

- codon. The same probes were used to identify targeted clones and genotyping.
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 11. Pregnant females were culled, and embryos were dissected in ice-cold phosphate-buffered saline (PBS). The surrounding yolk sac was collected for genotyping. Embryos were fixed for 30 min in 4% paraformaldehyde/PBS then incubated in X-Gal (1 mg/ml) at 30°C for revealing β -Gal activity. Stained embryos were washed in PBS, postfixed for 1 hour in 4% paraformaldehyde/PBS, dehydrated, and either embedded in paraffin before sectioning or dissected to remove the inner ear for whole-mount studies.
 12. N. A. Bermingham *et al.*, unpublished data.
 13. LM and TEM were carried out as previously described (29). Tissue preparation for SEM consisted of treatment with 1% OsO₄ in cacodylate buffer, dehydration, critical-point drying, sputter coating with gold, and examination in a JEOL 35S electron microscope.
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 20. For immunofluorescence, embryos were fixed for 1.5 hours in 4% paraformaldehyde/PBS at 4°C, sunk through 15% sucrose/PBS for 5 hours then through 30% sucrose/PBS overnight, and snap frozen in a 2-methylbutane dry ice bath. Fourteen-micrometer sections were cut on a cryostat and mounted on gelatin-coated slides. Sections were fixed on slides by dipping for 10 min in Streck tissue fixative (Streck Laboratories, Omaha, NE) and air drying. Sections were blocked in 10% normal goat serum and 0.3% Triton X-100 in PBS for 1 hour at room temperature (RT). Rabbit polyclonal antibody to calretinin (Chemicon International, Temecula, CA) was diluted 1:200 or rabbit antibody to myosin VI (a gift from T. Hasson, University of California, San Diego) was diluted 1:100 in blocking solution and incubated overnight on sections at 4°C. Sections were washed three times (20 min each) in PBS at RT. The secondary antibody, Alexa 488 (Molecular Probes, Eugene, OR), was diluted 1:400 in blocking solution. Sections were covered and incubated at RT for 2 hours before washing and mounting in Vectashield containing 4'6'-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA). For confocal microscopy, sections were treated with ribonuclease (25 μ g/ml) before counterstaining with propidium iodide (50 μ g/ml) and mounted in Vectashield without DAPI or were directly mounted in Vectashield containing TOTO-3 iodide (Molecular Probes). Stained sections were viewed under a Bio-Rad 1024 confocal microscope.
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 30. We thank R. Atkinson, D. Trikka, and V. Brandt for assistance and helpful discussions and T. Hasson for the myosin VI antibody, and we acknowledge the support of the Electron Microscopic Facility of the University of

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Crystal Structure of the Z α Domain of the Human Editing Enzyme ADAR1 Bound to Left-Handed Z-DNA

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The editing enzyme double-stranded RNA adenosine deaminase includes a DNA binding domain, Z α , which is specific for left-handed Z-DNA. The 2.1 angstrom crystal structure of Z α complexed to DNA reveals that the substrate is in the left-handed Z conformation. The contacts between Z α and Z-DNA are made primarily with the "zigzag" sugar-phosphate backbone, which provides a basis for the specificity for the Z conformation. A single base contact is observed to guanine in the syn conformation, characteristic of Z-DNA. Intriguingly, the helix-turn-helix motif, frequently used to recognize B-DNA, is used by Z α to contact Z-DNA.

Since the elucidation of the structure of left-handed Z-DNA in 1979 (1), the question of a biological role for this conformation has remained in the forefront of research in the field. The discovery of a Z-DNA binding activity for double-stranded (ds) RNA adenosine deaminase (ADAR1) constituted an important step (2). ADAR1 deaminates adenine in pre-mRNA, yielding inosine, which codes as guanine. Such codon changes result in alternative forms of the translated protein. The NH₂-terminus of ADAR1 includes a domain, Z α , which is responsible for high-affinity binding to the Z conformation of DNA. This was originally shown by a band-shift assay (3), where the binding withstood a challenge of a 10,000-fold mass excess of nonspecific B-DNA competitor (2, 4). Circular dichroic and Raman spectroscopic measurements have confirmed the specificity of Z α containing peptides for Z-DNA (4–6). Z α is the prototype for a family of related peptides, which share common amino acid sequence motifs (4). We have now cocrystallized a proteolytically defined Z α domain [residues 133 to 209 (7, 8)] of human ADAR1 with the 6–base pair (bp) DNA fragment d(TCGCGCG)₂. The structure was determined by single isomorphous replacement including anomalous scattering (SIRAS) and was refined to a

resolution of 2.1 Å (Table 1).

The monomeric Z α domain binds to one strand of the palindromic dsDNA. The conformation of the DNA substrate is very similar to the canonical Z-DNA structure determined by Wang *et al.* (1, 9) [root mean square deviation (rmsd) 0.80 Å, including all atoms in the duplex]. A second monomer related by the twofold axis of the DNA oligomer binds to the opposite strand of the DNA; however, the two proteins do not interact with each other. In the asymmetric unit there are three independent Z α -DNA complexes (10).

The Z α domain has a compact α/β architecture containing a three-helix bundle (α 1 to α 3) flanked on one side by a twisted antiparallel β sheet (β 1 to β 3) (Fig. 1). The NH₂-terminal helix α 1 (Ser¹³⁴ to Leu¹⁵⁰) is followed by the short-strand β 1 (Ala¹⁵⁵ to Thr¹⁵⁷), which positions the COOH-terminal β hairpin (β 2: Leu¹⁸⁵ to Ala¹⁸⁸; β 3: Pro¹⁹³ to Ile¹⁹⁷) by means of two hydrogen bonds. This arrangement of a β hairpin with exactly two hydrogen bonds to a third β strand is a common feature of helix-turn-helix (HTH) proteins with α/β topology. Helices α 2 and α 3 (Ala¹⁵⁸ to Leu¹⁶⁵ and Lys¹⁶⁹ to Lys¹⁸²) reside between β 1 and β 2 and form the HTH motif. Aliphatic residues from the three helices, together with Trp¹⁹⁵ in strand β 3, interdigitate and form a hydrophobic core. The secondary structure reported in a nuclear magnetic resonance experiment indicates that the unbound protein in solution adopts a very similar fold (11).

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The contact between $Z\alpha$ and the DNA strand consists of a single continuous recognition surface (Fig. 2). This surface, which is

complementary to the DNA in terms of shape and electrostatic nature, consists of residues from helix $\alpha 3$ and the COOH-terminal β

hairpin. All the polar interactions in the complex are with the sugar-phosphate backbone. Direct contacts are made between Lys¹⁶⁹, Lys¹⁷⁰, Asn¹⁷³, Arg¹⁷⁴, and Tyr¹⁷⁷ in helix $\alpha 3$ as well as Thr¹⁹¹ and five consecutive phosphates. In addition water-mediated phosphate contacts are observed. Lys¹⁶⁹, Asn¹⁷³, and Trp¹⁹⁵ coordinate two ordered water molecules embedded within the protein-DNA interface in all three independent complexes. These waters are completely shielded from the surface. Further, Thr¹⁹¹ and Arg¹⁷⁴ also bind to the furanose oxygens of G2 and G6, respectively. This ensemble of residues forms an extensive network of hydrogen bonds; four of the five consecutive phosphate groups are grasped by at least two hydrogen bond donors.

In concert with these polar interactions, the crystal structure reveals a striking complementarity in the van der Waals surfaces of the protein and the DNA. Peptide backbone atoms of helix $\alpha 3$ (Lys¹⁷⁰, Arg¹⁷⁴) contact the ribose of G4. The face of the aromatic ring of Tyr¹⁷⁷ makes a close van der Waals contact with the exposed carbon 8 of G4. This interaction is possible only if this base is in the syn conformation and is therefore specific for Z-DNA. The phenolic side chain of Tyr¹⁷⁷ is almost perpendicular to the G4 base. Further, the aromatic ring of Trp¹⁹⁵ is perpendicular to the Tyr¹⁷⁷ ring from the other side and buttresses it from within the protein core. Similar systems of mutually perpendicular aromatic rings in an edge-to-face orientation have been shown to stabilize other protein-ligand interactions (12). In $Z\alpha$, Trp¹⁹⁵, positioned precisely within the hydrophobic core, maintains two important indirect DNA contacts, via Tyr¹⁷⁷ and via a water to the backbone. This central role of Trp¹⁹⁵ explains the detrimental effect of Trp¹⁹⁵ to Ala (W195A) and Trp¹⁹⁵ to Tyr (W195Y) mutations (13) on both protein stability and DNA binding as well as the tryptophan fluorescence quenching observed as a result of Z-DNA binding (5, 11).

Pro¹⁹² and Pro¹⁹³ in the COOH-terminal β -hairpin form another important van der Waals interaction with the DNA. The pyrrolidine rings of both residues pack against the sugar-phosphate backbone from phosphate 2 to phosphate 3. In contrast to the predominant trans peptide linkage, Pro¹⁹² forms a cis peptide bond, thereby positioning the β -hairpin loop against the DNA surface. The absolute conservation of Pro¹⁹² within the known homologues of $Z\alpha$ (4) underlines the importance of this local structure.

Finally, an array of well-defined waters is positioned on the edge of the protein-DNA interface. The total structure, including this bonded water network on the protein-DNA interface, is nearly identical in all three inde-

Table 1. Crystallographic data. Recombinant $Z\alpha$ (residues 133 to 209 of hADAR1) was overproduced and purified from *Escherichia coli* as described (7). Single crystals of the protein-DNA complex were obtained by the hanging-drop vapor diffusion method with ammonium sulfate used as precipitant [for details see (26)]. The crystals belong to space group P4₂,2 with cell dimensions $a = b = 85.9$ Å, $c = 71.3$ Å. Three protein-DNA complexes form the asymmetric unit. Derivatives were prepared by substituting 5-iodouracil for thymine 1 (iodo-1) and 5-iodocytosine for cytosine 6 (iodo-6), although only the latter served as a suitable derivative. Diffraction data were collected from cryocooled crystals on a rotating anode x-ray source with an R-Axis IIc image plate detector and reduced with the HKL program package (20). Iodine sites were located and initial SIRAS phases were calculated with SOLVE (20). Solvent flattening and noncrystallographic symmetry (NCS) averaging with DM (20) gave a readily interpretable electron density map. Because the iodo-1 derivative diffracted to higher resolution than the original native, it was used for model building and refinement with X-PLOR (20). NCS restraints were not used during refinement, and water molecules were added to the three complexes independently. A total of 158 of 161 nonglycine residues lie in most-favored regions of the Ramachandran plot. The remaining three residues lie in additional favored regions [calculated with Procheck (20)].

Data collection and SIRAS phasing statistics			
	Native	Iodo-1	Iodo-6
DNA	d(TCGCGCG)	d(⁵ UCGCGCG)	d(TCGCG ⁵ CG)
Data coverage (Å)	40–2.4	20–2.1	20–2.8
R_{merge}^* (%)	5.2	7.4	12.0
Number of unique reflections	10896	29702	12566
Completeness (%)	99.2	99.9	99.9
Redundancy	6.6	9.8	6.9
R_{iso}^\dagger (%)	—	—	22.3
$R_{\text{cullis}}^\ddagger$ (%)	—	—	57.0
Iso. phasing power§	—	—	1.34
Anom. phasing power	—	—	1.06
Figure of merit, SIRAS	0.490	—	—
Figure of merit, DM	0.822	—	—

Refinement statistics (6–2.1 Å)					
			Protein	DNA	Water
$R_{\text{cryst}}^\parallel$ (%)	22.7				
$R_{\text{free}}^\parallel$ (%)	26.5				
Reflections in working set	13772	rms from ideality:			
Reflections in test set	1531	Bond lengths (Å)	0.006	0.004	
N_{atoms}	1898	Bond angles (°)	1.12	0.67	
N_{waters}	244	Average B-factor (Å ²)	32.9	21.9	43.0
		rms B-factors (Å ²)	3.31	3.19	

* $R_{\text{merge}} = \sum_i |I_h - \bar{I}_h| / \sum_i I_h$, where I_h is the mean intensity for the reflection. $\dagger R_{\text{iso}} = \sum |F_{\text{PH}} - F_p| / \sum F_p$. $\ddagger R_{\text{cullis}} = \sum |F_{\text{PH}} \pm F_p| - F_{\text{H, calc}} / \sum |F_{\text{PH}} \pm F_p|$ for centric reflections only. \S Phasing power = $\text{rms}(F_{\text{H, calc}}) / \text{rms}(F_{\text{H, calc}})$ (phase-integrated lack of closure). $\parallel R_{\text{cryst}} = \sum |F_p - F_{p(\text{calc})}| / \sum F_p$. R_{free} is the same as R_{cryst} but calculated on 10% of data selected evenly over the resolution range and excluded from refinement. $||$ Between covalently bonded atoms.

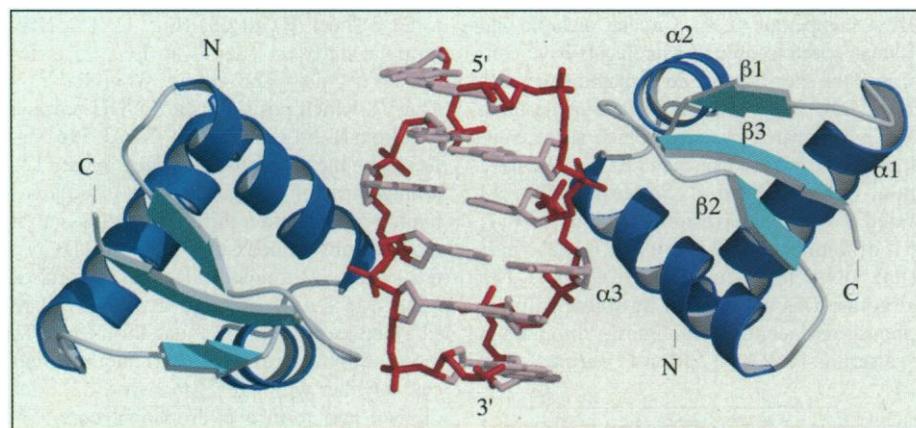


Fig. 1. Overview of the $Z\alpha$ domain bound to left-handed Z-DNA. Residues 134 to 198 of two symmetry-related $Z\alpha$ monomers and the 6-bp DNA duplex d(CGCGCG)₂ are shown. Labels indicate NH₂- (N) and COOH-termini (C) of the proteins as well as helices ($\alpha 1$ to $\alpha 3$) and strands ($\beta 1$ to $\beta 3$) (27).

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pendent complexes in the asymmetric unit (14).

Z α belongs to the family of HTH proteins with α/β topology (15) (Fig. 3C). The interaction of HTH proteins with right-handed DNA has been well characterized. Typically, the second helix of the HTH motif, called the recognition helix, is cradled in the major groove. There, the base edges expose a characteristic pattern of potential hydrogen bond donors and acceptors, which allow for base-specific recognition. In the family of HTH proteins with α/β topology, DNA contacts are also made between residues in the first helix of the HTH motif and the phosphate backbone. In variants of the family, additional contacts extend the interaction surface (16). One example is illustrated by the structure of hepatocyte nuclear factor HNF-3 γ bound to B-DNA (17) (Fig. 3A, right), where contacts include residues in two extended loops whose importance and spatial arrangement gave rise to the name "winged-helix" motif.

The mode of interaction of Z α with DNA (Fig. 3A, left) is markedly different from that of B-DNA binding HTH proteins, reflecting the fact that, unlike B-DNA, Z-DNA has no major groove and a very deep, nearly inaccessible minor groove. In Z α , recognition helix $\alpha 3$ is positioned on the outer surface of the DNA, with residues of the first two helical turns contacting the DNA. These residues are in the same positions as $\alpha 3$ residues used for B-DNA binding by the catabolite gene activator protein CAP and HNF-3 γ (17) (Fig. 4B). In contrast to B-DNA binding HTH proteins with α/β topology, the first helix of the HTH motif in Z α is not involved in DNA contacts. Instead, the COOH-terminal β -hairpin contributes a second set of interactions. This β -hairpin is structurally well defined and is the region that deviates to the greatest extent from other HTH proteins with α/β topology. The cluster of highly conserved residues in the hairpin is specific to the Z α family (Fig. 4A) and is necessary for Z-DNA binding (4, 11). Unlike most structurally defined B-DNA binding HTH proteins, the interaction seen with Z α is conformation specific rather than sequence specific. The base contact to carbon 8 of G4 described above specifies the syn as opposed to the anti conformation of this base, but any base in the syn conformation at that position would expose a similar surface. These findings provide a structural basis for biochemical results, which describe a conformation specific binding behavior for the Z α domain (6).

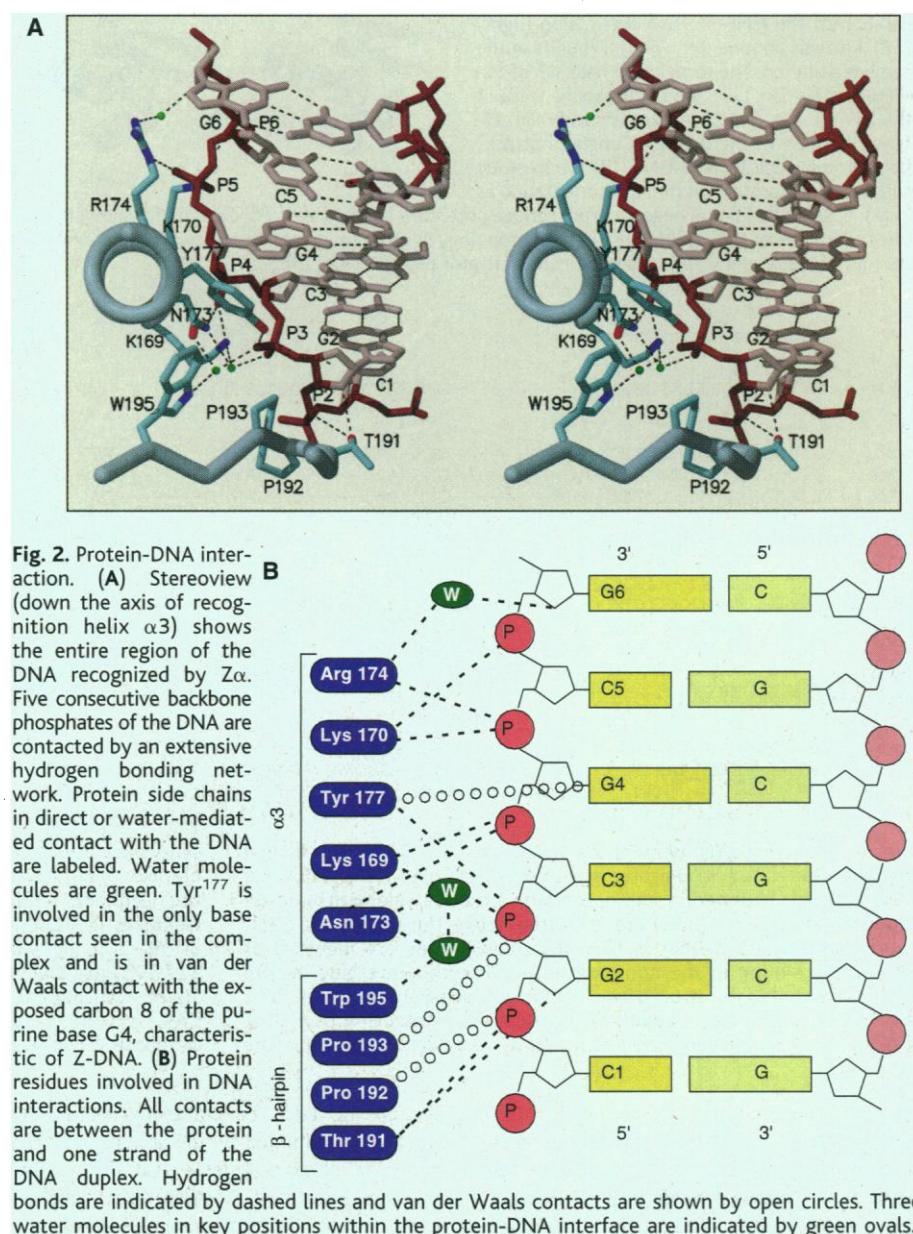
The structural basis for sequence specific binding of B-DNA using the HTH motif is well established for many prokaryotic gene regulatory proteins and eukaryotic homeodomains [reviewed in (18)]. However, recently published HTH protein structures show

marked variations of the originally described interaction with B-DNA. The endonuclease Fok I, the ribosomal protein L11, and the homeodomain protein bicoid are examples in which the HTH motif is used in a noncanonical way to bind B-DNA or even to interact specifically with RNA (19). Here, we describe the interaction between a HTH protein, Z α , with Z-DNA. Apparently, variations in the binding site of HTH proteins can result in very different substrate specificities.

A reduction in solvent-exposed surface area is thought to contribute significantly to the free energy of association (16). When Z α binds Z-DNA, 420 \AA^2 of solvent-accessible protein surface area is lost (calculated with X-PLOR) (20), significantly less than in other specific protein-DNA complexes (21). However, there is extensive electrostatic and

van der Waals complementarity, which may compensate in part (Fig. 3B, left), explaining the high affinity of Z α for Z-DNA (22). Furthermore, two Z α domains bind to the same Z-DNA duplex, albeit without contacts between the monomers, as shown in this crystal structure. In ADAR1, there is a second sequence, Z β , which is very similar to Z α (Fig. 4A). These sequences are separated by a duplicated 49-amino acid module. The linker tethers the two regions together, and a bipartite Z-DNA binding domain, Zab, has been proposed (7). This would effectively double the interaction surface area. The coupling of subdomains to enhance DNA binding affinity and specificity has been seen in the bipartite OCT-1 and PAX-6 domains (17).

Z-DNA is formed transiently behind a moving RNA polymerase, stabilized by the



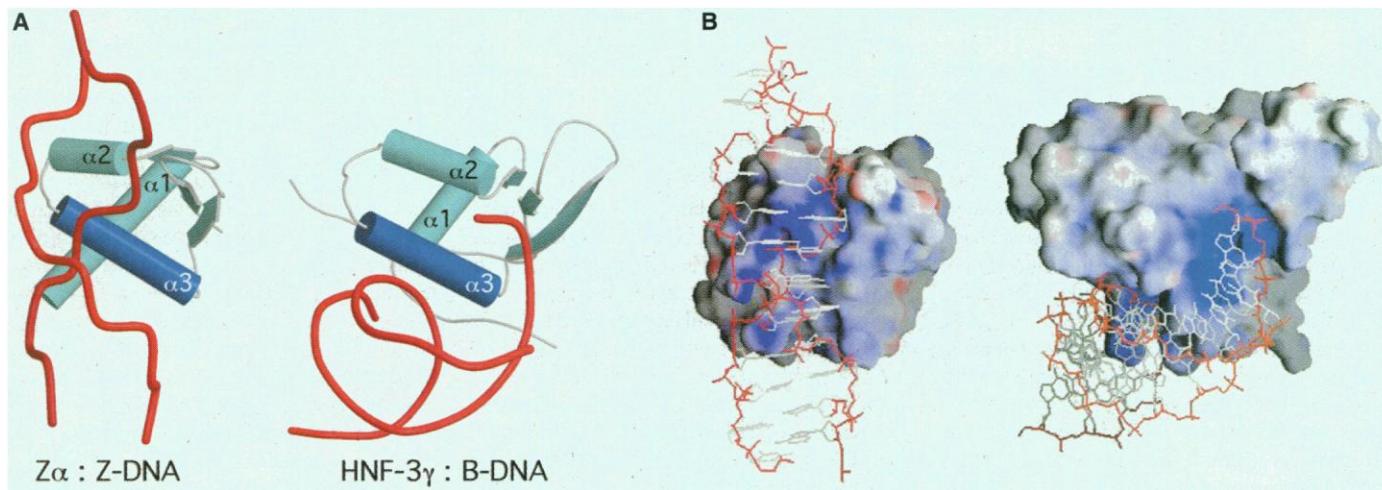


Fig. 3. Comparison with other HTH proteins. (A) Schematic of the Z α -DNA complex (left) compared with the HNF-3 γ -DNA interaction (right) (17). Proteins are oriented with HTH units in the same orientation. The recognition helix α 3 of the HTH motif is dark blue and the backbone trace of the DNA is red. The DNA axes are radically different because of the different "angle of attack" both proteins use to bind DNA. (B) Electrostatic surfaces of the proteins in the same orientation as in (A). Positive (blue) and negative (red) surface potentials are indicated. (C) Schematics showing the close similarity in topology of the Z α domain (left) and three members of the HTH family with α/β topology in order of increasing structural deviations from left to right: metallothionein repressor SmtB, globular domain of histone H5, and catabolite gene activator protein CAP (73).

	α 1	β 1	α 2	α 3	β 2	β 3
	134 140 150		160	170 180		190 199
hZ α	SIYQDQEQRIKLFLEEL	-----GEGKATT-----	AHDLGKGL	-----GTP-KKEINRVL	YSLAKKGG-LQKEA-----	-----GTPPLWKIAV
A						
mZ α	SISQSPEQKVLNRLEEL	-----GEGKATT-----	AHVLAREL	-----RIP-KRDINRILY	SLEKKGG-LHRGR-----	-----GKPLWLSLVP
rZ α	SISQNPQKVLNRLEEL	-----GEGKATT-----	AYALAREL	-----RTP-KKDINRILY	SLERKGG-LHRGV-----	-----GKPLWLSLVP
bZ α	TISQDQEQRTLELLDEL	-----GDGKATT-----	ARDLARKL	-----QAP-KKDINRVL	YSLAEKGG-LHQEA-----	-----GSPPLWRATV
xZ α 1	TVSHDLLENNLLTFFKE	-----IGTKTFT-----	AKALAWQF	-----KVE-KKRINHFLY	TFETKGL-LCRYP-----	-----GTPPLWRVFS
xZ α 2	SVSRPLENILLTFFFRG	-----QGDTQFT-----	AKALAWQF	-----KVK-KKHINFLY	KFQTKGL-LCKNS-----	-----GTPPLWKISS
hZ β	MAEIKEKICDY	-----LFNVSDDS	ALNLAKNI	-----GLTKARDINAVL	IDMERQGD-VYRQG-----	-----TTPPIWHLTD
mZ β	MAEIKEKICDY	-----LFNVSNS	ALNLAKNI	-----GLTKARDVTSVL	IDLERQGD-VYRQG-----	-----ATPPIWYLTLD
rZ β	MAEIKEKICDY	-----LFNVS	SKSS	-----ALNLAKNI	-----GLAKARDVNAV	LIDLERQGD-VYREG-----
bZ β	MAEIKEKICDH	-----LFNVS	SSSS	-----ALNLAKNI	-----GLTKARDVNAV	LIDLERQGD-VYRQG-----
xZ β 1	MAGNKEKVCEF	-----LYNSPST	-----TLIIRKNV	-----GISKLPELNQIL	NTLEKQGE-ACKAS-----	-----TNPVKWTLTD
xZ β 2	MATNSAKVCEF	-----LYNSPST	-----PFIIRKNV	-----GISKMPELTQ	ILNTLEKQGE-ACKAS-----	-----TNPVKWTLTA
E3L	MSKIYIDERSNAEIVCEA	-----IKTIGIEGAT	-----AAQLTRQL	-----NME-KREVNKAL	YDLQRSAM-VYSSD-----	-----DIPPRWFMTT
B						
SmtB	<44>DPNRLRLLSLLAR	-----seIC	-----VGDLAQAI	-----GVS-ESAVSHQL	RSRLRNLRL-VSYRk	-----ggrhVYVYQL
H5	<27> ySEMIAAAIRAekserg	-----GSS	-----RQSIQKYI	ksykvghn-aDLQIKLS	IRRLAAGV-LKQTK	-----gvgaSGSFRL
CAP	<137> qVTGRIAQTLNLa-kqpdamthp	qgmqIRIT	-----RQEIGQIV	-----GCS-RETVGR	ILKMLEDQNL-ISA	-----hGKTIVV
HNF3 γ <123>	yISLITMAIQQApqk	-----mlt	seiYQWIMDLF	-----pyyren-QQRWQNS	SIRHLSfndcfv	kvarspdkpgkgsywalh

Fig. 4. Sequence alignment of Z α homologs (Z α , Z β , E3L) (A) and structurally related HTH proteins with α/β topology (SmtB, H5, CAP, HNF-3 γ) (B). Numbering and secondary structural elements for Z α from human ADAR1 (hZ α) are indicated above the sequences. Residues that contact Z-DNA in hZ α are green if they are identical or chemically similar in the homologs; otherwise they are blue. In (B), residues situated in structurally equivalent positions between hZ α and HTH proteins are in uppercase letters. DNA contacts with B-DNA (taken from published complex crystal structures) are red. The

sequences are as follows: hZ α , hZ β , ADAR1 *Homo sapiens*, accession number (acc) GenBank U18121; mZ α , mZ β , ADAR1 *Mus musculus*, acc GenBank AF052506; rZ α , rZ β , ADAR1 *Rattus norvegicus*, acc GenBank U18942; bZ α , bZ β , ADAR1 *Bos taurus* (5); xZ α 1, xZ β 1, dsRAD-1 *Xenopus laevis*, acc GenBank U88065; xZ α 2, xZ β 2, dsRAD-2 *X. laevis*, acc GenBank U88066; E3L *Vaccinia virus* Western Reserve strain, acc GenBank S64006; SmtB, acc Protein Data Bank (PDB) 1SMT; CAP, acc PDB 1CGP; H5, acc PDB 1HST; HNF-3 γ , acc Nucleic Acid Database PDT013.

negative supercoiling in its wake (23). ADAR1 has three dsRNA binding domains and it requires dsRNA to function. This dsRNA is frequently made in pre-mRNA by pairing intron and exon sequences; therefore,

the editing must be completed before the introns are removed by the splicing machinery (24). It is postulated that the Z-DNA binding domain of ADAR1 targets the enzyme to actively transcribing genes (25), as

they are the predominant sites for Z-DNA formation (23). It is likely that Z-DNA binding is evolutionarily maintained, because the residues that contact Z-DNA in Z α are well conserved within the family of Z α homo-

logs, including the second Z-DNA binding domain of ADAR1, Z β , and the viral protein E3L (Fig. 4A).

The unique zigzag pattern of the sugar-phosphate backbone and the anti-syn alternation of the bases are characteristic of Z-DNA and distinguish it from other nucleic acid structures. The Z α domain has a tailored fit, which recognizes both features specifically. This binding can be generalized to other related left-handed Z-DNA binding HTH proteins and may provide a way to further explore the biological role(s) of Z-DNA.

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8. The protein construct used here was originally named Za (7). It is structurally homogeneous, unlike other published Z α peptides. For simplicity, here we use the generic name Z α instead of Za.
9. Guanine nucleotides are in the syn conformation characteristic of Z-DNA, with C3'-endo sugar puckers. In contrast to crystals containing Z-DNA without protein, the DNA in this crystal does not form continuous helices; instead it is stacked 3' to 3' with the stacked molecules slightly displaced laterally. In pure DNA crystals the phosphate group in G4pC5 is seen in two alternate conformations, Z $_I$ and Z $_II$ [A. J. Wang *et al.*, *Science* **211**, 171 (1981); R. V. Gessner, C. A. Frederick, G. J. Quigley, A. Rich, A. H. Wang, *J. Biol. Chem.* **264**, 7921 (1989)]. In the structure described here, this phosphate group is uniformly in the Z $_I$ conformation as are all other phosphates at GpC steps. The Z $_II$ conformation does not allow the interaction with Lys¹⁷⁰.
10. Two complexes (I, II) are related by a noncrystallographic twofold axis, and the third (III) resides on a crystallographic dyad. Some amino acids at the NH₂- and COOH-termini of the protein are disordered. The structure is defined for residues 134 to 198 of the Z α monomer in complex I, 136 to 199 in complex II, and 134 to 199 in complex III. The DNA structure is well resolved except for the 5'-dT overhang, which appears to be disordered.
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13. Single-letter abbreviations for amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
14. Rmsd is 0.93 Å between complex I and II, 1.07 Å between complex II and III, and 0.76 Å between complex I and III, including all protein and DNA atoms, even though the three complexes were built and refined entirely independently.
15. When the Z α domain is superimposed on other proteins in the family, the similarity is most striking with the metallothionein transcriptional repressor SmtB (17) (rmsd 44 C α atoms 0.79 Å), the catabolite gene activator protein CAP (17) (rmsd 43 C α atoms 0.94 Å), and the related winged-helix class of proteins, such as hepatocyte nuclear factor HNF-3 γ (17) (rmsd 30 C α atoms 2.36 Å) and histone H5 (17) (rmsd 39 C α atoms 1.28 Å).
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Specific Coupling of NMDA Receptor Activation to Nitric Oxide Neurotoxicity by PSD-95 Protein

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The efficiency with which N-methyl-D-aspartate receptors (NMDARs) trigger intracellular signaling pathways governs neuronal plasticity, development, senescence, and disease. In cultured cortical neurons, suppressing the expression of the NMDAR scaffolding protein PSD-95 (postsynaptic density-95) selectively attenuated excitotoxicity triggered via NMDARs, but not by other glutamate or calcium ion (Ca²⁺) channels. NMDAR function was unaffected, because receptor expression, NMDA currents, and ⁴⁵Ca²⁺ loading were unchanged. Suppressing PSD-95 blocked Ca²⁺-activated nitric oxide production by NMDARs selectively, without affecting neuronal nitric oxide synthase expression or function. Thus, PSD-95 is required for efficient coupling of NMDAR activity to nitric oxide toxicity, and imparts specificity to excitotoxic Ca²⁺ signaling.

Calcium influx through NMDARs plays key roles in synaptic transmission, neuronal development, and plasticity (1). Excessive Ca influx triggers excitotoxicity (2), damaging neurons in diverse neurological disorders (3). Rapid Ca²⁺-dependent neurotoxicity is trig-

gered most efficiently when Ca²⁺ influx occurs through NMDARs, and cannot be reproduced by loading neurons with equivalent quantities of Ca²⁺ through non-NMDARs or voltage-sensitive Ca²⁺ channels (VSCCs) (4). This suggests that Ca²⁺ influx through NMDAR channels is functionally coupled to neurotoxic signaling pathways.

We hypothesized that lethal Ca²⁺ signaling by NMDARs is determined by the molecules with which they interact. The NR2 NMDAR subunits bind to PSD-95/SAP90 (5), chapsyn-110/PSD-93, and other members of the membrane-associated guanylate

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