Genesis, a Winged Helix Transcriptional Repressor with Expression Restricted to Embryonic Stem Cells*

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A novel member of the winged helix (formerly HNF-3/Forkhead) transcriptional regulatory family, termed Genesis, was isolated and characterized. Putative translation of the complete cDNA revealed the winged helix DNA binding domain to be centrally located within the protein, with regions on either side that contain known transcriptional regulatory motifs. Extensive Northern analysis of Genesis found that the message was exclusively expressed in embryonic stem cells or their malignant equivalent, embryonal carcinoma cells. The Genesis transcript was down-regulated when these cells were stimulated to differentiate. DNA sequences that Genesis protein would interact with were characterized and were found to contain a consensus similar to that found in an embryonic stem cell enhancer sequence. Co-transfection experiments revealed that Genesis is a transcriptional repressor. Genesis mapped to mouse chromosome 1p31, a site of nonrandom abnormalities in germ cell neoplasia, neuroblastoma, and acute lymphoblastic leukemia. Genesis is a candidate for regulating the phenotype of normal or malignant embryonic stem cells.

Development is controlled by regulatory genes acting as genetic switches to stimulate cascades of gene expression, producing tissue and organismal phenotypes. In Drosophila these regulatory genes are often transcriptional regulators that not only initiate the gene cascade resulting in that embryonic stage's phenotypic change but also direct expression of the regulators important in the next embryonic stage (reviewed in Refs. 1 and 2). Although the temporal regulatory cascades involved in expression of genes during mammalian embryonic development is less well understood, it is likely that a similar mechanism also exists whereby transcriptional regulators play important roles in lineage commitment and stage progression (reviewed in Refs. 3–5).

These transcriptional regulators fall into related families based on conserved structure in their DNA binding domain (reviewed in Refs. 6 and 7). One such family that has been strongly implicated in developmental regulation is the winged helix family (reviewed in Ref. 8). Termed winged helix based on its three-dimensional structure when bound to DNA (9), this family was formerly called HNF-3/Forkhead (HFH)1 after its original members (10–13). The winged helix domain of the hepatocyte nuclear factor 3 (HNF-3) proteins mediates binding to specific DNA sequences having the consensus A/A/T/RTT/G/T/RTRY (14).

Several members of this family are essential for normal Drosophila development (12, 15). In addition, members of this family participate in mammalian oncogenesis. Qin is a retrovirally transduced murine oncogene (16), and the oncoprotein in human rhabdomyosarcoma is a fusion between pax5 and a winged helix gene (17, 18). The human leukemia translocation t(X;11) involves a winged helix gene (19). A rat and mouse nude mutation disrupts the winged helix gene wbn (20, 21).

It has also been demonstrated that the HNF-3 proteins are critical factors in maintaining the potential for proper differentiation in in vitro models of differentiation (22, 23). For example, HNF-3β mutant mice cannot form the node or notochord appropriately, producing defects in neurotube and somite organization (24, 25). These mutants also fail to form tissues arising from the gut endoderm, including liver lung, pancreas, and intestine. Finally, forcing the expression of HNF-3β in transgenic mice in the hindbrain results in conversion of this structure to the floorplate, with subsequent activation of a number of floorplate markers (26). Thus, there are several lines of evidence that the winged helix family function as genetic regulators of development.

We previously cloned three members of the winged helix family, H-3, H-8, and 5-3, from hematopoietic cells by polymerase chain reaction amplification using degenerate oligonucleotide primers to extremely conserved regions of the DNA binding domain (27). One of these, 5-3, was also expressed in an embryonal carcinoma cell line. Using the 5-3 cDNA as a probe under low stringency we isolated several winged helix family members from a PCC4 embryonal carcinoma cDNA library.

1 The abbreviations used are: HNF, hepatocyte nuclear factor; HFH, HNF-3/Forkhead; CAT, chloramphenicol acetyltransferase; FKH, Forkhead; kb, kilobase pair(s).
Genesis, an Embryonic Transcriptional Regulator

One of the new cDNA clones encoded a novel member of the winged helix transcription factor family. Because this gene’s expression was restricted to embryonic stem cells and their malignant equivalent, the gene was termed Genesis. Genesis binds to DNA sequences similar to those found in known embryonic stem cells enhancers. In co-transfection assays with binding sites linked to a chloramphenicol acetyltransferase (CAT) reporter construct Genesis acted as a transcriptional repressor. Therefore, Genesis represents a transcriptional repressor in the winged helix family with regulatory activity early in embryonic differentiation.

MATERIALS AND METHODS

Cloning of Genesis—One million phage plaques of the PCC4 murine embryonal carcinoma cDNA library (Stratagene, La Jolla, CA) were screened as described (28) with the full-length cDNA insert from human 5-3, a winged helix gene expressed in primitive embryonic cells (27). Two cDNA inserts were obtained that encoded Genesis. One was termed 3A and the other 4C. Both clones were sequenced on both strands using Sanger dideoxy double strand sequencing. Ambiguities were resolved with denazidazole nucleotides, and formamide sequencing gels. A new clone had a minimal amount of additional sequence on the 3′-untranslated region, while the coding regions of both clones were identical. The sequence of 3A was found to be unique to GenBankTM and has been deposited under accession number U41047.

Expression Analysis—Poly(A)+ RNA was isolated from cell lines using the MicroFastTrack kit (Invitrogen, San Diego, CA) according to the manufacturer’s instructions. Total RNA was isolated from dissected murine embryonic and adult tissues using Trizol reagent according to the manufacturer’s instructions (Life Technologies, Inc.). RNA was denatured, size-fractionated by electrophoresis on formaldehyde agarose gels, and capillary-blotted to a nylon membrane (28). Blots were hybridized in 40% formamide, 10% dextran sulfate, 4 × SSPE, 2 × Denhardt’s solution, 0.1% SDS, and 100 μg/ml of sheared salmon sperm DNA at 50 °C for 24 h. Blots were then washed to a final stringency of 0.1 × SSPE and 0.1% SDS at 65 °C and exposed to autoradiography.

Mobility Shift DNA Binding Analysis—In vitro translated full-length Genesis protein was synthesized using the TNT kit (Promega, Madison, WI) in the presence of [35S]methione per the manufacturer’s instructions. Genesis protein synthesis was assessed by SDS-denaturing gel electrophoresis, and autoradiography. Full-length protein was used because of the evidence that for some transcription factors portions of the protein outside of the putative DNA binding domain changed the DNA binding sensitivity and specificity (14). Mobility shift analysis took place as we previously described (29). Briefly, 5 μl of the Genesis translation reaction was incubated with 1 ng of [35S]Flabeled duplex oligonucleotide in 25 μl of binding buffer for 15 min at room temperature. Binding buffer consisted of 10 mM Tris-Cl (pH 7.9), 50 mM NaCl, 5 mM MgCl2, 1 μM dithiothreitol, 5% glycerol, 0.8 μg/ml poly(dI-dC), 25 ng/ml random duplex 20-mer oligonucleotide, and 0.2 μg/ml albumin. Where appropriate, 100 ng of unleveled duplex oligonucleotide was added to the binding reaction to assess whether Genesis DNA binding could be specifically competed away. Samples were then electrophoresed on a prerun 4% nondenaturing polyacrylamide gel (acrylamide: bisacrylamide, 40:1) in recirculating 0.25 × TBE at 10 V/cm. The gel was dried and exposed to autoradiography. Sequences to which Genesis bound were used to search the Eukaryotic Promoter Database of GenBankTM.

Transfection Assays—The full-length Genesis cDNA was subcloned into the EcoRI site of pRS, an expression vector using the rat β-actin promoter (30). This subclone was termed P6-Gen. Four number 3 binding sites were concatamerized in forward orientation in the XbaI site 5′ of the TATA-CAT reporter gene (31, 32). This reporter construct was termed Gen-CAT. HeLa cervical carcinoma and 293 embryonic kidney cells were obtained from the American Type Culture Collection (Bethesda, MD) and maintained in Dulbecco’s modified Eagle’s medium with 10% heat-inactivated fetal calf serum and antibiotics. Expression and reporter plasmids were co-transfected into log phase HeLa or 293 cells using the calcium phosphate method according to the manufacturer’s instructions (Life Technologies, Inc.). Transfection efficiencies were normalized with SV40-Gal, a β-galactosidase expression vector (Promega). Cells were harvested 48 h after transfection, washed in phosphate-buffered saline, resuspended in 250 mM Tris-Cl, and lysed by three cycles of freezing and thawing. After normalization for β-galactosidase activity (33), CAT activity was assayed as previously reported (34). Each transfection was performed at least three separate times to obtain the mean and standard error.

Chromosomal Mapping—Genesis was mapped using interspecific mouse back-cross analysis. Interspecific back-cross progeny were generated by mating (C57BL/6J × Mus spretus) F1 females with C57BL/6J males as described (35). A total of 205 N2 mice were used to map the Genesis locus. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and probe hybridization were all performed essentially as described (36). Hybrid-N+ nylon membrane was used for all blots. The entire Genesis cDNA was radioabeled by nick translation and used as a probe. Blots were washed to a final stringency of 0.5 × SSC, 0.1% SDS at 65 °C. A major fragment of 6.2 kb was detected in BglII-digested C57BL/6J DNA, and a major fragment of 14.0 kb was detected in BglII-digested M. spretus DNA. The presence or absence of the 14.0 kb BglII M. spretus-specific fragment was followed in back-cross mice.

A description of the probes and restriction fragment length polymorphisms for two of the loci linked to Genesis, the Jun proto-oncogene and the cytochrome P450α gene Cypdα10, has been published (37). The map position for the phosphoglucomutase type 2 gene (Pgm2) has not been reported for our back-cross analyses. A 380-base pair XbaI/PstI murine Pgm2 cDNA fragment (the generous gift of N. Bahary, Rockefeller University) was radioabeled by nick translation for use as a probe. Southern blots were washed at a final stringency of 0.25 × SSC, 0.1% SDS, at 65 °C. A strongly hybridizing fragment of 3.0 kb and two weakly hybridizing fragments of 8.4 and 15.0 kb were detected in XbaI-digested C57BL/6J DNA. A strongly hybridizing fragment of 8.8 kb and two weakly hybridizing fragments of 5.5 and 9.6 kb were detected in XbaI-digested M. spretus DNA. Restriction fragment length polymorphism analysis was also performed using the TaqI restriction enzyme. Fragments of 1.0, 1.5, 2.8, 4.4, 8.6, and 19.0 kb were detected in TaqI-digested C57BL/6J DNA. Bands of 1.0, 1.6, 1.9, 3.2, and 8.6 kb and one weakly hybridizing band of 3.0 kb were detected in TaqI-digested M. spretus DNA. The presence or absence of the 8.8-kb XhoI M. spretus-specific fragment and the 1.9- and 3.2-kb TaqI M. spretus-specific fragments, which co-segregated, were followed in back-cross mice. The XhoI and the TaqI data were combined. Recombination distances were calculated as described (38) using the computer program Spretus Madness. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

RESULTS

Sequence Analysis—Genesis was cloned from a PCC4 murine embryonal carcinoma cDNA library using 5-3 CDNA as a probe under low stringency. 5-3 is a member of the winged helix family that was expressed preferentially but not exclusively in hematopoietic and embryonic cells (27). Six clones were obtained, two of which were murine 5-3, and four were uncharacterized members of the winged helix family. Two of these four uncharacterized clones were found to be identical, and subsequent expression analysis revealed that this gene was expressed only in very primitive embryonic cells. Therefore, this gene was termed Genesis.

The Genesis cDNA had 2225 nucleotides, which when translated had an open reading frame of 465 amino acids (GenBankTM accession number U41047, Fig. 1). Allowing for a poly(A) tail, this sequence probably represents the entire cDNA, since the size of the transcript by Northern analysis is 2.4 kb. The conserved winged helix domain starts at amino acid 129 in the protein and extends to amino acid 229. This is consistent with the rest of the family: almost all of the other members also have the winged helix domain in the central portion of the protein (8). The Genesis winged helix domain is identical to that of another member that we previously isolated, HFH-2, at the amino acid level (39). The winged helix DNA binding domain of HFH-2 was isolated by polymerase chain reaction from genomic DNA, indicating that the winged helix domain existed as a single exon, but the entire cDNA was isolated, consistent with the need for isolation. The cDNA sequence of HFH-2 cDNA was probably due to the extremely restricted expression of this gene (HFM-2, GenBankTM accession number L13202). Fig. 2 compares the Genesis winged helix domain with the
there are 17 alanines, and from amino acids 437–464 there are 12 alanine residues. There is evidence that alanine-rich regions may be an important structural mediator of repression seen in embryonic transcriptional regulators (44). In summary, the sequence analysis of Genesis revealed a conserved winged helix DNA binding domain in conjunction with several known transcriptional regulatory regions.

Expression Analysis—The expression pattern of Genesis was investigated using poly(A)⁺ Northern analysis (Fig. 3). Genesis was expressed as an approximately 2.4-kb message only in cells of very primitive embryonic origin. Genesis transcripts were present in D3 embryonic stem cells and Tera1, P19, N-Tera2D1, A33, and 1618 embryonal carcinoma cells. Based on the size of the message in Northern analysis and the presence of a good polyadenylation signal, it is likely that the sequence reported here represents the entire cDNA.

Next, the expression of Genesis during embryonal carcinoma differentiation was studied. When Tera-1 or P19 embryonal carcinoma cells were exposed to retinoic acid at 10⁻⁶ M for 24 h, Genesis expression was markedly reduced. Retinoic acid induces these cells to differentiate toward neurons (45, 46). Genesis transcripts were not observed in any hematopoietic cell lines, including EL4 T-cell leukemia, FDCP1 myeloid leukemia, A20 B-cell leukemia, BAF3 erythroleukemia, H7 myeloid leukemia, and HL60 promyelocytic leukemia cells. Treating HL60 cells with retinoic acid to induce granulocytic differentiation did not induce Genesis expression. Genesis was not expressed in 1141 yolk sac carcinoma cells, Bewo choriocarcinoma cells, or TM4 sertoli cells. In addition, Genesis expression was not observed in the murine fibroblast cell line 3T3.

Next, the expression of Genesis at various stages during murine development was investigated. Tissues were dissected from day 18, neonatal, and adult mice, and total RNA was prepared for Northern analysis. Genesis expression was not expressed in any of these tissues at any stage of development. Specifically, Genesis was not expressed in embryonic, neonatal, or adult brain, gut, heart, kidney, liver, lung, muscle, skin, spleen, or thymus. It was also not expressed in adult aorta, uterus, or testis. Probing for 18 S ribosomal RNA and murine FKH-3-3 after probing for Genesis showed that loaded RNA was intact and abundant.

Finally, D3 embryonic stem cells were removed from leukemia inhibitory factor and grown without substrate adherence to promote differentiation to embryoid bodies. Between 4 and 8 days after this differentiation protocol, Genesis expression decreased, and by 9 days of differentiation, transcripts are undetectable by Northern analysis. Thus, Genesis appears to be expressed preferentially in embryonic stem cells or their malignant equivalent. When these cells differentiate, either in vitro or in vivo during embryogenesis, expression is down-regulated.

DNA Binding Specificity—Mobility shift analysis was used to study the DNA binding specificity of Genesis. Full-length Genesis protein was synthesized in vitro, and the in vitro translated product of both cDNA clones (3A and 4C) was approximately 50 kDa, in agreement with the predicted protein size of approximately 50 kDa. In binding experiments, the DNA binding domain of the translated protein was used in the subsequent mobility shift assays. Previous studies with the HFH-2 winged helix DNA binding domain had revealed a number of DNA sequences that Genesis was likely to bind to (34). Radiolabeled double-stranded oligonucleotides of these and other sequences were incubated with Genesis and then electrophoresed on nondenaturing polyacrylamide gels to visualize the slowly migrating protein-DNA complexes.

Fig. 4 shows the mobility-shifted radiolabeled oligonucleo-
Fig. 2. Comparison of the DNA binding domains of the mammalian members of the winged helix transcription factor family. The three helices and two wings of the DNA binding domain are overlined (9). The family members are arranged in decreasing order of homology to HNF-3α, the original member of the family. The percentage homology to HNF-3α is on the right. The similarity between Genesis and 5-3, the probe used to isolate Genesis, should be noted. Also, Genesis belongs to a subgroup of winged helix genes that all share a unique S(G/N/R)R... (R/E)KFPA motif within the DNA binding domain. The references for these sequences are as follows: HNF-3α (10), HNF-3β and γ (51, Forhead 11), βh 1–6 (57), BF-1,2 (39, 58), 5-3 and H8 (27), MF-1,2 (59), HNF 1–8 (39). C57BL/6J and M. spretus DNAs were digested with several restriction enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms using a Genesis cDNA probe. The 14-kb probe has been typed for over 2100 loci that are well distributed among all autosomes as well as the X chromosome. The mapping results indicate that the M. spretus, C57BL/6J, and M. spretus loci in back-cross mice.

**Chromosomal Mapping**—The mouse chromosomal location of Genesis was determined by interspecific back-cross analysis using progeny derived from matings of (C57BL/6J × M. spreitus) F1 × C57BL/6J mice. This interspecific back-cross mapping panel has been typed for over 2100 loci that are well distributed among all autosomes as well as the X chromosome (35). C57BL/6J and M. spreitus DNAs were digested with several restriction enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms using a Genesis cDNA probe. The 14-kb Bgl III M. spreitus restriction fragment length polymorphism was used to follow the segregation of the Genesis locus in back-cross mice. The mapping results indicate that Genesis is located in the central region of mouse chromosome 4, linked to Jun, Pgm2, and Cypr4a10 (Fig. 6). This portion of murine chromosome 4 is syntenic to human chromosome 1p31, a region of multiple nonrandom chromosomal abnormalities in human neoplasia. Although 143 mice were analyzed for every marker and are shown in the segregation analysis, up to 176 mice were typed...
for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The most likely gene order and the ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci are as follows (centimorgans 2.2): Jun (5/174); Genesis (0/176); Pgm2 (14/176); Cyp4a10. The recombination frequencies (expressed as genetic distances in centimorgans ± S.E.) are as follows: Jun (2.9 ± 1.3); Genesis/Pgm2 (8.7 ± 2.2); Cyp4a10. No recombinants were detected between Genesis and Pgm2 in 176 animals typed for both markers, suggesting that the two loci are within 1.7 centimorgans of each other (upper 95% confidence limit).

**DISCUSSION**

**Genesis**, an Embryonic Transcriptional Regulator

*Genesis* is a novel member of the winged helix transcriptional regulator family (previously HNF-3/Forkhead, or HFH). We have recently cloned the human genomic locus of *HNF-3* and partial sequencing of this showed exonic amino acid sequences. The sequence is derived from the embryonic stem cell-specific enhancer, similar to HFH-2 (14). The E2 sequence is derived from the embryonic stem cell-specific enhancer, which *Oct-3* interacts with (48). The transthyretin (TTR) and HNF-3 β sequences come from promoter regions of those genes (32).

**TABLE I**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Sequence</th>
<th>Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>AATTGTTTATTTT</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>AAAAATTGCTTT</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>TTATTTTATTTT</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>GTTTGTTTTTTT</td>
<td>++</td>
</tr>
<tr>
<td>12</td>
<td>TTATGTTTATTTT</td>
<td>++</td>
</tr>
<tr>
<td>25</td>
<td>CATTGGTTTATTT</td>
<td>+</td>
</tr>
<tr>
<td>E2</td>
<td>TTTACAAATTCCTTTAATTTA</td>
<td>+</td>
</tr>
<tr>
<td>TTR</td>
<td>TTGACTAAGCTAAATTTTAAAA</td>
<td>+</td>
</tr>
<tr>
<td>HNF-3β</td>
<td>CCCTGTTTGTATT</td>
<td>-</td>
</tr>
</tbody>
</table>

**FIG. 4.** Mobility shift analysis of the DNA binding specificities of *Genesis* protein. Radiolabeled duplex oligonucleotides were incubated with *in vitro* translated *Genesis* protein and then electrophoresed on a nondenaturing polyacrylamide gel. Oligonucleotide bound to protein has a slower mobility than free oligonucleotide. Where 100-fold unlabeled specific oligonucleotide as competitor was added, the lane is designated with a plus sign, while lanes without such competition are designated with a minus sign. The sequence of each oligonucleotide used in these assays is listed in Table I. Sequence length and, therefore, gel mobility differs between oligonucleotide probes.

**FIG. 3.** Northern analysis of the expression of *Genesis*. The murine embryonic and adult normal tissues are total RNA, and all others are poly(A) + RNA. To control for integrity and abundance of total RNA, an 18 S ribosomal RNA probe was used, while for poly(A) + RNA a β-actin probe was used as a control. A, the absence of expression of *Genesis* in a wide variety of normal embryonic, neonatal, and adult murine tissues. FKH 5-3 is a winged helix transcriptional regulator closely related to *Genesis* in the conserved DNA binding domain, but it differs outside that domain. Both 5-3 and 18 S were probed for after *Genesis* was probed for on these blots. Blots were washed to a stringency of 65 °C and 0.1 × SSC. The same radiolabeled *Genesis* cDNA probe was used on blots in B that produced strong signal. B, the expression of *Genesis* in a wide variety of murine and human cell lines. *Genesis* is expressed only in embryonic stem cells or their malignant equivalent, embryonal carcinoma cells. When *Genesis* is expressed, the intensity of its signal rivals actin.
are most commonly, but not exclusively, expressed in embryonic cells (8). MF-2 and HFH-B2, which may represent the mouse and human homologues, respectively, of the same gene, are expressed in murine embryonic somites and head mesenchyme. 5-3 is most highly expressed in differentiating N-Tera-2D cells. FKH-2 and HFH-6, which also may represent the mouse and human homologues, respectively, of the same gene, are expressed in murine D3 embryonic stem cells, brain, lung, and gut. Thus, this family may regulate sets of genes that are important in development. The shared amino acid sequence in this subfamily may mediate specific DNA binding to regulatory motifs within these genes. 

Significantly, Genesis was found to be expressed only in embryonic stem cells or their malignant equivalent, embryonal carcinoma cells. In addition, expression was quite high, often equivalent to that seen with the actin control. Despite extensive Northern analysis of normal and neoplastic tissues, Genesis expression was tightly restricted to primitive cell types. Genesis was also not expressed in accessory cells in the gonads, such as Sertoli cells from the testis. When stimulated to differentiate by several mechanisms, Genesis expression markedly declined in these cells. Retinoic acid exposure appeared to lower expression of Genesis much more rapidly than removal of leukemia inhibitory factor.

Interestingly, Genesis was not expressed in cell lines derived from extraembryonic testicular malignancies, such as a yolk sac or choriocarcinomas. These tumors are considered more differentiated than embryonal carcinomas. When embryonal carcinomas have differentiated elements mixed in with the more malignant embryonal cells, they are termed teratocarcinomas, with the slower growing, nonmetastatic, differentiated cells called teratoma. Cell lines derived from teratocarcinomas generally consist of the more malignant embryonal cells, since they rapidly overgrow the differentiated elements. Although often called teratocarcinoma cells because of their origin, they consist of embryonal carcinoma cells. When treated with retinoic acid, these embryonal cell lines differentiate to teratoma-like cells, with mainly neural attributes. Genesis expression declines rapidly with such treatment, further evidence that Genesis expression is correlated with the pluripotent, more primitive state of embryonic cells.

The specific DNA sequences to which Genesis could bind were also investigated. They were consistent with what we had previously found for HFH-2 (14). This indicated that amino acids adjacent to the winged helix DNA binding domain of Genesis did not change the specificity of DNA binding, as they have for other winged helix genes (14). These sequences share an A-T-rich consensus, A/AT/TG/T/TTGGTTT, that is found in a number of developmentally regulated promoters. Genesis binds to a sequence that Oct-3 can also bind to, the E2 embryonic stem cell enhancer sequence. This is a retinoic acid-responsive ES cell enhancer through which Oct-3 can activate transcription. For neural differentiation to take place, Oct-3 transcriptional activity must decline (49). Since Oct-3 and Genesis can share specific DNA binding sequences, they may also share genetic regulatory function.

One of the sequences that Genesis bound to, sequence 3, was concatamerized in front of a TATA-CAT reporter construct. This was used in co-transfection assays to examine the transcriptional regulatory properties of Genesis. Genesis was found to markedly repress CAT reporter activity from these binding sites in a dose-dependent manner. Thus, it appears that Genesis is a transcriptional repressor. This is the first winged helix gene described that functions as a transcriptional repressor.
All others characterized for transcriptional activity thus far have been activators (10, 32, 50–53). Since Genesis DNA binding sites are similar to those of other winged helix genes (14), perhaps Genesis may act antagonistically to transcriptional activation by other winged helix genes. HNF-3β is an excellent candidate for this, since it is up-regulated by retinoic acid and is required for early events in embryogenesis (26).

Significantly, the consensus sequence of Genesis is found in several regulatory regions of genes that are activated early in neurogenesis, such as the D2 dopamine receptor, acetylcholinesterase, HoxB2 and Hox3, and retinoic acid receptor β genes (14). Since Genesis rapidly declines when embryonal carcinoma cells are differentiated toward neurons by treatment with retinoic acid, it is possible that Genesis must be down-regulated for such differentiation to occur. Since Genesis is a transcriptional repressor, it is possible that Genesis prevents the expression of genes responsible for a more mature phenotype, perhaps even other winged helix activators, since there are sequences similar to those that Genesis can bind in the promoters of some winged helix genes (14). Genesis may therefore play a role in the pluripotent state of primitive embryonic cells by transcriptionally repressing differentiation. It may complement Oct-3, repressing maturation genes, while Oct-3 activates genes responsible for maintaining the pluripotent state. Formal proof of this awaits gain-of-function and loss-of-function experiments in embryonic stem cells.

Genesis was mapped to mouse chromosome 4. We have compared our interspecific map of chromosome 4 with a composite mouse linkage map that reports the map location of many murine genes (54, 55). The tight linkage map that reports the map location of many murine genes (54, 55). The tight linkage map that reports the map location of many murine genes (54, 55).

There are a number of reported translocations involving this region in human germ cell neoplasia (mainly testicular carcinomas), neuroblastoma, lymphoma, and especially acute lymphoblastic leukemia (56). It is possible that abnormalities of this gene could play a role in testicular oncogenesis, perhaps by preventing normal maturation of germ cells. A rationale could also be made for involvement of Genesis in neuroblastoma.

The rapid down-regulation of Genesis when embryonal carcinoma differentiate to neurons implies that maintenance of Genesis expression would be detrimental for neuronal maturation. Either of these possibilities would seem most likely if Genesis were aberrantly expressed off a translocated constitutive promoter (6). This possibility will be explored by assessing the genomic structure of Genesis with fluorescence in situ hybridization and Southern analysis on tumor samples bearing cytogenetic abnormalities in this region. There is certainly precedent for winged helix genes to be involved in oncogenesis (16–19). However, it is also possible that Genesis down-regulation during differentiation is not central to the genetic mechanism for stage progression from pluripotentiality to commitment, but rather an epiphemonephon, caused by the maturation of the cells. It should also be noted that the protooncogene jun is located in this same region and would also be a candidate for disruption in these malignancies.

In summary, Genesis is a novel winged helix transcriptional repressor with expression restricted to primitive embryonic stem cells or their malignant equivalent. It may play an important role in the decision of normal or neoplastic embryonic stem cells to differentiate and/or proliferate.

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