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DNA Mimic Proteins: Functions, Structures, and Bioinformatic Analysis

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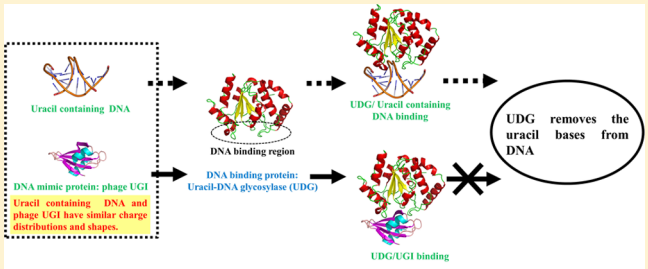
ABSTRACT: DNA mimic proteins have DNA-like negative surface charge distributions, and they function by occupying the DNA binding sites of DNA binding proteins to prevent these sites from being accessed by DNA. DNA mimic proteins control the activities of a variety of DNA binding proteins and are involved in a wide range of cellular mechanisms such as chromatin assembly, DNA repair, transcription regulation, and gene recombination. However, the sequences and structures of DNA mimic proteins are diverse, making them difficult to predict by bioinformatic search. To date, only a few DNA mimic proteins have been reported. These DNA mimics were not found by searching for functional motifs in their sequences but were revealed only by structural analysis of their charge distribution. This review highlights the biological roles and structures of 16 reported DNA mimic proteins. We also discuss approaches that might be used to discover new DNA mimic proteins.



An example of how the phage UGI DNA mimic protein can act as a competitive inhibitor for UDG

There are many examples of mimicry in the macroscopic world of plants and animals, but the concept of mimicry also extends to molecular levels. The most well-known examples of mimicry in the cell are enzyme inhibitors. These inhibitors possess chemical properties remarkably similar to those of the normal substrate of their target enzymes, such that competitive inhibition occurs when the "fake" substrate competes with the normal substrate for the active site on the enzyme. This kind of inhibitor includes the sugar-mimicking glycosidase inhibitors and peptide-mimicking protease inhibitors.[1,2] Compared to other ways of altering enzyme activity, e.g., protein conformational change and post-translational modification, mimicking strategies are relatively simple and direct.

Research in the past decade has revealed several examples of proteins that imitate the substrates of DNA binding proteins. DNA mimic proteins directly occupy the DNA binding sites of these proteins to control their DNA binding activity.[3,4] DNA mimic proteins have been found in the cells of prokaryotes and eukaryotes as well as in bacteriophages and in a eukaryotic virus.[3,5] They are involved in many DNA regulation mechanisms, such as the restriction−modification system,[6,7] the DNA repair activity of uracil-DNA glycosylase,[8−13] transcriptional control,[14−16] DNA packaging,[5,17−19] protection of gyrase from antibiotics,[20] P53 activity control,[21] and recombination.[22,23] In this review, we look at 16 possible DNA mimic proteins for which annotated functions and protein structures have so far been described (Table 1 and Figure 1).

CHARACTERISTICS OF DNA MIMIC PROTEINS

For effective mimicry, DNA mimic proteins must have some characteristics that resemble those of a DNA molecule. B-DNA has two regular, helical strands of negative charge distribution, each formed by phosphate groups. Although amino acids, which are the building blocks of proteins, have properties very different from those of the nucleic acid building blocks of DNA, DNA mimic proteins are able to mimic this specific charge distribution with the two negatively charged amino acids: aspartic acid (Asp or D) and glutamic acid (Glu or E). While the known DNA mimic proteins have different structures and their shapes are also very different (see Figure 1), the surface of all of these proteins has distinct negative charge distributions. However, the single-strand mimics DinI and P53 (residues 33−60) have only a single row,[21,22] while Gam, which can function as a double-strand or single-strand DNA mimic, has both.[23] These specialized surface charges can be found on the entire

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Table 1. Sixteen Previously Identified DNA Mimic Proteins[a]

DNA mimic protein	targeted DNA binding protein	targeted DNA function	PDB entry	year of release of PDB data	MW (kDa)[b]	pI[b]
		Viruses				
UGI (*Bacillus* phage PBS2)	uracil-DNA glycosylase	DNA repair	1UGI	1999	9.4	4.1
Ocr (enterobacteria phage T7)	type I restriction enzymes	restriction	1S7Z	2004	13.7	3.9
Gam (bacteriophage λ)	RecBCD	recombination	2UUZ	2007	11.7	5.0
ICP11 (white spot syndrome virus)	histone proteins	nucleosome assembly	2ZUG	2008	9.2	4.2
p56 (*Bacillus* phage ϕ29)	uracil-DNA glycosylase	DNA repair	2LE2	2011	6.5	4.2
		Bacteria				
DinI (*Escherichia coli* BL21)	RecA	recombination	1GHH	2001	9.0	4.7
HI1450 (*Haemophilus influenzae* PittGG)	nucleoid-associated protein HU	DNA packaging	1NNV	2004	12.6	4.0
Mfpa (*Mycobacterium tuberculosis*)	DNA gyrase	topology	2BM4	2005	20.3	5.5
NuiA (*Nostoc* sp.)	nonspecific nuclease	phosphodiester bond digestion	2O3B	2006	15.1	4.4
ArdA (*Enterococcus faecalis*)	type I and II restriction enzymes	restriction	2W82	2009	19.1	3.9
CarS (*Myxococcus xanthus*)	CarA transcription repressor	transcription	2KSS	2010	9.3	4.1
DMP19 (*Neisseria meningitidis*)	NHTF transcription repressor	transcription	3VJZ	2012	18.5	4.4
DMP12 (*N. meningitidis*)	nucleoid-associated protein HU	DNA packaging	3W1O	2013	13.9	4.6
SAUGI (*Staphylococcus aureus*)	uracil-DNA glycosylase	DNA repair	3WDG	2014	13.3	4.5
		Eukaryotic Animals				
TAFII230 (residues 11−77) (*Drosophila*)	RNA polymerase	transcription	1TBA	1999	7.0[c]	4.0[c]
P53 transactivation domain (residues 33−60) (*Homo sapiens*)	replication protein A	single-strand binding	2B3G	2005	3.1[c]	3.2[c]

[a]Functional DNA mimicry has been experimentally demonstrated for all of the DNA mimic proteins listed here. [b]The theoretical molecular weights (MW) and isoelectric points (pI) are calculated from the amino acid sequences of DNA mimic proteins using ProtParam (http://web.expasy.org/protparam/). [c]MW and pI are based on the DNA mimic regions of TAFII230 (residues 11−77) and P53 (residues 33−60).

exposed surface of the white spot syndrome virus ICP11 and *Haemophilus influenzae* HI1450 proteins,[5,17] on a one-dimensional surface of proteins such as *Neisseria* DMP19 and bacteriophage PBS2 UGI,[8,16] or on just a special region of proteins such as eukaryotic TAFII230 (residues 11−77) and P53 (residues 33−60).[14,21] Figure 2A compares the negative charge distribution of two of these different types of DNA mimic proteins (ICP11 and DMP19) with B-form double-stranded DNA at the same scale. The surface charges of DNA mimic proteins and their targets are usually complementary to each other (Figure 2B), suggesting that charge−charge interaction plays an important role in the DNA mimic's ability to bind to its targets. Two single-strand DNA mimic proteins, Gam and P53,[21,23] use an additional hydrophobic interaction, whereby aromatic amino acids mimic unpaired bases on the DNA. Because of these characteristics, DNA mimic proteins usually have high binding affinities for their respective targets (Table 2).

It should also be noted that although DNA mimic proteins have DNA-like charge distributions and shapes, they do not exhibit DNA-like structural flexibility. For example, when the three well-studied uracil DNA-glycosylase inhibitors (UGI, SAUGI, and p56) form complexes with their corresponding uracil DNA-glycosylases (UDGs), only very slight conformational changes are seen.[8,11,13] A somewhat larger change occurs in the NuiA−NucA complex: although binding to NuiA causes only a few minor side chain rearrangements on NucA, on the DNA mimic itself, α-helix 4 and several loops are affected. However, the conformations of bound versus unbound NuiA are still close (root-mean-square deviation fit of 3.1 Å).[27]

VIRAL DNA MIMIC PROTEINS VERSUS HOST DEFENSE MECHANISMS

In this section, we look at five reported viral DNA mimic proteins, all of which use different means to play an important role against the defense systems of the host.

Ocr. Ocr (overcome classical restriction) is a DNA mimic protein from bacteriophage T7 that inhibits the activity of the type I R/M system (restriction and modification).[25] In bacteria, the R/M system acts as a defense mechanism against phage infection and other types of DNA invasion. This system consists of a methyltransferase that methylates a specific site on "friendly" DNA to prevent cleavage by a restriction endonuclease.[28] In this way, host DNA is protected by the sequence-specific base modifications introduced by the DNA methyltransferase (MTase), whereas invading DNA (e.g., a bacteriophage genome) that lacks the appropriate base modifications will be cleaved by the restriction endonuclease. To protect its genomic DNA, a bacteriophage must therefore use some methods to counter the R/M system. Bacteriophage T7 Ocr is a small, acidic protein (13.7 kDa with pI 3.9) encoded by gene 0.3, which inhibits all type I R/M enzymatic DNA processing, i.e., both the DNA modification and the DNA cleavage steps.[25] The crystal structure of Ocr shows that the size of each Ocr monomer is equivalent to ∼12 bp of double-stranded DNA, while the surface charge distribution is a very good match to that of the DNA phosphate group backbone.[6] The Ocr homodimer forms a croissant shape that mimics the 33.6° bent B-form DNA, and this allows it to be captured by the type I R/M enzyme with a strong binding affinity in the picomolar range.[25,26] This model was confirmed for the

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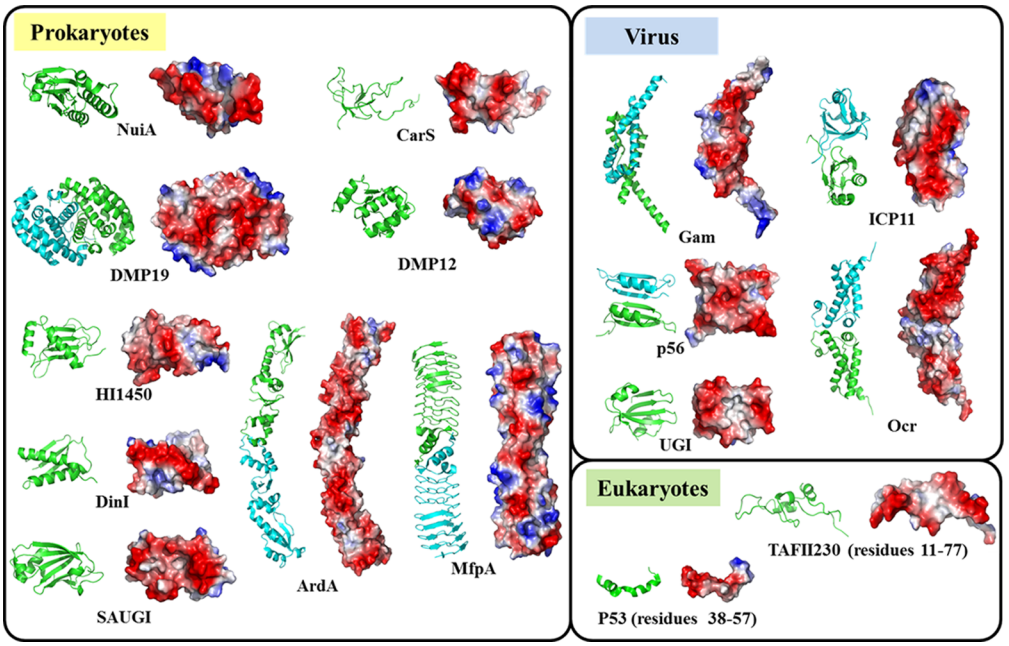

Figure 1. Tertiary structures (ribbon diagrams) and surface charge distributions of 12 published DNA mimic proteins. All of these proteins have double rows of negative charges except for DinI and P53 (single row) and Gam (double rows and an additional single row). In bacteria and viruses, DNA mimicry is performed by small, acidic proteins, while in the two known eukaryotic DNA mimics, the mimicry is performed by a motif or domain within the protein. The shapes of DNA mimic proteins can be very different. For example, *Mycobacterium tuberculosis* MfpA uses a right-handed β-helix to form a rodlike shape, while bacteriophage Ocr has a croissantlike shape that mimics bent double-stranded DNA. Meanwhile, *Escherichia coli* DinI, *Haemophilus influenzae* HI1450, and white spot syndrome virus ICP11 all have a globular protein surface. The secondary structures seen in these DNA mimic proteins include all known secondary structure types, i.e., α-helices, β-strands, and peptide loops, and structural homology searches via the DALI Web site reveal no consistent features. Because the amino acid sequences are also thought to be unique (no primary structure similarity has yet been reported for the published DNA mimic proteins), it is hard to identify DNA mimic proteins by using bioinformatic searches.

M.EcoKI type I R/M enzyme−Ocr binding complex by image reconstruction from electron micrographs.[29]

UGI and p56. UGI from *Bacillus* phage PBS2 is another example of a phage DNA mimic protein that acts as a uracil-DNA glycosylase inhibitor.[30] *Bacillus* phage PBS2 contains a double-stranded DNA genome that is unusual in that it incorporates uracil nucleotides instead of thymine nucleotides. This special genetic code of PBS2 prevents the phage genome from being attacked by the R/M system. However, bacteria also contain a DNA repair enzyme called uracil-DNA glycosylase (UDG) that acts to remove uracil bases from damaged DNA in a repair process that has two steps. (1) UDG docks on the DNA uracil site, and (2) it flips the uracil base out of the stacked double-stranded DNA. PBS2 therefore uses UGI to counter this repair mechanism, which would otherwise lead to the inactivation of the PBS2 genome and prevent viral replication.[8,30] Functionally, UGI blocks UDG by irreversibly forming a UDG−UGI complex. It is interesting to note that UGI mimics not only the shape and charge distribution of DNA but also the specific "flipped" state that uracil-DNA adopts after being bound by UDG. This additional level of mimicry allows UGI to block UDGs from a wide range of sources, from prokaryotic cells to eukaryotic cells and viruses, while still being highly specific. Thus, UGI targets the UDGs from *B. subtilis*, *E. coli*, *Micrococcus luteus*, *Saccharomyces cerevisae*, rat liver, herpes simplex virus (HSV), and humans, but it does not target other common DNA-metabolizing enzymes or even other DNA glycosylases.[8]

Recently, a novel uracil-DNA glycosylase inhibitor, p56, was found in *Bacillus* phage ϕ29.[9,10] Although the structures of dimeric p56 and monomeric UGI are quite different, dimeric p56 also forms a stable complex with *Bacillus* or HSV UDG in a 2:1 molar stoichiometry.[11,12] Unlike phage PBS2, the genomic DNA of phage ϕ29 does not contain uracil nucleotides, but Serrano-Heras hypothesized that p56 might knock down this UDG activity to prevent uracil nucleotides from appearing in the replicative intermediates during phage replication.[9]

Gam. Lambda phage Gam is a DNA mimic protein that inhibits all the known enzymatic activities of the bacterical protein complex RecBCD, which is an exonuclease and helicase that also acts as a defense mechanism against bacteriophage infection.[31] RecBCD works by digesting the linear viral DNA that is present during replication of the virus or results from the action of restriction enzymes, and Murphy showed that Gam acted to prevent RecBCD from binding to DNA.[32] The crystal structure of Gam suggests it may mimic both single-stranded and double-stranded DNA.[23] The N-terminal α-helix 1 of Gam might act as a single-strand DNA mimic that enters either the 3′ channel on RecB or the 5′ channel of RecC/RecD, while the shape, dimensions, and negative charge of the Gam dimerization domain led Court et al. to further suggest that, like Ocr, Gam might use this domain to mimic the charge and shape of the double-stranded DNA.[23] However, the actual structure of the Gam−RecBCD complex still needs to be clarified experimentally to confirm this hypothesis.

ICP11. White spot syndrome virus (WSSV), a double-stranded DNA virus that causes a lethal disease in shrimp, expresses the nonstructural protein ICP11 at the late stage of viral replication.[33] The role of ICP11 as a DNA mimic was suggested by structural analysis, which showed that the ICP11

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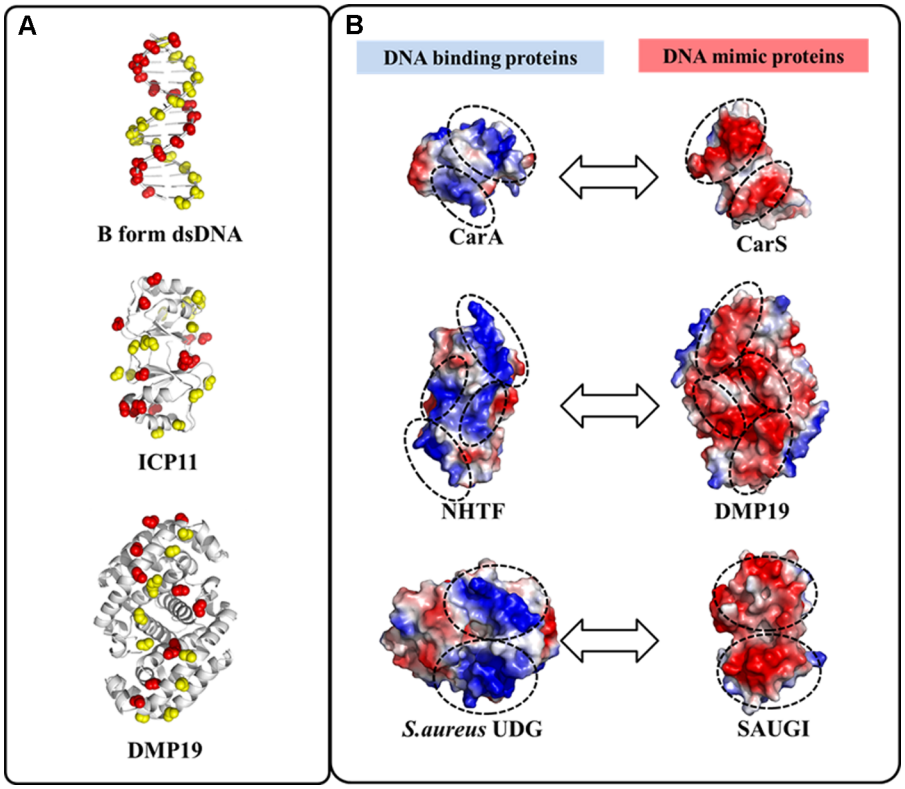

Figure 2. Specialized configuration of the surface charges on the surface of DNA mimic proteins. (A) These space-filling models show how the phosphate groups on the DNA backbone of B-form double-stranded DNA are mimicked by the negatively charged carboxyl groups of ICP11 and DMP19. The two negatively charged rows in each molecule are colored red and yellow, respectively. (B) Matching surface configuration of DNA mimic proteins and their target DNA binding proteins. Surface charges for the first two pairs were obtained from the individual proteins of the proposed binding models, while the *S. aureus* UDG/SAUGI surface charges were obtained from the actual complex.

Table 2. Binding Affinities of DNA Mimic Proteins and Their Respective Targets

DNA mimic protein	targeted DNA binding protein	binding affinity (K_D, equilibrium constant)	refs
	Binding Affinities Determined by Surface Plasmon Resonance (BIAcore)		
UGI (*Bacillus* phage PBS2)	*S. aureus* uracil-DNA glycosylase	0.69 nM	13
DinI (*E. coli* BL21)	*E. coli* RecA	0.86 μM	24
Mfpa (*M. tuberculosis*)	*E. coli* DNA gyrase	0.46 μM	20
SAUGI (*S. aureus*)	*S. aureus* uracil-DNA glycosylase	1.20 nM	13
	Binding Affinities Determined by Isothermal Titration Calorimetry (ITC)		
Ocr (enterobacteria phage T7)	*E. coli* M.EcoKI	<100 pM[a]	25, 26
p56 (*Bacillus* phage ϕ29)	*Bacillus subtilis* uracil-DNA glycosylase	16.00 nM	10
HI1450 (*H. influenzae* PittGG)	*H. influenzae* HUα	3.00 μM	18
DMP12 (*N. meningitidis*)	*N. meningitidis* HU	2.82 μM	19
CarS (*M. xanthus*)	*M. xanthus* CarA N-terminal domain (residues 1−79)	12.00 nM	15

[a]This binding is too tight to be accurately measured by ITC.

dimer has two rows of negatively charged spots that approximately match, in scale and configuration, the arrangement of the phosphate groups in the two opposing strands of DNA (Figure 2).[5] Functionally, ICP11 prevents DNA from binding to histone proteins H2A, H2B, H3, and H2A.x. In WSSV-infected shrimp hemocytes and ICP11-expressing HeLa cells, the predominantly cytoplasmic colocalization of ICP11 with histone H3 and histone H2A.x suggests that ICP11 prevents these histones from translocating into the nucleus where they would normally participate in nucleosome assembly or fulfill some other histone function. Although the exact purpose of ICP11's DNA mimicry is not yet clear, the high expression level of this protein suggests that it is likely to be important in WSSV replication. It may be that by disrupting the host's nucleosomes, ICP11 affects the epigenetic control of gene expression. If so, it might also have the presumably unwanted consequence of inducing cell death.[5] Histone proteins and their derived fragments also have antimicrobial activity in vertebrates and invertebrates, and many studies have focused on the defensive role of H2A and H2B. For example, in the Pacific white shrimp *Litopenaeus vannamei*, enzymatic cleavage of histone H2A produces active peptides that can completely inhibit the growth of Gram-positive bacteria.[34] Thus, it is also possible that ICP11's ability to bind to histone proteins might be a direct attempt to interfere with the host's defenses.

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BACTERIAL DNA MIMIC PROTEINS ACT AS INTERNAL CONTROL FACTORS

Approximately half of the reported DNA mimic proteins were found in bacteria. DNA mimic proteins such as ArdA,[7] HI1450,[17,18] and MfpA[20] are conserved in many different species, suggesting that they are important to prokaryotes. Unlike the viral DNA mimic proteins, the bacterial DNA mimics act not only as inhibitors but also as regulators. They are involved in many internal response controls.

DinI. *E. coli* DinI is a bacterial protein that inhibits the activity of RecA and limits the response of the SOS mutagenesis system to DNA damage.[24,35] The solution structure of DinI provided some insight into how it might achieve RecA inactivation by acting as a single-strand DNA mimic protein.[22] The C-terminal α-helix of DinI contains six negatively charged Asp and Glu R.EcoKI residues, and these amino acids are arranged in an extended, negatively charged ridge. Because this ridge has an electrostatic character similar to that of the phosphate backbone of single-stranded DNA, DinI may use this α-helical region to compete with single-stranded DNA in binding to the L2 loop of RecA. In the model proposed by Ramirez et al.,[22] DinI displaces the single-stranded DNA from the active RecA nucleoprotein filament, inhibits the strand exchange reaction, and thus destabilizes filament formation. However, the actual mechanism of the RecA−DinI interaction is still in dispute. More recent reports have shown that the interactions of DinI with the RecA filament are concentration-dependent and may in fact result in either stabilization or depolymerization of the filament.[36,37] More studies will be needed to fully elucidate the functional effects of DinI on RecA filament formation.

HI1450 and DMP12. Homologues of the *H. influenzae* HI1450 protein are widely distributed and can be found in many different species, including *Salmonella typhimurim*, *Yersinia pestis*, *E. coli*, and *Vibrio vulnificus*.[17,18] HI1450 is weakly structurally homologous to UGI, while its double-stranded DNA-like surface charge distribution led Parsons to propose that it might act as a B-form DNA mimic protein.[17] Functionally, HI1450 prevents DNA from binding to the bacterial nucleoid-associated protein HU-α, and it may therefore have a role in controlling the chromosome-like structure in prokaryotes.[18] However, the full biological meaning of the HI1450−HU-α interaction has yet to be experimentally determined.

DMP12 is another DNA mimic protein that binds to HU, and it is found in *Neisseria* spp.[19] The DMP12 monomer has a specialized shape and charge distribution that resembles those of HU-bound DNA and is complementary to the HU protein dimer. In a competitive electrophoresis mobility shift assay (EMSA), DMP12 only partially disrupted the formation of HU−plasmid DNA complexes. The binding affinities determined by isothermal titration calorimetry also showed that the affinity of *Neisseria* HU for DMP12 is 4 times lower than it is for dsDNA. These findings suggest that although DMP12 and DNA share the same binding sites on the HU protein, DMP12 is more likely to act as a regulator than a competitive inhibitor. Because the DNA binding β-ribbon arms of bacterial HU protein are usually disordered and unstable, DMP12 may act to maintain the stability of the disordered regions of the unbound HU protein. This hypothesis was investigated using a limited trypsin digestion assay that showed *Neisseria* HU protein is sensitive to trypsin in the absence of DMP12 or dsDNA, while DMP12 and dsDNA both reduced the rate of HU protein degradation.

ArdA. Bacteria also express antirestriction proteins. Although these are functionally similar to the Ocr protein of phage T7, the roles played by these bacterial mimics are somewhat different. ArdA protein is very commonly found in conjugative plasmids and transposons in a wide array of prokaryotes.[38] Compared to Ocr, ArdA is a more effective inhibitor of the activity of the M.EcoKI type IA restriction enzyme in the type I R/M system, and this inhibition allows the horizontal transfer of mobile genetic DNA elements (e.g., plasmid DNA) between different bacterial cells.[39] It was recently shown that the rodlike structure of dimeric ArdA mimics ∼42 bp of bent B-form DNA, which is close in length to the normal DNA substrate of the M.EcoKI type IA restriction enzyme.[7] The carboxyl groups on the surface of the ArdA dimer also match the negatively charged phosphate groups of the DNA that is bound to the type I MTase core. Interestingly, the mutation that disrupts the dimeric structure of ArdA will affect ArdA's antimodification activity, but not its antirestriction activity.[40] This suggests that, in addition to the dimeric binding of ArdA to the core MTase, the ArdA monomer might also bind independently to the restriction subunit of the type I restriction enzyme. The ability of both Ocr and ArdA proteins to inhibit the type I R/M system despite their different structures and binding affinities is a striking reminder of the diversity found among DNA mimic proteins even when they fulfill similar functional roles.

MfpA. Fluoroquinolones are powerful antibiotics that prevent bacterial DNA from unwinding and duplicating by inhibiting the activity of DNA gyrase and topoisomerase IV. However, resistance to fluoroquinolones is a clinical problem in the treatment of *M. tuberculosis*. The MfpA gene/protein was first identified from fluoroquinolone-resistant strains of *M. tuberculosis*.[41] Functionally, MfpA protein binds to the DNA binding site of gyrase and protects it from fluoroquinolones.[42] Analysis of the crystal structure of MfpA revealed that the dimeric form presents as a right-handed quadrilateral β-helix and rodlike structure.[20] Although the pI of MfpA (5.5) is higher than those of other DNA mimic proteins, the pattern of distribution of the acidic amino acids on the protein surface still creates enough negatively charged spots to mimic a 30 bp B-form DNA.[20] MfpA is the first antibiotic-resistance protein that protects the antibiotic's target directly by binding to it, rather than by degrading the drug. Since this discovery, quinolone-resistance proteins QnrB1 [Protein Data Bank (PDB) entry 2XTW], AlbG (PDB entry 2XT2), and EfsQnR (PDB entry 2W7Z) have also been shown to have a similar structure, suggesting that they might use the same strategy against this related antibiotic family.[43−45] Interestingly, quinolone-related antibiotics such as the fluoroquinolones are a relatively recent invention, which raises the question of what the original function of MfpA might have been. It should be interesting to investigate other possible biological functions of MfpA and its homologues.

NuiA. Nuclease A (NucA) is a nonspecific endonuclease from *Anabaena* sp. that digests single- and double-stranded DNA and RNA in the presence of divalent metal ions.[46] Nonspecific nucleases are involved in a broad range of functions, including programmed cell death, defense, DNA replication, recombination, and repair, and in general, their activities must be carefully controlled to ensure that they do not become toxic to the cell.[27] NuiA is a specific inhibitor of NucA

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that uses the negatively charged amino acids located at the edge of its central β-sheet to mimic the phosphate backbone of NucA's DNA substrate. Additionally, the Thr135 residue on the C-terminus of NuiA is directly coordinated with the catalytic Mg^{2+} of the NucA active site, while the Glu24 residue of NuiA also extends into the active site, mimicking the charge of a scissile phosphate. NucA and NuiA thus form a tight 1:1 complex with picomolar affinity.[27]

CarS. In *M. xanthus*, CarA−CarS repressor−antirepressor interaction is involved in the transcriptional control of the carB operon that responds to the carotenogenesis induced by blue light.[47,48] In the dark, RNA polymerase cannot access the promoter on the carB operon because it is blocked by CarA, which binds cooperatively to a bipartite operator, one part of which overlaps with the −35 promoter region.[49] This inhibition by CarA is neutralized by the CarS antirepressor that is expressed in blue light.[47] CarS recognizes the MerR-type, winged-helix DNA-binding domain on the N-terminus of CarA, and structural analysis showed that CarS contains a polar solvent-exposed pocket that is filled with negatively charged residues.[15] This pocket mimics (with some slight distortion) the major groove of the operator DNA, and it binds to CarA recognition helix α2 with high affinity ($K_D \sim 10$ nM). This allows CarS to compete effectively against the operator DNA in trapping the CarA DNA binding domain.[15]

DMP19. *Neisseria* DMP19 is a conserved gene/protein that is found in the major hypervirulent lineages and serogroups of many *Neisseria* species that cause human disease.[50,51] Surface charge analysis revealed a double-stranded DNA-like negative charge distribution on one of the dimensional electrostatic surfaces of the DMP19 dimer.[16] Functional studies showed that DMP19 prevented a *Neisseria* transcriptional repressor (NHTF) from binding to its DNA target, which is a specific sequence in its own promoter region. The complementary surface charges of DMP19 and NHTF strongly suggest that a charge−charge interaction mediates the binding of these two proteins. Because NHTF is an autorepressor that down-regulates its own expression as well as the expression of downstream genes, there are likely to be critical situations in which more control is needed. DMP19 is thought to play a role in the further control of NHTF's autofeedback mechanism: by temporarily interfering with the binding of NHTF to its own promoter region, DMP19 also restores the expression of the downstream genes. NHTF belongs to the XRE transcription factor family, and it will be interesting to determine whether DMP19 can interact with other members of the same family.

SAUGI. The most recently reported example of a DNA mimic protein is *Staphylococcus* SAUGI. SAUGI is the third uracil-DNA glycosylase inhibitor to be identified, and the first to be found in a non-phage species.[13] Like the two other uracil DNA-glycosylase inhibitors (UGI and p56),[8−12] SAUGI acts by binding tightly to *Staphylococcus* UDG or human UDG with a nanomolar affinity. Although the amino acid sequences and structures of SAUGI, UGI, and p56 are different, all three of these uracil DNA-glycosylase inhibitors share a conserved UDG binding strategy that involves targeting their respective UDG's protruding residue (i.e., SAUDG Leu184, *E. coli* UDG Leu191, and *B. subtilis* UDG Phe191) with a hydrophobic pocket. The three uracil-DNA glycosylase inhibitors are also similar in that when they bind to their respective targets, they induce only very minor conformational changes in the UDGs. However, unlike UGI and p56, which help their respective phages to replicate by inhibiting host UDG activity, it is not clear why *Staphylococcus* would produce a protein that inhibits its own DNA repair system. Perhaps, like bacteria ArdA, which inhibits the bacteria's own restriction enzymes to allow the horizontal transfer of mobile genetic DNA elements,[38,39] SAUGI also acts to regulate UDG activity for some as yet unknown purpose. For instance, because it has been shown that the replication of DNA viruses can be blocked by increasing the amount of dUTP in the viral genome,[52,53] SAUGI's inhibition of UDG might serve an anti-phage function by increasing the amount of uracil on the phage genome.[13]

EUKARYOTIC P53 AND TAFII230 PROTEINS USE MOTIFS TO ACHIEVE DNA MIMICRY

Most DNA mimic proteins from bacteria and viruses use the entire protein to produce a DNA-like charge distribution on the protein surface. By contrast, the two eukaryotic examples that are presently known, P53 and TAFII230, use only a part of the protein to mimic DNA. Both of these proteins are involved in transcription control.

P53. The first of these eukaryotic proteins, P53, is a well-known tumor suppressor. When it is activated, it modulates the transcriptional regulation of genes involved in the cell cycle, DNA repair, and apoptosis.[54] P53 protein interacts with a number of targets, including DNA as well as other types of proteins. One of P53's targets is replication protein A (RPA), which is the major single-strand DNA binding protein of the eukaryotic nucleus.[55] The interaction between P53 and RPA suppresses homologous recombination and modulates Werner syndrome helicase activity.[56,57] Structural analysis of the complex structure formed by the N-terminal acidic transcription−activation domain (P53TAD) and RPA showed how these two proteins interact with each other.[21] A comparison of the complex formed by RPA and the P53TAD and that formed by RPA and single-stranded DNA showed that the negatively charged and hydrophobic amino acids on α-helix 1 of the P53TAD are able to mimic the phosphate backbone and bases of the RPA-bound single-stranded DNA. The P53TAD is in competition with single-stranded DNA to bind to RPA, and increasing the local concentration of single-stranded DNA causes the RPA−P53 complex to dissociate. Because single-stranded DNA is produced as a consequence of DNA damage, P53 may be using its single-stranded DNA mimic region to control the activity of RPA. Recently, P53 was also found to interact with positive cofactor 4 (PC4) via the same P53TAD.[58] PC4 is a transcriptional coactivator that mediates activator-dependent transcription of class II genes through interactions with the basal machinery.[59] The C-terminal DNA binding domain of PC4 binds tightly to both single-stranded DNA and to melted double-stranded DNA, but the P53TAD competes with this domain only for single-stranded DNA.[58] Other acidic transcription−activation domains can be found on herpes simplex virion protein 16 (VP16)[60] and eukaryotic transcriptional regulator Ap-2α.[61] The similarity of these domains to P53TAD suggests that these proteins might also use the same single-stranded DNA mimic strategy to modulate transcription.

TAFII230. One of the proteins in the eukaryotic TFIID complex also shows DNA mimic activity. The TFIID complex acts as a general transcription factor that is involved in eukaryotic transcription initiation, and it consists of the TATA box-binding protein (TBP) and several TBP-associated factors. The complex plays a central role in both positive and negative transcriptional control.[62] *Drosophila* TATA box-binding TAFII (TAFII230) is a 230 kDa protein that prevents TBP from

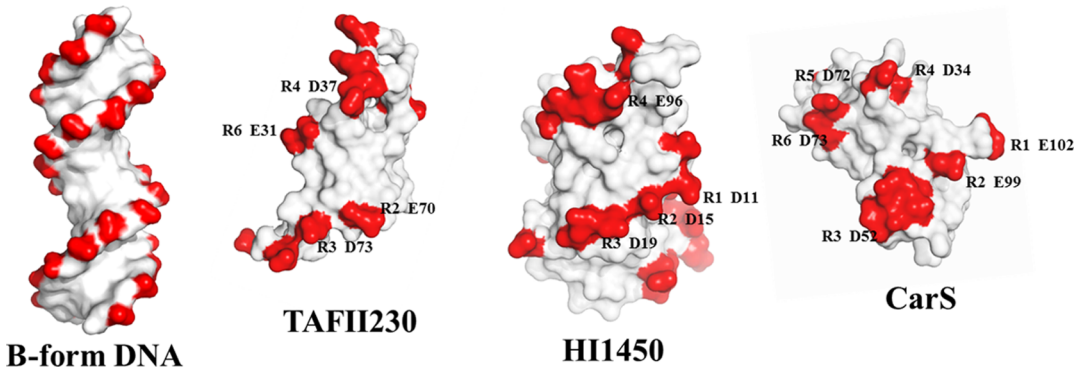

Figure 3. Conserved negative charge distribution and shape of three DNA mimic proteins. The locations of the conserved negatively charged spots match the locations of those of B-form double-stranded DNA on these three individual DNA mimic proteins. The six matching positions are labeled R1−R6.

binding to the TATA box. Structural analysis of the protein complex shows that the N-terminal domain of TAFII230 (residues 11−77) binds to TBP by mimicking the minor groove surface of the TATA box.[14] Because gene expression is shut down when TBP binds to the TATA box, the TAFII230−TBP interaction is likely to play an important role in the transcriptional control of the genes downstream of the TATA box.

PREDICTING NEW DNA MIMIC PROTEINS BY BIOINFORMATIC ANALYSIS

Although we have reviewed some of the ways in which how DNA mimics function, the lack of any conserved functional motif or domain on these 16 DNA mimic proteins means that this information is not immediately helpful when searching for new DNA mimic candidates. Additionally, the published DNA mimic proteins do not have a conserved structure, and in fact, they are often classified as proteins that have novel folding because of their lack of homology to any known structure. Conversely, even when these proteins contain the same secondary structures, these structures are arranged in different ways. Thus, for example, as shown in Figure 1 for DMP19, Gam, and Ocr, although each of these proteins consists entirely of α-helices, the actual folding of each respective protein is quite different. Although DNA mimic proteins from bacteria and viruses are usually small and acidic, this too is still not specific enough to identify new DNA mimic proteins because many other proteins, such as ferritin, SUMO proteins, and calmodulin, also have these two features. It seems likely that the only shared, universal feature of DNA mimic proteins is the negative charge distribution on their protein surface, and this is hard to predict without knowledge of the three-dimensional structure of the protein. Thus, while a handful of DNA mimic proteins have so far been reported, it is highly possible that the protein database already contains many unrecognized protein structures that mimic DNA. The fact that SAUGI was already identified as a DNA mimic protein is a case in point.[13]

A new approach that may potentially be useful for identifying DNA mimic proteins is to consider the tertiary structure of candidate mimic proteins. A comparison of the 16 reported DNA mimic proteins shows that the negatively charged spots of three of them (TAF230, HI1450, and CarS) have a similar pattern. A superimposition of these four DNA mimic proteins over double-stranded DNA shows how six of the negatively charged amino acids on these three DNA mimic proteins are aligned [R1−R6 (Figure 3)]. The alignment of these proteins seems to suggest that these sites must be conserved for the protein to perform DNA mimic activity. We also note that the shapes of the regions between the negatively charged rows resemble DNA grooves (Figure 3).

CONCLUDING REMARKS

Although the biological role of the DNA mimic protein OCR was first reported by William Studier more than 30 years ago,[63] there are still only a handful of known DNA mimics. Even so, DNA mimics have already been shown to have several potentially important applications. (1) The DNA mimic protein Ocr significantly increases transformation efficiencies of unmodified DNA in bacterial strains by inhibiting their R/M systems.[64] (2) While a monomeric mutant of the Ocr is already being used as a novel fusion tag to improve the solubility of recombinant proteins,[65] Ratner previously reported that Ocr protein binds to a column containing immobilized RNA polymerase,[66] which suggests that it might be possible to use Ocr as a new affinity tag. (3) The uracil-DNA glycosylase inhibitors UGI and SAUGI and the histone binding protein ICP11 are able to bind to their respective targets in mammalian cells, and this has potential applications in human research. Another example is the fluoroquinolone-resistance protein MfpA, which could become an important target in clinical treatment. It should also be possible to design peptide inhibitors for other disease-related DNA binding proteins such as NFκB by using a DNA mimic strategy.

As more DNA mimics are discovered, it seems likely that the number of potential applications will also increase. Some new candidate DNA mimics are already being reported, but there must be a vast number of other DNA mimic proteins that have not yet been identified and investigated. While the best empirical evidence of actual DNA mimicry is provided by solid cocrystal structural data for the interactions between DNA mimic proteins and their targets, an effective platform of bioinformatic screening would be still extremely useful for finding new DNA mimics. As we have shown here, the critical characteristics of DNA mimic proteins typically include a small size, a low pI, a DNA-like negative charge distribution on the protein's surface, and the conserved positions of the negatively charged amino acids on the surface that matches the DNA groove. Although the research into this family of novel control factors is still in its infancy, all of these characteristics are potentially useful for bioinformatic searches in the Protein Data Bank.

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ABBREVIATIONS

Ocr, overcome classical restriction; R/M system, restriction and modification system; UGI, phage PBS2 uracil-DNA glycosylase inhibitor; p56, phage ϕ29 protein of 56 amino acids; UDG, uracil-DNA glycosylase; HSV, herpes simplex virus; WSSV, white spot syndrome virus; ICP11, intracellular protein of 11 kDa; DinI, DNA damage-inducible protein I; M.EcoKI, methyltransferase subunit of the EcoKI restriction−modification system; R.EcoKI, restriction endonuclease subunit of the EcoKI restriction−modification system; MTase, DNA methyltransferase; NuiA, nuclease A inhibitor; NucA, nuclease A; MfpA, *Mycobacterium* fluoroquinolone resistance protein A; ArdA, anti-restriction protein type A; DMP19 and DMP12, *Neisseria* DNA mimic proteins of 19 and 12 kDa, respectively; NHTF, *Neisseria* hypothetical transcriptional factor; SAUGI, *S. aureus* uracil-DNA glycosylase inhibitor; SAUDG, *S. aureus* uracil-DNA glycosylase; TAFII230, transcription fator II D 230 kDa subunit.

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