint of T cell acute lymphoblastic leukemia has demonstrated a transcript for the candidate gene TCL3. Characterization of this gene from chromosome segment 10q24 revealed it to be a new homeobox, HOX11. The HOX11 homeodomain is most similar to that of the murine gene *Hlx* and possesses a markedly glycine-rich variable region and an acidic carboxyl terminus. HOX11, while expressed in liver, was not detected in normal thymus or T cells. This lineage-restricted homeobox gene is deregulated upon translocation into the T cell receptor locus where it may act as an oncogene.

SPECIFIC INTERCHROMOSOMAL TRANSlocations are repeatedly found in distinct types of malignancies (*1*). Chromosomal translocations found in lymphoid neoplasms provide the opportunity to identify new putative proto-oncogenes introduced into either the immunoglobulin loci of B cells or the T cell receptor (TCR) loci of T cells. For example, the t(14;18) of follicular B cell lymphoma revealed BCL-2 juxtaposed with the immunoglobulin heavy chain gene (*2*). Transgenic mice established the prospective oncogenic importance of this new gene in malignant lymphomagenesis (*3*). Similarly, the most frequent site of chromosomal aberration in T cell acute lymphoblastic leukemia (T-ALL) represents the α and δ subunits of the TCR at 14q11. Approximately 7% of T-ALLs possess the t(10;14)(q24;q11) (*4*). Rearrangements of the TCR were used to clone breakpoints within chromosome segment 10q24 that clustered around an evolutionarily conserved region (*5, 6*).

A conserved 1.3-kb Pvu II–Bam HI fragment of 10q24 origin (Fig. 1) recognized a transcript of the proposed TCL3 gene in t(10;14) T-ALLs (*6*) (Fig. 2). To identify the normal cellular lineage that expressed TCL3, we hybridized this probe to Northern panels of RNA from human and murine organs. Human liver was the only organ to contain demonstrable TCL3 RNA as did murine liver, attesting to its evolutionary conservation (Fig. 2). While normal liver contained TCL3, the hepatocellular carcinoma line HepG2 did not. TCL3 was not detected in thymus (Fig. 2), repeated thymus samples, resting T cells, or T cells activated with phytohemagglutinin (PHA) or

M. Hatano, C. W. M. Roberts, S. J. Korsmeyer, The Howard Hughes Medical Institute and the Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110.
M. Minden, The Princess Margaret Hospital, Toronto, Ontario, Canada.
W. M. Crist, St. Jude Children's Research Hospital, and the Department of Pediatrics, The University of Tennessee, Memphis, College of Medicine, Memphis, TN, and the Pediatric Oncology Group, St. Louis, MO.

*To whom communications should be addressed.

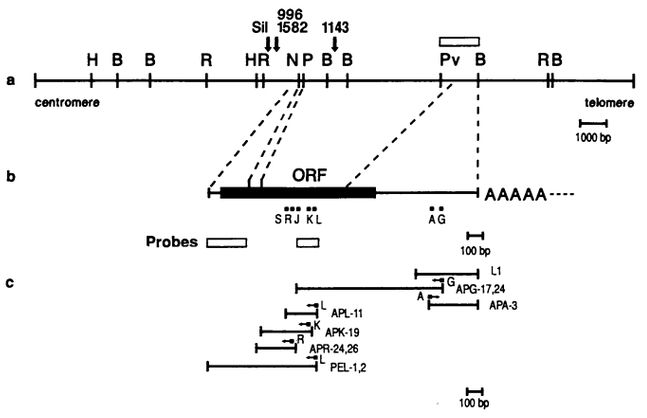

Fig. 1. Schematic of HOX11 = TCL3. (**a**) Restriction map of 20 kb of germline 10q24. Locations of the chromosomal breakpoints of ALL-Sil, T-ALL-996, 1582, and 1143 are noted by arrows. Three other breakpoints in the vicinity of T-ALL 996 as well as a t(7;10) that breaks ~10 kb further centromeric have been reported (*5*). B, Bam HI; H, Hind III; R, Eco RI; P, Pst I; Pv, Pvu II; N, Not I. (**b**) HOX11 cDNA in which the open reading frame (ORF) is indicated by a black box. Dashed lines indicate the location of HOX11 cDNA within the genomic map as determined by genomic sequence. The complete exon/intron boundaries are not all identified. The primers used to generate or assess anchored PCR or primer extension cDNA clones are indicated by letters. (**c**) Overlapping cDNA clones that were sequenced on both strands. The cDNA clones generated by anchored PCR with specific primers are indicated by the AP series. The cDNAs generated by primer extension with oligonucleotide L are denoted PEL-1,2.

PHA + PMA (phorbol 12-myristate 13-acetate) (*7*). Moreover, TCL3 was not present in αβ T cell lines (Hut 78 and Det), γδ T cell lines (Peer, MV, and 702), or $CD3^-$ T cell lines (8402, CEM, and HSB-2). Other tissues including placenta (Fig. 2), heart, lung, kidney, spleen, brain, ovary, adrenal, muscle, artery, salivary gland, and intestine also lacked TCL3 at the sensitivity of Northern analysis. In addition, monoblastic (U937), promyelocytic (HL-60), Burkitt lymphoma (Raji and BJAB), pre B cell (NALM-6), neuroblastoma (N2A), and embryonal stem cell (D3) lines revealed no TCL3 RNA.

Consequently, a cDNA library of human liver (*8*) was screened with the 1.3-kb Pvu II–Bam HI probe; an incomplete cDNA, L1, bearing a poly(A) tail and identical sequence to the genomic probe was identified (Fig. 1). Our goal was to examine the TCL3 transcript in t(10;14) T-ALL to determine if it was entirely of chromosome 10 origin or potentially chimeric. However, the only available RNA was of limited amount (10 μg total) from the leukemic cells of T-ALL-996 (Fig. 2). This prompted an anchored PCR-based cloning strategy in which the L1 cDNA was used to initiate cDNA cloning from t(10;14) ALL (*9*). Successive anchored PCRs yielded overlapping clones covering 1.6 kb of the message (Fig. 1). However, an upstream GC-rich sequence interfered with further extension. At that juncture, larger amounts of RNA (100 μg total), provided by one of us (M.M.), from the t(10;14)-bearing ALL-Sil line (*10*) enabled the creation of an L oligonucleotide–generated primer extension library (*9*) (L oligonucleotide primer is indicated in Fig. 1b). Two independent clones, PEL-1 and 2, completed the protein encoding portion of TCL3 (Fig. 1). The 5′ end of PEL-1 was placed within our genomic sequence of 10q24 and recognized TCL3 RNA (Fig. 2).

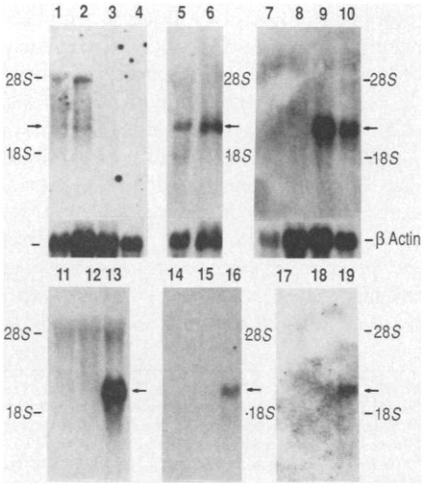

Fig. 2. Northern analysis of HOX11 (TCL3). Fifteen to 20 μg of total RNA prepared by a guanidine thiocyanate lysis procedure or 5 μg of poly(A)$^+$ RNA were denatured in formamide, separated by electrophoresis in agarose + formaldehyde gels, and transferred to Nitroplus 2000 (MSI). Probes were radioactively labeled by random priming or PCR and hybridized at 42°C in 50% (human) or 40% (mouse) formamide. Blots were washed twice at a final stringency of 55°C in 0.1× SSC + 0.1% SDS. Lane 1, human liver total RNA; lane 2, human liver poly(A)$^+$ RNA; lane 3, HepG2 poly(A)$^+$ RNA; lane 4, human placenta poly(A)$^+$ RNA; lane 5, mouse liver total RNA; lane 6, mouse liver poly(A)$^+$ RNA; lane 7, human thymus total RNA; lane 8, RPMI 8402 total RNA; lane 9, T-ALL-996 total RNA; lane 10, T-ALL-1582 total RNA. Lanes 1 to 10 were hybridized with the 1.3-kb Pvu II–Bam HI genomic probe. Reprobing with β-actin ensured that hybridizable RNA was present. Lanes 11, 14, and 17 contain human thymus total RNA; Lanes 12, 15, and 18 contain RPMI 8402 total RNA; lanes 13, 16, and 19 contain ALL-Sil total RNA. RNA in lanes 11, 12, and 13 was hybridized to the 1.3-kb Pvu II–Bam HI genomic probe; RNA in lanes 14, 15, and 16 was hybridized to a PCR-generated probe flanked by primers J to L (Fig. 1); RNA in lanes 17, 18, and 19 was hybridized to a 280-bp Not I probe from the 5′ end of PEL-1 cDNA. Transcripts measured approximately 2.5 kb, somewhat smaller than a previous estimate (*6*).

DNA sequence of the composite cDNA revealed an excellent Kozak consensus sequence (*11*) for an initiation codon and an open reading frame that encoded 330 amino acids of a new homeobox protein (Fig. 3). Upon the advice of the Nomenclature Committee of the Human Gene Mapping Workshops, TCL3 was renamed HOX11. The HOX11 gene in ALL-Sil resides on the der(14) chromosome and is of chromosome 10 origin (Fig. 1). Garnier-Robson program analysis (*12*) predicted α helices within the homeodomain. Overall, HOX11 is most similar to the murine homeobox gene *Hlx*, recently described by Allen *et al.* (*13*) (Fig. 4a). Thirty-one of the 60 amino acids in the homeodomain are identical in the HOX11 and *Hlx* products, including 9 of 10 within helix 3. Helix 3 possesses the highly conserved WFQ motif and, in the best studied proteins, is the region that most strongly confers DNA-binding specificity (*14, 15*). However, beyond the homeodomain, human HOX11 and murine *Hlx* are distinctly different genes and display a different tissue distribution (*13*). Homeodomains from k8, H2.0, c1, and Antennapedia also demonstrate similarity (*14*). Helix 1, especially the ELEK motif, is conserved among the homeotic proteins and is a region suspected of protein-protein interactions (Fig. 4a). The POU family of proteins, which includes Oct-1 and the yeast MATal and MATα2, was considerably more divergent (*14*).

Other salient features include a lysine-rich stretch bordered by prolines (PPKKKKP) at the NH_2-terminal portion of the homeodomain (Fig. 3). All homeodomain proteins to date have been located in the nucleus and this sequence highly resembles a nuclear localization motif. Alternatively, this may be analogous to the POU family that has a basic amino acid cluster NH_2-terminal of the POU homeodomain (*16*). However, HOX11 lacks the POU-specific domain of this family. HOX11 is only partially identical (TFPWM) with the conserved pentapeptide motif IYPWM often found in front of the homeodomain. HOX11 contains a portion of the Hep motif (FGIDQIL) also seen in the *Hlx* product (Figs. 3 and 4b) (*13*). The NH_2-terminal variable sequence of HOX11 had several extremely glycine-rich regions, some of which were interspersed with prolines. Glycine-rich regions have

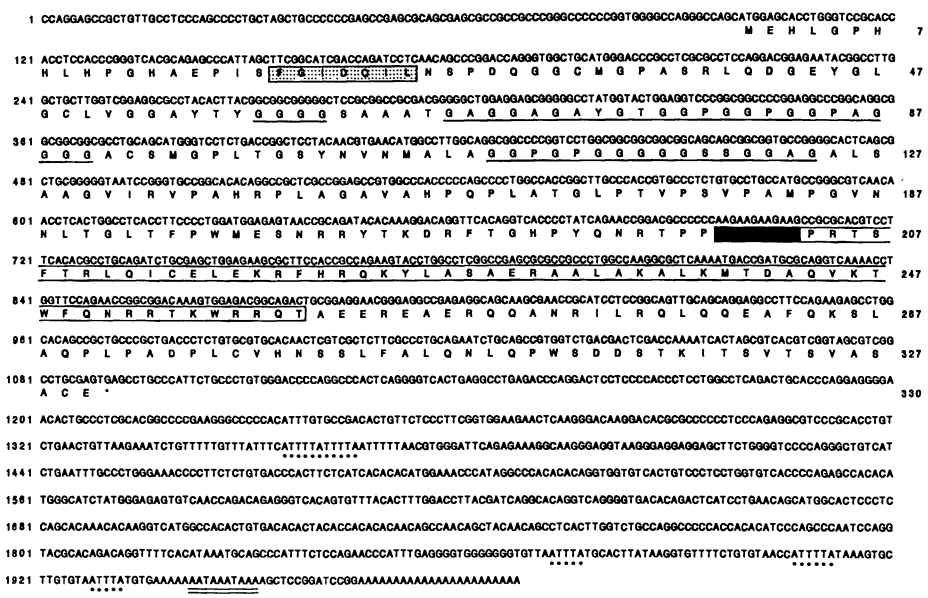

Fig. 3. Nucleic acid and protein sequence of HOX11. The homeodomain is open-boxed, with the NH_2-terminal basic cluster shaded. The partial Hep motif is stippled. Glycine-rich stretches are underlined; sequences that might mediate short mRNA half-life are noted by dots. Overlapping poly(A) addition signals are double underlined. The selected methionine initiation codon displays an ideal Kozak consensus sequence. Further primer extension sequencing covered 350 bp of the 5′ untranslated region upstream from this methionine and revealed two potential methionine codons. However, they both lacked a favorable Kozak consensus sequence and each was followed closely by an in-frame stop codon. In addition, an upstream stop codon was noted in the predicted frame of HOX11 (7). Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

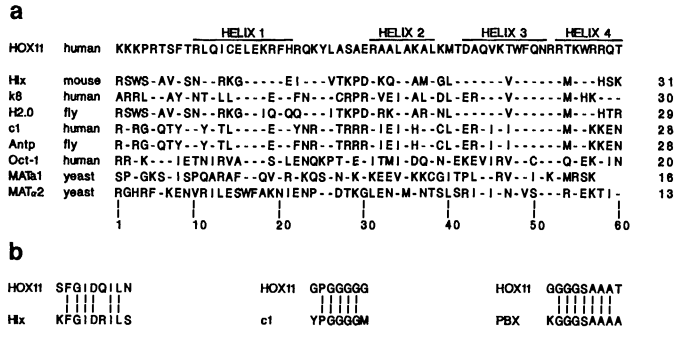

Fig. 4. (**a**) Comparison of HOX11 homeodomain with other homeobox genes. Dashes indicate identity with HOX11. The numbers on the right indicate the residues out of 60 that are identical with HOX11. Comparisons were done with the Bestfit program of the Genetics Computer Group software package (*26*). (**b**) Comparison of the Hep motif and two glycine-rich stretches outside the homeodomain between HOX11 and other homeobox genes (*13, 14*).

been noted in other homeoboxes including c1 (*14*), c13 (*17*), PBX (*18*), Ubx (*19*), and Dfd (*20*) (Fig. 4b). Whereas the overall protein (pI 10.3) and the homeodomain (pI 12.1) were basic, the COOH-terminus was acidic (pI 4.71). Serine and threonine residues were well represented in the COOH-terminus. Other homeobox proteins have acidic COOH-terminal domains rich in serine and threonine residues (*13, 14*). Such domains have been implicated in transcriptional activation (*21*).

The t(10;14) has revealed the first homeobox gene implicated in T cell neoplasia. In parallel, a substantial portion of pre B ALLs possess a t(1;19)(q23;p13.3) that creates a chimeric peptide between the E2A immunoglobulin enhancer–binding protein and a homeodomain from PBX (*18*). However, HOX11 is not highly similar to PBX. In ALL-Sil HOX11 is entirely of chromosome 10 origin, although a variant breakpoint that would interrupt HOX11 was noted (*6*).

A common theme is now emerging for the translocated genes in T cell leukemia. These genes, including HOX11, belong to classic transcription factor families. Ttg-1 or rhombotin from 11p15 is a nuclear protein with two Lim motifs also found in the Isl-1 transcription factor. Ttg-1 is expressed predominantly in the neural lineage rather than T cells (*22*). Moreover, 19p13 contributes a helix-loop-helix protein, Lyl-1, which is expressed highly in most hematopoietic lineages, but minimally in T cells (*23*). Chromosome segment 1p32 also translocates a helix-loop-helix protein (Scl, tal, or TCL5) normally expressed in early hematopoietic cells rather than in thymus or T cells (*24*). Thus many of these genes are intended for other lineages, but are apparently redirected to T cells by translocation into TCR loci.

Members of the homeobox family of proteins have been shown to direct pivotal steps in segmental development or to regulate cell type–specific differentiation (*14*). These DNA-binding transcription factors presumably mediate their effects by the induction or repression of target genes. Given the similarity of the homeodomains and their DNA-binding specificity, a remaining puzzle is how these factors focus their control (*14*). In that context, how could HOX11 contribute to T cell neoplasia? One thesis would hold that redirecting HOX11 to T cells activates an entire program of genes normally intended for another lineage. Alternatively, the role of HOX11 may be molecular mimicry in which it substitutes in a positive or negative fashion for a homeobox homolog expressed in T cells. The fact that homeobox proteins have been shown to dimerize (*14*) opens the possibility of heterodimer formation as well. The discovery of a deregulated homeobox gene, HOX11, in this T cell leukemia provides a model to assess these mechanisms.

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with 5′ anchor primers (AN primer plus ANpolyC at a 9:1 ratio) together with a specific 3′ primer (G, L, K, or R; see Fig. 1b). In contrast, the 3′ extended cDNA clone, APA-3, was obtained by using a specific 5′ primer, A, with a 3′ poly(T)primer (5′-GAGAGAGAGAGAGAGAGAACTAGTC-TCGAGT$_{(18)}$-3′). Thermal cycling was carried out for 36 cycles (95°C × 1 min, 60°C × 1 min, 72°C × 40 min for 1 cycle and 72°C × 5 min for 35 cycles) followed by a 15-min final extension at 72°C. DNA fragments >500 bp were size-selected by LMP agarose gel electrophoresis prior to second round PCR. PCR products were subcloned into Not I- and Sma I-digested plasmid. Recombinant plasmids were screened with probes of sequences found upstream to the specific PCR primers. An L and a random hexamer primer extension cDNA library constructed from ~1 μg of poly(A)$^+$ RNA from ALL-Sil yielded 1 × 10^5 recombinant plaques that were screened with a radioactively labeled J-K PCR-generated probe. The two longest inserts, PEL-1 and -2 were analyzed.

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Human Ultrasonic Speech Perception

MARTIN L. LENHARDT, RUTH SKELLETT, PETER WANG, ALEX M. CLARKE

Bone-conducted ultrasonic hearing has been found capable of supporting frequency discrimination and speech detection in normal, older hearing-impaired, and profoundly deaf human subjects. When speech signals were modulated into the ultrasonic range, listening to words resulted in the clear perception of the speech stimuli and not a sense of high-frequency vibration. These data suggest that ultrasonic bone conduction hearing has potential as an alternative communication channel in the rehabilitation of hearing disorders.

THE UPPER RANGE OF HUMAN AIR conduction hearing is believed to be no higher than about 24,000 Hz (*1*); nevertheless, there have been reports of humans hearing well into the ultrasonic range but only when the ultrasonic stimuli are delivered by bone conduction (*2–4*). Furthermore, ultrasonic bone conduction hearing in humans has been readily demonstrated in various conditions of auditory pathology, including sensorineural hearing loss and middle ear disorders (*2*). Ultrasonic stimulation of the skull with frequencies up to 108 kHz induces a perception of sound within the head without any sensation of cutaneous feeling (*2–4*), suggesting that these high frequencies are being processed in a modality other than the vibratory-somatosensory system.

In contrast to the excellent frequency discrimination found in the midsonic auditory range, ultrasonic perception has been described as having poor frequency-resolving ability (*3*). This interpretation originates from reports that the pitch elicited by ultrasonic stimulation is similar to the pitch of the highest air conduction frequency detectable, that is, 8 to 16 kHz (*4*). Because the perceived pitch of ultrasonic tones was associated with the upper limit of conventional air conduction hearing, these investigators assumed that the perceived audible sensation produced by ultrasonic stimuli consisted only of fixed pitch with monotonal quality. In addition, it was assumed that ultrasonic detection was a by-product of some form of cochlear processing that activated sensorineural elements within the classical auditory system (*4*) and, as such, offered little in the form of an additional communication channel.

We have tried to determine whether these early investigators might have overlooked the possibility that ultrasonic frequency discrimination exists and that ultrasonic hearing may, as a consequence, be capable of serving as a viable alternative communication channel, particularly for individuals with varying degrees of hearing loss. To explore this possibility, we obtained audiometric and ultrasonic thresholds (4,000 to 90,000 Hz) from ten normal, young adults (N_1) (19 to 26 years of age) (*5*). Standard audiometric testing revealed that these subjects had normal hearing (see Fig. 1A), and ultrasonic testing confirmed early reports that humans could perceive ultrasonic stimuli at least as high as 90,000 Hz (see Fig. 1B). Thresholds for tonal detection in the ultrasonic range ranged from 82 to 112 dB of acceleration depending on the stimulus frequency. When these same subjects were presented with a stimulus approximately 30 dB above threshold, they reported the tone as being loud and unpleasant.

In order to characterize the frequency discrimination, a pattern recognition task was then introduced. Audio rising or falling tones within the frequency limit of 2000 to 4000 Hz were randomly presented to these same subjects, who were instructed to identify only sweep direction. After obtaining an accuracy level of greater than 95%, ultrasonic frequency shifts were presented with the frequency limits of 25 to 32 kHz (*6*). These subjects displayed a mean 90% accuracy level (with a range of 80 to 100%) in detecting ultrasonic sweep direction, indicating that they were able to perceive changes in frequency. The inability of previous investigators to detect such changes within the ultrasonic range (*3, 4*) may be due to their use of a continuous tone or a small number of trials, or both, rather than the time-varying stimulus with extended practice used in the present study.

We next sought to determine the ability of humans to resolve frequency in the ultrasonic range, using a paradigm in which ten additional normal, young adult subjects (N_2) (23 to 25 years of age) were required to indicate just noticeable differences (JNDs) in pitch to tonal stimuli of 6, 11.2, 32, and 40 kHz (*7*). The subjects were able to discriminate changes in frequency, although there was a steady increase in the

M. L. Lenhardt, Department of Otolaryngology and Biomedical Engineering Program, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298, and Hearing Innovations Inc., 2451 E. Calle Los Altos, Tucson, AZ 85718.
R. Skellett, P. Wang, A. M. Clarke, Biomedical Engineering Program, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.