to previously determined structures of human [35], herpes simplex virus [36], *E. coli* [37] and Atlantic cod [38] UNGs. However, drUNG was shown to possess a very high catalytic efficiency on uracil containing substrate (mainly U:A and U:G base pairs) compared to human UNG, which was attributed to a high substrate affinity caused by a cluster of positively charged residues close to the DNA binding site [28]. The recent high-resolution co-crystal structure of drUNG-DNA (Fig. 1) confirmed a strong enzyme-substrate interaction caused by a high number of long-range electrostatic interactions at the protein-DNA interface [29].

Mismatch specific uracil DNA N-glycosylases (MUG) also remove uracil from DNA, but have been shown to possess high specificity for U:G base pairs and little activity on U:A and ssU substrates compared to UNG [39,40]. The crystal structure of drMUG (Fig. 1) showed high similarity to the previously determined structure of *E. coli* MUG [30,39]. However, its substrate specificity was found to be broader: it is able to process A:U and ssU, as well as the 'classical' MUG substrate G:U [40]. In addition, a novel catalytic residue was identified, Asp93, which had not been observed before and based on a phylogenetic analysis, the enzyme was classified into a new class of MUG/thymidine DNA glycosylase family MUG2 [30].

No structural information is so far available for the remaining UDGs from *D. radiodurans*, however the activity of drTmUDG and the two hypothetical UDGs (DR_0022 and DR_1663) have been assessed [41]. DrTmUDG was found to efficiently remove uracil from single-stranded DNA (ssU) and also to a lesser extent from U:G and U:A base pairs. The hypothetical UDG encoded by DR_0022, in contrast, shows no detectable UDG activity. The second hypothetical UDG encoded by DR_1663 has also been expressed and purified and preliminary results from activity measurements on uracil containing oligonucleotide substrates (U:A, U:G and ssU) indicate no uracil excision activity for this enzyme (Moe's laboratory, unpublished data). Based on inhibitory studies of UDG activity in *D. radiodurans* extracts using the DNA glycosylase inhibitor (Ugi), drUNG was proposed to represent the major UDG activity of *D. radiodurans*. This is in agreement with a comparative study of drUNG and drMUG, in which drUNG displayed 2600 times higher specific activity on a uracil containing substrate than did drMUG [30].

3-methyladenine DNA glycosylases II (AlkA) are monofunctional glycosylases with broad substrate specificity for alkylated bases, *e.g.* 3-methyl adenine (3meA), 7-methyl guanine (7meG) 1,N^6-ethenoadenine (εA) and hypoxanthine (Hx) [42–44] and belong to the Helix-hairpin-Helix (HhH) superfamily of DNA glycosylases [45]. Both *D. radiodurans* AlkA genes have been cloned and subjected to expression test, however no expression of the eukaryotic-like drAlkA1 was observed (Moe's laboratory, unpublished data). In contrast, drAlkA2 was expressed in amounts sufficient for purification and crystallisation purposes and its crystal structure was determined by experimental phasing (Fig. 1). It revealed that the conserved N-terminal domain observed in other bacterial AlkA structures [46] is absent in the *D. radiodurans* enzyme [31]. The core structure, composed of a two helical bundle HhH, is however similar, but with a wider DNA binding cleft. Activity assays revealed that drAlkA2 can efficiently process the common 3meA and 7meG AlkA substrates, but not εA and Hx. In addition, it possesses specificity for the AlkB dioxygenase substrates 1-methyladenine and 3-methylcytosine. Interestingly, no gene encoding an AlkB enzyme has so far been identified in the *D. radiodurans* genome, which may explain why drAlkA2 has evolved to be able to process both AlkA and AlkB substrates. Such broad substrate specificity could be accommodated by its wider DNA binding cleft and a highly accessible specificity pocket [31].

In addition to having two AlkAs, *D. radiodurans* possesses three bifunctional Endonuclease III enzymes (drEndoIII-1, -2 and -3), which are responsible for removal of oxidised pyrimidines. The crystal structures of two of the three EndoIIIs (drEndoIII-1 and drEndoIII-3) were determined experimentally (Fig. 1), taking advantage of the [4Fe–4S] clusters in the enzymes, and a reliable homology model of the third member, drEndoIII-2, was generated using *E. coli* EndoIII (>30% sequence identity) as a model [32]. DrEndoIII-3 had to be N-terminally truncated prior to crystallisation [47], but sequence analysis strongly indicates that the gene was most likely incorrectly annotated during the processing of the genome sequencing data [9]. The overall structures of drEndoIIIs, consisting of two all α-helical domains characteristic of enzymes from the HhH-GPD superfamily, are very similar to previously determined structures [48,49]. Several differences were nonetheless observed. Both drEndoIII-1 and -3 possess an additional helix αX inserted in domain 2 just before the conserved HhH motif. Also, compared with previous structures, the DNA binding cleft seems to be more open in the case of drEndoIII-1 and instead more closed and thus less accessible for drEndoIII-3. There are also several critical substitutions close to the active site in the case of drEndoIII-1 and in the DNA binding loops of drEndoIII-3 that could explain the differences observed in terms of enzymatic activity and substrate specificity. Such measurements have indeed shown that drEndoIII-2 is a robust enzyme with strong bifunctional activity against the typical EndoIII substrate, thymine glycol (Tg), while drEndoIII-1 only exhibits weak glycosylase and lyase activities on Tg-containing DNA. In the case of drEndoIII-3, no enzymatic activity has so far been detected on common EndoIII substrates, even though the catalytic residues are conserved both in sequence and positioning in the structure [32]. At present, it is still unclear why *D. radiodurans* possesses these three EndoIII enzymes. Having three EndoIII proteins with different catalytic efficiencies and potentially different substrate specificities within the cell most likely contributes to an improved DNA repair repertoire to survive oxidative DNA damage caused by either radiation or desiccation. This hypothesis is supported by the observation that a majority of the members of the *Deinococci* species also possess three EndoIIIs.

Table 2
List of *D. radiodurans* DNA glycosylases.

Base damage	Protein	ORF	Short name	DG activity	Reference
Uracil repair	Uracil-DNA N glycosylase	DR_0689	UNG	Yes	[28,29]
	Mismatch specific uracil DNA glycosylase	DR_0715	MUG	Yes	[30]
	Thermophilic DNA glycosylase	DR_1751	TmUDG	Yes	[41]
	Putative uracil DNA glycosylase	DR_0022	-	No	[41]
		DR_1663	–	No	(unpublished)
Methylation repair	3-methyladenine DNA glycosylase II	DR_2074	AlkA1	Not tested	–
		DR_2584	AlkA2	Yes	[31]
Oxidation repair	Endonuclease III	DR_2438	EndoIII-1	Yes	[32]
		DR_0289	EndoIII-2	Yes	
		DR_0928	EndoIII-3	No	
	Formamidopyrimidine DNA glycosylase	DR_0493	MutM	Yes	[51,125]
	A/G specific adenine DNA glycosylase	DR_2285	MutY	Yes	[50]

ORF: Open reading frame. DG: DNA glycosylase.