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Tissue, Developmental, and Tumor-Specific Expression of Divergent Transcripts in Wilms Tumor

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The Wilms tumor locus on chromosome 11p13 has been mapped to a region defined by overlapping, tumor-specific deletions. Complementary DNA clones representing transcripts of 2.5 (WIT-1) and 3.5 kb (WIT-2) mapping to this region were isolated from a kidney complementary DNA library. Expression of WIT-1 and WIT-2 was restricted to kidney and spleen. RNase protection revealed divergent transcription of WIT-1 and WIT-2, originating from a DNA region of <600 bp. Both transcripts were present at high concentrations in fetal kidney and at much reduced amounts in 5-year-old and adult kidneys. Eleven of 12 Wilms tumors classified as histopathologically heterogeneous exhibited absent or reduced expression of WIT-2, whereas only 4 of 14 histopathologically homogeneous tumors showed reduced expression. These data demonstrate a molecular basis for the pathogenetic heterogeneity in Wilms tumorigenesis.

WILMS TUMOR (WT) IS A HERITABLE embryonal renal malignancy with complex genetic (*1*) and pathological (*2*) features. Although earlier statistical and molecular genetic analyses implicated a single recessive oncogene locus in the etiology of WT (*3, 4*) recent studies suggest mutations in at least three loci (two mapping to 11p) can cause or predispose to the development of WT (*5, 6*). Heterogeneity also characterizes WT histopathology, such that these tumors can be categorized into: (i) intralobar WTs, which feature heterogeneous tumor histology and association with the WAGR (Wilms tumor, aniridia, genitourinary dysplasia, mental retardation) syndrome region on 11p13; or (ii) perilobar WTs, which are characterized by homogeneous histology and association with the Beckwith-Wiedemann syndrome mapped to 11p15 (*7*).

In Bonetta *et al.* (*8*) we describe the identification and mapping of three transcripts expressed in kidney tissues to a homozygous 175-kb WT deletion, using chromosome walking and yeast artificial chromosome (YAC) cloning. On the basis of data by Gessler *et al.* (*9*), it appears that one of these transcripts (WIT-3) maps outside the putative WT locus and thus seems less likely to be involved in WT. Here we report the biological characterization of cDNA clones corresponding to the two remaining transcripts, WIT-1 and WIT-2. These genes are transcribed in divergent directions from a single CpG island, and their expression shows a striking correlation with tumor histopathology.

Two phylogenetically conserved DNA fragments, AvH1 and E9, which detected distinct transcripts of 2.5 (WIT-1) and 3.5 kb (WIT-2), respectively, in human fetal kidney RNA (*8*), were used to isolate two corresponding cDNA clones, GB16 (WIT-1) and 31E1 (WIT-2). Southern blot hybridization and DNA sequencing confirmed that these cDNAs mapped to the homozygously deleted region in the WiT-13 cell line (*10, 11*). The WIT-1 transcript corresponding to GB16 is novel, whereas 31E1 identifies the same gene as two other previously described cDNAs (*12*). This gene encodes a potential zinc finger protein and is deleted in WiT-13 and in two other cases of sporadic Wilms tumor (*9, 12, 13*).

Using GB16 and 31E1 as probes for Southern blot hybridization of cloned genomic DNA, we found that the transcription units for these two genes were tightly linked. RNase protection analyses (*14*) determined that the WIT-1 mRNA was transcribed in a telomeric-to-centromeric direction, while transcription of the WIT-2 mRNA was in the opposite direction (Fig. 1) DNA sequencing mapped the 5′ termini of GB16 and 31E1 to a single genomic fragment of <3 kb. As both GB16 (2 kb) and 31E1 (2.7 kb) did not represent full-length cDNA clones, we used RNase protection analysis of the intervening genomic region to identify and map transcribed sequences corresponding to the uncloned 5′ regions of each gene. RNA probes complementary to both strands of genomic DNA were used to show that the most 5′ exons detected for each transcript were nonoverlapping and divergently mapped within approximately 600 bp. Sequence analysis revealed the presence of two putative TATA boxes and a CCAAT box within this intervening region (Fig. 1A) that could potentially function as a bidirectional promoter.

When we examined the sequence of GB16 (Fig. 1B), we found the WIT-1 transcript spanned CpG island 6, which we had identified previously by pulsed-field gel electrophoresis (*8, 15*). Although RNase protection experiments indicated GB16 contained only exon sequences, the largest open reading frame (ORF) discernible within the 2025-bp insert of GB16 was 276 bp (Fig. 1B). Subsequent isolation (from independent libraries) of three shorter cDNA clones

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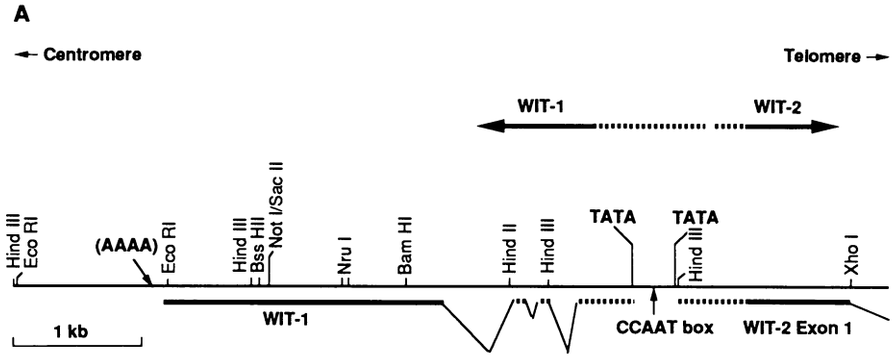
with identical 3′ ends and complete sequence overlap with GB16 confirmed that all these sequences were part of the WIT-1 transcriptional unit. Anchored polymerase chain reaction (PCR) (*16*) from the 3′ terminus of WIT-1 produced several clones with a 3′-terminal polyadenylate [poly(A)] tract, which extended the GB16 sequence by 114 bp. Sequencing of the genomic DNA downstream of GB16 revealed complete sequence agreement up to a series of more than 16 adenine residues. Although the presence of a genomic poly(A) tract and lack of a long ORF suggested WIT-1 might represent a processed pseudogene (*17*) or a human repeat element (*18*), the WIT-1 sequence is not represented in nucleic acid or protein data bases. Thus WIT-1 is a unique transcriptional unit that encompasses CpG island 6.

Since secondary tumors are rare in patients with WT, expression of genes implicated in Wilms tumorigenesis may be kidney-specific. To determine whether this was the case for WIT-1 or WIT-2, we analyzed RNA from different established cell lines and various tissues by Northern blot hybridization. Neither transcript was detectable in polyadenylated [poly(A)$^+$] RNA preparations of HeLa, Daudi, or G401 cells, but very low concentrations of WIT-2 could be detected in transformed human embryonic kidney 293 cells (*19*). Both transcripts were seen in fetal kidney and spleen RNA, but were absent from other fetal tissue RNAs (Fig. 2A). In general, the WIT-1 transcript was much less abundant than WIT-2, but was readily detected in poly(A$^+$) RNA.

The recapitulation of nephrogenesis seen in many WTs led us to examine the expression of WIT-1 and WIT-2 in kidneys from three different age groups on the assumption that developmentally regulated expression might be a feature of genes important for nephrogenesis. In Northern analyses, the two transcripts exhibited similar patterns of developmental expression. Both were most abundant in RNA from fetal kidney, but expressed at much lower levels in 5-year-old and adult kidney (Fig. 2B).

The gene corresponding to WIT-2 has been proposed as a candidate WT gene based on its deletion in WiT-13 (*13*). Confirmation of WIT-2 or WIT-1 as a WT gene, however, requires an indication that expression is altered in at least some tumors. Accordingly, we examined the gross structure and expression of the WIT-1 and WIT-2 genes in 32 cases of sporadic WT by Southern and Northern hybridization of DNA and RNA extracted from frozen tumor tissue. Although no genomic alterations were detected, a significant number of

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CACTAAGCCA GCGCTGGGGA ACTGACGTAC TCCTGCAGTC GCAGGGCGCT CCATGCCTCT CTGTCCTCTT  210
CTTTGTTGTG GGTAACGTTC TCTGCGGGAG ACCTGAGGTT TTCCAAGGGG GACATGCCAG CTACACTGGC  280
CTTGGCGCCC TGAGCTAAGC ACCAGGACTC ACAGCCTAGC ACGAAGGCAG GTAGCACCTT CACCCGCCGC  350
GGACGATAGG CGCTCTCCTG TACCTCCTTT AGCTCGCGAG GCCCGCCCTT CGCGAGGTCC CAGAGAAAAG  420
CAGGCTGTGG AAAACTGGCG CCCCCTTCTT TCACCCACCT TCTTACCCCT GTCAGCGCCG AGATCTGTAG  490
CAGAGGTTCC TGGTCTGAAC CACCGATTGG CAAAGAAAGC TGCAGATTAA ACTTCTCGTT TTACAGAGAA  560
GGAAACTGAG GCCCAGACAG CCGAAGGAGA GGCAGTCTAT GGAGCGCAGC GGTAAAGAGC AAGGGGTTGG  630
TGGCCCCAGA ATGGAACCTC AGCTCTGCCA GGTAACCAAC AGCTGTGTGA CCCTAGACGA GTTCCGCAGT  700
CTCTCTGAGC CTCAGTTTCC TTACTGGTCA AACGGGATAA TGGGATACTA GCGCCCACCT CATAGAGTTG  770
TTCTGAGGAT TAGATAGGAG AGCAGTGTGG GCCTTTGACT CAATCAACTT TACAGTTTTT GTTACTACTA  840
CTGTTTAAGG ACAGGAAACA AGTTAGTGGC AACGCGAGCT GAAACCCGGG TCTCTCAACG TCCAGTTGGA  910
GGTTTTACCC ACCACCCCTC TACCTGTTCA GAGCTAATGG G ATG CAG AGG CGA GGA CAG CCC      972
                                              M  Q  R  R  G  Q  P                7aa
CTG GAA AAC CAT GTG GCG TTG ATA CAC TGG CAA AGC GCA GGC ATC CCG GCC TCG AAG   1029
 L  E  N  H  V  A  L  I  H  W  Q  S  A  G  I  P  A  S  K                    26aa
GTG CAT AAT TAT TGC AAT ATG AAA AAA TCG AGG CTG GGT AGG AGC AGG GCA GTG AGG   1086
 V  H  N  Y  C  N  M  K  K  S  R  L  G  R  S  R  A  V  R                    45aa
ATT TCT CAA CCC TTA CTT TCA CCC CGG CGC TGT CCA CTG CAT CTG ACA GAG CGC GGA   1143
 I  S  Q  P  L  L  S  P  R  R  C  P  L  H  L  T  E  R  G                    64aa
GCT GGG CTG CTA CAG CCG CAA CCC CAG GGA CCA GTG CGC ACG CCT GGG CCG CCC TCC   1200
 A  G  L  L  Q  P  Q  P  Q  G  P  V  R  T  P  G  P  P  S                    83aa
GGG AGT CAC CCA GCG GCC GCG GAC AAC TGA      ATAAACACC CCAAAGCGCT GCGGTCGGTC   1259
 G  S  H  P  A  A  A  D  N  End                                              93aa
AAGGGCGGGG ACAGCCACAG TGCGCGCGGG GCCCGCAGGC CGTAATAAAG AGTGGCTCGA CCTCGCTGCT  1329
GCCTGTCGCG GGAGAGGAAC AAGCTTTCGA CTAGCGCCTC TCCCCGGGGC CCGCGCCCCG AGCCCCACGC  1399
GCAAGACAGC CCAACAGCTG TTCCTCCCCT CCCCGCCGAC TCCAACTCTT CGGAATCTGC CCACTCGGGG  1469
GCTGCAGGGC AAGTGTTTAG GATGGTTCCC AGCCCCGCGC TGCGCGGTGA AAATTTCAAC GTCATTCCTT  1539
CAATTAAAAA AAGGGGGGGG CAAGGGAGGG GCTTTGTGAT AACTACTCCC AGCTTCTTCT GATCATTTCA  1609
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CGCTGGGGTT ACTCGTCTGT TCTGATGCCA CCGCGAGATG GTCCCCGAGC TCCCCGAGAG TCCTCAGTGA  1819
AAGGATTCCG CGGCACTGCC TCTATTATTA TACCGTAAAT CTTTTTAAAT TCTGGAACTA ATTATATAGA  1889
GGATATGTCT CAATTTGTTC TGCATTAATG CCACAGTGGG GATGGAGGCC AGGCCGTGGC CAGAGCAGAT  1959
ACGTAGGCCC ATGAAATTGA TGAACTGAGA GTTGTCTTCC AGTCCTGAGC GCACCATCTG GAATTCCAGT  2029
CTGAGGTTAC AATTAGAACT CCTGACCCCA GATTCAACTT TGTAAACAAC AGGGGAAAAA AATGGGGAAA  2099
AGAAAAACCA CGAGCGAGGA AAAAAATTAT CTGCACCTGT                                   2139
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Fig. 1. (**A**) WIT-1 and WIT-2 are divergent transcripts. A detailed restriction map of the 6-kb genomic region encompassing all of WIT-1, the first exon of WIT-2, and the putative bidirectional promoter from which WIT-1 and WIT-2 are divergently transcribed (diverging arrows above the restriction map) is schematically diagrammed. The location of two TATA boxes and an intervening CCAAT box (as detected by DNA sequencing) within the putative promoter region is indicated. The AAAA indicates the position of a stretch of adenine residues on genomic sequence to which the 3′ end of WIT-1 maps. The spiked lines denote the location of intron sequences as determined by RNase protection and DNA sequencing. The broken lines below the restriction map mark the location of the terminal 5′ region of the WIT-1 and WIT-2 transcriptional units as predicted from RNase protection data. (**B**) Nucleotide sequence of overlapping cDNAs corresponding to the WIT-1 transcript. Positions 1 to 2025 represent the longest cDNA clone obtained (GB16). The sequence 3′ to position 2025 was obtained from PCR extension clones. Nucleotide 2139 is followed by a poly(A) tract. The deduced amino acid sequence of the longest ORF (position 951 to 1227) is indicated (GenBank accession number M37983).

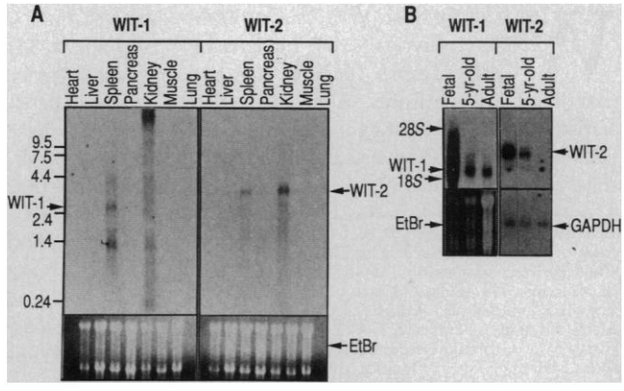

Fig. 2. Northern blot analysis of WIT-1 and WIT-2 expression in normal tissues. Total RNA (10 μg) isolated from frozen tissues (*28*) were separated by electrophoresis on a 1% agarose-formaldehyde gel, transferred onto nylon Gene Screen membranes, and hybridized with GB16 or 31E1 DNA probes radiolabeled by random priming. Hybridizations were carried out at 65°C in 1% bovine serum albumin, 0.5 M sodium phosphate, 1 mM EDTA, and 7% SDS (*29*). When detection of low amounts of transcript was required, 1 to 5 μg of poly(A)$^+$ RNA was used. The positions of hybridizing bands in relation to RNA markers are indicated. (**A**) The hybridization of cDNA clones GB16 and 31E1 to transcripts of 2.5 kb and 3.5 kb in select fetal tissues is shown. The WIT-1 and WIT-2 blots were exposed for 8 and 3 days, respectively. (**B**) Decreasing amounts of WIT-1 and WIT-2 transcripts in poly(A)$^+$ RNA of fetal, 5-year-old, and adult kidneys is shown. In the left panel, ethidium bromide fluorescence of the RNA gel indicates overloading (>5 μg) of 5-year-old and adult kidney RNA. In the right panel, less than 1 μg of poly(A)$^+$ RNA was loaded; hence, hybridization to glyceraldehyde phosphate dehydrogenase [GAPDH (*30*)] was used to monitor RNA loading. In this hybridization, the WIT-2 transcript was detected in adult kidney RNA upon prolonged autoradiographic exposure.

tumors exhibited striking differences in the expression of WIT-1 and WIT-2. From a total of 32 tumors, 16 showed no detectable WIT-1 transcript (*20*). For WIT-2, a varied range of expression was evident, as shown in Fig. 3. In total, 14 tumors expressed WIT-2 at amounts comparable to those seen in normal fetal kidney RNA, whereas 7 tumors showed reduced amounts of WIT-2 mRNA. In 11 tumor samples, no WIT-2 transcript was detectable. In most of the WT samples we analyzed, the presence of WIT-1 transcripts correlated with the presence of WIT-2 transcripts. In other words, WIT-1 transcripts were present in tumor samples with significant concentrations of WIT-2 mRNA and absent in samples with little or no WIT-2 (Fig. 3). Exceptions to this coordinate pattern of WIT-1 and WIT-2 expression were seen in four tumor samples, including WiT-38 (shown in Fig. 3).

The analysis of tumor RNA concentrations together with the tissue and developmental regulation of WIT-1 and WIT-2 expression suggest that theses genes are coordinately regulated during normal kidney development and tumorigenesis. Beckwith and co-workers have found an association between the 11p13-associated WAGR syndrome and the occurrence of WT with heterologous elements, such as skeletal muscle not normally found in embryonic kidney (*7*). These tumors are associated with potential precursor lesions termed intralobar nephrogenic rests (ILNR). In contrast, tumors without heterologous elements are frequently associated with perilobar nephrogenic rests (PLNR), lesions that are also seen in patients with the Beckwith-Wiedemann syndrome. This syndrome has been mapped to chromosome 11p15 (*6*). Hence, only a subset of WT with specific histology would be predicted to show aberrant expression of the WT genes on 11p13. Prompted by this prediction, we performed a double-blind analysis comparing the expression data described above with a histopathological classification of the 32 tumors (*21*). We found the differences in expression of WIT-1 and WIT-2 in WT directly reflect differences in the histopathological phenotypes of these tumors. Of 12 WT classified as histopathologically heterogeneous (designated het in Fig. 3), 11 exhibited no or low expression of WIT-2 by Northern analysis. In contrast, of 14 WTs classified as histopathologically homogeneous (designated hom in Fig. 3), only 4 showed reduced amounts of WIT-2 expression. Thus, sporadic tumors with an 11p13-associated histopathological phenotype also exhibit aberrant expression of WIT-2.

Divergent transcription initiating from DNA sequences spanning 3 kb or less has been described for a number of genes (*22–25*). The close physical location of the 5′ most exons detected for WIT-1 and WIT-2 and their very similar patterns of expression suggest they are transcribed divergently from a common promoter, or at the very least share some upstream regulatory sequences. The WIT-1 sequence is reminescent of transcribed elements upstream of the proto-oncogenes, c-*mos* and c-H-*ras*, and the epsilon globin gene (*23–25*). It has been shown that differential expression of some of these elements are vital in regulating the expression of their associated genes in specific cellular and developmental contexts (*23, 24*). Thus, deregulated expression of WIT-2 could potentially result from mutations in either WIT-1 or WIT-2. Unlike the WiT-13 tumor, none of the tumors analyzed here showed any gross DNA rearrangements (including those that expressed little or no WIT-2 transcript). However, the majority of tumors may carry mutations in other WT genes or subtle alterations that would not be detected by Southern analysis. A more sensitive analysis, such as PCR amplification, will be necessary to detect small deletions or point mutations within the WIT-1 or WIT-2 genes. A more intriguing possibility to explain deregulated expression arises from our observation that the WIT-1 gene sequence spans a CpG island. These islands have been shown to bind constitutively expressed cellular factors when they are methylated at CpG (*26*). Because of data that suggest a role for genomic imprinting in Wilms tumorigenesis (*27*), it is tempting to speculate that methylation may have a central role in regulating both WIT-1 and WIT-2 gene expression.

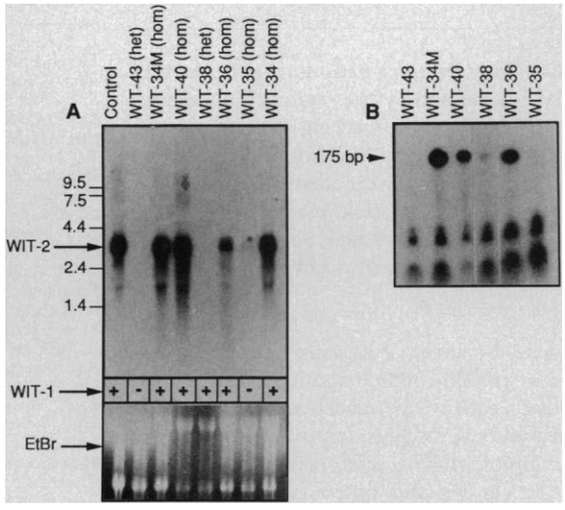

Fig. 3. (**A**) Northern blot analysis of total RNA from WT tissue. Variable expression of a normal-sized 3.5-kb WIT-2 transcript in different WT tissue is shown in this representative Northern blot. Comparable amounts of WIT-2 are seen in tumors 34, 40, a metastasis from 34 (34M), and the normal fetal kidney sample. Tumors 43 and 38 show no detectable amounts of WIT-2. The blot was overexposed to detect the signal in tumor 35. An ethidium bromide fluorescence stain of the corresponding RNA gel is shown. The expression of WIT-1 in the same tumor samples (separate experiment) is also indicated (+/−). The histopathological classification of the tumor is indicated as heterologous (het) or homologous (hom). For this analysis, tumor tissues were collected and stored as described (*31*). RNA extraction and Northern hybridization were performed as in Fig. 1A, using a 1.8-kb fragment of 31E1 as a probe for WIT-2. (**B**) RNase protection analysis of total RNA from WT tissue. As the WIT-1 transcript was not readily detected by Northern hybridization of total RNA from WT samples, RNase protection (*14*) was also performed on 10 μg of total RNA with a 175-bp probe representing nucleotides 1 to 175 of WIT-1 (Fig. 1B). Presence of the protected fragment of WIT-1 is indicated.

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11. Probes AvH1 and E9 were used to screen a total of 2.5×10^6 clones from a human fetal kidney cDNA library. From this initial screen, a single clone GB16 was isolated with AvH1, whereas E9 yielded seven overlapping clones. GB16 and 31E1 (the longest cDNA clone isolated with E9) hybridized to DNA from normal human lymphoblast and an 11p-specific somatic cell hybrid J1-11, but not to DNA from the WT cell line WiT-13. Sequencing of genomic DNA corresponding to all of GB16 and 800 bp of 31E1 (including eight intron-exon boundaries) confirmed that both the WIT-1 and WIT-2 transcripts and their corresponding cDNA clones were derived from the 11p13 region deleted in WiT-13.
12. 31E1 has a complete poly(A)$^+$ terminus and an incomplete 5′ terminus, which is 361 bp longer than the reported WT33 cDNA (*13*), but is 272 bp shorter than the reported LK15 cDNA (*9*). A stretch of 51 nucleotides present in LK15 (*9*) but absent from the WT33 cDNA sequence is also present in 31E1. In vitro translation of RNA synthesized from the 31E1 cDNA results in a protein of

about 50 kD. Thus, the 51-nucleotide stretch common to 31E1 and LK15 is not a cloning artifact. The isolation of WT33 from a pre-B cell cDNA library and the origin of LK15 and 31E1 from separate fetal kidney cDNA libraries suggest that the WIT-2 gene may be alternatively spliced in different tissues.
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31. Tissues were processed for molecular analysis as follows. Whole fetal kidneys and samples of other fetal organs were resected from 12- to 20-week-old fetuses and snap frozen and stored in liquid nitrogen or at −70°C. The 5-year-old and adult transplant donor kidneys, previously perfused with Ringer's lactate solution and kept on ice, were dissected, and slices of cortex were snap frozen in liquid nitrogen and stored. Tumor tissues and adjacent areas of normal kidney were obtained within 1 to 2 hours after nephrectomy, and snap frozen in liquid nitrogen with or without prior embedding in O.C.T. compound (Tissue-Tek, Miles Scientific, Naperoille, IL). Alternatively, tumor tissue was resuspended as a coarse mince in cryoprotectant [10% dimethyl sulfoxide (DMSO), 20 to 50% fetal bovine serum, α-minimum essential medium (MEM)], frozen first at −70°C, and subsequently transferred to liquid nitrogen storage.
32. We thank R. Worton for advice and encouragement; R. Weksberg, A. R. Reeve, and R. J. Wilkins for helpful discussions; R. McInnes and G. Bell for cDNA libraries; and members of the B.R.G.W. laboratory for support and encouragement. This work was supported by a grant from the National Cancer Institute of Canada (NCIC) to B.R.G.W. and H.Y., and a March of Dimes Birth Defects Foundation grant to A.P.F. A.H. was the recipient of a Steve Fonyo NCIC studentship, C.E.C. a Medical Research Council of Canada Scholarship, L.B. an Ontario Graduate Studentship, and M.J.C. an award by the Ter Meulen Fund, The Royal Netherlands Academy of Arts and Sciences.

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Wilms Tumor Locus on 11p13 Defined by Multiple CpG Island-Associated Transcripts

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Wilms tumor is an embryonal kidney tumor involving complex pathology and genetics. The Wilms tumor locus on chromosome 11p13 is defined by the region of overlap of constitutional and tumor-associated deletions. Chromosome walking and yeast artificial chromosome (YAC) cloning were used to clone and map 850 kilobases of DNA. Nine CpG islands, constituting a "CpG island archipelago," were identified, including three islands that were not apparent by conventional pulsed-field mapping, and thus were at least partially methylated. Three distinct transcriptional units were found closely associated with a CpG island within the boundaries of a homozygous DNA deletion in a Wilms tumor.

THE DISTAL HALF OF CHROMOSOMAL band 11p13 has attracted considerable interest as a target for positional cloning because patients with 11p13 deletions develop four abnormalities comprising the WAGR syndrome: Wilms tumor (WT), an embryonal malignancy of the kidney; aniridia, or hypoplasia of the iris; genitourinary dysplasia, including kidney and genital malformations; and mental retardation (*1*). Sporadic, non-WAGR Wilms tumor affects that a gene isolated from 11p13 is the WT gene, by virtue of its location, although its 1 in 10,000 children and represents a putative tumor-suppressor model fulfilling Knudson's two-hit hypothesis, the paradigm of which is retinoblastoma (*2*). However, as we and others have shown, the etiology of Wilms tumor is complex and involves an additional locus at 11p15, and in familial cases a locus on another chromosome (*3*). Two laboratories have proposed expression was unaltered in Wilms tumors (*4, 5*). In this and the accompanying report (*6*), we describe the presence and location of multiple transcribed sequences from this region, including two that show altered expression in some Wilms tumors.

Our starting point in these efforts was S1 (D11S37), a random DNA segment within a region homozygously deleted in WiT-13, a sporadically occurring Wilms tumor (*7*). We had previously set the upper size limit of the WiT-13 deletion at 375 kb, on the basis of mapping of random clones isolated from chromosome- or band-specific libraries (*8*). To define the boundaries of the WiT-13 deletion and to identify regions for more intensive screening for the genitourinary and mental retardation genes, which have been mapped close to WT (*9*), we used yeast artificial chromosome (YAC) cloning, thereby generating a complete physical map of the region, unhampered by DNA methylation of genomic human DNA.

To obtain YACs with human genomic DNA inserts from this region, we synthesized oligonucleotides on the basis of the DNA sequence of S1, and used these as primers to screen a human YAC library (*10*) by polymerase chain reaction (PCR) and filter hybridization (*11*). Southern (DNA) blot hybridization confirmed that two clones, designated yF12 and yG6, included S1 in their sequence, while only yG6 included probe AvH1, which had been isolated by genomic walking and was located 65 kb telomeric from S1. This indicated that the two YACs have only a small region of overlap and thus span a relatively large region of DNA. The YAC clones were mapped by pulsed-field gel electrophoresis (PFGE), by means of partial digestion conditions with a set of eight rare-cutting re-

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