Pruning DNA: Structure-Specific Endonucleases (XPF/Rad1/Mus81)

The crystal structure of Hef endonuclease, an archaeal member of the XPF/Rad1/Mus81 family, reveals a type II restriction enzyme-like active site and a bifurcated dimer interface essential for DNA cleavage. These findings provide a focal point for future study of this family of structure-specific endonucleases.

In the 50 years since Watson and Crick discovered the DNA double helix, our knowledge about the complexity of DNA structures has grown considerably, from right-handed A and B form DNA to left-handed Z form DNA, from duplex to triplex and quadruplex, from straight to extended to coiled in a nucleosome. In addition to serving as a genetic blueprint, DNA undergoes replication and recombination as well as repair in response to endogenous and exogenous damage. During DNA metabolism, double-stranded DNA inevitably forms three-way (replication fork) and four-way (Holliday junctions, bubbles (melted base pairs), flaps (single-stranded branch), or broken ends with single-stranded extensions. These irregular structures must be correctly processed by helicases and nucleases to successfully complete DNA replication, recombination, and repair [1].

Two different families of structure-specific endonucleases have been identified, each of which processes DNA bubbles, flaps, and single-stranded extensions of a unique polarity, 5′ or 3′: XPF, a structure-specific endonuclease essential for nucleotide excision repair, cleaves DNA 5′ to bulky adducts or UV-damaged bases after the local duplex is partially unwound [2]. Inactivation of XPF leads to extreme sensitivity to UV light and xeroderma pigmentosum (XP), which is characterized by a high frequency of skin cancer [3]. The ortholog of XPF in budding yeast (S. cerevisiae) is known as Rad1 [4]. Mus81, an XPF-related nuclease conserved from yeast to human, on the other hand, appears to be essential for meiotic DNA recombination and cell cycle checkpoint signaling [5]. These endonucleases often form heterodimers, XPF pairing with ERCC1, Rad1 with Rad10, and S. cerevisiae Mus81 with Mms4. The resulting dimeric enzymes are specialized in “pruning” downstream branch, flap, and bubble structures by incision near a double- and single-stranded junction [6]. Irregular structures 5′ to a canonical DNA duplex are processed by the XPG/FEN-1 family of endonucleases [7], whose sequences differ remarkably from those of the XPF/Rad1/Mus81 family.

Despite the biological importance of the structure-specific XPF/Rad1/Mus81 family of endonucleases and 25 years of biochemical analysis of their roles in DNA repair [8], it is only now that Nishino, Morikawa, and their colleagues have determined the crystal structure of an archaeal homolog, Hef endonuclease from Pyrococcus furiosus, presented in this issue of Structure [9]. Hef forms a homodimer instead of a heterodimer and is composed of three structural domains, an SF2-like helicase domain at the N terminus (residues 1–546) [10], the XPF- and Mus81-like endonuclease in the middle (residues 547–681), and two helix-hairpin-helix (HhH) motifs at the C terminus (residues 682–763). Although the helicase domain structure appears to be conserved in XPF and Rad1, the sequence motifs essential for catalysis are altered and result in an inactive helicase. The C-terminal HhH motifs are present not only in XPF, Rad1, and Mus81, but also in some dimeric partners, such as with ERCC1 and Rad10. The HhH motifs are shown to be important for dimer formation and DNA recognition [11].

The crystallographic analysis reveals that the Hef endonuclease domain is unrelated to FEN-1 [12–14] but adopts a tertiary structure similar to that of the type II restriction enzymes. The signature motifs of this nuclease family, ERKX3D, with an extension of GDXn at the N terminus (GDXnERKX3D), can be nicely superimposed with the PDXxD(D/E)XX motif of the type II restriction enzymes. To locate the magnesium ion essential for catalysis, Nishino and colleagues also prepared Hef...
nuclease crystals in the presence of similar but heavier divalent cations, Mn²⁺ or Ca²⁺. As in type II restriction enzymes, the conserved acidic residues participate in coordinating a divalent metal ion that probably stabilizes the transition state and facilitates catalysis. The conserved lysine residue is thought to deprotonate the nucleophile water molecule for phosphodiester bond breakage. The conserved Arg sandwiched between Glu and Lys is unique in the structure-specific endonucleases and directly coordinates the divalent cation with its carbonyl oxygen atom. In addition, this Arg residue forms a salt bridge with a Glu side chain, which in turn coordinates the divalent cation through a water molecule. Mutational analyses of these conserved residues have firmly established their roles in catalysis [9].

The surprising finding is that the Hef nuclease domain forms a dimer, and this dimer interface is conserved in the XPF/Rad1/Mus81 family. A point mutation that disrupts this dimer interface only dissociates the nuclease domain, whereas the full-length Hef still dimerizes through the C-terminal HhH domain. Nishino et al. made an additional C-terminal deletion to disrupt dimerization of the HhH domain and found that the mutant Hef is monomeric and its nuclease activity is reduced by more than 100-fold despite an intact active site. The monomeric mutant protein is less stable than the native dimeric protein but retains specific binding of a branched DNA substrate. The dramatic reduction in catalytic activity indicates that dimerization is essential for coordinating substrate binding and DNA cleavage, perhaps by achieving a specific conformational state that facilitates phosphodiester bond breakage. Although Hef only makes a nick in dsDNA adjacent to a 3’ bubble, branch, flap, or single-stranded tail (ref), it may require the dimeric nuclease domain to bind substrate instead of needing two active sites for double-stranded cleavage. It is possible that when associated with a DNA substrate, Hef becomes asymmetric and only one of the two active sites is engaged in catalysis. Alternatively, Hef may make double incisions if a suitable substrate is provided as suggested by Nishino et al. (2003).

Based on the sequence conservation, domain arrangement, and dimeric nature of Hef, XPF, Rad1, and Mus81, Morikawa and colleagues propose that Hef is likely ancestral to its eukaryotic homologs. Because the eukaryotic nucleases form homodimers instead of homodimers, there is the obvious question as to what the conserved dimerization interface of the nuclease domain in XPF, Rad1, and Mus81 interacts with. It was proposed previously based on sequence similarity that XPF and ERCC1 may be derived from a common ancestor [19]. Even though ERCC1 does not contain nuclease activity, it retains the inactive nuclease domain, through which ERCC1 may interact with XPF. One XPF mutation that results in XP is located in the putative nuclease dimerization domain. Interestingly, no XP mutation has been found in ERCC1, and XPF-ERCC1 can be dissociated by deletion in the HhH domain alone. In summary, the crystal structure of the Hef nuclease redefines the functionally important regions in XPF, Rad1, and Mus81, and provides a platform for studying the mechanism and substrate specificity of this family of endonucleases.

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