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The Krüppel-associated Box (KRAB)-Zinc Finger Protein Kid-1 and the Wilms' Tumor Protein WT1, Two Transcriptional Repressor Proteins, Bind to Heteroduplex DNA*

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Zinc finger proteins of the Cys_2His_2 class represent a large group of DNA-binding proteins. A major subfamily of those proteins, the Krüppel-associated box (KRAB) domain-containing Cys_2His_2-zinc finger proteins, have been described as potent transcriptional repressors. So far, however, no DNA-binding sites for KRAB domain-containing zinc finger proteins have been isolated. Using a polymerase chain reaction-based selection strategy with double- and single-stranded DNA, we failed to reveal a binding site for Kid-1, one member of KRAB-zinc finger proteins. Binding of Kid-1 both to single- and homoduplex double-stranded DNA was negligible. We now present evidence that Kid-1 binds to heteroduplex DNA. Similar to Kid-1, the non-KRAB-zinc finger protein WT1 also bound avidly to heteroduplex DNA (both the −KTS and +KTS splice variant of WT1), whereas the POU domain protein Oct-6, the ets domain protein Ets-1 and the RING finger of BRCA-1 did not bind to heteroduplex DNA. Binding of WT1 to heteroduplex DNA was markedly reduced in naturally occurring mutants. The recognition of certain DNA structures by transcriptional repressor proteins may therefore represent a more common phenomenon than previously thought.

DNA-binding proteins are essential for the utilization of genetic information. They take part in the replication of the genome, in transcription of DNA into mRNA and in the repair of the genome. In these processes the interaction with DNA takes place in a variety of ways. Interaction with sequence-specific DNA is a common and well established phenomenon. There are many examples of proteins binding to a specific sequence of double-stranded DNA. In contrast, sequence-specific interaction with single-stranded DNA seems to occur more rarely. An example for such an interaction would be YB-1, a member of the Y box family of transcription factors, which binds to a region of single-stranded DNA spanning the X box of the major histocompatibility class II DRA promotor (1).

Interaction independent of a specific sequence is another possibility, but so far only a few sequence-independent, structural motifs have been described as binding sites. Poly(ADP-ribose) polymerase and DNA-dependent protein kinase are essential elements of the cellular response to DNA damage (for reviews see, Refs. 2 and 3). Whereas poly(ADP-ribose) polymerase binds to DNA containing single-strand breaks, DNP-PK binds to double-strand DNA breaks, and both do so independently of the DNA sequence. In the case of single-strand DNA breaks, the DNA molecule assumes a V-shaped conformation, which is recognized by poly(ADP-ribose) polymerase. Other examples of protein/DNA interaction based on structural characteristics of the DNA molecule can be found in the recognition of cruciform DNA by the high-mobility group protein HMG-1 (4), the tumor suppressor protein p53 (5), and the transcriptional regulator complex SWI-SNF (6).

Transcription factors as a subclass of DNA-binding proteins play an important role during growth and differentiation of cells and organs. Several families of transcription factors have been described, one of which is the family of zinc finger proteins (for recent reviews, see Refs. 7 and 8). So far, however, binding sites have been characterized for only a subset of zinc finger proteins. "Classical" zinc finger proteins like Sp1, Egr-1, and WT1 have been shown to recognize sequence-specific double-stranded DNA (*e.g.* Ref. 9). One large subfamily of zinc finger proteins is characterized by the presence of a highly conserved motif, the so-called KRAB-A domain or FBP (10, 11), which has been identified as a potent transcriptional repressor domain (12–15). Because the KRAB-A domain is present in approximately one-third of the large number of Cis_2His_2-zinc finger proteins (10) (there are several hundred genes coding for those proteins in the mammalian genome (16)), it represents a very important paradigm of transcriptional repression. The identification of the KRAB-A domain as a transcriptional repressor motif was soon followed by the cloning of proteins that interact with the KRAB-A domain (17–19), but up to this point no DNA-binding site for a KRAB-zinc finger protein could be identified. Only in the case of the human KRAB-zinc finger protein ZNF74 has an affinity for RNA homopolymers been described (20).

Kid-1 is a 66-kDa protein with 13 Cis_2His_2-zinc fingers at its COOH terminus. The zinc fingers are clustered in two groups of four and nine zinc fingers and are separated by a 32-amino acid spacer (21). The Kid-1 protein also contains a KRAB-A domain at its NH_2 terminus. Here we demonstrate binding of the zinc finger region of Kid-1 to heteroduplex DNA. Another transcriptional repressor protein, the Wilms' tumor protein WT1, also binds to heteroduplex DNA. This function is impaired in naturally occurring mutants of WT1.

MATERIALS AND METHODS

Expression and Purification of Proteins in Escherichia coli—In the case of Kid-1 and WT1, recombinant plasmids encoding fusion proteins with glutathione *S*-transferase were constructed using the plasmid pGEX-KG (22). Details are available from the authors upon request.

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The recombinant proteins were purified from bacterial extracts according to standard protocols (23).

Oligonucleotides—The binding site selections were carried out using the random oligonucleotide 5′-CTT GAT CCT AAG ATT CCC TG-N_{15}-AGG CTC AAA GCT GAA TTA CT-3′ and the PCR[1] primers 5′-CTT GAT CCT AAG ATT CCC TG-3′ and 5′-AGT AAT TCA GCT TTG AGC CT -3′, the random oligonucleotide 5′-CTT GAT CCT AAG ATT CCC TG-N_{35}-AGG CTC AAA GCT GAA TTA CT-3′ and the same PCR primers mentioned before, and the random oligonucleotide 5′-GAT CCT AAG ATT CCC TGT CGA C-N_{75}-GTC GAC TCC CTT TAG TGA GGG TT-3′ and the PCR primers 5′-CTT GAT CCT AAG ATT CCC TG-3′ and 5′-ATT AAC CCT CAC TAA AGG GA-3′. Heteroduplex DNA with a bubble of 25 nucleotides consisted either of the oligonucleotides ss1 5′-CCT CAC TAA AGG GAG TCG AC***T ACC TTT GAA ATC GAA AGG CGC CTC*** GGA TCC AGG GAA TCT TAG GA-3′ and ss2 5′-TCC TAA GAT TCC CTG GAT CC***T CTT ATA AGG GAT CGG GAC CCT TGC*** GTC GAC TCC CTT TAG TGA GG-3′ or of oligonucleotides ss3 5′-AAG ACT GAG TTC ACC GGA GC***A TTA TCC CCG TTG CGT AAC CGA GAA*** GCT TTA CGC AAG TGG AAG AC-3′ and ss4 5′-GTC TTC CAC TTG CGT AAA GC***G GAG ATT GGC ATA CCA TTT TCG CCG*** GCT CCG GTG AAC TCA GTC TT-3′ (noncomplementary nucleotides are bold and italic). When a homoduplex of the same length was used, oligonucleotide ss1 was substituted with an oligonucleotide fully complementary to oligonucleotide ss2. The structure of heteroduplex ss1/ss2 was corroborated by digests with restriction enzymes cutting either in the double-stranded region or in the bubble. *Ava*II recognizes a site in the bubble region of ss2 and in the homoduplex (5′-GGACC-3′), this site is cut only in the homoduplex. *Sal*I (5′-GTCGAC-3′) and *Bam*HI (5′-GGATCC-3′) recognize sites in the double-stranded region of the heteroduplex immediately adjacent to the bubble, these enzymes are able to cut the heteroduplex DNA, although less efficiently than the homoduplex (the lower efficiency probably results from the close location to the bubble). The heteroduplex with a bubble of 7 nucleotides consisted of the oligonucleotides ss5 5′-CCT CAC TAA AGG GAG TCG AC***T ACC TTT*** GGA TCC AGG GAA TCT TAG GA-3′ and ss6 5′-TCC TAA GAT TCC CTG GAT CC***C CCT TGC*** GTC GAC TCC CTT TAG TGA GG-3′ (noncomplementary nucleotides are bold and italic). The oligonucleotide with the oct-binding site had the sequence 5′-CTG AGC AAA ACA CCA CCT GGG TA***A TTT GCA T***TT CTA AAA TAA G-3′ (the octamer motif is bold and italic), the oligonucleotide with the ets-binding site had the sequence 5′-TGA ATA TTT TGT AA***T TTC CT***A GTC TTG-3′ (the ets-binding site is bold and italic), and the oligonucleotide with the Egr-1/WT1-binding site had the sequence 5′-CGC CCT ***CGC CCC CGC*** GCC GGG-3′ (the Egr-1/WT1-binding site is bold and italic); the oligonucleotides containing the octamer motif, the ets-binding site and the Egr-1/WT1-binding site were regular homoduplex DNA. Homoduplex and heteroduplex DNA were prepared by mixing equimolar amounts of both strands, heating to 80–85 °C in a water bath, and subsequent slow cooling to room temperature.

Binding Site Selection—The selection was carried out according to already published protocols with minor modifications (24). Approximately 1 μg of purified protein and 100–200 ng of selected and PCR-amplified oligonucleotide were used for each round of selection. To produce single-stranded oligonucleotide, a second PCR reaction was run with the reverse primer only after having precipitated the double-stranded PCR product with ethanol and ammonium acetate.

Gel Shift Assay—Gel shifts were performed essentially as described previously (24). When competition experiments were performed, a 5-, 25-, and 125-fold molar excess of unlabeled oligonucleotide was added to the protein together with the labeled binding site. When the zinc finger structure was to be disrupted, a combination of 10 mM EDTA, pH 8.0, and 0.1 mM 1,10-phenanthroline (final concentrations) was added to the binding buffer. The 1,10-phenanthroline was added from a 20 mM stock in ethanol; as a negative control, an equal amount of ethanol was added to the binding buffer.

RESULTS AND DISCUSSION

The KRAB-Zinc Finger Protein Kid-1 Binds to Heteroduplex DNA—To define a binding site for the KRAB-zinc finger protein Kid-1, we performed a PCR-based selection strategy with double-stranded DNA and various portions of the Kid-1 zinc finger domain. Binding site selections with the four-zinc finger cluster and a 15-bp random oligonucleotide, with the nine-zinc finger cluster and a 35-bp random oligonucleotide, and finally with all 13 zinc fingers of Kid-1 and a 75-bp random oligonucleotide did not result in the isolation of a binding site for any of the zinc finger constructs. We therefore also initiated a binding site selection with single-stranded DNA and the 13-zinc finger construct of Kid-1. Although we noticed a weak but consistent shift throughout the selection process, we were unable to define a consensus sequence. The observation of a weak shift even with nonselected single-stranded DNA prompted us to try a gel shift with heteroduplex DNA. Heteroduplex DNA occurs as a result of "bubble formation" during transcription of DNA into RNA, when the double-stranded DNA is melted so that RNA polymerase II can read the template strand, and may therefore serve as a natural substrate for transcriptional repressor proteins. A heteroduplex DNA with 20 paired nucleotides at the 5′- and 3′-end and a bubble of 25 nucleotides in the middle was designed randomly, consisting of single-stranded oligonucleotides ss1 and ss2. Care was taken that the nucleotides facing each other in the bubble consisted of pyrimidine·pyrimidine and purine·purine base pairs, to avoid non-Watson/Crick base pairs. Such a heteroduplex DNA was shifted efficiently by a fusion protein between the 13 zinc fingers of Kid-1 and glutathione *S*-transferase (GST), whereas either single-stranded oligonucleotide was only barely shifted (Fig. 1*A*). When the 13-zinc finger construct was incubated with homoduplex DNA consisting of the single-stranded oligonucleotide ss2 used in the heteroduplex DNA, and another fully complementary single-stranded oligonucleotide, no shift could be detected (Fig. 1*B*). A heteroduplex DNA with the same paired sequences at either end but only a bubble of 7 nucleotides in the middle could not be shifted as efficiently as the heteroduplex DNA with a bubble of 25 nucleotides (Fig. 1*B*). Glutathione *S*-transferase could not shift any of the DNA molecules just described (Fig. 1, *A* and *B*).

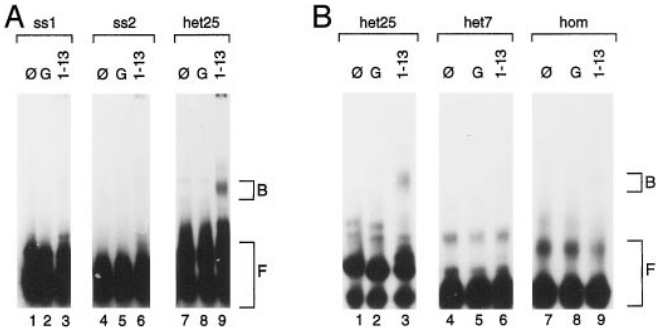

FIG. 1. *A*, a fusion protein consisting of GST and the 13 zinc fingers of Kid-1 binds to heteroduplex DNA, but only inefficiently to single-stranded DNA. Whereas two single-stranded oligonucleotides of 65 nucleotides in length (*ss1, ss2*) are not recognized by the fusion protein (*lanes 3* and *6*), the heteroduplex DNA formed between these two single-stranded oligonucleotides (*het25*) is shifted (*lane 9*). GST alone does not shift any of the DNAs tested (*lanes 2, 5*, and *8*). *B*, a fusion protein consisting of GST and the 13 zinc fingers of Kid-1 binds to heteroduplex DNA with a 25-nucleotide bubble, but not to heteroduplex DNA with a bubble of 7 nucleotides or homoduplex DNA. Homoduplex DNA of 65 nucleotides in length (*hom*) consisting of single-stranded oligonucleotide ss2 and a fully complementary oligonucleotide, as well as a heteroduplex oligonucleotide with a 7-nucleotide bubble (*het7*), are not shifted by the Kid-1 13-zinc finger domain (*lanes 6* and *9*). GST again does not bind to any of the DNA molecules (*lanes 2, 5*, and *8*). *F*, free DNA; *B*, bound DNA; *ø*, no protein added; *G*, GST; *1–13*, fusion protein between GST and the 13 zinc fingers of Kid-1; *ss1* and *ss2*, single-stranded oligonucleotides 1 and 2, respectively; *het25* and *het7*, heteroduplex DNA with a 25- and 7-nucleotide bubble, respectively; *hom*, homoduplex DNA consisting of ss2 and a fully complementary oligonucleotide.

To further demonstrate the specificity of the interaction be-

[1] The abbreviations used are: PCR, polymerase chain reaction; bp, base pair(s); GST, glutathione *S*-transferase; KRAB, Krüppel-associated box; WT1, Wilms' tumor protein 1.

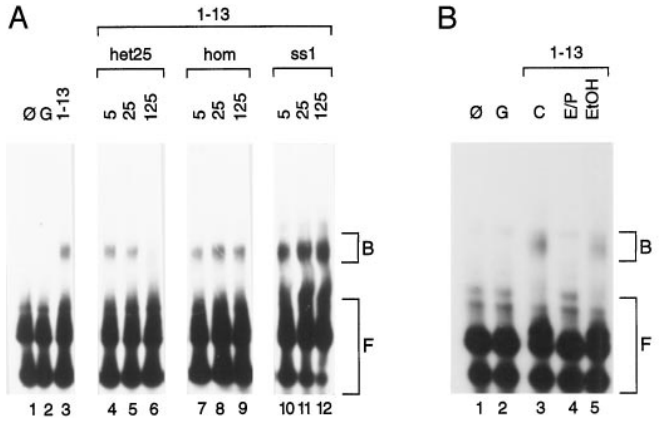

FIG. 2. *A*, the gel shift between radiolabeled heteroduplex DNA and the 13 zinc fingers of Kid-1 can be disrupted by an excess of unlabeled heteroduplex DNA, but not homoduplex or single-stranded DNA. Gel shift experiments were performed with radiolabeled heteroduplex DNA ss1/ss2 (25-nucleotide bubble) and the fusion protein between GST and the 13-zinc finger domain of Kid-1. When the binding between radiolabeled heteroduplex DNA and the 13 zinc fingers of Kid-1 was competed with a 5-, 25-, and 125-fold molar excess of unlabeled heteroduplex DNA (*het25*, *lanes 4–6*), homoduplex DNA (*hom, lanes 7–9*), and single-stranded oligonucleotide ss1 (*lanes 10–12*), only an excess of heteroduplex DNA had an effect. *B*, an intact zinc finger structure is important for the binding of Kid-1 to heteroduplex DNA with a 25-nucleotide bubble. Preincubation of the fusion protein between GST and the 13 zinc fingers of Kid-1 with a combination of 10 mM EDTA and 0.1 mM 1,10-phenanthroline resulted in the complete abrogation of the shift (*lane 4*). Treatment with ethanol, the solute for 1,10-phenanthroline, had no effect (*lane 5*). *F*, free DNA; *B*, bound DNA; *ø*, no protein added; *G*, GST; *1–13*, fusion protein between GST and the 13 zinc fingers of Kid-1; *C*, no pretreatment; *E/P*, pretreatment with EDTA and 1,10-phenanthroline; *EtOH*, pretreatment with Ethanol.

tween Kid-1 and heteroduplex DNA, competition experiments were performed with the 13 zinc fingers of Kid-1 and a radiolabeled heteroduplex oligonucleotide containing the 25 nucleotide bubble. The shift was competed out by a 125-fold molar excess of the heteroduplex oligonucleotide with the 25 nucleotide bubble, whereas a homoduplex oligonucleotide and a single-stranded oligonucleotide of the same length had no effect on the shift (Fig. 2*A*). We then tested Kid-1 with a second heteroduplex DNA containing a 25-nucleotide bubble. This second heteroduplex consisted of single-stranded oligonucleotides ss3 and ss4, which were obtained by scrambling oligonucleotides ss1 and ss2, respectively. Incubation of the 13 zinc fingers of Kid-1 with this second large heteroduplex again resulted in an efficient shift (data not shown).

To control for the role of the zinc fingers, we incubated the fusion protein between GST and the 13 zinc fingers of Kid-1 with a combination of EDTA, a divalent cation chelator, and 1,10-phenanthroline, a Zn^{2+}-chelating agent, before performing a gel shift with heteroduplex DNA. This resulted in the complete abrogation of the shift. Prior incubation of the protein with an equal volume of ethanol, the solvent for 1,10-phenanthroline, did not influence binding of Kid-1 to heteroduplex DNA, thus again attesting to the specificity of the shift (Fig. 2*B*).

Another set of experiments was carried out to determine which portion of the Kid-1 zinc finger domain contributed to the shift. Fusion proteins between GST and the four-zinc finger cluster of Kid-1, between GST and the nine-zinc finger cluster, and between GST and the complete zinc finger domain of Kid-1 were used in gel shift assays with heteroduplex DNA containing a 25-nucleotide bubble. All three fusion proteins shifted the heteroduplex DNA (Fig. 3), whereas no shift could be detected when the corresponding single-stranded oligonucleotides were used (data not shown).

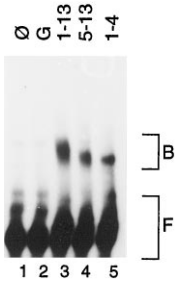

FIG. 3. **Both zinc finger domains of Kid-1 are able to bind to heteroduplex DNA.** GST (*lane 2*) and GST-fusion proteins with the four-zinc finger domain (*lane 5*), the nine-zinc finger domain (*lane 4*), and all 13 zinc fingers of Kid-1 (*lane 3*) were incubated with the heteroduplex DNA ss1/ss2 (containing a 25-nucleotide bubble) and run on a polyacrylamide gel. All three fusion proteins created a shift, whereas GST did not. *F*, free DNA; *B*, bound DNA; *ø*, no protein added; *G*, GST; *1–13*, fusion protein between GST and the 13 zinc fingers of Kid-1; *5–13*, fusion protein between GST and the nine-zinc finger cluster of Kid-1; *1–4*, fusion protein between GST and the four-zinc finger cluster of Kid-1.

The Transcriptional Repressor Protein WT1 Also Binds to Heteroduplex DNA, but Oct-6, Ets-1, and the RING Finger of BRCA-1 Do Not—Kid-1 belongs to the zinc finger class of transcription factors, and we therefore wanted to know whether other zinc finger proteins and members of other classes of transcription factors also bound to heteroduplex DNA. The POU domain protein Oct-6 (25) and the ets domain protein Ets-1 (26) bind to homoduplex DNA sequences of 8 nucleotides and 6 nucleotides in length, respectively. Neither protein, however, bound to heteroduplex DNA when examined in a gel shift assay (Fig. 4). The specificity of the shift by the Kid-1 zinc fingers was further demonstrated by the inability of another zinc finger structure, the RING finger domain of BRCA-1, to bind to heteroduplex DNA (data not shown).

The Wilms' tumor protein WT1 also belongs to the family of Cys_2His_2-zinc finger proteins, it contains four zinc fingers at its COOH terminus. Several naturally occurring splice variants of WT1 have been described with different DNA binding specificities (for a recent review, see Reddy and Licht (27)). A binding site selection with the splice variant lacking the tripeptide KTS between the third and fourth zinc finger of WT1 resulted in the isolation of a binding site that closely resembles that of the immediate-early zinc finger protein Egr-1 (28). WT1, which does not belong to the subfamily of KRAB-zinc finger proteins, has been found to act as a transcriptional repressor in transient transfection experiments (*e.g.* Refs. 29–31). When a fusion protein between GST and all four zinc fingers of WT1 was incubated with the Egr-1-binding site, a strong gel shift could be detected only with the splice variant lacking the tripeptide KTS between the third and fourth zinc finger, but not with the WT1+KTS form (Fig. 4). Surprisingly, both WT1−KTS and WT1+KTS bound strongly to heteroduplex DNA consisting of single-stranded oligonucleotides ss1 and ss2 (Fig. 4). Similar to Kid-1, WT1−KTS and WT1+KTS also bound strongly to the second large heteroduplex consisting of oligonucleotides ss3 and ss4 (data not shown). The specificity of those gel shifts was demonstrated by competition experiments. In the case of the WT1−KTS splice variant, successful competition was carried out with an excess of both unlabeled heteroduplex DNA and Egr-1-binding site (Fig. 5, *A* and *B*). The gel shift of the heteroduplex DNA by WT1+KTS could only be competed by an excess of unlabeled heteroduplex DNA, but not Egr-1- binding site (Fig. 5*C*). Binding of WT1−KTS and WT1+KTS to homoduplex DNA consisting of one of the oligonucleotides used in the heteroduplex DNA and a fully complementary oligonucleotide was very weak (data not shown).

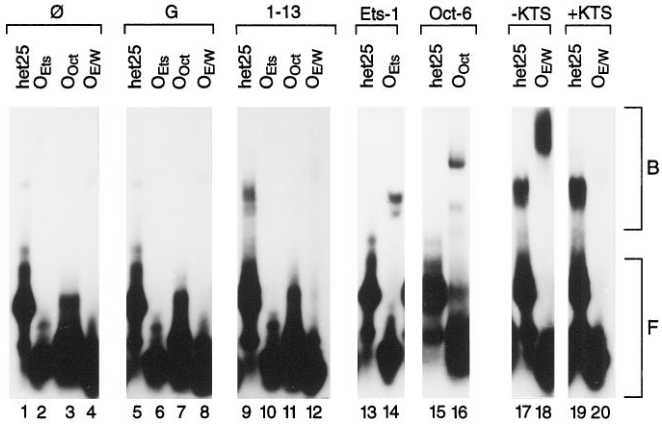

FIG. 4. **The ets domain protein Ets-1 and the POU domain protein Oct-6 do not bind to heteroduplex DNA, but WT1 does.** Heteroduplex DNA ss1/ss2 with a 25-nucleotide bubble (*het25*) was incubated with GST, with the 13 zinc fingers of Kid-1, with the ets domain protein Ets-1, with the POU domain protein Oct-6, and with WT1−KTS and WT1+KTS. The 13 zinc fingers of Kid-1 only bind to the heteroduplex DNA but not to any of the other binding sites (lanes 9–12). Ets-1 (*lanes 13* and *14*) and Oct-6 (*lanes 15* and *16*) only bind to their respective binding site but not to heteroduplex DNA. WT1−KTS binds to both the Egr-1/WT1-binding site and heteroduplex DNA (*lanes 17* and *18*), whereas WT1+KTS binds to heteroduplex DNA only (*lanes 19* and *20*). *F*, free DNA; *B*, bound DNA; *ø*, no protein added; *G*, GST; *1–13*, fusion protein between GST and the 13 zinc fingers of Kid-1; *Ets-1*, chicken Ets-1 protein; *Oct-6*, murine Oct-6 protein; −KTS, fusion protein between GST and the four zinc fingers of WT1 without the KTS tripeptide; +*KTS*, fusion protein between GST and the four zinc fingers of WT1 with the KTS tripeptide; *het25*, heteroduplex oligonucleotide with a 25-nucleotide bubble; O_{Ets}, oligonucleotide with an ets-binding site; O_{Oct}, oligonucleotide with octamer motif; $O_{E/W}$, oligonucleotide with an Egr-1/WT1-binding site.

To define the structural requirements for the binding of WT1 to heteroduplex DNA, we made use of naturally occurring WT1 mutant proteins. Both a WT1+KTS mutant harboring a deletion of zinc finger 3 (32) as well as a mutant protein with a missense mutation in zinc finger 3 found in a patient with Denys-Drash syndrome (R394W) showed decreased binding to heteroduplex DNA (Fig. 5*D*). This suggests that zinc finger 3 is involved in this interaction.

The results described above demonstrate for the first time the binding of a KRAB-zinc finger protein to DNA. Surprisingly, it is DNA structure that is recognized by Kid-1, because two unrelated heteroduplex DNAs with bubbles of 25 nucleotides are shifted with the same efficiency. Together with the finding that another transcriptional repressor protein, WT1, also binds to heteroduplex DNA without obvious sequence specificity, this represents an important new paradigm. Although this particular DNA structure, heteroduplex DNA, resembles the "transcription bubble," which can be found when RNA polymerase II is transcribing a gene, it appears unlikely that the transcription bubble serves as the natural recognition site. Kid-1 bound only inefficiently to heteroduplex DNA with a 7-nucleotide bubble, a size that approximates the region of the transcription bubble not covered by RNA polymerase II. Further experiments are needed to clarify where those recognition sites are located in the nucleus and how they are recognized in addition to their structure.

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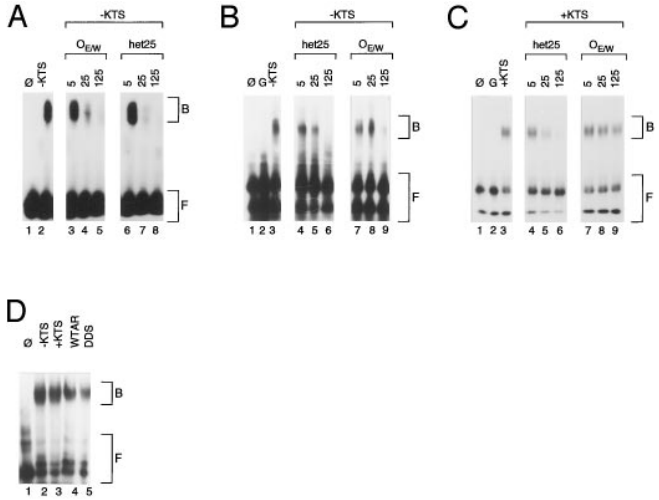

FIG. 5. *A–C*, competition experiments with WT−KTS and WT1+KTS. *A*, WT−KTS was incubated with a radiolabeled oligonucleotide containing an Egr-1/WT1-binding site ($O_{E/W}$) and increasing amounts of the unlabeled oligonucleotide with an Egr-1/WT1-binding site (*lanes 3–5*) or unlabeled heteroduplex DNA ss1/ss2 with a 25-nucleotide bubble (*het25, lanes 6–8*). The shift can be very effectively competed already with a 25-fold molar excess of either oligonucleotide. *B*, WT−KTS was incubated with radiolabeled heteroduplex DNA ss1/ss2 containing a 25-nucleotide bubble, and increasing amounts of unlabeled het25 (*lanes 4–6*) or the oligonucleotide containing the Egr-1/WT1-binding site (*lanes 7–9*). Both kinds of unlabeled DNA successfully competed out the shift. *C*, WT+KTS was incubated with radiolabeled heteroduplex DNA ss1/ss2, and increasing amounts of unlabeled heteroduplex DNA (*lanes 4–6*) or unlabeled oligonucleotide containing an Egr-1/WT1-binding site (*lanes 7–9*). The shift could only be competed with an excess of heteroduplex DNA. *D*, gel shift experiments using GST fusion proteins with naturally occurring mutants of WT1. 50 ng each of WT−KTS, WT+KTS, WTAR (a mutant with a deletion of zinc finger 3), and a mutant WT1 protein from a Denys-Drash syndrome (*DDS*) patient (R394W) were incubated with radiolabeled heteroduplex DNA ss1/ss2 and subjected to a gel shift. Both the WTAR and R394W mutant protein created a weaker gel shift. *F*, free DNA; *B*, bound DNA; *ø*, no protein added; *G*, GST; *WTAR*, fusion protein between GST and WT1 with a deletion of zinc finger 3; *DDS*, fusion protein between GST and WT1 with mutation R394W in zinc finger 3; *het25*, heteroduplex oligonucleotide with a 25 nucleotide bubble; $O_{E/W}$, oligonucleotide with an Egr-1/WT1-binding site; −KTS, fusion protein between GST and the four zinc fingers of WT1 without the KTS tripeptide; +*KTS*, fusion protein between GST and the four zinc fingers of WT1 with the KTS tripeptide.

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