Immunogenetics (1997) 46: 509–515

ORIGINAL PAPER

Michael Schorpp · Michael Hofmann · T. Neil Dear
Thomas Boehm

Characterization of mouse and human nude genes

Received: 1 May 1997

Abstract The differentiation of primitive epithelial precursor cells in the thymic primordium into subcapsular, cortical, and medullary epithelial cells of the mature thymus requires the activity of the nude gene product Whn. Whn is also required for proper keratinization of the hair shaft. We determined the nucleotide sequence of a 58 kilobase region on mouse chromosome 11 that encompasses the mouse nude gene and part of the two neighboring genes, encoding a sodium/dicarboxylate co-transporter and the retinal protein 4. Using cross-hybridization, the human orthologue of the mouse nude gene was isolated. The human WHN protein also consists of 648 amino acids, 85% of which are identical to the mouse protein. Like the mouse gene, the human gene consists of eight coding exons and utilizes two alternative first exons in a tissue-specific fashion. Sequences upstream of the two alternative first exons display promoter activity in heterologous reporter assays. Whereas both promoters appear to be active in skin (albeit at different levels), only the most upstream element is active in the thymus, indicating that transcriptional activity of the *whn* gene is subject to complex regulation. Nucleotide sequence database comparisons reveal that among other winged-helix genes, the *HTLF* and *HTLFL1* genes are most closely related to *whn*, although the exon/intron structure of the human *HTLF* gene in the DNA binding domain differs from that of *whn*.

Introduction

Loss-of-function mutations in the mouse *w*inged-*h*elix-*n*ude (*whn*) gene, which encodes a forkhead/winged helix transcription factor (Nehls et al. 1994), are associated with the nude phenotype of hairlessness and congenital athymia (Pantelouris 1968). The *whn* gene was recently isolated by positional cloning (Nehls et al. 1994; Segré et al. 1995) and was verified as the nude gene by the identification of deleterious mutations on the original nude alleles in mouse (Nehls et al. 1994) and rat (Nehls et al. 1994; Segré et al. 1995; Schüddekopf et al. 1996; Huth et al. 1997), and by use of a targeted disruption of *whn* (Nehls et al. 1996). Our recent studies suggest that *whn* is responsible for the initiation and maintenance of the differentiated phenotype of thymic epithelial cells (Nehls et al. 1996). Reversal of the skin abnormalities but not of the thymic dysplasia in *nu/nu* mice transgenic for a cosmid spanning the *whn* gene has suggested that the transcriptional regulation of the *whn* gene is complex (Kurooka et al. 1996). We therefore undertook an extensive analysis of the mouse nude locus to clarify the *whn* gene structure and to identify the genes flanking it on either side.

Two groups of human diseases are associated with aberrations in the epithelial compartment of the thymus. First, thymomas are believed to arise from malignant transformation of epithelial rather than lymphoid cells in the thymus (Debono et al. 1996; Hammond et al. 1991). Second, thymic dysplasia, a heterogeneous group of diseases including Nezelof syndrome (Nezelof 1992), is a rare but recognized cause of immunodeficiency (Brooks et al. 1994). As *whn* is a candidate gene for these diseases, we also isolated and characterized the human homologue of the rodent nude gene.

The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank nucleotide sequence databases and have been assigned the accession numbers Y11739-11746, and Y12488

M. Schorpp* · M. Hofmann* · T. N. Dear · T. Boehm (✉)
Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280,
D-69120 Heidelberg, Germany

* Both contributed equally to this work and should be considered joint first authors.

Materials and methods

Tissues and cell lines

Mouse skin and thymus tissues were taken from Balb/c mice. Human thymus samples were kindly provided by B. O. Boehm, University of Ulm, Germany. The mouse thymic epithelial cell line 427.1.86-B5 (here referred to as 427) was kindly provided by B. Knowles, The Jackson Laboratory (Faas et al. 1993).

DNA and RNA biochemistry

Standard procedures for DNA and RNA purification, genomic cloning, cDNA cloning, library screening, RT-PCR, determination of promoter activity, and DNA sequence determination have been described earlier (Schlake et al. 1997).

Chromosomal localization of the human WHN gene

Chromosomal localization of the human *WHN* gene was obtained by polymerase chain reaction (PCR) analysis of genomic DNAs from hybrid mapping panels. Primer sequences were (5′ to 3′): HS39: TGCTCTCGGTCACTCCTGAG; HS40: TACCTGTCAAACAA-GAGCCAAG, which amplify an approximately 350 base pair (bp) fragment of the *WHN* gene from human, but not mouse or hamster DNA. PCRs were performed in 20 µl reactions containing 50 mM KCl, 10 mM Tris-HCl pH 9, 0.1% Triton X-100, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1 unit *Taq* polymerase, 25 ng genomic DNA, and 0.4 µM primer each with a thermocycling protocol of 94 °C - 15 s, 58 °C - 30 s, 72 °C - 1 min for 35 cycles. Initially, the Genebridge 4 RH panel (Walter et al. 1994) was used but despite seemingly unequivocal results we were unable to obtain a localization. Consequently, the chromosome localization was determined using the NIGMS human/rodent somatic cell hybrid mapping panel (Coriell Cell Repositories, Camden, NJ). The localization was subsequently refined using the Stanford G3 panel (Schuler et al. 1996) obtained from Research Genetics (Huntsville, AL). Results were analysed using the database at http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl.

Similarity search

Derived protein sequences of *whn* and *HTLF* genes were used to search for similarity against translations of the EST database at the National Center for Biotechnology Information. One previously undescribed gene, designated *HTLFL1* (for HTLF-like 1), was discovered both in human (ESTs with accession numbers AA259017, H23770, W87352, AA017388, AA017394, AA017395) and mouse (ESTs with accession numbers AA016382, AA036484) cDNA sequences. The derived protein sequences in the presumed DNA binding domain are identical for human and mouse.

Results

Identification of genes flanking the mouse whn gene

Following preliminary analysis using P1 recombinants (Nehls et al. 1994), a detailed restriction map of the mouse *whn* gene and flanking regions was established (Fig. 1A). This analysis was based on the construction of a minimal tiling path of overlapping λGET clones derived from partial *Sau* 3AI digests of P1 clones spanning the *whn* gene (Nehls et al. 1995). The same set of clones was used for nucleotide sequence determination (accession number Y12488). A region of about 58 kilobases (kb) was covered in this analysis, spanning the entire *whn* gene. The sequence information was subsequently used to locate the previously described STS markers *D11Bhm52* and *D11Bhm53*, and a number of exon fragments isolated using the λGET system (Nehls et al. 1995, and unpublished data). This led to the unequivocal identification of the genes flanking the *whn* locus. Upstream of *whn*, a sodium/dicarboxylate co-transporter gene was identified by comparison with human (accession number U26209), rat (accession number U51153), and rabbit (Pajor 1995) cDNAs. Downstream of *whn*, the retinal gene 4 was detected by comparison with human and rat cDNA clones (Higashide 1996). Whereas the sodium/dicarboxylate co-transporter is transcribed from the same strand as *whn*, the retinal gene 4 has an opposite transcriptional polarity. Because the orientation of the *whn* gene relative to the chromosome is known (Nehls et al. 1995), that of sodium/dicarboxylate-transporter and retinal gene 4 could also be inferred (Fig. 1A).

The *whn* gene itself spans about 30 kb, with comparatively large introns separating the two alternative first exons 1a and 1b (see below) from the first coding exon 2. The initiation methionine occurs in exon 2. Eight coding exons give rise to a predicted protein of 648 amino acids; the translational stop codon occurs in exon 9 and is followed by about 1.1 kb of untranslated sequence preceding the polyadenylation signal sequence. The intergenic distance between the sodium/dicarboxylate co-transporter gene and *whn* is about 10 kb, that between *whn* and retinal gene 4 is about 8 kb.

Identification of a human WHN homologue

A cDNA library prepared from mRNA of human infant thymus was screened under low-stringency conditions to obtain cDNAs of the human *WHN* homologue. A comparison between the derived amino acid sequence of the presumptive human *WHN* gene (accession number for cDNA Y11739) and the mouse and rat *whn* genes revealed 85% sequence identity among all three species (Fig. 2). In particular, in the DNA binding domain (Schlake et al. 1997), 90 of 92 amino acids, and in the transcriptional activation domain (Schüddekopf et al. 1996), 54 of 55 amino acids, are identical in all three species.

Subsequently, the corresponding genomic locus was obtained from a human P1 library. Sequence comparisons of genomic and cDNA clones established the exon/intron structure of the human *WHN* gene and revealed complete concordance with the rodent *whn* genes (Fig. 1B); intron sizes differed slightly between mouse and human *whn* genes. Like the mouse *whn* gene, the human *WHN* gene comprises 8 coding exons (accession numbers Y11741 to Y11746) and also contains the two alternative first exons (accession number Y11740). The DNA binding domain of about 100 amino acids is encoded by three exons (exons 5, 6 and 7), the transcriptional activation domain of about 50 amino acids is encoded by 2 exons (exons 8 and 9). Several sequence polymorphisms were detected betwen the cDNA and the genomic sequences, two of which lead to amino acid changes, residue 283 (Val > Ala in exon 6) and residue 599 (Ala > Pro in exon 9). It is of interest that at these two positions the amino acids coded by the genomic sequence are identical to those in the mouse and rat protein.

Although these data strongly suggested that the human equivalent of the mouse nude gene was cloned, it was important to verify this conclusion by analysis of the chromosomal context of the human *WHN* gene. In a first experiment, the chromosomal localization of the *WHN* gene

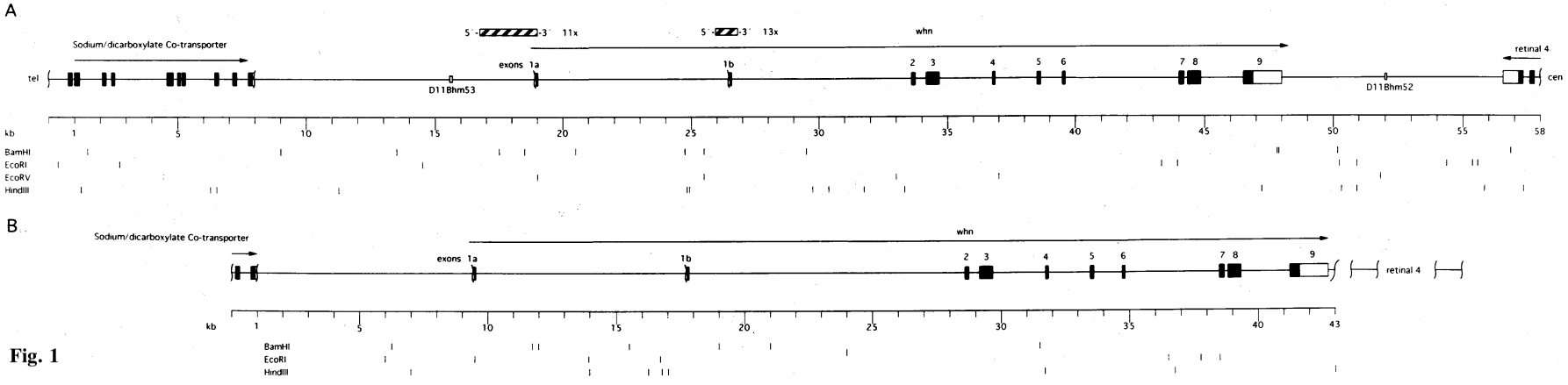

Fig. 1

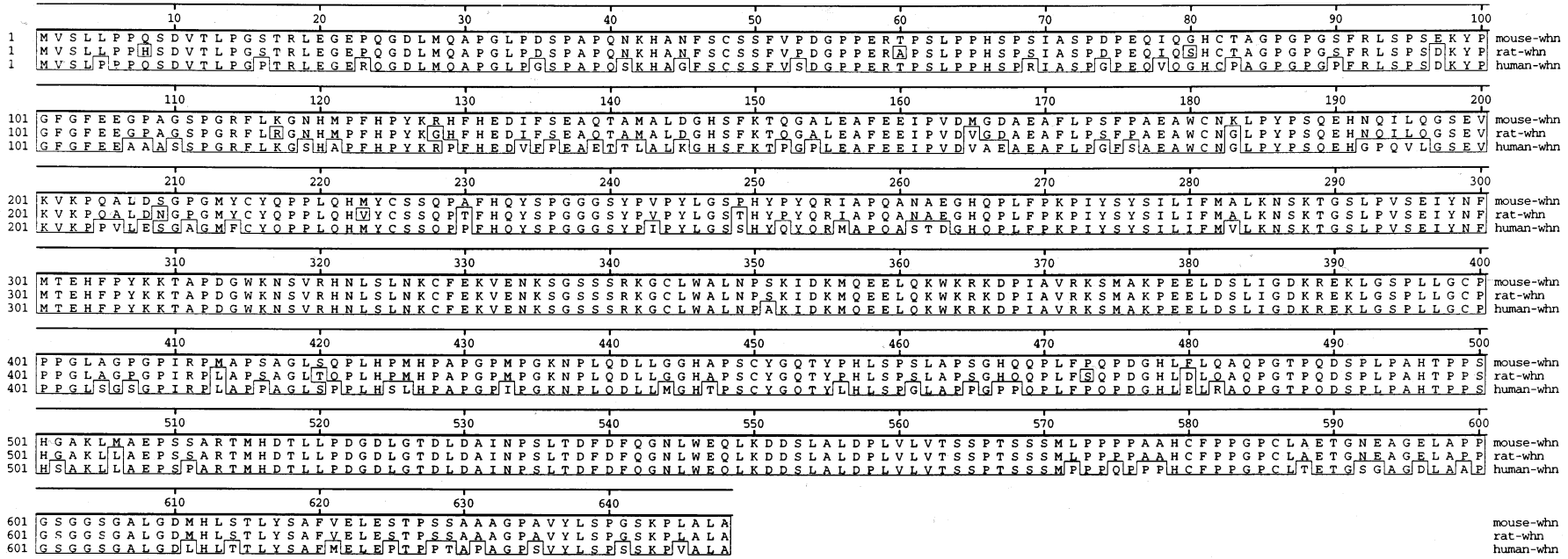

Fig. 2

Fig. 1A, B Structure of mouse and human *whn* genes and flanking genomic regions. **A** Restriction map of the mouse *whn* locus (for nucleotide sequence and exact location of exons, restriction sites, etc., see sequence and annotations in accession number Y12488). The location and orientation of fragments used for determination promotor activity is indicated with the fold stimulation of activity of reporter gene product. The transcriptional orientation of all three genes is indicated by the *arrow*; the telomere of chromosome 11 is to the *left*. **B** Structure of the human *WHN* locus. The orientation and distance to *WHN* of the retinal gene 4 in human DNA has not between determined

Fig. 2 Amino acid sequence comparisons of human, mouse, and rat *whn* genes. The DNA binding domain comprises residues 271 to 362, the transcriptional activation domain comprises residues 509 to 563. Note that sequence polymorphisms between the human *whn* cDNA and the human *WHN* gene contained on genomic P1 clones lead to amino acid changes at positions 283 (V>A) and 599 (A>P)

was determined. A mono-chromosomal somatic cell hybrid panel was used to ascertain that the human *WHN* gene was located on chromosome 17, which shares syntenic regions with mouse chromosome 11. More detailed mapping using the Stanford Human Genome Center radiation hybrid panel suggests that the human *WHN* gene maps to 17q11-12 (data not shown). The human *WHN* gene, like the mouse *whn* gene (Nehls et al. 1995), is therefore closely linked to the neurofibromatosis-1 gene (located on human chromosome 17q11). In a second set of experiments, the extent of short-range synteny was evaluated. Hybridization analyses using P1 recombinant clones were initially used to verify the presence of human equivalents of the sodium/dicarboxylate co-transporter gene and the retinal gene 4, respectively, in the vicinity of the human *WHN* gene. Long-range PCR experiments using primers derived from the 3′ untranslated region of the human sodium/dicarboxylate co-transporter gene and exon 1a of *WHN* and nucleotide sequence analysis showed that the human homologue of the sodium/dicarboxylate co-transporter gene is located about 7.5 kb upstream of human exon 1a in the same orientation as the mouse gene relative to *WHN*. The exact distance and orientation of the human retinal gene 4 to *WHN* was not determined, although partial sequencing confirmed the presence of the human retinal gene 4 homologue on the P1 clones also containing the sodium/dicarboxylate co-transporter and *WHN* genes (data not shown). This strongly suggests that the overall genomic context of the human *WHN* gene is identical to that of the mouse *whn* gene.

Identification of alternative first exons of the whn gene

Mouse *whn* cDNA clones generated from skin mRNA contained two different 5′ ends, suggesting the presence of alternative first exons in the *whn* gene (data not shown). Restriction mapping revealed that these two exons (designated exons 1a and 1b, respectively) are situated about 15 kb and about 7 kb, respectively, upstream of *whn* exon 2, which represents the first coding exon of the *whn* gene. In the genomic sequence, exons 1a and 1b are both demarcated at their 3′ end by a *bona fide* splice donor site. The longest cDNAs emanating from exon 1a contain 73 nucleotides from this exon (nt 19025 to 19097 in accession number Y12488); the longest cDNAs from exon 1b contain 26 nucleotides from this exon (nt 26583 to 26608 in accession number Y12488).

As the longest available human *WHN* cDNA clone from the thymus library terminated in exon 2 upstream of the ATG initiation codon, it was important to determine whether the human genome also contained the two alternative first exons identified in the mouse *whn* gene. Indeed, low-stringency hybridization analysis revealed similarities to mouse exons 1a and 1b in human DNA that were subsequently confirmed by sequencing (Fig. 1B).

The tissue-specificity of alternative first-exon usage was determined by reverse transcriptase RT-PCR using primers from exons 1a and 1b, and exon 2, respectively. Using mRNA isolated from mouse thymus and skin, 1a-2 transcripts were observed in both tissues, whereas 1b-2 transcripts were detected in skin only. In addition, only 1a-2 transcripts were detected in the mRNA isolated from the thymic epithelial cell line 427 (Faas et al. 1993). For the human gene, only thymus mRNA was analyzed; in keeping with the results from the mouse, only cDNAs of 1a-2 type were detected (Fig. 3A).

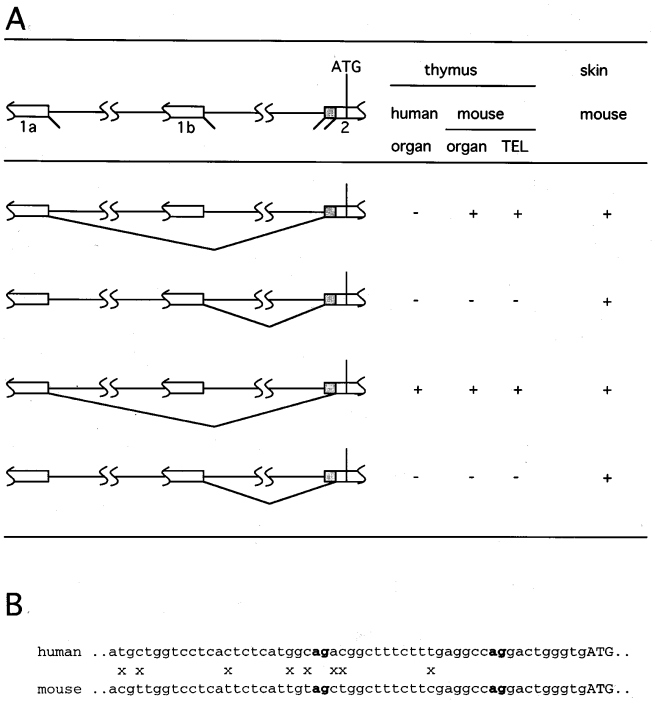

Fig. 3A, B Alternative splicing patterns involving the 5′ exons of the human and mouse *whn* genes. **A** The *top panel* gives a schematic of the *whn* partial gene structure, with exons indicated as *boxes*, and introns as *lines*. The splice donor and acceptor sites are indicated as is the location of the initiation codon (ATG). The four cDNA forms detected for *whn* are displayed *underneath*, and their presence (+) or absence (–) in cDNAs prepared from mRNA extracted from the different sources indicated in the *right-hand* columns. *TEL*, thymic epithelial cell line 427. **B** Partial nucleotide sequences of human and mouse *whn* genes at the 5′ end of exons 2. The experimentally verified splice acceptor sites are indicated (ag in *boldface*). Sequence mismatches are *highlighted* by x. The initiation codon (ATG) is in *uppercase*

In addition to alternative first exons, a second type of splice variation was observed at the splice acceptor junction of exon 2 in the mouse (Fig. 3A). Transcripts were observed in which the splice acceptor junction of exon 2 occurred after either of two AG dinucleotides located upstream of the ATG initiation codon (Fig. 3A); the upstream site (Fig. 3B) conforms better to an extended splice acceptor consensus sequence (Mount 1982) and appears to be more frequently used than the downstream site. Nevertheless, both splice sites are located upstream of the initiation codon, so that no change in reading frame occurs with these variations. Splice variants using both alternate splice acceptors were observed for transcripts containing exons 1a and 1b in mouse tissues. In human thymus tissue, only the more distal site was used in the 1a-2 type cDNAs analyzed.

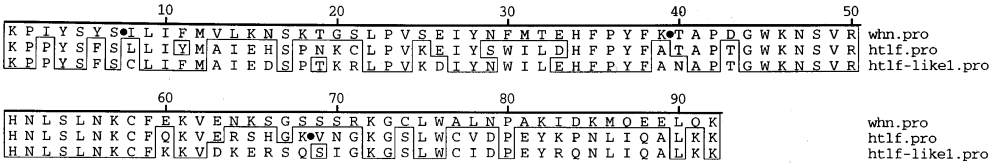

Fig. 4 Protein sequence alignment of DNA binding domains of human *HTLF*, *HTLFL1*, and *WHN* genes. The location of introns is indicated by *dots*, the exon structure of *HTLFL1* is not known

The above results suggested the presence of two individual promotor regions upstream of exons 1a and 1b, respectively. Therefore, several restriction fragments from the mouse *whn* gene were cloned upstream of a promoterless luciferase reporter construct. These fragments contained the regions of strong (>80%) nucleotide sequence conservation between human and mouse *whn* genes upstream of, and including, exons 1a and 1b, respectively. These regions comprise about 500 bp for exon 1a, and about 300 bp for exon 1b.

When cloned upstream of a promoterless luciferase vector, such fragments (indicated in Fig. 1A) increased luciferase activity by a factor of more than 10, after transient transfection into COS7 and BHK cells, respectively. Unfortunately, promoter activity of these fragments could not be reliably assessed in the thymic epithelial cell line 427, because its transfection proved very inefficient despite the use of a variety of transfection procedures. It appears therefore that, although sequences upstream of exon 1a and exon 1b do have promoter activity in a heterologous system, more elaborate experiments will be required to establish the molecular basis of their tissue-specific utilization.

Whn-related genes in the human and mouse genome

Database comparisons reveal that the human genes most closely related to *whn* are *HTLF*, located on human chromosome 2p16 (Li et al. 1992), and a previously undescribed gene, designated HTLF-like 1 (*HTLFL1*), identified from the collection of EST sequences in the GenBank database. In the presumed DNA binding domains, *HTLF* and *HTLFL1* are 77% identical at the protein level; *whn* and the *HTLF* or *HTLFL1* genes share 54% of amino acids in that region. This result prompted us to determine whether *HTLF/HTLFL1* and *whn* could have been derived by an ancient gene duplication event. To evaluate this possibility, we determined the exon/intron structure for the DNA binding domain of the human *HTLF* gene. The results summarized in Fig. 4 indicate that the DNA binding domain of *HTLF* is interrupted by one intron, which is located in a different location than either of the two introns in the *whn* DNA binding domain. The next intron in the *HTLF* gene occurs 10 amino acids after the last residue (lysine) of the DNA binding domain, again differing in phase and location from the organization of the *whn* gene. The existence of a common precursor of *HTLF* and *whn* thus remains uncertain.

Discussion

Is the human WHN gene a paralogue or an orthologue of the mouse nude gene?

The isolation of homologous genes from different species always raises the question as to whether a homologue is a paralogue (i.e., related by duplication), or an orthologue (i.e., related by vertical descent). Several criteria can be used to decide between these possibilities. First, the number of *whn*-like genes in the genome may be considered. Our previous results indicated that there is only one *whn*-like gene in the genome of mouse (Schlake et al. 1997) and human (unpublished data) and we show here that they possess an identical genomic structure: that is, their number, size, location, and phase of introns are identical. Second, conservation of short-range synteny may be taken as an argument for orthology. Indeed, our present data indicate that the two genes flanking the *whn* gene in the mouse and human genome are identical. Third, a reasonably high degree of sequence identity of derived protein sequences can be expected. About 85% of amino acids in the human WHN protein are identical to the mouse whn protein and both proteins are identical in length (648 aa). The DNA binding and transactivation domains that have been localized in the mouse whn protein (Schüddekopf et al. 1996; Schlake et al. 1997) are also present in the human protein. Fourth, functional criteria can be used to make a decision between paralogue and orthologue. The identity of the rat homologue of the mouse nude gene was proved by the identification of deleterious mutations in the *whn* gene of nude rats (Nehls et al. 1994; Segré et al. 1995; Schüddekopf et al. 1996; Huth et al. 1997). However, no phenotype identical to nude mice has yet been described in human beings; therefore, we cannot use this criterion in the present case. Nevertheless, the available information strongly suggests that we have identified the human orthologue of the mouse nude gene. The results described in this paper therefore form the basis for a comprehensive analysis of a possible involvement of the *whn* gene in human thymic dysplasia syndromes (Nezelof 1992; Brooks et al. 1994) and human thymomas (Debono et al. 1996; Hammond et al. 1991).

The exon/intron structure of the DNA binding domain of whn is unique among forkhead/winged-helix proteins

The genomic structure of the human and mouse *whn* genes is notable for the fact that the presumptive DNA binding

domain is encoded in three exons. In most other genes of the forkhead/winged-helix class of transcription factors, the DNA binding domain is encoded in one exon (Kaestner et al. 1994, 1995, 1996), although exceptions with one intron exist, as shown here for the human *HTLF* gene. The forkhead/winged-helix proteins most closely related to the *whn* gene are *HTLF* and *HTLFL1*, where an amino acid identity of about 54% is observed in the DNA binding domain. Our results show that the exon/intron structure across the DNA binding domain of the human *HTLF* gene is different from that of *whn* genes. It is interesting to note, however, that the location of the first intron in the *HTLF* binding domain occurs in phase 0, and is equivalent to the location of an extension of the first wing of the DNA binding domain in the *whn* orthologue from *Lampetra planeri* (Schlake et al. 1997). Nevertheless, although considerable sequence similarity exists between *whn* and *HTLF*, no definitive conclusion about their evolutionary relationship can yet be made.

The significance of alternative first exons

Our results indicate that the expression of the 1a-2 to 9 or 1b-2 to 9 types of *whn* mRNAs is tissue-specific. It is of interest to note that mRNAs isolated from mouse skin contain both forms; although no precise quantitation has been done, about 75% of *whn* cDNAs from a primary plating of a mouse skin cDNA library are of the 1b-2 structure. In thymus, however, only the 1a-2 form is observed. It thus appears likely that the *whn* gene possesses two distinct partially redundant promoter elements. Indeed, promoter activity has been detected upstream of exons 1a and 1b. Our present results thus provide a molecular explanation for the incomplete rescue of the nude phenotype observed with a cosmid clone spanning the *whn* gene lacking exon 1a (Kurooka et al. 1996). The skin phenotype is at least partially rescued, presumably due to the activity of the downstream 1b promoter in transgenic *nu/nu* animals. In contrast, the characteristic thymic dysplasia is still present, most likely due to the absence of the thymus-specific promoter 1a. In this context, it will be interesting to study the organization of promoter elements of the *whn* gene in lower vertebrates (Schüddekopf et al.1996; Schlake et al.1997) that possess a thymus but no hair (for example zebrafish) or neither of these tissues (for example, lamprey). It is conceivable that the residual activity of promoter 1a in skin represents an evolutionary remnant of a primordial *whn* promoter. This promoter may have initially controlled *whn* expression in both thymus and skin in mammals. Subsequent alterations in the transcriptional control region of *whn* may then have led to the creation of the second promoter 1b, containing strictly skin-specific control elements. The *whn* gene structure thus represents an interesting example of how a gene can be assigned to new functions without changing its copy number (Piatigorsky et al. 1991).

Acknowledgments We are grateful to Dr. M. Nehls and Dr. D. Pfeifer for help in the initial stages of this project and thank M. Huth and K. Matena for expert technical assistance. We also thank Dr. B. O. Boehm for human thymus samples and Dr. R. Gaynor for the cDNA nucleotide sequence of human *HTLF*. Financial support was provided by the Deutsche Forschungsgemeinschaft.

References

Brooks, E. G., Klimpel, G. R., Vaidya, S., Keeney, S. E., Raimer, S., Goldman, A. S., and Schmalstieg, F. C. Thymic hypoplasia and T-cell deficiency in ectodermal dysplasia: case report and review of the literature. *Clin Immunol Immunopathol 71:* 44–52, 1994

Debono, D. J. and Loehrer, P. J. Thymic neoplasms. *Curr Opin Oncol 8:* 112–119, 1996

Faas, S. J., Rothstein, J. L., Kreider, B. L., Rovera, G., and Knowles, B. B. Phenotypically diverse mouse thymic stromal cell lines which induce proliferation and differentiation of hematopoietic cells. *Eur J Immunol 23:* 1201–1214, 1993

Hammond, E. H. and Flinner, R. L. The diagnosis of thymoma: a review. *Ultrastruct Pathol 15:* 419–438, 1991

Higashide, T., Murakami, A., McLaren, M. J., and Inana, G. Cloning of the cDNA for a novel photoreceptor protein. *J Biol Chem 271:* 1797–1804, 1996

Huth, M., Schlake, T., and Boehm, T. Transposon-induced splicing defect in the rat nude gene. *Immunogenetics 45:* 282–283, 1997

Kaestner, K. H., Hiemisch, H., Luckow, B., and Schütz, G. The *HNF-3* gene family of transcription factors in mice: gene structure, cDNA sequence, and mRNA distribution. *Genomics 20:* 377–385, 1994

Kaestner, K. H., Monaghan, A. P., Kern, H., Ang, S.-L., Weitz, S., Lichter, P., and Schütz, G. The mouse *fkh-2* gene. *J Biol Chem 270:* 30029–30035, 1995

Kaestner, K. H., Schütz, G., and Monaghan, A. P. Expression of the winged helix genes *fkh-4* and *fkh-5* defines domains in the central nervous system. *Mech Develop 55:* 221–230, 1996

Kurooka, H., Segré, J. A., Hirano, Y., Nemhauser, J. L., Nishimura, H., Yoneda, K., Lander, E. S., and Honjo, T. Rescue of the hairless phenotype in nude mice by transgenic insertion of the wild-type *Hfh11* genomic locus. *Int Immunol 8:* 961–966, 1996

Li, C., Lusis, A. J., Sparkes, R., Tran, S.-M., and Gaynor, R. Characterization and chromosomal mapping of the gene encoding the cellular DNA binding protein *HTLF*. *Genomics 13:* 658–664, 1992

Mount, S. M. A catalogue of splice junction sequences. *Nucleic Acids Res 10:* 459–472, 1982

Nehls, M., Pfeifer, D., Schorpp, M., Hedrich, H., and Boehm, T. New member of the winged-helix protein family disrupted in mouse and rat nude mutations. *Nature 372:* 103–107, 1994

Nehls, M., Lüno, K., Schorpp, M., Pfeifer, D., Krause, S., Matysiak-Scholze, U., Dierbach, H., and Boehm, T. YAC/P1 contigs defining the location of 56 microsatellite markers and several genes across a 3.4-cM interval on mouse chromosome 11. *Mamm Genome 6:* 321–331, 1995

Nehls, M., Kyewski, B., Messerle, M., Waldschütz, R., Schüddekopf, K., Smith, A. J. H., and Boehm, T. Two genetically separable steps in the differentiation of thymic epithelium. *Science 272:* 886–889, 1996

Nezelof, C. Thymic pathology in primary and secondary immunodeficiencies. *Histopathology 21:* 499–511, 1992

Pajor, A. M. Expression cloning of a renal sodium/dicarboxylate cotransporter. *J Biol Chem 270:* 5779–5785, 1995

Pantelouris, E. M. Absence of thymus in a mouse mutant. *Nature 217:* 370–371, 1968

Piatigorsky, J. and Wistow, G. The recruitment of crystallins: new functions precede gene duplication. *Science 252:* 1078–1079, 1991

Schlake, T., Schorpp, M., Nehls, M., and Boehm, T. The nude gene encodes a sequence-specific DNA-binding protein with homologs in organisms that lack an anticipatory immune system. *Proc Natl Acad Sci USA 94:* 3842–3847, 1997

Schüddekopf, K., Schorpp, M., and Boehm, T. The *whn* transcription factor encoded by the nude locus contains an evolutionarily conserved and functionally indispensable activation domain. *Proc Natl Acad Sci USA 93:* 9661–9664, 1996

Schuler, G. D. et al. A gene map of the human genome. *Science 274:* 540–546, 1996

Segré, J. A., Nemhauser, J. L., Taylor, B. A., Nadeau, J. H., and Lander, E. S. Positional cloning of the nude locus: genetic, physical and transcription maps of the region and mutations in the mouse and rat. *Genomics 28:* 549–559, 1995

Walter, M. A., Spillett, D. J., Thomas, P., Weissenbach, J., and Goodfellow, P. N. A method for constructing radiation hybrid maps of whole genomes. *Nat Genet 7:* 22–28, 1994