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Breaking the Rules: Protein Sculpting in NEIL2 Regulation

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The canonical DNA glycosylase role is global base damage repair but includes functions in epigenetic gene regulation, immune response modulation, replication, and transcription. In this issue of *Structure*, Eckenroth et al. (2020) present the NEIL2 glycosylase structure. Its catalytic domain flexibility differentiates it from most other glycosylases and suggests novel regulatory mechanisms.

Critical for genome maintenance, DNA repair pathways face two major mechanistic challenges. First, DNA repair intermediates can be more toxic than the DNA damage itself. Thus, many eukaryotic DNA repair proteins remain bound to their product and act as chaperones to prevent diversion off pathway. Commonly interacting through intrinsically disordered regions at the N and C termini, downstream proteins then promote product release (Liu and Roy, 2002). Second, DNA repair proteins must distinguish uncommon occurrences of DNA damage from undamaged DNA, whether within double-stranded (ds) or single-stranded (ss) DNA in replication forks or transcription. For recognition, DNA repair proteins test for their specific DNA damage through distortion. The first enzymes in the base excision repair (BER), of which NEIL2 is a member, often recognize their target, DNA base damage, by destabilized base stacking (Mullins et al., 2019; Parikh et al., 1998). NEIL2 acts on 5-hydroxyuracil (5-hU), abasic sites, 5-guanidinohydantoin (Gh), Spiroiminodihydantoin (Sp), and 4,6-diamino-5-formamidopyrimidine (FapyA) in ssDNA preferentially over dsDNA. Such base damages are predicted to disrupt base stacking by disturbing the base structure or mispairing. To find their target damage substrate, glycosylases bend and/or flip nucleotides out from the double helix, prior to specific recognition of the base damage (Parikh et al., 1998).

In this issue of *Structure*, Eckenroth and coworkers (2020) present the first NEIL2 structure. Although it is DNA-free and the exact structural mechanisms underlying its glycosylase activity were not revealed, it is clear from crystal structure and the small-angle X-ray scattering (SAXS) data that NEIL2 is unlike most BER glycosylases. Typically, the BER enzyme's catalytic core acts as an inflexible mold, testing for the characteristics of the DNA to be distorted (Parikh et al., 1998; Mullins et al., 2019). Intrinsically disordered regions are typically in the termini. BER crystal structures of both monofunctional (Uracil N-Glycosylase/UNG, Alkylation DNA glycosylase/AlkA) and bifunctional glycosylases (8-Oxoguanine DNA Glycosylase/OGG1, endonuclease III/NTH, Nei Like DNA Glycosylase 1/NEIL1, and NEIL3) show little change between DNA-free and DNA-bound structures (Figure 1) (Mullins et al., 2019). Local shifts in loops and closing to pinch the damaged strand and bend DNA occur, but the protein core fold remains mostly unchanged with a root-mean-square deviation (RMSD) of less than 3 Å.

NEIL2 breaks this rigidity rule. In the article by Eckenroth et al. (2020), the crystal structure and SAXS of the DNA-free enzyme indicate that the two lobes of its catalytic core are not in a catalytically competent orientation, as compared to its paralogs, NEIL1 and NEIL3 structures (Mullins et al., 2019). This flexibility is distinct from more common disordered termini that tether partner proteins. By breaking the catalytic core, NEIL2 is by default inactive until the catalytic domains come together in the proper orientation. Thus, NEIL2 would require a rare conformational change within the catalytic core to achieve catalytic competency. Its bacterial ortholog Endonuclease VIII (Nei), as noted by Eckenroth and coworkers (2020), also shows a requirement for significant reorganization.

Why NEIL2 requires such a regulatory mechanism remains enigmatic. The NEIL protein family is exceptional to other repair enzymes in its links to non-repair-related functions in epigenetic gene activation, modulation of immune response, replication, and transcription (Bacolla et al., 2020; Chakraborty et al., 2015; Hazra et al., 2002; Schomacher et al., 2016), so perhaps NEIL2 needs greater regulation. One potential reason for flexibility is to mechanistically recognize its diverse damaged DNA substrates. Only when its targeted DNA substrate is bound will the enzyme transform into a catalytically competent conformation. NEIL2 works on damage preferentially in ssDNA and bubble DNA, and the energetic cost of nucleotide flipping of undamaged DNA versus damaged DNA is not as severe as in paired dsDNA, thus setting the need for a clever substrate recognition mechanism. NEIL3 also acts on damage in ssDNA, but its catalytic core is intact. Eckenroth and co-workers (2020) reason that catalytic core flexibility allows tethering of partner proteins, while still allowing specific DNA binding. Perhaps these proteins license the glycosylase activity to occur at specific locations as exemplified by the interaction and stimulation of NEIL2 activity by YB-1 transcription factor (Das et al., 2007). Yet, NEIL2 has activity similar to that of NEIL1 and NEIL3 *in vitro* in the absence of partner proteins, suggesting that the DNA substrate is sufficient to reorient the catalytic domains, putting partner protein interactions

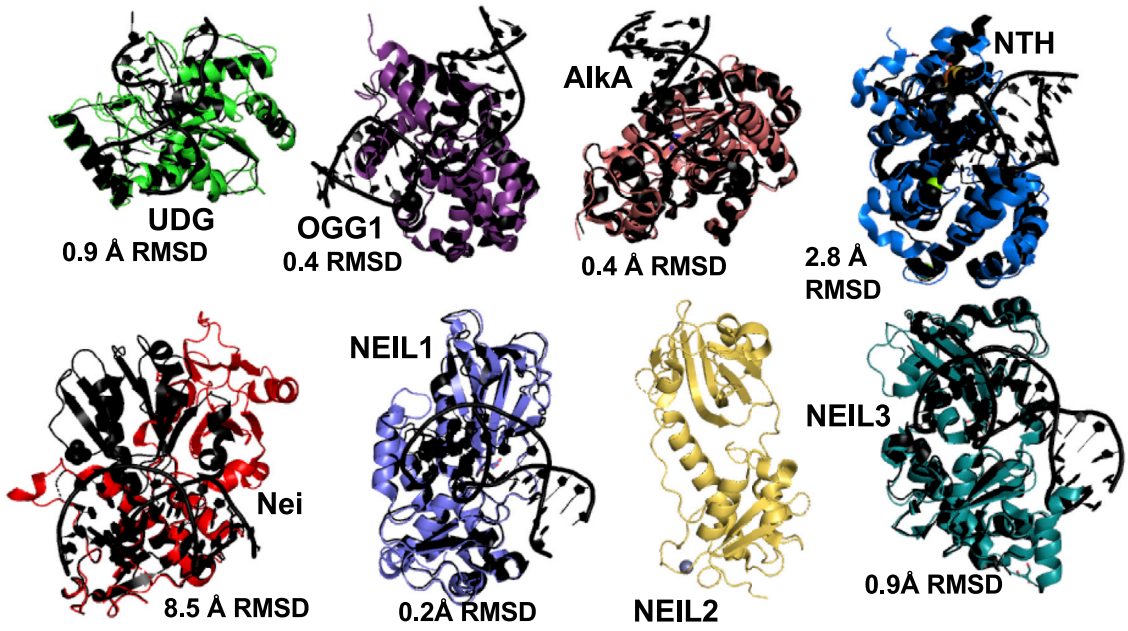

Figure 1. NEIL2 Catalytic Domain Flexibility Breaks the Rule from Most DNA Repair Glycosylases
To identify damaged bases, DNA repair glycosylases typically provide rigid frames to sculpt DNA into a distorted conformation, often with damage nucleotides flipped out. DNA-free NEIL2, in contrast, would require a major rearrangement of its catalytic domains to be catalytically competent. Shown are DNA-free glycosylase structures (color) overlaid on DNA-bound structures (black). UNG (PDB: 1AKZ/4SKN), 3-methyladenine DNA glycosylase AlkA (PDB: 1PVS, 1DIZ), OGG1 (PDB: 3FHF, 3KNT), NTH (PDB: 4UNF,1P59), Nei (PDB: 1Q3b, 2EA0), NEIL1 (PDB: 1TDH, 5ITR), NEIL2 (PDB: 6VJI), NEIL3 (PDB: 3TWK, 3TWM).

as a means to raise or lower glycosylase activity, but not for absolute activation. NEIL2 may have additional non-enzymatic roles where glycosylase activity is purposefully disengaged, as observed for XPG nuclease (Sarker et al., 2005). This NEIL2 structure, breaking the rules for a rigid and preformed catalytic core, suggests that exploration of NEIL2 regulation will be interesting in the years to come.

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