

Visualization of Cell-defending Nonspecific Nucleases in DNA Recognition and Cleavage

A variety of nonspecific nucleases take part in cell defense by degrading foreign nucleic acid molecules in a sequence-independent manner to protect the intactness of host genomes in prokaryotes and eukaryotes. We co-crystallized a couple of sugar nonspecific nucleases with DNA and solved the crystal structures of the protein/DNA complexes by X-ray diffraction methods. These structures not only provide a structural basis for nonspecific DNA recognition and cleavage but also offer a solid foundation for the better understanding of the molecular mechanisms of nonspecific endonucleases involved in the protection of bacterial cells.

Protein-nucleic acid interactions play many important cellular roles, including regulating DNA replication, controlling gene expression and DNA house-keeping. A wealth of information regarding how proteins select DNA target sites has been discovered through biochemical, structural and statistical analysis in site-specific DNA-binding proteins, such as transcription factors. However, non-specific protein/DNA interactions have not been so well studied due to the small number of resolved three-dimensional structures of non-specific protein/DNA complexes. This is likely because it is more difficult to find an oligonucleotide which can bind to the nonspecific DNA-binding protein in an ordered fashion. The lack of high-resolution structures of protein/DNA complexes for nonspecific proteins hampers our overall understanding for protein-DNA recognition.

DNA endonucleases provide good opportunities to investigate sequence-independent recognition between proteins and DNA. The most well studied endonucleases in bacteria are the type II restriction enzymes. These restriction enzymes bind DNA non-specifically, but cleave only short palindromic DNA sequences in the presence of Mg^{2+} at specific sites. The major biological function of restriction enzymes is to cleave foreign unmethylated DNA and thereby protect the host methylated genome. In prokaryotic organisms, a variety of other types of endonucleases involved in the protection of bacterial cells have been identified but differ from restriction enzymes in that they cleave DNA in a sequence-independent manner. We show examples of two types of cell-defending sugar-nonspecific nucleases here. Current knowledge about the crystal structures of nucleases and nuclease/DNA complexes, structural basis for nonspecific DNA/RNA binding and hydrolytic mechanisms are briefly discussed.

The first example is a family of periplasmic

or extra-cellular nucleases, such as Vvn from *Vibrio vulnificus*. Vvn is a periplasmic enzyme which protects the cell by preventing the uptake of foreign DNA molecules (see Figure 1). Vvn and its homologues all contain a signal peptide located at the N-terminus and eight strictly conserved cysteine residues. The signal peptides are cleaved off during the transportation of the nuclease from the cytoplasm to periplasm, resulting in mature proteins with an average size of about 25 kD. This family of endonucleases is capable of digesting both DNA and RNA and are only active in the oxidized form.

The crystal structure of Vvn has been resolved by the MAD method and it bears a novel mixed α/β topology containing four disulfide bridges.

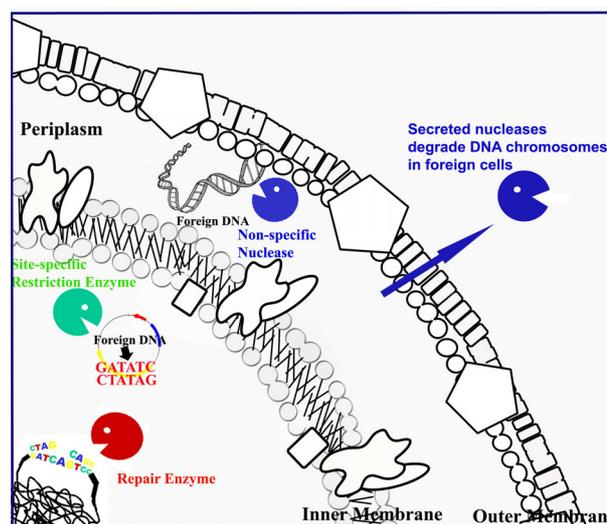


Fig. 1: A cross section of a bacterial cell shows that different types of nucleases constitute a secure network for host defense against foreign nucleic acids. In the host cell, restriction enzymes cleave foreign unmethylated DNA site-specifically in cytoplasm and periplasmic nucleases degrade foreign nucleic acids nonspecifically in cell wall. Host cells also secrete nuclease-type toxins to degrade nucleic acids in foreign cells to increase host survival advantage.

Vvn binds a magnesium ion at its active site and cleaves a phosphodiester bond at its 3' side, producing DNA fragments containing a 3'-OH and a 5'-phosphate. Vvn is not active under reducing conditions, suggesting that it is not folded when it is expressed in the cytoplasm. In this way, host genomes escape from the digestion by Vvn, which becomes folded and targets only foreign DNA molecules after it is transported into periplasm under oxidized conditions.

Nuclease-type bacteriacins represent another class of nonspecific endonucleases that are involved in protection of bacterial cells. Examples include colicins from *Escherichia coli* and pyocins from *Pseudomonas aeruginosa*. These toxins are secreted endonucleases which digest nucleic acids randomly in foreign and target cells to induce cell death, thereby improving host cells survival advantage during times of stress (see Figure 2). In other words, these bacteria adopt an aggressive offensive approach for the purpose of cell defense and survival. The nuclease-type colicins share high sequence identity, containing three functional domains: receptor binding, membrane translocation and cytotoxic nuclease domains. Under stressful conditions, the SOS response is turned on and colicins and immunity proteins are co-expressed, and secreted from the host cell as heterodimeric complexes. This group of colicin/immunity complexes then searches for other bacteria possessing the outer membrane cobalamin transporter, BtuB. After entering the periplasm of the target cell, ColE7 is likely cleaved to produce an enzyme containing only the nuclease domain of

ColE7 which then traverses across the inner membrane and enters the cytoplasm of target cells. The nuclease domain of the colicin then degrades all nucleic acids that it encounters, including RNA and DNA without sequence specificity, making it an efficient cell killer.

The immunity protein binds to the nuclease domain of colicin and inhibits its nuclease activity to protect the host cell from the cytotoxic activity of a colicin. The crystal structures of the nuclease domain of ColE7 with or without their bound immunity proteins have been resolved. These complex structures explain how an immunity protein binds its cognate colicin with high affinity and specificity. The nuclease domain of colicin and pyocin contains an HNH motif, which consists of two anti-parallel β -strands and one α -helix with a centrally located divalent metal ion. A zinc ion has been identified in the HNH motif of ColE7 and this zinc ion is required for its nuclease activity.

The two cell-defending sugar-nonspecific nucleases, Vvn and ColE7, have been crystallized with a double-stranded DNA molecule (a palindromic 8 base-pair oligonucleotide) and the crystal structures of the complexes have been resolved (see Figure 3). Vvn/DNA diffracted to a resolution of 2.3 Å and nuclease-ColE7/DNA diffracted to a resolution of 2.5 Å by a Quantum 4 CCD detector at NSRRC SPRING8 beam line BL12B2. Although the amino acid sequence and overall fold of Vvn and ColE7 are completely different, the two nucleases actually have some common DNA binding and cleavage features. Firstly, both Vvn and ColE7 have a concaved and basic surface that binds DNA at the

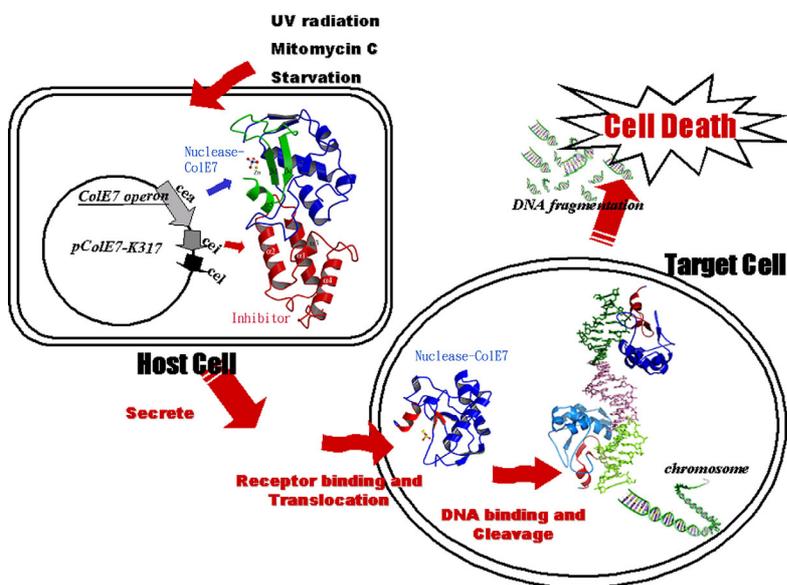


Fig. 2: The nuclease-type toxin ColE7 is expressed in host cells and cleaves nucleic acid molecules in foreign cells. In the host cell, ColE7 and its inhibitor Im7 are co-expressed and secreted as a hetero-dimeric complex. ColE7 contains three functional domains, receptor binding, membrane translocation and nuclease domains. After ColE7 binds to the receptor on foreign cells, ColE7 is cleaved during translocation. Only the nuclease domain of ColE7 reaches the cytoplasm of foreign cells for nucleic acid degradation.

minor groove, similar to most of the nonspecific DNA-binding proteins which almost always bind double-stranded DNA at minor grooves. This is because binding at the relatively narrow minor groove is advantageous in avoiding sequence-dependent recognition with DNA bases which are otherwise more accessible and more diverse in chemical features in major grooves.

Another feature common to both Vvn and ColE7 is that their active sites contain a similar $\beta\beta\alpha$ -metal topology, which has been observed in the active sites of several endonucleases, including the *I-PpoI*, *Serratia* nuclease and Phage T4 Endonuclease VII. The topological resemblance in the active sites of all these sequence-specific, nonspecific or structural-specific nucleases indicates that the $\beta\beta\alpha$ -metal motif is a general fold for endonuclease activity, but probably not responsible for site-specific recognition. Vvn and ColE7 are also similar in that both nucleases contact DNA primarily at phosphate atoms but make little contact with bases and ribose groups. This result suggests that nonspecific nucleases contact DNA much more via DNA backbones as compared to site-specific nucleases, very likely for the purpose of avoiding any sequence-dependent recognition.

How are nucleic acids hydrolyzed by nonspecific nucleases? The crystal structure of Vvn in complex with a un-cleaved and a cleaved DNA demonstrates clearly how DNA is hydrolyzed.

There are two protein/DNA complexes in one asymmetric unit of the monoclinic $P2_1$ crystal. A closer look at the electron density maps (see Figure 4) in the active site region surprisingly shows that one of the complexes is the enzyme/substrate complex and the other is the enzyme/product complex in which the phosphate backbone is cleaved. These two structures provide an ideal case to elucidate the hydrolytic mechanism.

Figure 4 shows the proposed single-metal ion hydrolysis mechanism mediated by Vvn. The His80 functions as a general base to activate a water molecule for nucleophilic attack to the scissile phosphate. This general base residue makes a hydrogen bond to a backbone carbonyl group which not only constrains the side chain conformation but also increases the pKa of His80, making it a better base for water activation. In the crystal structure of Vvn and Vvn/DNA complex, the nucleophilic water is visible, ideally located at the opposite site of the cleaved 3'O-P bond for the in-line attack. The metal ion is bound to the scissile phosphate to stabilize the phosphoanion transition state, as well as positioning and activating a water molecule to donate a proton to the leaving 3'-oxygen. A basic residue, Arg99, re-orientates its side chain conformation and makes a hydrogen bond to the cleaved phosphate in the enzyme/product complex, presumably to stabilize the cleaved DNA product for decelerating the reverse reaction.

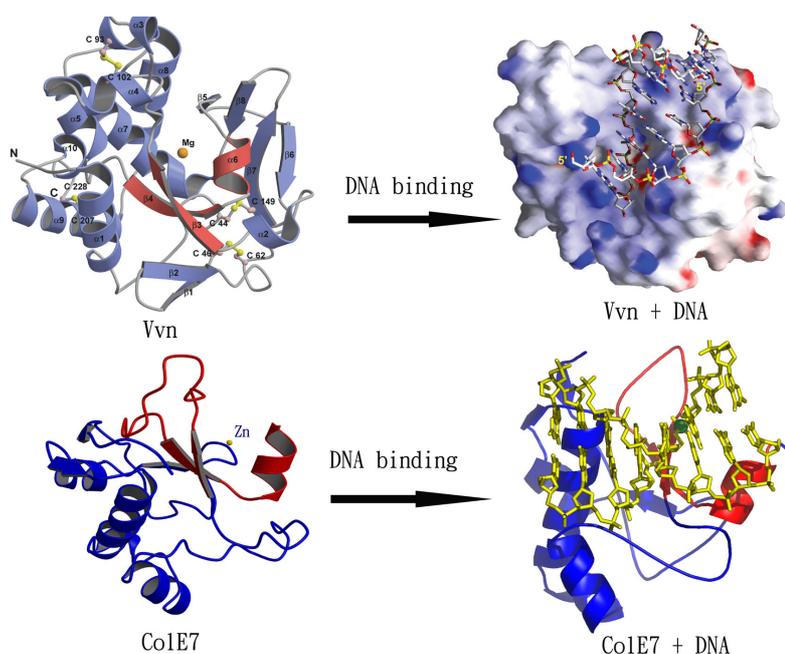


Fig. 3: The crystal structures of two bacterial sugar-nonspecific nucleases, Vvn (PDB: 1OUO), Vvn/DNA (PDB: 1OUP), Nuclease-ColE7 (PDB: 7CE1) and Nuclease-ColE7/DNA (PDB: 1PT3). The active sites of the two enzymes containing a common $\beta\beta\alpha$ -metal topology are displayed in red. Upon DNA binding, the $\beta\beta\alpha$ -metal fold faces the minor groove and induces DNA bending slightly toward the major groove.

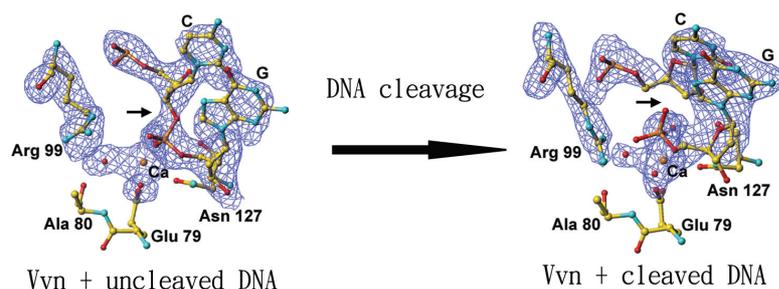
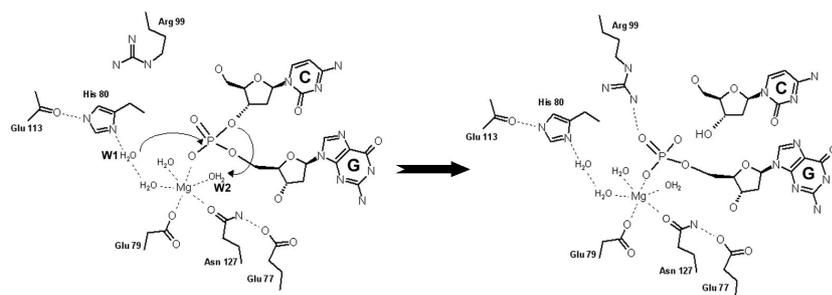


Fig. 4: Vvn was crystallized with a uncleaved DNA (substrate) and a cleaved DNA (product). Crystal structures of the enzyme/substrate and enzyme/product complexes demonstrate the hydrolytic mechanism catalyzed by Vvn. The His80, polarized by a backbone carbonyl group, functions as the general base to activate the water molecule for nucleophilic attack to the scissile phosphate. The Mg^{2+} is bound to the scissile phosphate to stabilize the phosphoanion transition state and the Mg^{2+} -bound water molecule provides a proton to the leaving 3'-oxygen.



The structural study for sugar-nonspecific nucleases in complex with DNA demonstrates that protein crystallography is one of the most powerful techniques in elucidating the role of protein molecule in cells and providing the structural basis for protein structure-and-function relationship. The three-dimensional structures of these proteins in complex with DNA not only reveal how nonspecific nucleases recognize DNA without sequence specificity, but also demonstrate how these enzymes cleave DNA at atomic level. This information will be useful in understanding and designing proteins which can recognize and manipulate genes to carry out a variety of biological functions.

BEAMLINES

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