Protein-Nucleic Acid Interactions

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Topic:

- Methods for studying proteins-DNA interactions
- DNA structure
- DNA recognition by helix-turn-helix motifs
- DNA recognition by eukaryotic transcription factors
- Site-specific vs. non-specific interactions
  - Restriction Enzymes
  - Non-specific nucleases

References: "Introduction to Protein Structure" 2nd Edition,
by Branden, C.and Tooze, J. G
Garland Publishing, Inc. USA.
Chapters 7-10

Methods for studying protein-DNA interactions

Ref: http://www.biochem.arizona.edu/classes/bioc568/protein_dna_interactions.htm

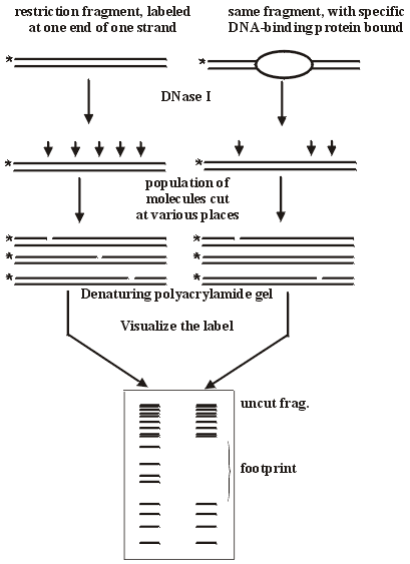
DNase I footprint

A restriction fragment containing a specific binding site is labeled at one end, usually with ^{32}P.

Molecules are treated lightly with DNase I, which makes single-strand breaks (nicks) in the DNA. A small amount of enzyme is used so that there is an average of <1 nick/strand.

The reaction is stopped, the DNA is denatured, and the mixture is run on a denaturing polyacrylamide gel. The distribution of radioactivity is visualized by autoradiography or by the use of a Phosphorimager.

The result is a ladder of bands, representing the various sites at which DNase I cuts. Where the protein was bound, there is a gap in the ladder -- the **footprint.**



The CI repressors of lambdoid phages bind cooperatively to adjacent binding sites. Cooperative binding occurs between dimers bound at two of the sites, a mode of binding termed pairwise cooperativity.

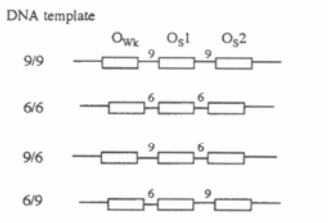


Figure 2. Templates used for test of extended cooperativity. All three templates contained the same three sites, as indicated, and differed in the spacing between the sites.

Ref: Liu, Z. and Little, J. W.
JMB 1998, 278, 331-338.

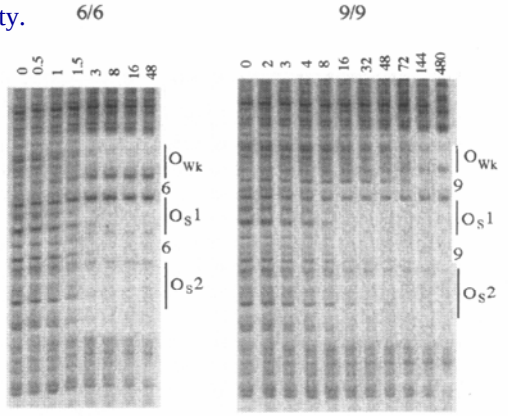


Figure 3. DNase I footprinting of the 6/6 and 9/9 templates. Footprinting analysis was carried out over a range of CI levels; concentrations (nM in monomer) are indicated above each lane. The positions of O_{Wk}, O_S1 and O_S2 in each template are shown by the brackets beside the lanes; positions were determined from Maxam-Gilbert sequencing reactions (not shown).

When used properly, footprint can be used to measure dissociation constants. One carries out a series of binding reactions over a range of protein concentrations, and determines the fraction of the binding site that is occupied at each protein concentration. The dissociation constant is equal to the concentration of protein when 50% of the binding site is occupied, as we now discuss. To do this, the DNA concentration in the assay must be far lower than the dissociation constant for the interaction. This constraint arises directly from the definition of the dissociation constant:

Kd = [P] [DNA] / [P/DNA]

where [P] and [DNA] are the concentrations of the free DNA-binding protein and binding site, respectively, and [P/DNA] is the concentration of the complex. At 50% occupancy, [DNA] = [P/DNA], and Kd = [P]. However, we only know [P] if the total amount of DNA is so small that it does not significantly deplete the pool of free P; in other words, [P/DNA]<<[P].

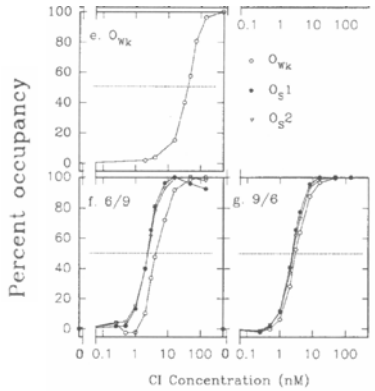


Figure 4. Binding curves for CI on all templates. Percentage occupancy at each CI concentration was determined by analysis of DNase I footprinting gels such as those shown in Figure 3. Symbols are the same for all plots, as shown. Certain points represent the average of duplicate samples from the same experiment. The broken horizontal line indicates 50% occupancy, a value that represents K_{app}, the apparent affinity of CI for each site. For a few points at low repressor concentrations, small negative values were obtained by this analysis; this reflects a slight uncertainty in assessing zero percentage occupancy in the samples lacking CI.

Table 1. Apparent dissociation constants for templates with three operators

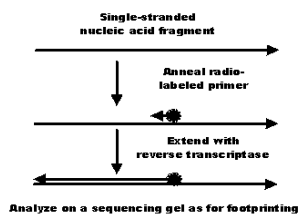
DNA template	Operators O_{wk}	O_s1	O_s2
6/6	1.4	1.3	1.3
9/6	2.9	2.3	2.5
6/9	4.3	2.4	2.4
9/9	38	3.0	3.6
O_s1-6 bp-O_s2	–	4.3	3.7
O_s1-9 bp-O_s2	–	3.4	3.4
O_{wk}	41	–	–

Values were taken from the binding curves shown in Figure 4, and represent the concentration of CI (nM monomer) required to give half-maximal occupancy of the operator site listed at the top. We estimate conservatively that the apparent dissociation constants have an uncertainty of +20%/−17% (see Materials and Methods).

This study shows that reducing the spacing between adjacent operators might allow cooperative interaction among three dimers, a mode of extended cooperativity.

Refinements to footprinting methodology- DMS Methylation

Other agents that break or damage DNA can be used in addition to DNase I. Dimethyl sulfate (DMS) methylates G residues, creating an adduct that can be broken chemically. Typically, complexes are formed, then treated with DMS, then the reaction is stopped. The position of the breaks is then determined by primer extension. This approach has several advantages. First, it can be carried out under conditions (*e.g.*, absence of magnesium) in which DNase I is inactive. Second, it can be done on covalently closed, supercoiled DNA, which often binds proteins differently than linear DNA.



Nature Structural & Molecular Biology **11**, 1223 - 1229 (2004)

Structure of human POT1 bound to telomeric single-stranded DNA provides a model for chromosome end-protection

Ming Lei, Elaine R Podell & Thomas R Cech

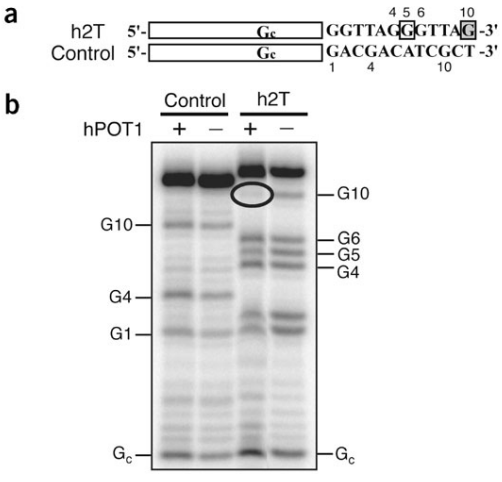
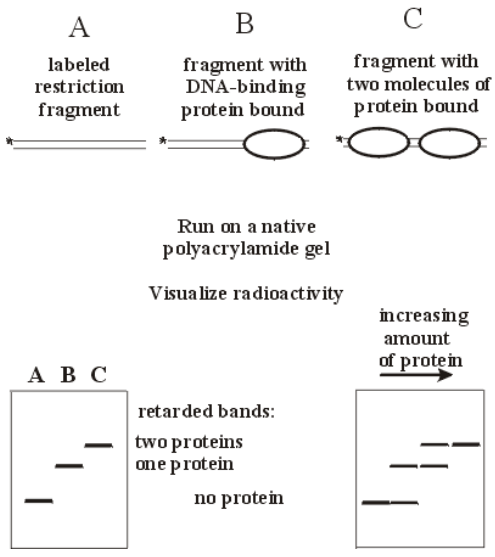


Figure 4. Telomeric DNA end-binding by hPOT1.
(**a**) Schematic representation of the methylation footprint patterns generated by hPOT1. The nucleotide numbering of h2T and the control oligonucleotide is at the top of the DNA sequences. Squares with white and gray shading represent moderate and extensive protection from methylation, respectively. (**b**) The 5'-labeled h2T and control oligonucleotide were incubated with or without hPOT1V1, and subjected to DMS methylation and pyrrolidine cleavage. The methylation footprint patterns were analyzed by urea-PAGE. Gc, a G residue in the 5' nontelomeric sequence serves as an internal control for loading differences. The black ellipse denotes the heavy protection of G10. The experiment was repeated with hPOT1V2 with equivalent results (data not shown).

Gel Mobility Assay



EMSA (Electrophoresis Mobility Shift Assay)

Prepare a labeled restriction fragment containing one or more binding sites for a specific DNA-binding protein.

DNA molecules to which proteins bind move more slowly in the gel and are retarded relative to the sample with no protein.

Refinements to the gel mobility assay

1. Complex mixtures of proteins

This assay can be carried out in the presence of **complex mixtures of proteins**, either in crude extracts or with partially purified components.

2. Supershift assay

When one uses a complex mixture of proteins, it's not clear which one is binding to the DNA. When an antibody is available that interacts with a protein of interest (call it the antigen), one can ask whether a particular shifted band contains the antigen by having a second incubation that includes the antibody. If the complex shifts further up in the gel (the "supershift"), this is evidence that the antigen was present in the initial complex.

3. Competition experiments

Addition of unlabeled DNA can compete with the labeled DNA for the protein. This assay is done when there is a limited amount of protein, so that it can be saturated.

Competition experiments can be done for several reasons:

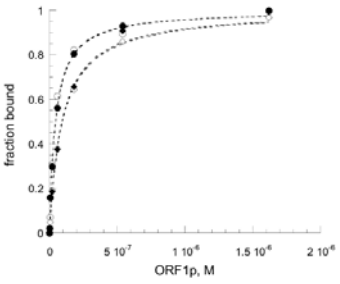
a. If you want to ask whether a particular protein is in the complex, you can add a known high-affinity site for that protein. If the protein is in the complex, it should be competed away; if not, then addition of the competitor has no effect.

b. If you want to determine which of the bases in the DNA are important, you can add competitor DNA with changes in particular bases. If the base is important, then the mutant template will have less effect than the same amount of the wild-type template.

Nitrocellulose filter-binding assay

This assay is no longer used widely, but it is rapid and simple, and can give a lot of information. It would be used for relatively detailed analysis of a particular protein-DNA interaction, for example.

Step	Comments
Incubate labeled DNA with protein	Allow enough time to allow the system to reach equilibrium
Filter the mixture through a filter disk made of nitrocellulose	Proteins bind to nitrocellulose, but DNA does not. Any DNA that is retained on the filter is there because it is interacting with the protein. A nylon membrane can be added to bind the free DNA.
Dry the filters and count	



High-affinity, Non-sequence-specific RNA Binding by the Open Reading Frame 1 (ORF1) Protein from Long Interspersed Nuclear Element 1

J. Biol. Chem. Vol. 278, Issue 10, 8112-8117, March 7, 2003.

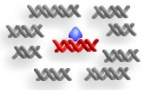
Binding of ORF1p to sense and antisense 40-nt transcripts. Nitrocellulose filter binding assay using the same dilution series of ORF1p with 10 pM ( ) and 40 pM ( ) of sense 40 RNA or 10 pM ( ) and 40 pM ( ) of antisense 40 RNA in binding buffer containing 410 mM NaCl. The K_{Dapp} from these data are 4.5×10^{-8} and 9.4×10^{-8} for sense and antisense transcripts, respectively. R2 is >0.99 for all curve fits.

Method	Advantages	Disadvantages and limitations
Footprinting	1. It is an equilibrium method 2. Other agents besides DNase I can be used 3. It localizes the binding site to within a few bp.	1. Tedious, technically demanding 2. DNase I can only be used under conditions that support its activity (presence of Mg^{++} and Ca^{++} ions).
Gel mobility assay	1. Technically easy 2. Gives some information about the mass of the protein bound 3. Can reveal multiple complexes 4. Can be used to measure DNA bending	1. Not really an equilibrium method, but can give info about relative affinities 2. Does not localize binding site
Filter-binding assay	1. Quick and easy 2. Can measure on- and off-rates	1. Does not localize binding site
Genetic analysis	1. Helps identify important bases in the binding site 2. With site-directed mutagenesis, can test models based on x-ray structures.	Analysis of protein is hard to interpret except in conjunction with x-ray crystallographic data
X-ray crystallo-graphy	1. Can give structural information at atomic resolution 2. Can give extremely explicit testable models for specificity of interaction.	1. Not for everyone! Requires a lab dedicated to this approach. 2. Not all DNA-binding proteins can be solved; many are "floppy". One solution is to use fragments or domains of protein.

Chromatin Immunoprecipitation (ChIP)

Chromatin immunoprecipitation, or ChIP, refers to a procedure used to determine whether a given protein binds to or is localized to a specific DNA sequence *in vivo*.

DNA-binding proteins are crosslinked to DNA with formaldehyde in vivo.



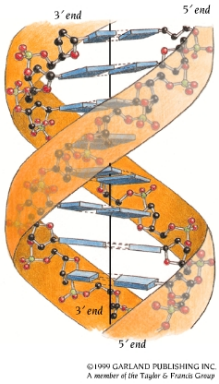
Isolate the chromatin. Shear DNA along with bound proteins into small fragments.

Bind antibodies specific to the DNA-binding protein to isolate the complex by precipitation. Reverse the cross-linking to release the DNA and digest the proteins.

The identity of the DNA fragments isolated in connection with the protein can then be determined by **PCR** using specific primers for the region that the protein in question is hypothesized to bind DNA. Alternativly when it is interesting to find where the protein binds accross the whole genome **DNA microarray** is uesd (ChIP on chip or ChIP-chip).

http://www.bio.brandeis.edu/haberlab/jehsite/chip.html

DNA Structure



B-DNA

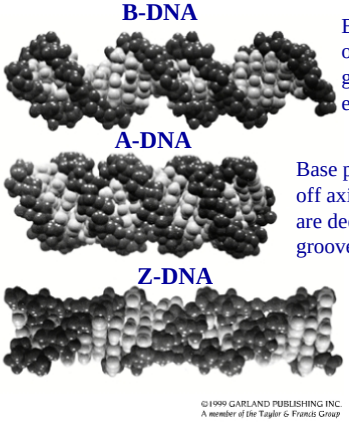
Base pairs stack on the axis- grooves are of equal depth.

A-DNA

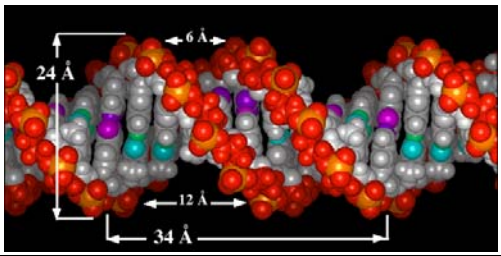
Base pairs are displaced off axis- major grooves are deeper and minor grooves are shallower.

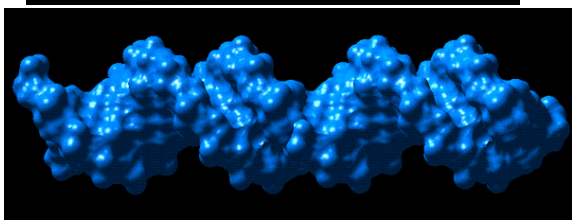
Z-DNA

Left-handed. CGCGCG- first structure solved in 1979.



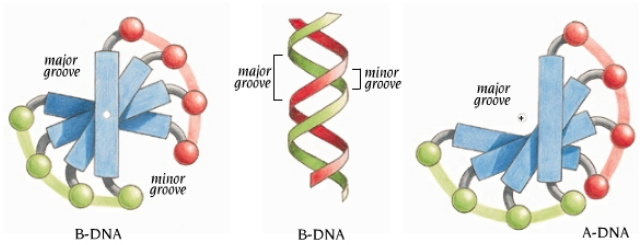
A-DNA occurs as a dehydrated form of DNA, the A-form is also the preferred conformation of RNA. Z-DNA can occur at stretches of alternating G and C bases and may be important for controlling replication. But by far the most important physiological conformation is B-DNA.

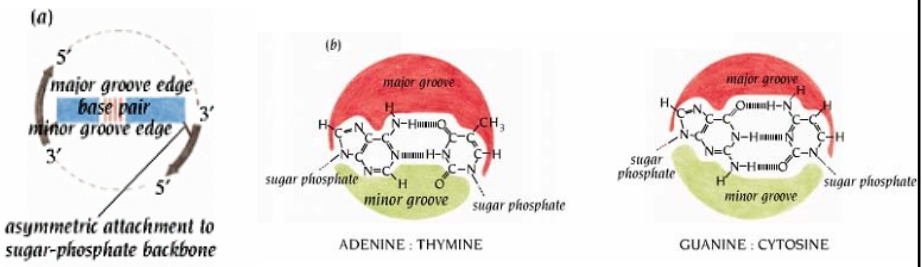


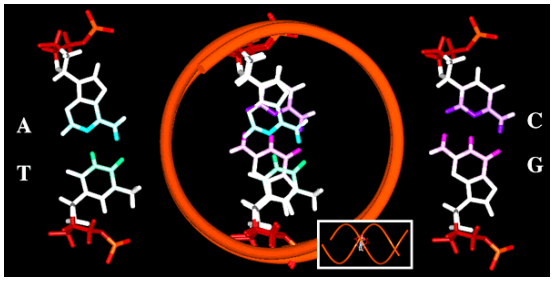


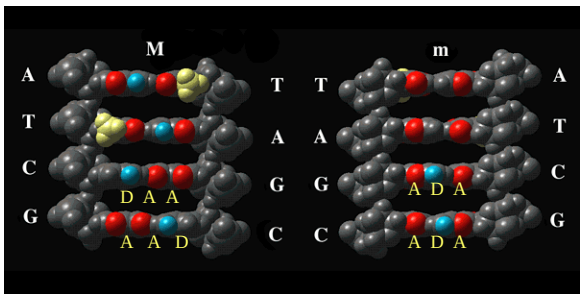
Since the base pairs are attached asymmetrically to the backbone, one groove between the strands is wider than the other. These are called the major and the minor groove. Both grooves provide opportunities for base-specific interactions, but the major groove is better suited for that task and more often observed as the primary binding site for proteins.

Major groove and minor groove



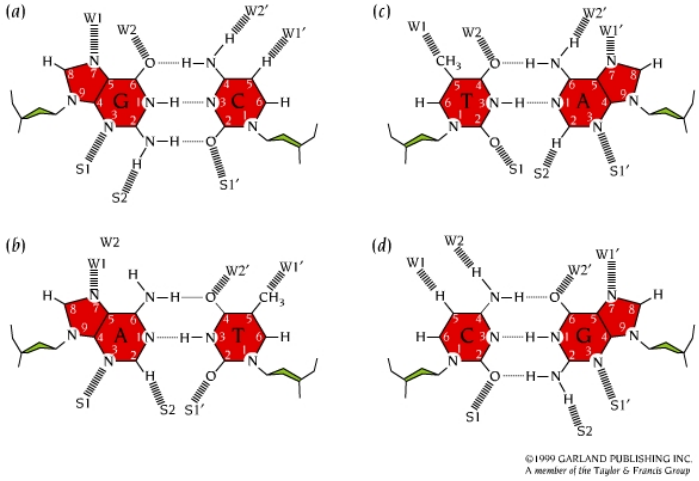




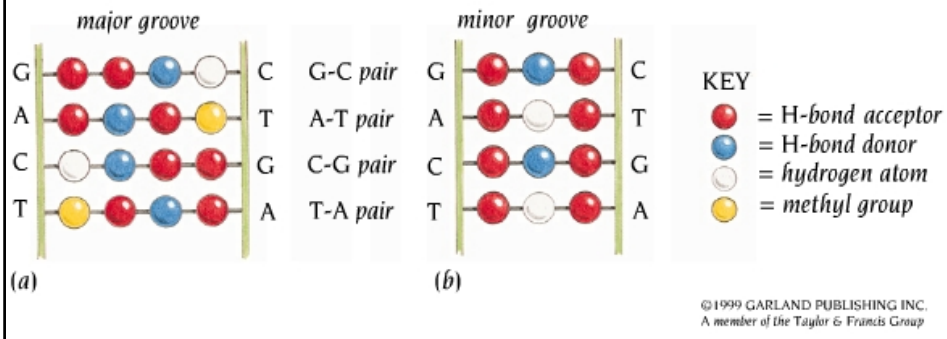


This is well illustrated, when one considers the hydrogen bond donors, hydrogen bond acceptors and methyl groups, accessible at the bottoms of the major and minor grooves of B-DNA. The se functional groups provide ample opportunity for sequence-specific interaction of proteins with DNA. Additionally, the idealized B-DNA backbone is quite significantly distorted in real-life and the phosphate backbone conformation itself will thus display sequence information.

Sequence-specific recognition



W: recognition sites at major groove
S: recognition sites at minor groove



Proteins prefer to bind DNA at major grooves likely because:
(1) more space and
(2) more chemical features
presented by the major groove.

Ideal B-DNA
Rise = 3.4 Angstrom

Tilt

Roll

Roll > 0° bends towards major groove
Roll < 0° bends towards minor groove

Twist < 34°, DNA is unwound
Twist > 34°, DNA is over wound

Twist

Propellor Twist

Figure 1.13 Tilt, roll, twist, and propeller twist. Tilt refers to the angle between two planes of base pairs. It is an angle of deflection in the direction of hydrogen bonds between two base pairs. The tilt angle opens or closes toward the phosphate backbone. Roll angle refers to the angle of deflection of two planar base pairs in a direction perpendicular to the direction of the hydrogen bonds between the base pairs. Twist refers to the rotation of one base pair with respect to another. Propeller twist refers to the angle of roll of one base relative to the other within a pair of hydrogen bonded bases.

Helix Parameters

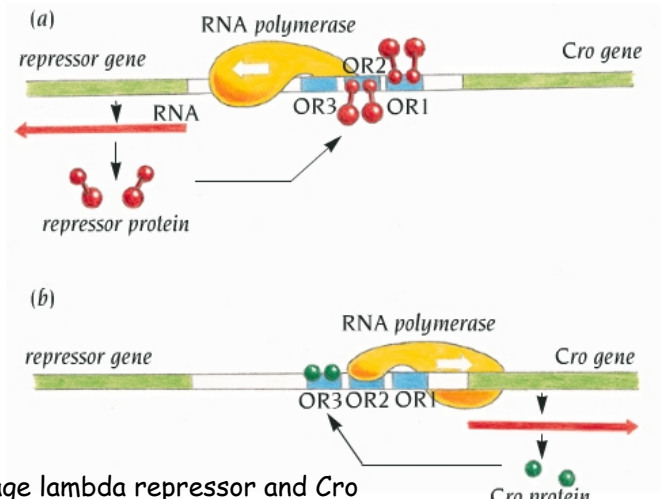
Parameter	A-DNA	B-DNA	Z-DNA
Helix sense	Right	Right	Left
Residue per turn	11	10 (10.5)[a]	12
Axial rise (Å)	2.55	3.4	3.7
Helix pitch (°)	28	34	45
Base pair tilt (°)	20	−6	7
Rotation per residue (°)	33	36 (34.3)[a]	−30
Diameter of helix (Å)	23	20	18
Glycosidic bond configuration			
dA, dT, dC	anti	anti	anti
dG	anti	anti	syn
Sugar pucker			
dA, dT, dC	C3′ endo	C2′ endo	C2′ endo
dG	C3′ endo	C2′ endo	C3′ endo
Intrastrand phosphate–phosphate distance (Å)			
dA, dT, dC	5.9	7.0	7.0
dG	5.9	7.0	5.9

[a]Values in parentheses are the residues per turn and rotation per residue for B-form DNA as it exists in solution of physiological ionic strength. Other values are taken from X-ray diffraction data.

DNA recognition in procaryotes by helix-turn-helix motifs

Lyzogenic phase

Lytic phase



Bacteriophage lambda repressor and Cro proteins operate a genetic switch

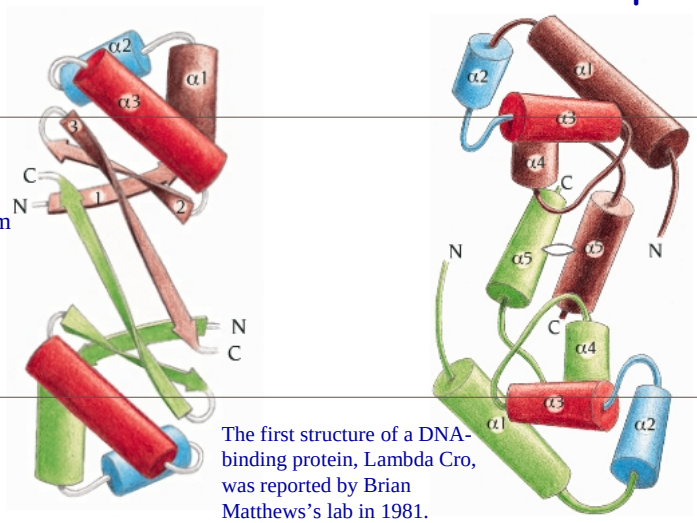
Table 8.1 The nucleotide sequences of the three protein-binding regions OR1, OR2, and OR3 of the operator of bacteriophage lambda

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
OR1	5′	T	A	T	C	A	C	C	G	C	C	A	G	T	G	G	T	A	3′
	3′	A	T	A	G	T	G	G	C	G	G	T	C	A	C	C	A	T	5′
OR2	5′	T	A	A	C	A	C	C	G	T	G	C	G	T	G	T	T	G	3′
	3′	A	T	T	G	T	G	G	C	A	C	G	C	A	C	A	A	C	5′
OR3	5′	T	A	T	C	A	C	C	G	C	A	A	G	G	G	A	T	A	3′
	3′	A	T	A	G	T	G	G	C	G	T	T	C	C	C	T	A	T	5′

Palindromic base pairs that are most frequent at the two ends are green, and the pseudo-twofold symmetry axis is indicated by a red dot.

Lambda Cro

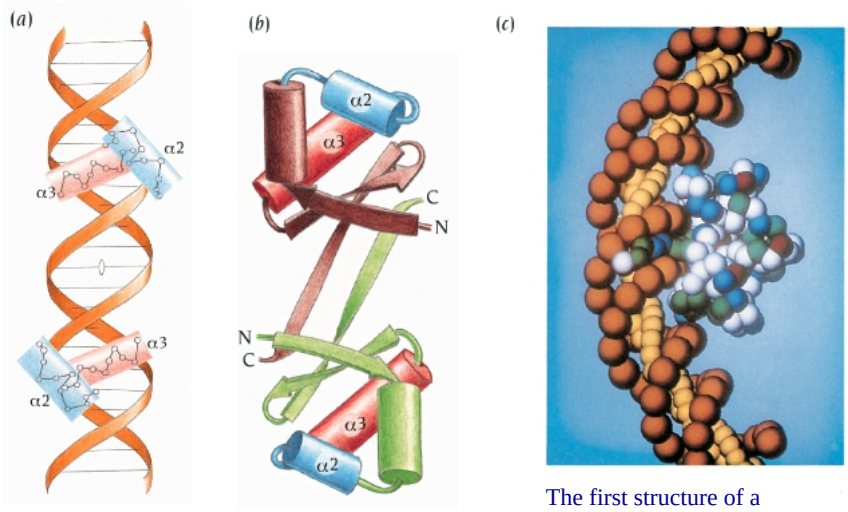
Lambda repressor



The first structure of a DNA-binding protein, Lambda Cro, was reported by Brian Matthews's lab in 1981.

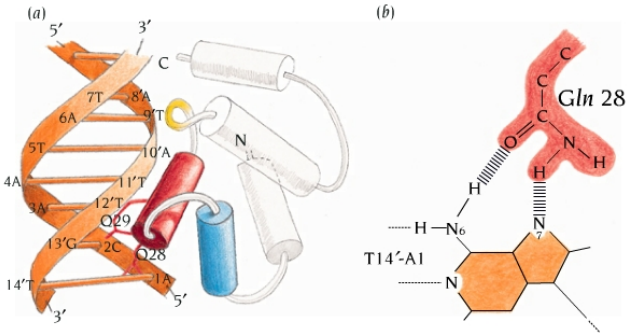
1. Both are dimers
2. α2 and α3 are the helix-turn-helix motifs
3. α3 helices are separated by a distance of 34 Angstrom

The helix-turn-helix DNA binding motif in lambda Cro bound to DNA



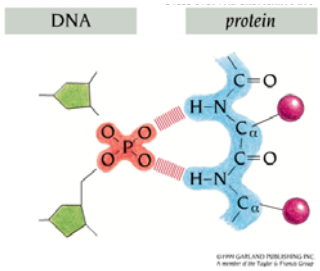
The first structure of a protein/DNA complex, 434 repressor/DNA, was solved by Steve Harrison's lab in 1987.

Specific and nonspecific interactions

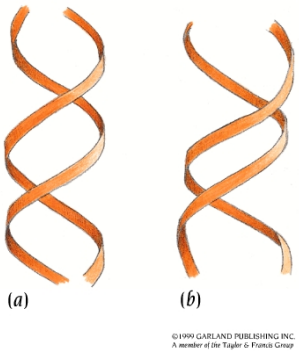


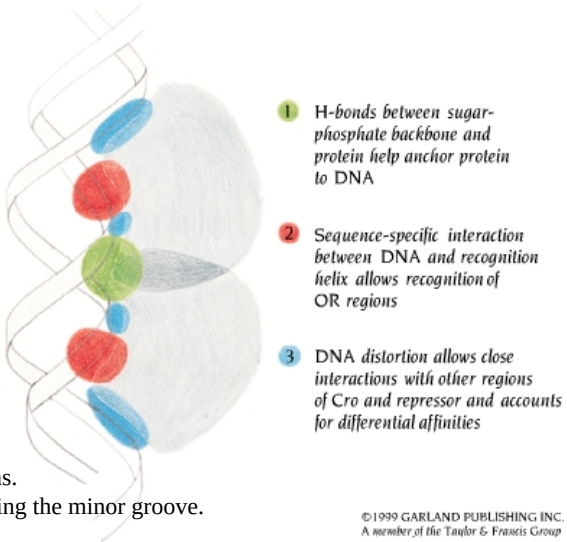
Sequence-specific interactions

434 repressor/DNA complex



Nonspecific protein-DNA interactions



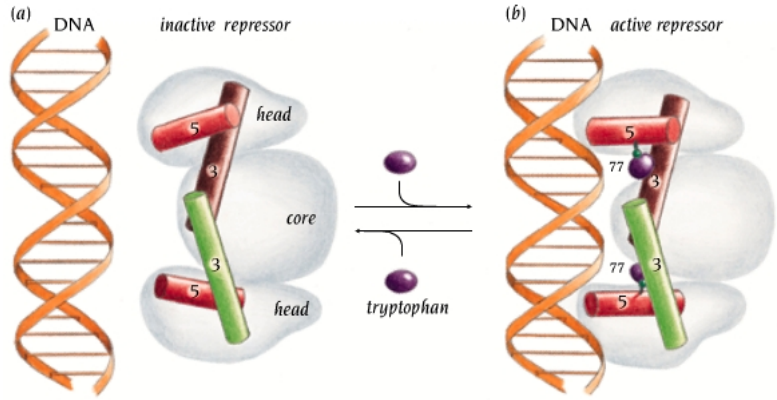


The B-DNA is distorted when Cro or repressor bind to operator regions.
DNA bends toward protein narrowing the minor groove.

Why repressor binds OR1 with higher affinity?
Ability of DNA to undergo specific structural change (repressor cannot induce OR3 to distort which is required for tight binding).

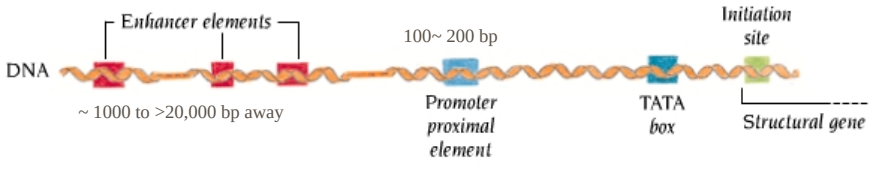
DNA binding is regulated by allosteric control

Allosteric effectors bind at sites distant from functional binding sites but cause a conformational change which alters the DNA binding affinity.

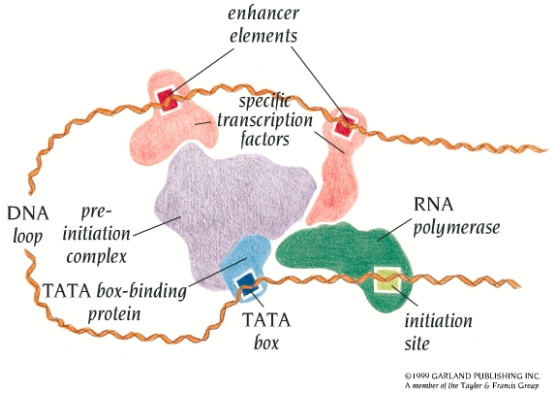


Trp repressor regulates the synthesis of L-Trp by a "negative feedback loop". When L-tryptophan binds to Trp repressor, the heads change positions to produce the active form of the repressor, which binds to DNA.

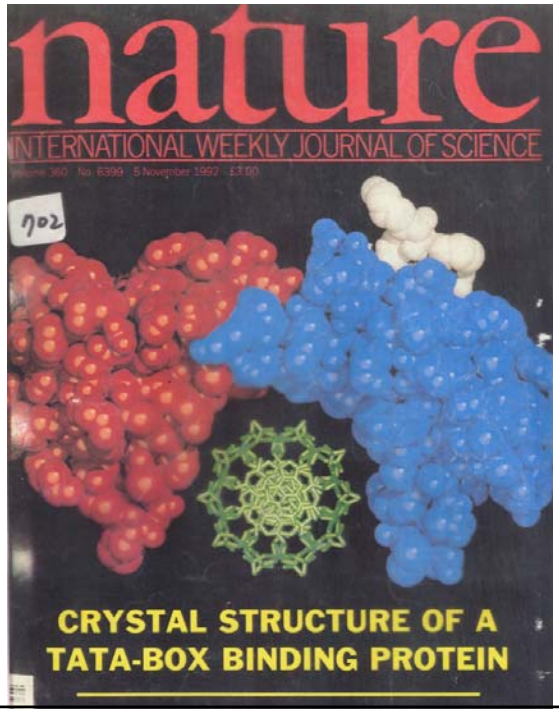
DNA recognition by eukaryotic transcription factors



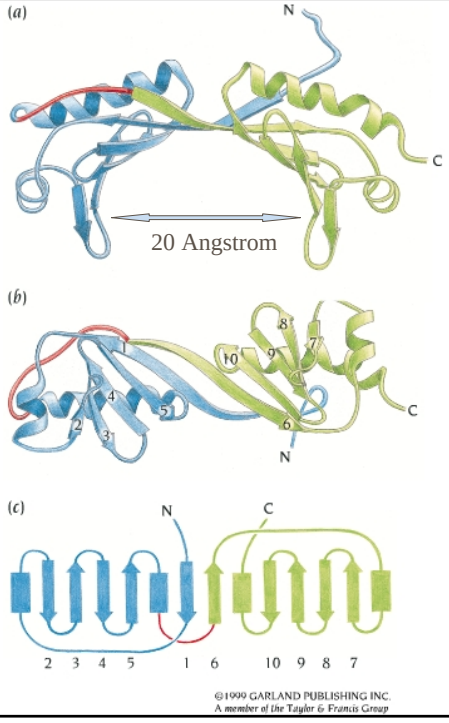
Transcriptional activation



1992 Steven Burley reported the crystal structure of TATA-box binding protein

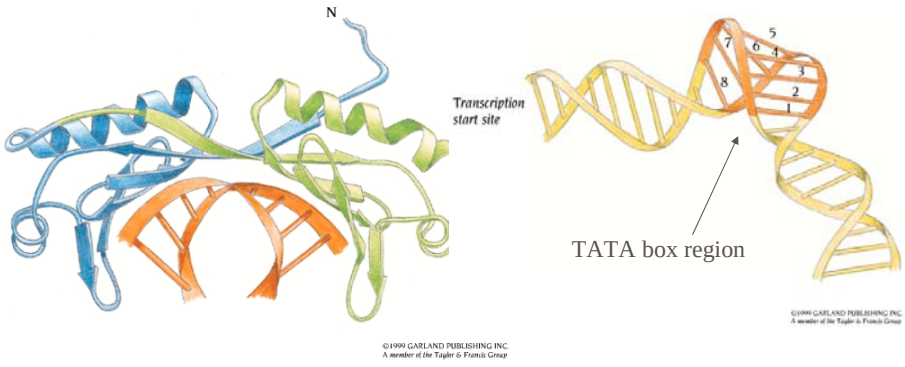


Crystal structure of the TATA-box binding protein with a saddle-like topology



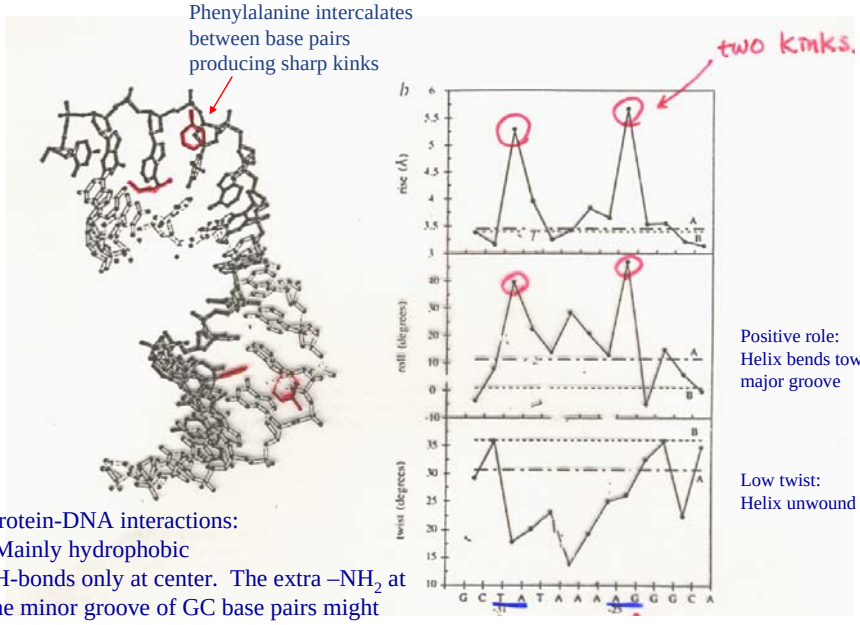
In 1993, the crystal structure of TATA-box binding protein in complex with DNA was resolved by Paul Sigler (yeast) and Steven Burley's (Arabidopsis) laboratories.

TBP binds in the minor groove and induces large structural changes in DNA



DNA is bended for ~ 100 degree.

Phenylalanine intercalates between base pairs producing sharp kinks

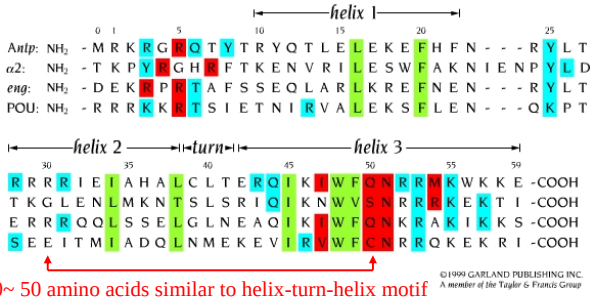


Positive role: Helix bends toward major groove

Low twist: Helix unwound

Protein-DNA interactions:
-Mainly hydrophobic
-H-bonds only at center. The extra $-NH_2$ at the minor groove of GC base pairs might interfere with binding.
-TATA sequence is more flexible for bending.

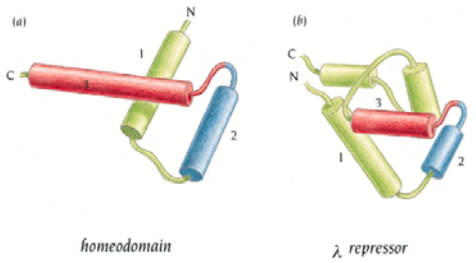
Homeodomain proteins are involved in the development of many eucaryotic organisms



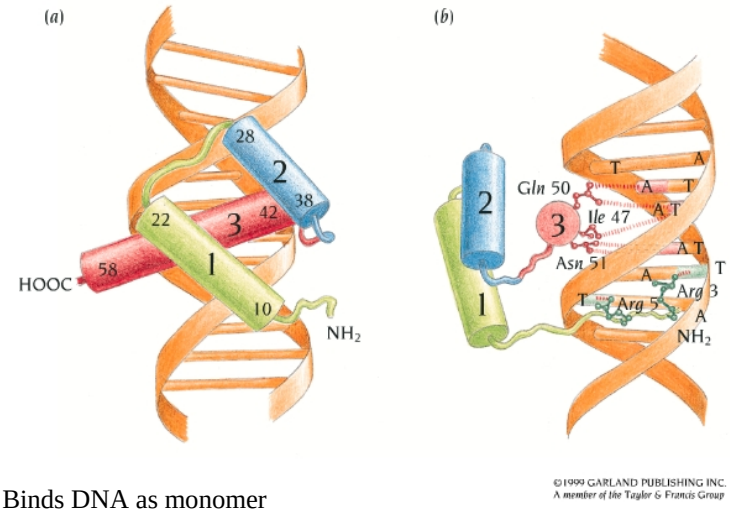
30~ 50 amino acids similar to helix-turn-helix motif

~180 bp DNA first discovered in Drosophila. Mutation in these regions produces homeotic transformation.

Red: specific
Blue: non-specific

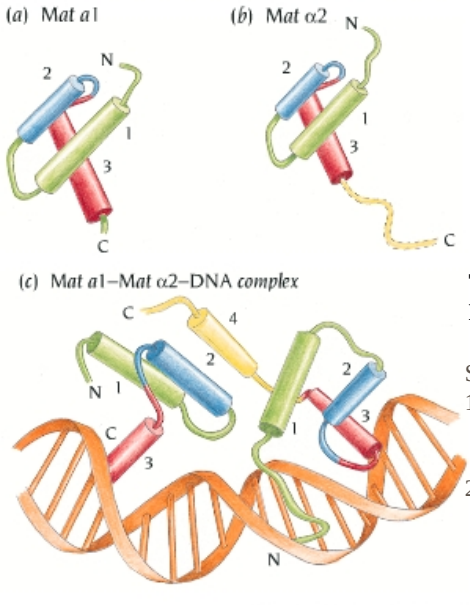


Recognition helix of homeodomain is longer than in the one in the helix-turn-helix motif



Binds DNA as monomer
Recognition helix binds in the major groove and the N-terminal tail binds at the minor groove.
Helix-3: specific interactions; N-terminal tail: nonspecific interactions

In vivo specificity of homeodomain transcription factors depends on interactions with other proteins



Mat α2/Mat a1
Mat α2/ MCM1
Specify two cell-types in yeast

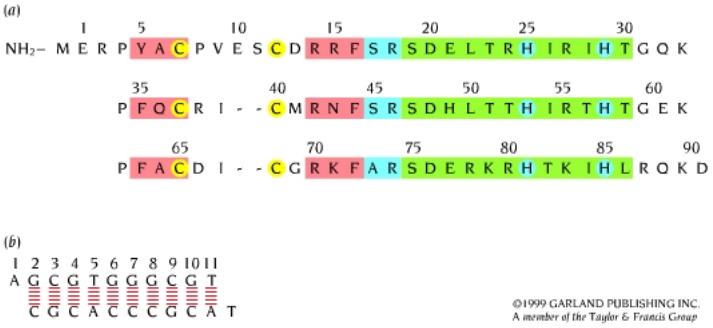
The structure of the yeast Mat a1-Mat α2-DNA complex

Strategies for increasing specificity:
1. Heterodimer interface specifies the precise spacing between the two binding sites.
2. Increasing the length of DNA recognition sequence.

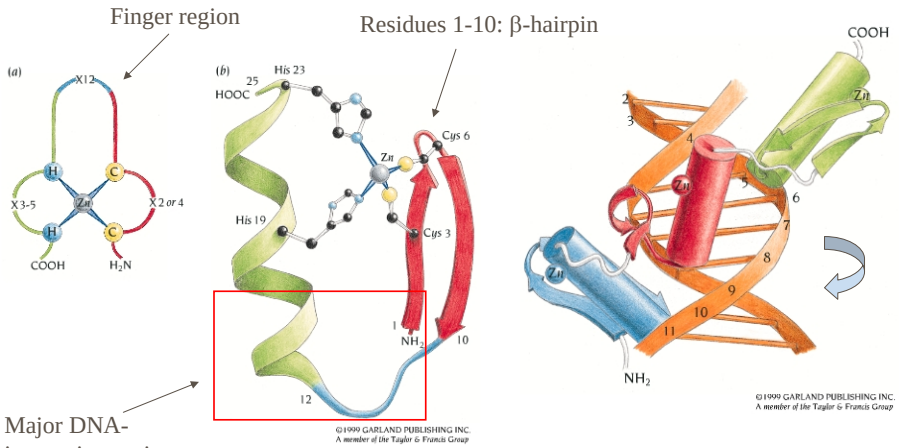
Examples of several DNA binding motifs:

Classic Zinc Fingers

Firstly discovered in TFIIIA containing nine repeats of 30-amino acid motif with conserved C-C-H-H sequence.

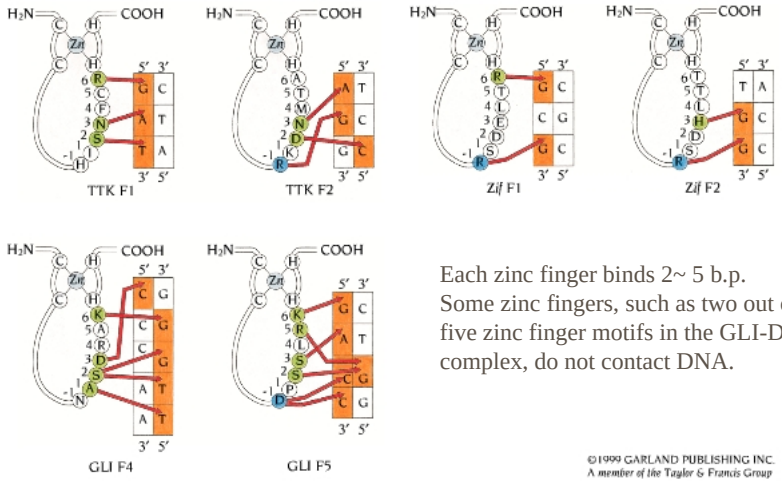


Amino acid sequence of a fragment of the Zif268 proteins that contains three zinc fingers



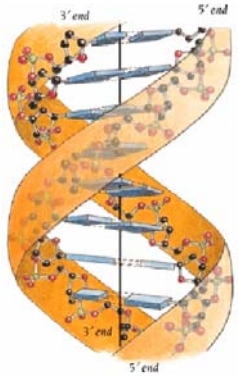
Alpha helix pointing into major groove
Rotation ~96 deg (3 x 32)
Translation 10 A (3 x 3.4)

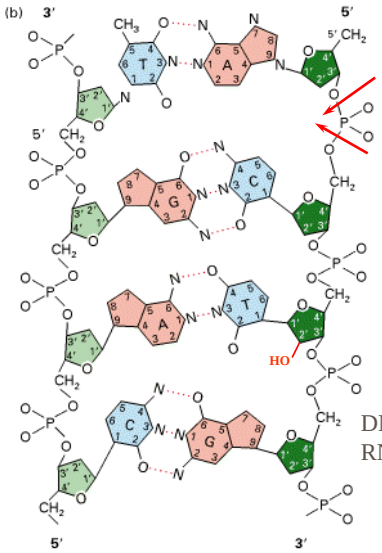
The finger region of the classical zinc finger motif interacts with DNA
But there is no simple rule that governs which bases the fingers contact



Each zinc finger binds 2~ 5 b.p.
Some zinc fingers, such as two out of five zinc finger motifs in the GLI-DNA complex, do not contact DNA.

Nucleases are a group of enzymes capable of hydrolyzing nucleic acids by breaking the phosphodiester bonds.





Nucleases cleave at 3'-side or 5'-side of the phosphodiester linkage.

DNase: deoxyribonuclease
RNase: ribonuclease

Structure and function of restriction endonucleases

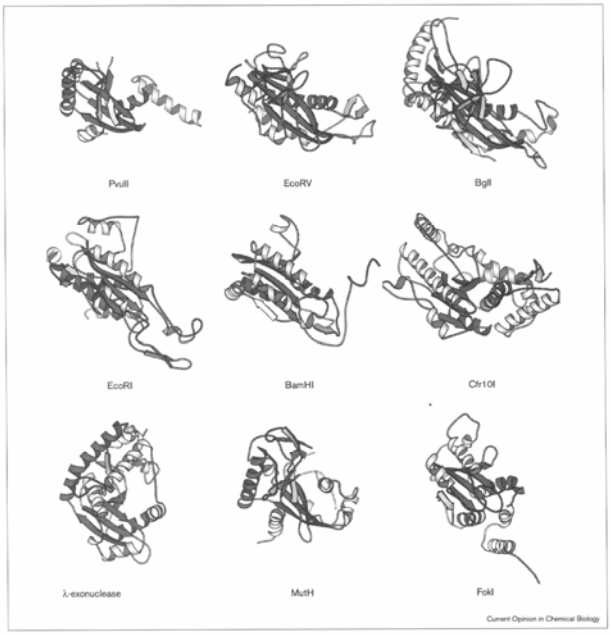
- Each protein of a pair of restriction endonuclease and modification methylase binds specifically and with high affinity to the same small 4-8 bp DNA sequence.

- More than 3000 restriction endonucleases discovered to date belong to the type II class, which recognize and cleave short palindromic DNA sites, requires only Mg^{2+} for optimal activity.

-Bacterial restriction enzymes cleave at the recognition sequence at least 10^6 times faster than at any other DNA sequences.

-Lack of sequence similarity among the type
- II restriction enzymes.

Fig. 1. The DNA recognition sequences of *Bam*HI, *Eco*RI, *Pvu*II, and *Eco*RV. The sites of cleavage are indicated by arrowheads.



Ribbon diagrams of the monomers of the type II restriction endonucleases PvuII, EcoRV, BglI, EcoRI, BamHI, Clr10I and the structurally related family members λ-exonuclease, MutH and FokI. The darker shaded regions correspond to the catalytic core that is conserved among all of these proteins. Each structure is displayed in a similar orientation. The figure was created with MOLSCRIPT [36].

-All the type II restriction endonucleases have a structurally similar catalytic core that consists of a five-stranded β-sheet flanked by two α-helices.

-This conserved catalytic core brings together three essential charged residues, typically two acidic residues and one lysine residue

Ref: Kovall, R. A. and Matthews, B. W. Curr. Opin. Chem. Biol. 1999, 3, 578-583.

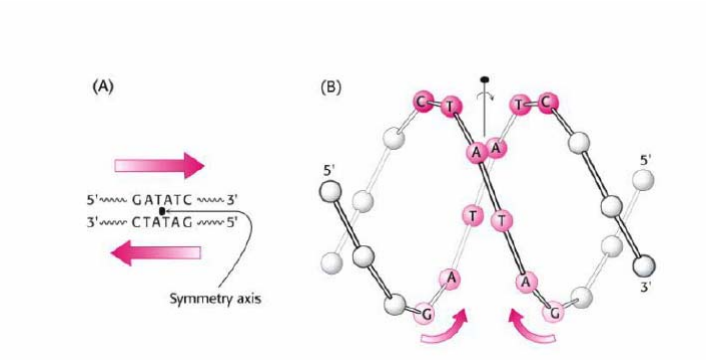
*Eco*RV - example

a. Characteristics

(1) Cleaves double stranded DNA with recognition site 5'-GATATC-3' (cleaves in middle of TA) on both strands

(2) Exists as a homodimer

(3) Each dimer binds 2 Mg^{2+} ions; each Mg^{2+} is coordinated by 2 Asp residues, 3 H_2O molecules and the phosphate oxygen from DNA backbone



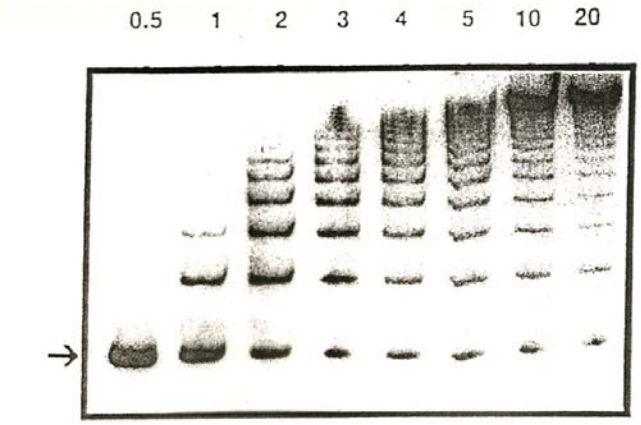


Fig. 3. Gel shifts of DNA binding by *Eco*RV. A 235 bp fragment of DNA, that contained one *Eco*RV site, was mixed with varied concentrations of *Eco*RV restriction enzyme (nM, given above each lane). The binding buffer was identical to the reaction buffer for *Eco*RV, except that it contained EDTA in place of $MgCl_2$. The DNA was then analysed by electrophoresis through polyacrylamide. The mobility of the free DNA is arrowed on the left of the gel. Reprinted, with permission, from *Biochemistry* (Taylor *et al.*, 1991); copyright (1991) American Chemical Society.

The crystal structure of *Eco*RV endonuclease in complex with its cognate and non-cognate DNA fragments

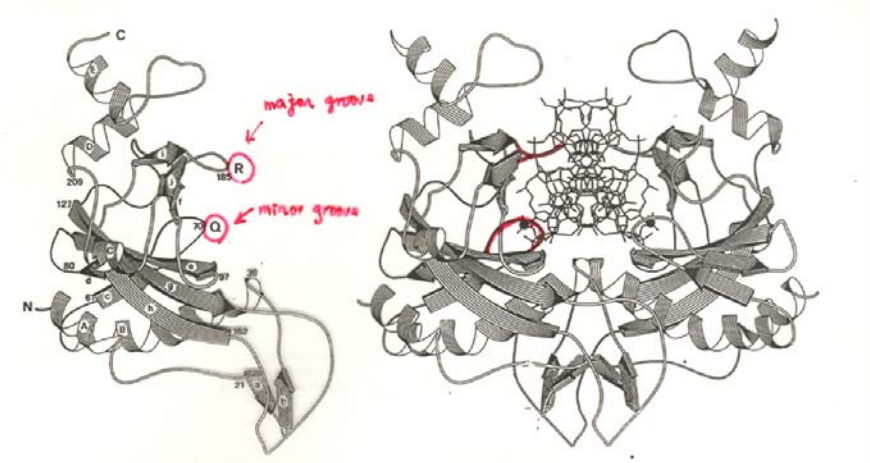
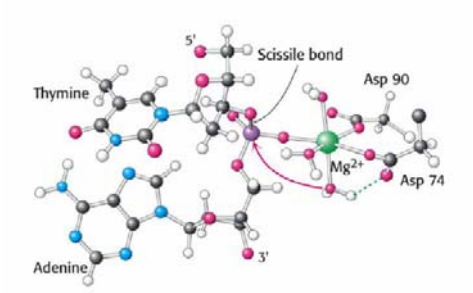
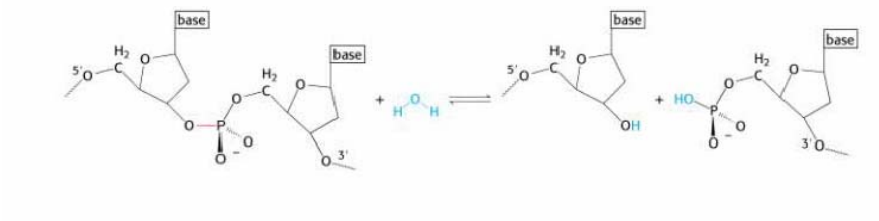


Fig. 2. *Eco*RV ribbon diagrams (Priestle, 1988). **(Left)** Monomer structure (subunit A of 1RVE) with α-helices and β-strands labelled according to Figure 1. N and C mark the N- and C-termini of the protein, Q and R the location of the Q-turn (residues 68–71) and recognition loop (residues 182–187) respectively, both important for DNA interaction. In addition the approximate positions of selected residues are indicated with residue numbers. **(Right)** Dimer structure (crystallographic dimer of 3RVE) with a stick model of the bound cognate DNA fragment. The two symmetrically disposed scissile phosphodiester groups are emphasized by black circles.

http://www.worthpublishers.com/lehninger3d

Mechanism- enzyme uses an in- line direct attack of a metal produced hydroxide on phosphorus

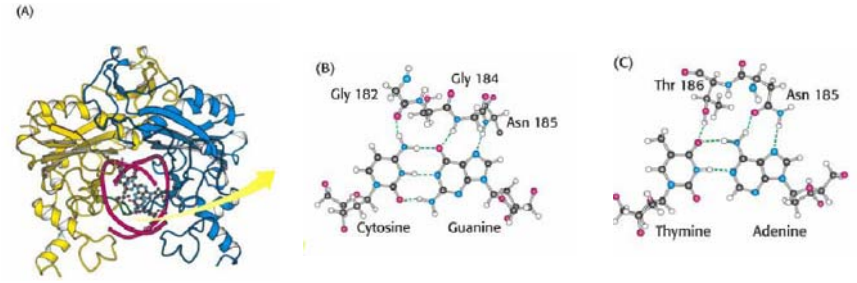




How does *Eco*RV only cleave at recognition site in cognate DNA?

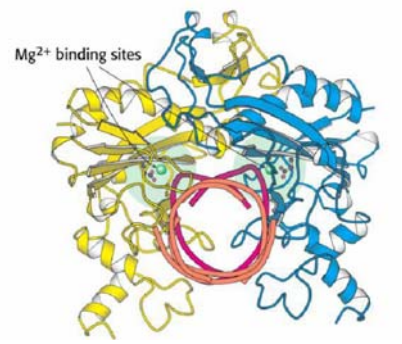
Cognate DNA

(a) Double stranded DNA binds in active sites- interactions between GA-TC and enzyme. No interactions between enzyme and interior TA.
(b) DNA structure is distorted (kinked) by hydrogen bonds to active site residues (TA is particularly easy to distort).
(c) Distorted DNA allows phosphate oxygen at TA to bind to Mg^{2+}
(d) DNA is hydrolyzed



Non-cognate DNA

(a) Double stranded DNA binds
(b) If proper hydrogen bonds do not form and if no TA interior sequence, the DNA does not kink
(c) Phosphate oxygen not brought into correct position for Mg^{2+} to bind
(d) No DNA hydrolysis
(e) Lack of "kinking" accounts for 10^6-fold specificity of cognate DNA hydrolysis as compared to noncognate DNA



Non-specific DNA: CGAGCTCG

Specific DNA: GGGATATCCC

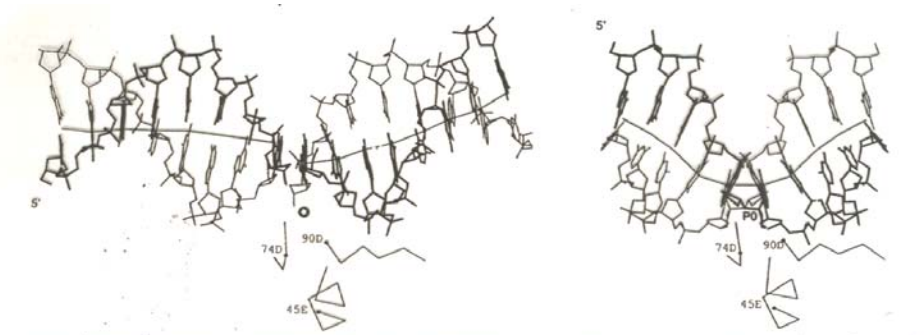
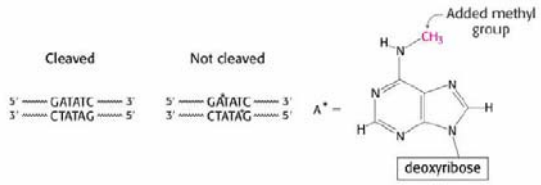


Fig. 5. Non-cognate and cognate DNA conformations. Side view of non-cognate (left) and cognate (right) DNA in their respective complexes, seen perpendicular to the quasi-twofold or twofold axis. The α-carbon positions of segments 38−45 and 73−76 and 90−95 (subunit A in 2RVE, subunit C in 3RVE) serve as common reference frames (r.m.s. error in superimposition = 0.73 Å). The DNA strands interacting predominantly with the represented protein subunits (E1−S1 interaction, Table II) are drawn in thicker lines. The scissile phosphodiester group of the cognate DNA is marked P0 just underneath the scissile O3′-P bond. The corresponding position with respect to the protein subunit of the non-cognate complex is indicated by a white star. The lines marking the paths of the helical axis are drawn to illustrate the small and large central kinking in the two complexes.

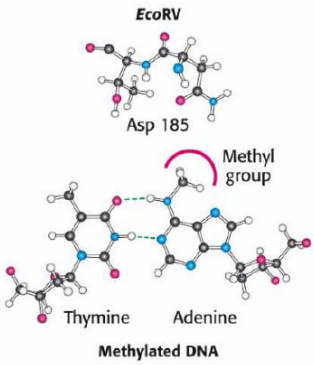
Why does *Eco*RV not hydrolyze host DNA?

(1) GATATC sequence is found often in *E. coli* genome
(2) As host DNA is synthesized, methylase methylates adenines bases



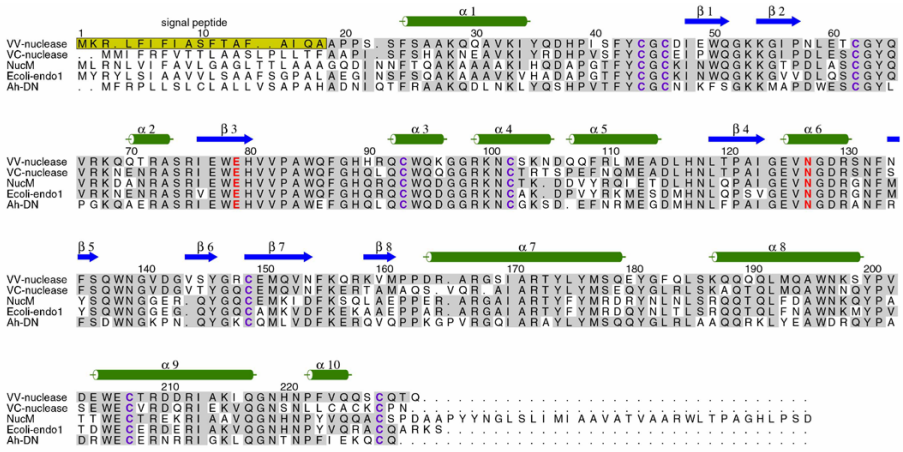
(3) Methyl group blocks a key hydrogen bond that forms between DNA and enzyme active site

(4) Proper kinking does not take place and DNA is not hydrolyzed

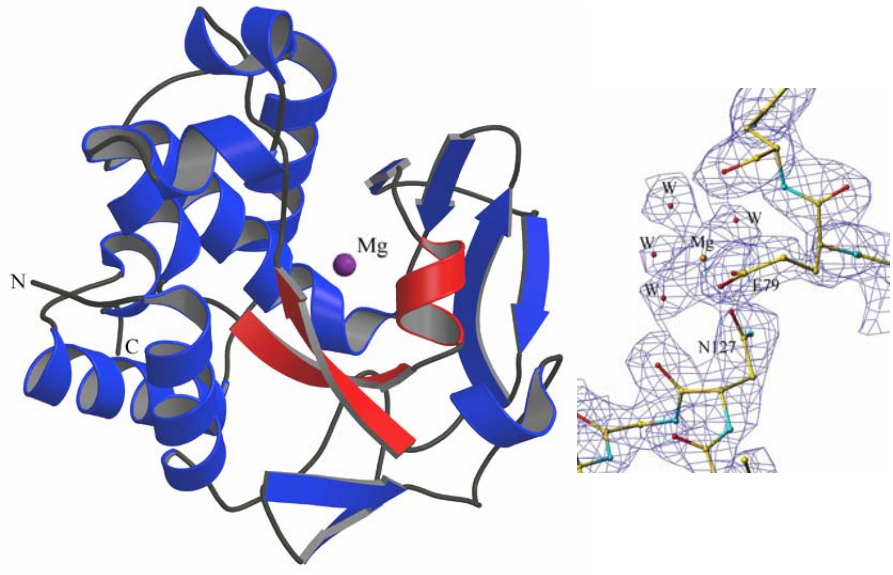


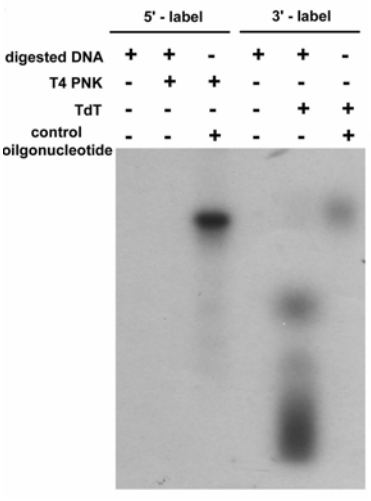
VVN- A periplasmic nuclease of *Vibrio Vulnificus* (創傷弧菌)

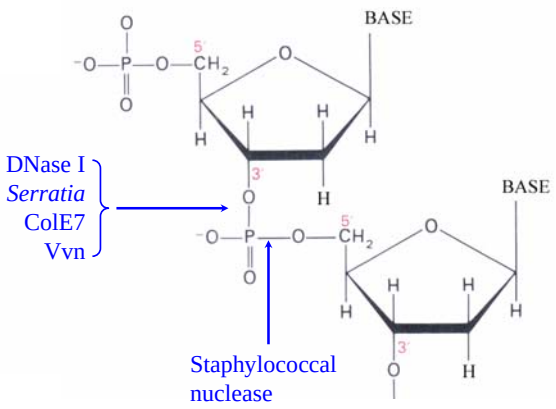
- *V. Vulnificus* is a bacterium in the same family of *Vibrio cholerae* (霍亂弧菌).
- Cloned in Lien-I Hor's lab in Cheng-Kung University.
- 18-amino-acid signal peptide + 214 amino acid residues.
- Likely involved in preventing the uptake of foreign DNA.



Crystal structure of Vvn at 2.3 Å resolution

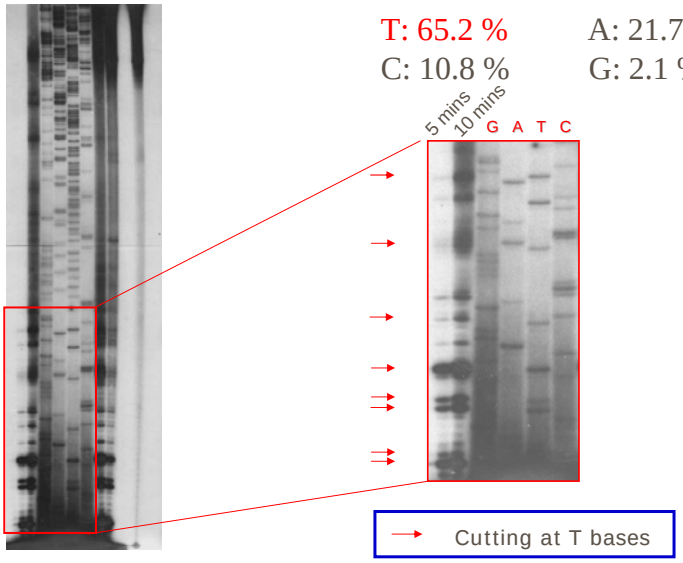




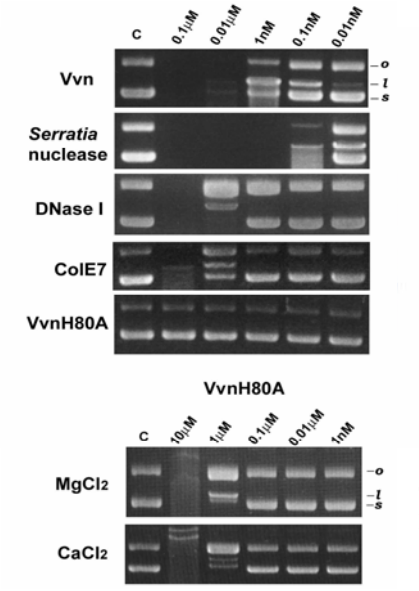


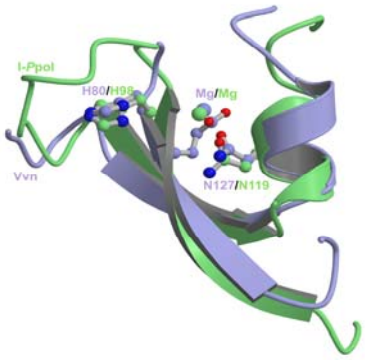
T4 PNK: T4 polynucleotide kinase
TdT: terminal deoxynucleotidyl transferase

Nuclease-ColE7 cleaves at the 3'-side of a phosphodiester bond with a preference to make nicks at thymine bases in dsDNA

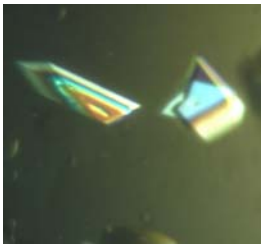


Superimposition of the active sties of I-*Ppo*I and Vvn

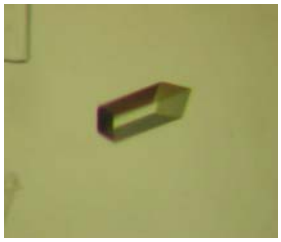




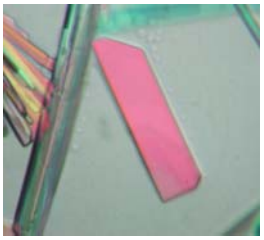
The average rms difference: 1.1 Å.
His80 in Vvn likely functions as a general base.



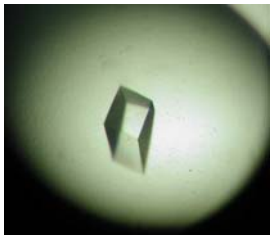
nuclease-ColE7+8-bp DNA+EDTA



Vvn-H80A+8-bp DNA+Ca^{2+}



nuclease-ColE7+16-bp DNA+EDTA

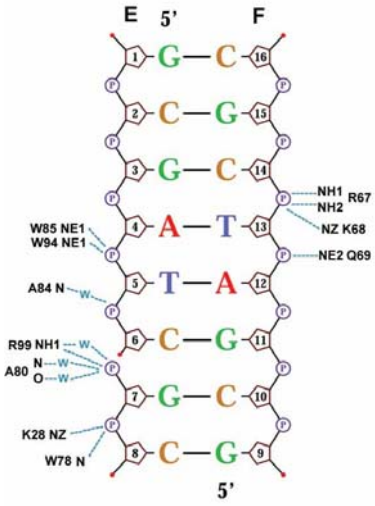


Vvn-H80A+16-bp DNA +Ca^{2+}

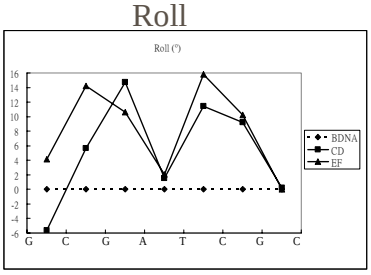
Crystal structure of Vvn/DNA complex at a resolution of 2.3 Å

Li, C.-L., et al. ***EMBO J.*** (2003) 22, 4014-4025.

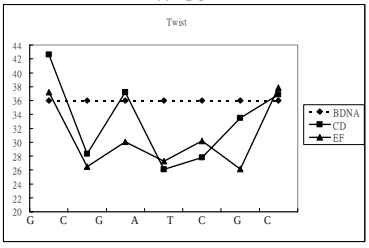
Only the DNA phosphate backbones make hydrogen bonds with Vvn, suggesting structural basis for its sequence-independent recognition of DNA and RNA.



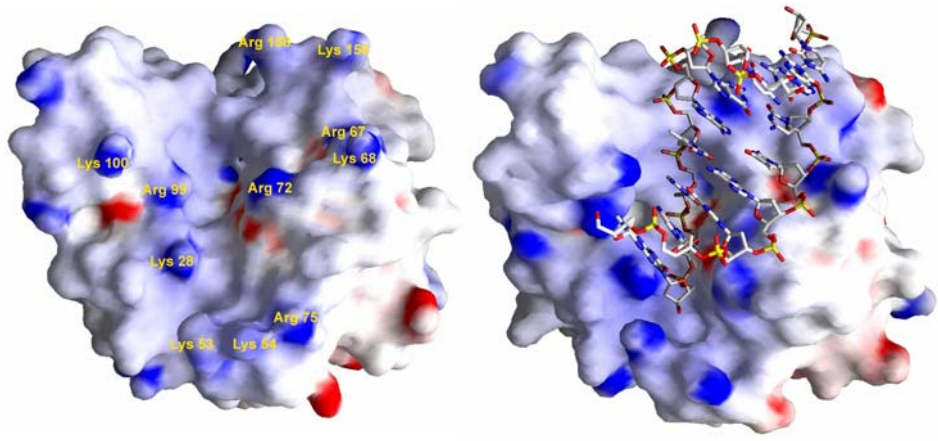
Roll



Twist



This structural study suggests that Vvn hydrolyzes DNA by a general single-metal ion mechanism, and indicates how non-specific DNA-binding proteins may recognize DNA.



Nonspecific nucleases interact with DNA primarily at backbones but not at bases, providing the structural basis for sequence-independent recognition

	Hydrogen Bond to DNA			Nonbonded contact to DNA				
	Base	Backbone	Total	Base	Backbone	Total	Backbone/ Base	Km
Non-specific nuclease								
DNaseI	3	18	21	0	2	2	6.7	$3x10^{-4}$ M
ColE7	3	14	17	0	2	2	5.3	$1x10^{-7}$ M
Vvn	0	3	3	0	11	11	>7	NA
Site-specific nuclease								
Homing endonuclease								
I-*Ppo*I	27	55	82	4	10	14	2.1	$1x10^{-11}$ M
PI-*Sce*I	11	26	37	5	21	26	2.9	NA
I-*Cre*I	21	43	64	12	28	40	2.1	NA
Restriction enzyme								
*Bgl*II	25	54	79	0	24	24	3.1	NA
*Eco*RV	12	17	29	5	22	27	2.3	$1x10^{-9}$ M
*Bam*HI	13	25	38	8	15	23	1.9	$4x10^{-9}$ M

Hsia, K.-C. et al, *Curr. Opin. Struct. Biol.* 15, 126-134 (2005).