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Genetics

HEN1 and *HEN2*: A subgroup of basic helix–loop–helix genes that are coexpressed in a human neuroblastoma

(basic helix–loop–helix protein/transcription factor)

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ABSTRACT **An important family of regulatory molecules is made up of proteins that possess the DNA-binding and dimerization motif known as the basic helix–loop–helix (bHLH) domain. The bHLH family includes subgroups of closely related proteins that share common functional properties and overlapping patterns of expression (e.g., the MyoD1 and achaete-scute subgroups). In this report we describe *HEN1* and *HEN2*, mammalian genes that encode a distinct subgroup of bHLH proteins. The *HEN1* gene was identified on the basis of cross-hybridization with *TAL1*, a known bHLH gene implicated in T-cell acute lymphoblastic leukemia. *In situ* fluorescence hybridization was used to localize the human *HEN1* gene to chromosome band 1q22. *HEN1* and *HEN2* are coexpressed in the IMR-32 human neuroblastoma cell line, and they encode highly related proteins of 133 and 135 residues, respectively, that share 98% amino acid identity in their bHLH domains. These data imply that the bHLH protein subgroup encoded by *HEN1* and *HEN2* may serve important regulatory functions in the developing nervous system.**

The basic helix–loop–helix (bHLH) domain is found in a growing family of proteins, many of whose members have been implicated in the control of cell growth and differentiation (1). This motif includes a 50- to 60-amino acid sequence that has the potential to form two amphipathic α-helices separated by an intervening loop. The bHLH proteins also contain clusters of basic residues at the amino-terminal flank of the bHLH domain that potentially mediate sequence-specific DNA recognition. Several bHLH proteins are believed to serve as transcriptional regulatory factors based on their ability to bind the E-box motif (CANNTG) found in eukaryotic transcription enhancers (1–9). Recognition of the palindromic E-box consensus sequence is apparently mediated by bHLH protein dimers in the form of either a homodimer or a heterodimer composed of two different bHLH proteins (1, 2).

The bHLH proteins all have a moderate degree of sequence homology that is likely to be required for adoption of the domain's three-dimensional structure (1). Nevertheless, distinct subgroups of bHLH proteins can be defined that share exceptional amino acid homology within the bHLH domain. Moreover, the members of a particular subgroup generally exhibit overlapping patterns of expression and possess similar functional properties. For example, the myogenic subgroup includes four highly related bHLH proteins (MyoD1, myogenin, Myf-5, and MRF4/herculin/Myf-6), each of which is transcribed in a muscle-specific fashion and can mediate myogenic conversion of multipotential mesodermal cells (4, 5). Similarly, the achaete-scute complex encodes four homologous bHLH proteins (AS-C T3, T4, T5, and T8) that are specifically expressed in developing neural tissue and are required for neurogenesis in both the central and peripheral nervous systems of *Drosophila* (9–13). Certain subgroups of bHLH proteins can also be defined on the basis of a common pathogenic property. For example, the *TAL1*, *TAL2*, and *LYL1* genes are each altered as a consequence of tumor-specific chromosome abnormalities found in patients with T-cell acute lymphoblastic leukemia (T-ALL) (14–22). The proteins encoded by these genes display >80% amino acid identity in their bHLH domains and thus constitute a subgroup of bHLH proteins specifically implicated in human T-cell leukemogenesis (22). In this report we describe another subgroup of highly related bHLH proteins specified by the *HEN1* and *HEN2* genes[§]; these proteins are coexpressed in cells of neural derivation, and therefore they may serve a regulatory function during formation of mammalian nervous tissue.

MATERIALS AND METHODS

DNA Analysis and Cloning. DNA analysis and cloning were conducted by standard procedures (23). The λ phage library of human genomic DNA from the RPMI8402 cell line has been described (24). The libraries of mouse genomic DNA (NIH 3T3 cells) and human cDNA (fetal brain) were obtained from Stratagene. RNase protection assays were performed with the RPA kit (Ambion, Austin, TX).

***In Situ* Fluorescence Hybridization to Chromosomes.** Human metaphase cells were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. The *HEN1* probe (λANT9) is a 14.7-kilobase genomic DNA fragment cloned in the λ2001 phage vector. *In situ* fluorescence hybridization was performed as described (25). Biotin-labeled probes were prepared by nick-translation using Bio-11-dUTP (Enzo Diagnostics, New York). Hybridization was detected with fluorescein-conjugated avidin, and chromosomes were identified by staining with 4′,6-diamidino-2-phenylindole dihydrochloride (Sigma).

RESULTS

Identification and Chromosomal Localization of the *HEN1* Gene. The human *TAL1* gene encodes a bHLH protein that has been implicated in the development of T-ALL (15). In order to isolate the murine homolog of *TAL1*, a λ phage library of mouse genomic DNA was screened by low-

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Abbreviations: bHLH, basic helix–loop–helix; T-ALL, T-cell acute lymphoblastic leukemia.

[§]The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M97506 (mouse *Hen1*), M97507 (human *HEN1*), and M97508 (human *HEN2*)].

stringency hybridization with λcZ1, a human *TAL1* cDNA fragment (15). Each hybridizing phage clone was then examined by nucleotide sequence analysis for the presence of an open reading frame that encodes a potential bHLH domain. One of the clones identified in this manner (λM15; Fig. 1*a*) contains a 133-residue open reading frame with two potential translation initiators (methionine codons 1 and 8) and a 56-residue bHLH domain that shares 61% amino acid identity to that encoded by human *TAL1* (Fig. 1*b*). However, since the remainder of the reading frame is not homologous to *TAL1*, this locus is unlikely to be the murine equivalent of the human *TAL1* gene. Instead, it would appear to represent a distinct bHLH gene, designated *Hen1* upon the advice of the Nomenclature Committee of the Human Genome Mapping Workshops. To confirm this possibility, a λ phage library of human genomic DNA was screened by hybridization with a murine probe that includes the *Hen1* open reading frame (M15BB; Fig. 1*a*). Six hybridizing clones were isolated that share a common restriction pattern distinct from that of the human *TAL1* locus (λANT1, -2, -6, -7, -8, -9). Sequence analysis of one such clone (λANT6; Fig. 1*a*) revealed it to be the human equivalent of *Hen1*; hence, as illustrated in Fig. 1*b*, the size and character of the *Hen1* reading frame are strictly conserved in the human and mouse genomes (124 of 133 identical amino acids).

The distinction between *HEN1* and *TAL1* was reinforced by chromosomal mapping of the human *HEN1* locus. A genomic DNA fragment (ANT6XH; Fig. 1*a*) was used as a probe in Southern hybridization analysis with DNAs from a panel of 17 human–hamster somatic cell hybrids containing randomly segregated human chromosomes (26). The hybridization of ANT6XH was perfectly concordant with chromosome 1 and randomly associated (18–65% discordance) with every other human chromosome. To localize the *HEN1* gene

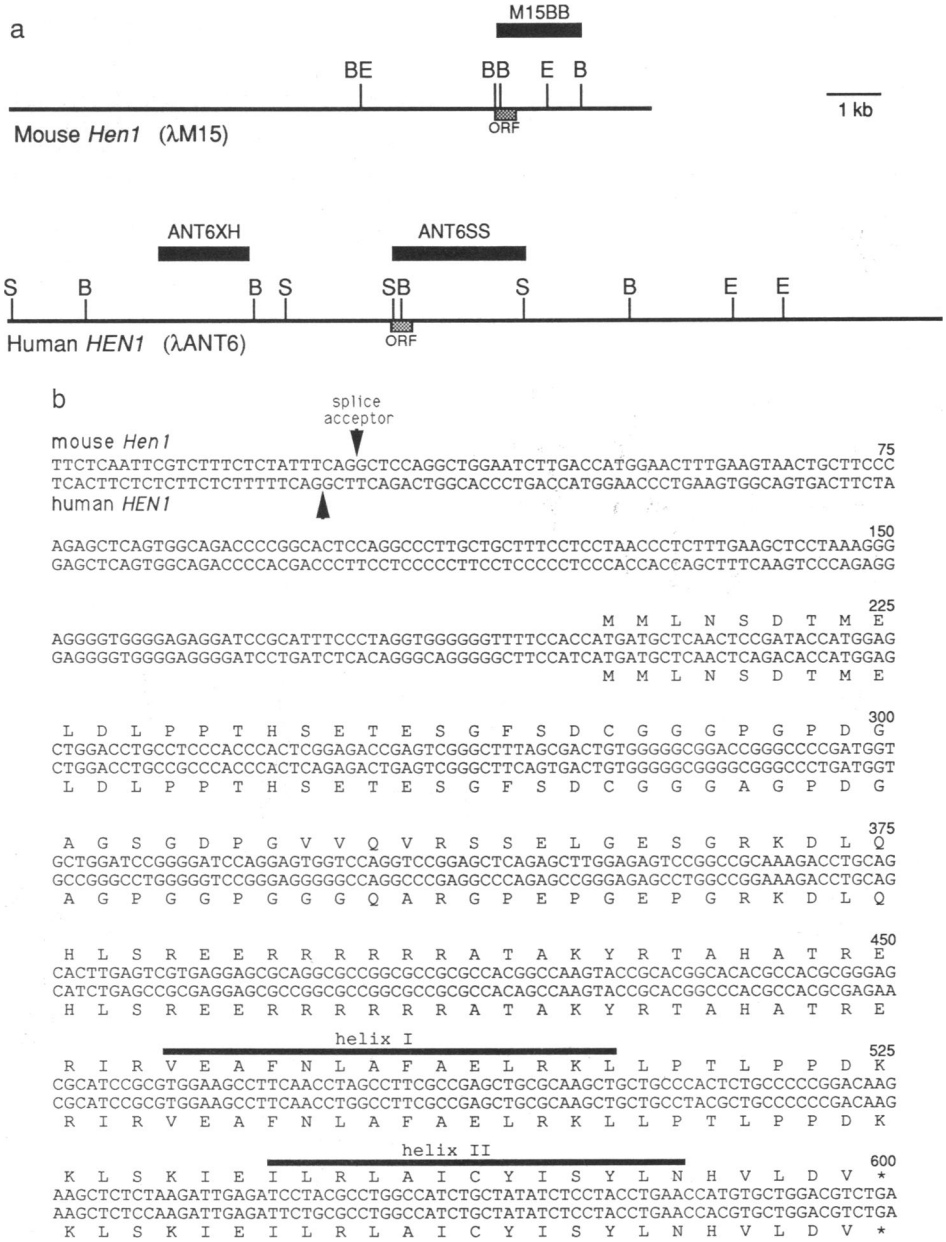


FIG. 1. Murine and human *HEN1* genes. (*a*) Restriction maps of recombinant clones representing murine (λM15) and human (λANT6) *HEN1* genomic DNA. The stippled box in each map corresponds to the *HEN1* open reading frame (ORF). *HEN1* genomic DNA probes are denoted by closed boxes above the maps (M15BB, ANT6XH, and ANT6SS). Restriction sites: B, *Bam*HI; E, *Eco*RI; S, *Sac* I. (*b*) The sequences of mouse and human genomic *HEN1* are compared. The open reading frame of 133 amino acids is preceded by a potential splice acceptor (vertical arrowhead) and terminates with an amber codon (asterisk). kb, Kilobases.

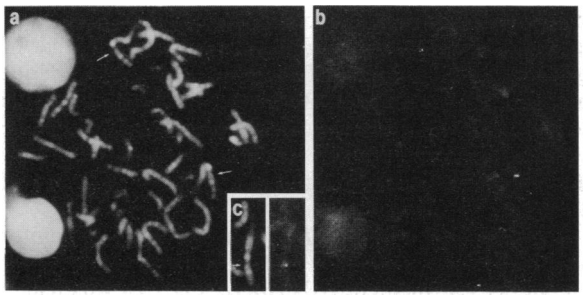
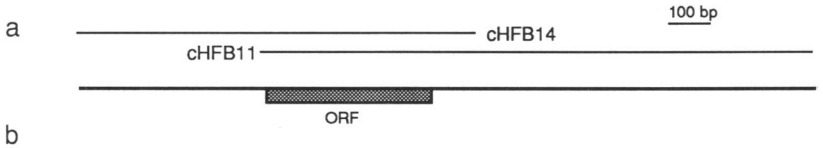


FIG. 2. (*a* and *b*) *In situ* hybridization of a biotin-labeled *HEN1* probe to human metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes. (*a*) Counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride. The chromosome 1 homologues are identified with arrows. (*b*) Detection of the probe with fluorescein isothiocyanate-conjugated avidin; specific labeling was observed at 1q22. (*c*) Partial karyotype of a chromosome 1 homologue illustrating specific labeling at 1q22 (arrow).

regionally on chromosome 1, we performed *in situ* fluorescence hybridization of biotin-labeled *HEN1* probes to normal human metaphase chromosomes. Hybridization of the λANT9 probe resulted in specific labeling of only chromosome 1 (Fig. 2). Specific labeling of band 1q22 was observed on one (two cells), two (six cells), three (nine cells), or all four (eight cells) chromatids of the chromosome 1 homologues in 25 cells examined. In some cells, the signal appeared to be at the junction of bands 1q21 and 1q22. Similar results were obtained in a second hybridization experiment using this probe. Thus, the *HEN1* gene is localized to the long arm of chromosome 1, band 1q22. In contrast, the *TAL1* gene is known to reside on the short arm of chromosome 1 within bands 1p32–34 (15–20).

***HEN1* and *HEN2*: A Discrete Subgroup of bHLH Genes.** In order to isolate human *HEN1* cDNA clones, a cDNA library of poly(A)-selected RNA from a fetal human brain was screened by hybridization with the *HEN1* genomic DNA probe ANT6SS (Fig. 1*a*). In addition to several *HEN1* clones, two overlapping clones with a unique restriction pattern were also obtained (Fig. 3*a*). Sequence analysis revealed that these clones potentially encode a bHLH protein that is distinct from but highly homologous to HEN1 (Fig. 3*b*). This protein (designated HEN2) is similar in length to HEN1 (133 vs. 135 amino acids) and includes a bHLH domain that is nearly identical to that encoded by *HEN1* (55 of 56 residues) (Fig. 4). The amino acid alignments in Fig. 4 show that HEN1 and HEN2 also share a highly charged sequence, including five tandem arginines, at the amino-terminal flank of the bHLH domain; this sequence is not found in TAL1 or the related bHLH proteins TAL2 and LYL1 (14–22). Although the *HEN1* gene was isolated on the basis of nucleic acid cross-hybridization with *TAL1*, it is clear that HEN1 and HEN2 constitute a discrete subgroup of bHLH proteins that is distinct from the leukemogenic subgroup comprised of TAL1, LYL1, and TAL2. The three members of the LYL/TAL subgroup share at least 80% amino acid identity within their 56-residue bHLH domains (22), while the bHLH motifs



b

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CCCGCCCCCGTGAAAACCCAGATGTATTCGTTGTTCGAAATCGCTCGTTTGTCCGGGAAGGGGACGAAGGGAGGG   75
AGGAGGAATAGCGCGGTTGGCATTTGGCCGGAAGAGGCACAAAAACCCAGTCGCGACCCCACAGGCCTGCGGTTG
TTCCCCAGGCCCAGGGCCACAGGCCCGCTGTGGCCGTCTGCAAGTCGAGGTGTCCAGTCTAGTATGCGGCCTGGG  225
TCAACCGCGTCCCCGCCGGCTCCCGCGCCAGGGCGAGGCGCAGGGCTCGCGCCCTCGGGCCAGATCCTATCGGCG
CCCTCCCGCGTCGCGCGCCGGCCTGCTGTGGGGAGGGGGCCCGGGCCGCGCGTCCGCTGGGAGGACCGGCGTGGT  375
AGGCCTCGGCAGCCATCCTTCCAGCAAAATACCCTGATTCCTTTTACTGATTAATTTTCCGACCCCTCCTCAGCC

      M  M  L  S  P  D  Q  A  A  D  S  D  H  P  S  S  A  H  S  D  P  E  S
TCCAAAATGATGCTGAGTCCGGACCAAGCAGCAGATTCGGACCATCCCAGCTCGGCGCACTCGGATCCGGAGTCC  525

 L  G  G  T  D  T  K  V  L  G  S  V  S  D  L  E  P  V  E  E  A  E  G  D  G
CTGGGCGGCACGGACACCAAGGTGCTCGGCAGCGTGTCGGACCTGGAGCCGGTGGAGGAGGCCGAGGGCGACGGC  600

 K  G  G  S  R  A  A  L  Y  P  H  P  Q  Q  L  S  R  E  E  K  R  R  R  R  R
AAGGGCGGCAGCCGAGCCGCGCTCTACCCGCACCCGCAGCAGCTGAGCCGCGAGGAGAAGCGCCGCCGCCGGCGC  675

 A  T  A  K  Y  R  S  A  H  A  T  R  E  R  I  R  V  E  A  F  N  L  A  F  A
GCCACGGCCAAGTACCGCTCGGCCCACGCCACCCGCGAGCGCATCCGCGTGGAAGCCTTCAACTTGGCCTTCGCC  750

 E  L  R  K  L  L  P  T  L  P  P  D  K  K  L  S  K  I  E  I  L  R  L  A  I
GAGCTCCGCAAATTGCTGCCCACGCTGCCCCCGGACAAGAAGCTCTCCAAGATCGAGATCCTGCGCCTGGCCATC  825

 C  Y  I  S  Y  L  N  H  V  L  D  V  *
TGCTACATCTCCTATCTCAACCACGTCCTGGACGTGTAGGGCGGGGTGCGCCGCGGGGGGCCGCCTGTCCCGGCG  900
TCCGCGAAACGCTACGGATCCGGACGGGCGCCGTCGCTCGGGTGGCGGAGGCTGCGGTGGCCCAGCGAGACGGCC
GCCTCGAGAGACGGCCGGGGAAGAAGCTCGCTACCTGTTCTCTCTGGTCAGCAGGGCGACGAGGGAAGGTTTCCG 1050
ACGCAGGCAGGTGGGGTTGTCTGGCGAGCCGGGGGGCTCGCAGGGGGGATTTTCCAGGCCAGGAGAACCTCGTGG
AGACACCCGAGATGAGCCACTTCTGGGGCTCCCCAAGGGTTGTGGTTTTAACCCACTGGAGACTTTGAGTTCTCC 1200
TACATTCATCCGCCACAAATCTTAATGTGTTGAAAGAATGAGGGCAGAAGTTCAGAGTATGCAGGCTCATAACTT
TAAGCTATCTGGAATTTTTCTAATGGACAAATCACCAAACACTGGGAAGGAGGAGAGAGGAGGGATCTCTCCCAT 1350
AGAGATGGAAGGCACAGAAAGGTGCGACTGGAAACTTTCCTTTCTGTTGTGAAAATAAAGATTATATTAGAATCC
AAATAAACCCTGAATATTTAGTAAGTTTAAAAAGACAAATTGAGAACAAGTAGACACACTGTGGGAGGATCTGAG 1500
CTTGGTGATATTTTTATCCCCAAGATATGACACCAAGGTAGACAGGAAAATCCATATTTAAATGGAAACTTTAAT
ATCTGATTACTTTTGCAGGCGAAGTGCCCCTTTATATATCCAAAAACATCTATTTGTGACCTTAAACATGTGGAC 1650
CCATAGGTGCAGTTAGAAAAAGACAACCTATTTTTATTTATGTTAGAAGGAGTAGAGTATTTTTTTCAAGACATT
TATTTTTCAGAGTGGTGATACTTTTACTTTGGATACTCTGTGCCAATTTATTTATAGTCAAGTGTTTACACTTTT 1800
TCCTGTGGAATAATTATGTCTAACTTTTTACGTGTTTGTTGAGATTATACTGTGGTCTTTCTTTCTGCTCTAATT
ATATTGCACTTGTATAACAAATTTCCCACTTCTCCCTGTTTCTAAACATATTTTATATATTAAGATGTTTGTTCT 1950
TGAAAGGTTCTTTTTGTTGTGAGATCAGCAACACTAGCACTTCACTATTATAGTTTTTTAAAAAGTGTATTATTCT
TATCTGTAGTTATGTCTGAGAAGTCCTTTACAAGCTGTTTATAAGGAGAGTAGCTTCTTTGTGTGTGTGATGTTT 2100
GTGCGTGTGATGAATTTAGCAAATTAAGTTATTTTCCTAAAAAAAAAAAAAAAAAA                    2138
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FIG. 3. Nucleotide sequence of human *HEN2* cDNAs. (*a*) The *HEN2* cDNA clones cHFB11 and cHFB14 are aligned, and the position of the open reading frame (ORF) is indicated. (*b*) The composite nucleotide sequence of the *HEN2* clones cHFB11 and cHFB14 is illustrated. The *HEN2* reading frame of 135 amino acids is preceded by an in-frame ocher stop codon (underlined) and terminates with an amber codon (asterisk). bp, Base pairs.

a

```
                    basic          helix I                    helix II
HEN1  MMLNSDTMELDLPPT  HSETESGFSDCGGGAGPDGAGPGGPGGGQARGPEPGEPGRKDLQHLSREER 66
      +++  +       +   ++  ++         +                              + +++++
HEN2  MMLSPDQAADSDHPSSAHSDPESLGGTDTKVLGSVSDLEPVEEAEGDGKGGSRAALYPHPQQLSREEK 68

                    basic          helix I                    helix II
HEN1  RRRRRATAKYRTAHATRERIRVEAFNLAFAELRKLLPTLPPDKKLSKIEILRLAICYISYLNHVLDV 133
      ++++++++++ ++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
HEN2  RRRRRATAKYRSAHATRERIRVEAFNLAFAELRKLLPTLPPDKKLSKIEILRLAICYISYLNHVLDV 135
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b

```
                    basic          helix I                    helix II
HEN1  RRRRRATAKYRTAHATRERIRVEAFNLAFAELRKLLPTLPPDKKLSKIEILRLAICYISYLNHVLDV
HEN2  ----------S-----------------------------------------------------------
TAL1  TDGPHTKVVR-IFTNS---W-QQNV-G--------I--H--------N------MK--NF-AKL-ND
TAL2        MTRKIFTN----W-QQNV-S---K----I--H--------N-T----MR--NF-VKV-GE
LYL1  EGHQPQKVARRVFTNS---W-QQNV-G----------H---R----N-V----MK--GF-VRL-RD
c-MYC -SSDTEENVK-RT-NVK--Q-RNEEKRS-FA--DQI-E-ENNE-AP-VV--KK-TA--LSVQAEEQK
MyoD1 CK-KTTN-DR-K-ATN---R-LSKV-E--ET-KRCTSSN- NQR-P-V----N--R--EG-QAL-RD
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FIG. 4. Human HEN1 and HEN2 proteins. (*a*) Amino acid alignments of HEN1 and HEN2; residues that comprise the basic region and amphipathic α-helices of the bHLH motif are indicated. Identical residues are indicated by a plus sign between the sequences. (*b*) The bHLH motifs of HEN1 and HEN2 are compared with those of other bHLH proteins, including TAL1, TAL2, LYL1, c-MYC, and MyoD1. A dash indicates amino acid identity with the sequence of HEN1. The first amino acid residue of the TAL2 bHLH sequence corresponds to the initiator methionine (Y. Xia and R.B., unpublished results).

of HEN1 and HEN2 are about 98% identical to each other. In contrast, each of the LYL/TAL proteins has no more than 61% amino acid identity with the bHLH domains of either HEN1 or HEN2.

Restricted Coexpression of *HEN1* and *HEN2* in Neuroblastoma Cells. A panel of 24 human cell lines representing a spectrum of different tissue types was examined for RNA expression of *HEN1* and *HEN2*. Of these, only the neuroblastoma line IMR-32 had detectable levels of *HEN1* mRNA (27). Surprisingly, IMR-32 was also the only cell line in our panel to express *HEN2* gene transcripts. Transcription of either *HEN1* or *HEN2* was not found in the other neuroblastoma lines tested (SK-N-MC and SK-N-SH). As illustrated in Fig. 5*a*, IMR-32 cells harbor polyadenylylated transcripts of 3.0 and 2.9 kilobases that can be detected by Northern hybridization with *HEN1* and *HEN2* probes, respectively. Because of the sequence homology shared by these genes, it was necessary to ensure that the apparent coexpression of *HEN1* and *HEN2* in IMR-32 cells was not an artifact due to cross-hybridization. Therefore, ribonuclease protection assays were performed using radiolabeled antisense RNA probes derived from *HEN1* and *HEN2* sequences that are highly divergent. As shown in Fig. 5*b*, a 211-nucleotide segment of the radiolabeled *HEN1* probe was protected from ribonuclease digestion by hybridization with IMR-32 RNA; the protection was clearly mediated by *HEN1* gene transcripts since this probe shares limited nucleotide sequence homology with *HEN2* (69 of 211 identical residues). Similarly, a 233-nucleotide segment of the radiolabeled *HEN2* probe was protected by hybridization with IMR-32 RNA (Fig. 5*b*); in this case, ribonuclease protection was *HEN2*-specific because the probe shares only moderate sequence homology with *HEN1* (129 of 233 identical residues). Hence, the *HEN1* and *HEN2* genes are coexpressed in IMR-32 cells in a highly restricted, tissue-specific fashion.

DISCUSSION

The data described above suggest that HEN1 and HEN2 constitute a distinct subgroup of tissue-specific bHLH proteins. Clusters of basic amino acids are apparent at the amino-terminal flanks of the HEN1 and HEN2 helix–loop–helix domains (Fig. 4); hence, unlike the negative-regulatory helix–loop–helix proteins such as Id (5), HEN1 and HEN2 may potentially serve as DNA-binding proteins. Although murine *Hen1* genomic DNA (λM15; Fig. 1*a*) was identified on the basis of nucleic acid cross-hybridization with human *TAL1*, HEN1 and HEN2 appear to be functionally unrelated to the leukemogenic subgroup of bHLH proteins composed of TAL1, TAL2, and LYL1. The cross-hybridization between *Hen1* and *TAL1* is reflected by a modest degree of amino acid homology between the bHLH domains encoded by these genes (Fig. 4). Nevertheless, the HEN1/HEN2 proteins and the leukemogenic proteins display qualitatively greater levels of bHLH identity within their subgroups (>98% and 80%, respectively) than between the subgroups (<61%). Although the *TAL1*, *TAL2*, and *LYL1* genes are each

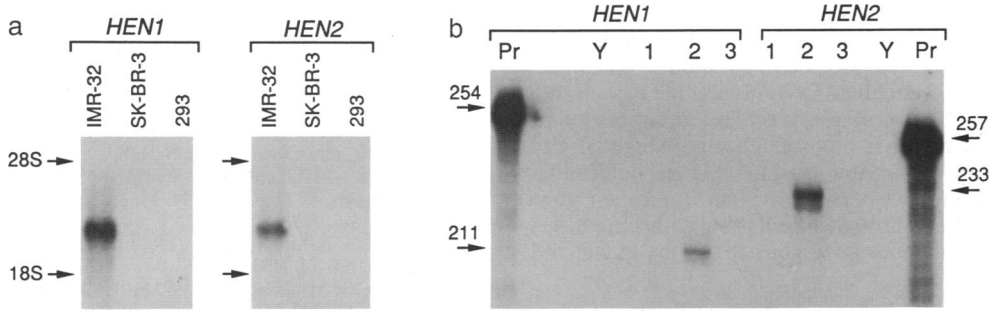

FIG. 5. Transcription of the human *HEN1* and *HEN2* genes. (*a*) Northern hybridization of human *HEN1* (ANT6SS) and *HEN2* (cHFB4) probes with 2-μg aliquots of poly(A)-selected RNA from the IMR-32, SK-BR-3, and 293 cell lines. (*b*) ^{32}P-labeled RNA probes were generated by *in vitro* transcription of human *HEN1* or *HEN2* genomic DNA. The radiolabeled probes were annealed with 10 μg of either yeast tRNA (lanes Y) or total RNA from the SK-N-MC (lanes 1), IMR-32 (lanes 2), or SK-N-SH (lanes 3) cell lines. After RNase treatment and denaturing polyacrylamide gel electrophoresis, radiolabeled RNA fragments were detected by autoradiography. Aliquots of untreated RNA probe were also fractionated (lanes Pr). The human *HEN1* probe includes 211 antisense nucleotides (residues 165–375, Fig. 1*B*), and the *HEN2* probe includes 233 antisense nucleotides (residues 751–983, Fig. 3*B*).

involved in chromosomal rearrangements in patients with T-ALL (14–22), Southern hybridization analysis of 60 T-ALL specimens has failed to uncover tumor-specific alterations of either the *HEN1* or *HEN2* loci (L.B. and R.B., unpublished data). *In situ* fluorescent hybridization revealed that the human *HEN1* gene is located at chromosome 1, band 1q22 (Fig. 2). The murine equivalent of *HEN1* (termed *NSCL*) was recently identified and mapped to a region of the mouse genome that is syntenic with human chromosome bands 1q12–23 (28). Although the cytogenetic breakpoints of several tumor-specific chromosome abnormalities lie near or within band 1q22, none of these include the chromosome defects known to recur in patients with T-ALL (29–31). Hence, there is no evidence to suggest that *HEN1* or *HEN2* contributes to T-ALL formation in a manner akin to the *TAL1*, *TAL2*, and *LYL1* genes.

Some bHLH proteins are expressed in a tissue-specific manner while others are ubiquitously expressed in a broad spectrum of tissues and cell types (2). In our survey of 24 established cell lines, transcription of the human *HEN1* and *HEN2* genes was restricted to a single neuroblastoma line, IMR-32 (Fig. 5). A neural pattern of expression was also recently reported for the murine homologue of *HEN1* (28). Several tissue-specific bHLH proteins have been shown to regulate critical decisions regarding cell-type determination within the cellular lineage in which they are expressed. For example, each of the myogenic bHLH proteins (MyoD1, myogenin, Myf-5, and MRF4/herculin/Myf-6) can induce myoblast conversion of multipotential fibroblasts (4, 5). Likewise, genetic studies of *Drosophila* indicate that the achaete-scute bHLH proteins control neuroblast formation from primordial neuroectodermal tissue (9–13). In view of the regulatory roles played by the myogenic and achaete-scute subgroups of bHLH proteins, it seems reasonable to propose that HEN1 and HEN2 are also involved in the control of cell-type determination, possibly within the developing nervous system. Similar functions have been suggested for *MASH1* and *MASH2*, two bHLH genes that encode mammalian homologues of the achaete-scute proteins (32).

It has been proposed that myogenic bHLH proteins, such as MyoD1, direct cell-type determination by activating expression of a repertoire of subordinate muscle-specific genes (4–6). The myogenic bHLH proteins presumably activate muscle-specific transcription of subordinate genes by binding E-box elements (CANNTG) within their cis-acting regulatory sequences. Likewise, the achaete-scute proteins are believed to control expression of subordinate genes required for neuroblast conversion by direct recognition of their associated E-box sequences (9). *In vitro* studies show that the myogenic and achaete-scute proteins [designated class B bHLH proteins by Murre *et al.* (2)] do not effectively recognize the E-box motif, apparently due to inefficient bHLH homodimerization (2). However, in the presence of certain ubiquitously expressed proteins (class A bHLH proteins) such as the *E2A*-encoded E47 polypeptide, the tissue-specific class B bHLH proteins form heterodimers (e.g., MyoD1/E47) that bind the E-box sequence with high affinity (2). If *HEN1* and *HEN2* are involved in cell-type determination within the developing nervous system, then their gene products may function through interaction with ubiquitous bHLH proteins in a manner analogous to the myogenic and achaete-scute proteins.

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