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Structure of the Wilms Tumor Suppressor Protein Zinc Finger Domain Bound to DNA

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The zinc finger domain of the Wilms tumor suppressor protein (WT1) contains four canonical Cys_2His_2 zinc fingers. WT1 binds preferentially to DNA sequences that are closely related to the EGR-1 consensus site. We report the structure determination by both X-ray crystallography and NMR spectroscopy of the WT1 zinc finger domain in complex with DNA. The X-ray structure was determined for the complex with a cognate 14 base-pair oligonucleotide, and composite X-ray/NMR structures were determined for complexes with both the 14 base-pair and an extended 17 base-pair DNA. This combined approach allowed unambiguous determination of the position of the first zinc finger, which is influenced by lattice contacts in the crystal structure. The crystal structure shows the second, third and fourth zinc finger domains inserted deep into the major groove of the DNA where they make base-specific interactions. The DNA duplex is distorted in the vicinity of the first zinc finger, with a cytidine twisted and tilted out of the base stack to pack against finger 1 and the tip of finger 2. By contrast, the composite X-ray/NMR structures show that finger 1 continues to follow the major groove in the solution complexes. However, the orientation of the helix is non-canonical, and the fingertip and the N terminus of the helix project out of the major groove; as a consequence, the zinc finger side-chains that are commonly involved in base recognition make no contact with the DNA. We conclude that finger 1 helps to anchor WT1 to the DNA by amplifying the binding affinity although it does not contribute significantly to binding specificity. The structures provide molecular level insights into the potential consequences of mutations in zinc fingers 2 and 3 that are associated with Denys-Drash syndrome and nephritic syndrome. The mutations are of two types, and either destabilize the zinc finger structure or replace key base contact residues.

Keywords: DNA binding; nucleic acid recognition; X-ray crystallography; NMR; residual dipolar coupling

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Abbreviations used: WT, Wilms tumor; WT1, Wilms tumor suppressor protein; zf, zinc finger; NOE, nuclear Overhauser enhancement; NOESY, NOE spectroscopy; HSQC, heteronuclear single quantum coherence; DDS, Denys-Drash syndrome; RDC, residual dipolar coupling.

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Introduction

Wilms tumor (WT) or nephroblastoma is a pediatric kidney cancer that was first described by Max Wilms in 1899.[1] It is one of the most frequently occurring solid tumors of childhood, affecting about 1 in 10,000 children, typically between ages two and four years.[1] WT serves as a paradigm for understanding the relationship between loss of developmental control and gain of tumorigenic potential.[2] In particular, loss of function of tumor suppressor genes has been implicated in the development of WT; the Wilms tumor suppressor gene *WT1* on chromosome 11p13 was the second tumor suppressor gene to be cloned after the retinoblastoma gene RB-1.

The Wilms tumor suppressor gene encodes a DNA-binding protein containing four Cys_2His_2 zinc fingers.[3,4] The Cys_2His_2 zinc finger (zf) motif is employed by a diverse array of transcription factors that play an important role in cellular signal transduction. Over 1000 copies of this motif have been identified in vertebrate genomes, making it one of the predominant mediators of sequence-specific protein–DNA interactions. In addition to its C-terminal DNA-binding domain, the Wilms tumor protein also contains a proline/glutamine-rich N terminus, activation and repression domains, nuclear localization signals, and at least two self-association domains.[5–8]

The cellular expression pattern of *WT1* is tissue-specific and also depends on the growth stage of the organism. WT1 is essential for normal urogenital development, and mutations in the *WT1* gene have been associated with diseases, such as Denys-Drash,[9] WAGR,[10] and Frasier[11] syndromes. A combination of alternative splicing, alternative translation start sites, and RNA editing leads to the expression of at least 24 different isoforms that share four C-terminal zinc fingers and an N-terminal proline/glutamine-rich regulatory region.[12,13] Of particular interest are two alternative splice sites at the end of exon 9 that lead to insertion of three amino acids (KTS) after the glycine in the canonical TGEKP linker sequence between zinc fingers 3 and 4.[13] This alternative splice site is highly conserved during evolution and is found in all vertebrates. The relative abundance of these splice forms is constant; developmental abnormalities are associated with altered ratios of +KTS and −KTS isoforms.[13–15] Mounting evidence indicates that +KTS and −KTS isoforms perform distinct biological functions and differ in their nucleic acid binding properties. The −KTS isoform binds DNA sequence-specifically and appears to function primarily in transcriptional regulation; more than 30 putative target genes have been identified, the majority of which contain the EGR-1 consensus site in their promoters.[1,16] The more abundant +KTS splice variant binds RNA, associates preferentially with components of the pre-mRNA splicing machinery and appears to function in post-transcriptional regulatory processes.[12,13,17,18] NMR chemical shift mapping[19] and relaxation experiments[20] show that insertion of the KTS sequence modulates DNA binding through isoform-specific DNA-induced conformational changes, and leads to increased linker flexibility and loss of DNA binding by zf4, hence providing a molecular basis for the differential affinity of +KTS and −KTS variants for DNA.[20]

The amino acid sequences of the Wilms tumor zinc fingers are homologous to those of other well-characterized zinc finger domains (Figure 1(a)). The Cys and His ligands are invariant, and the core hydrophobic residues and linker motifs are all highly conserved. The linkers perform a "snap-lock" helix-capping function to stabilize the complexes of these multi-finger proteins with DNA.[21] Fingers 2–4 of WT1 are closest in sequence (65% identity) to fingers 1–3 of zif268 (EGR-1); although finger 1 of WT1 contains the canonical zinc finger sequence motif (Ar-X-C-$X_{2\text{-}4}$-C-X_3-Ar-X_5-L-X_2-H-$X_{3\text{-}4}$-H), it has a lower homology with the other WT1 or Zif268 fingers in the characteristic base recognition motif at the tip of the finger (Figure 1(a)).

Initial experiments to identify WT1 binding sites showed that the protein preferentially binds to DNA sequences related to the EGR-1 consensus binding site 5′-GCG-(T/G)GG-GCG-3′.[22,23] Subsequent binding site selection studies have demonstrated that the highest affinity DNA binding site for WT1 has a consensus sequence of 5′-GCG-(T/G)GG-GAG-(T/G)(T/G/A)(T/G)-3′.[23–27] The high affinity binding sequence has been identified in the promoters of two WT1-responsive genes.[16] Sequence-specific DNA recognition is mediated by fingers 2–4. Finger 1 plays only a secondary role in DNA binding; it contributes only modestly to binding affinity and specificity and does not significantly protect the DNA in footprinting experiments.[24,25,27,28] Deletion of finger 1 results in only a twofold to fivefold decrease in binding affinity, depending on the nucleic acid sequence.[27] A systematic analysis of mutant DNA consensus sequences indicates specific recognition of most base-pairs, with the wild-type sequences showing the highest affinity for WT1–KTS.[26]

In order to elucidate the structural basis for differences in DNA binding between the three-finger Zif268 and the four-finger WT1 proteins and to obtain insights into the structural basis by which Denys-Drash and other disease-associated mutations affect DNA binding, we determined the structure of the C-terminal zinc finger domain of the WT1–KTS splice form bound to 14 bp and 17 bp DNA oligonucleotides containing the consensus EGR-1 binding site (Figure 1(b)), the binding site found in the majority of target gene promoters. The shorter 14 bp oligonucleotide was previously used in NMR dynamics studies.[19] The structure of the complex of zf1-4 with the 14 bp DNA duplex obtained using X-ray crystallography shows that zinc fingers 2–4 make base-specific contacts in the