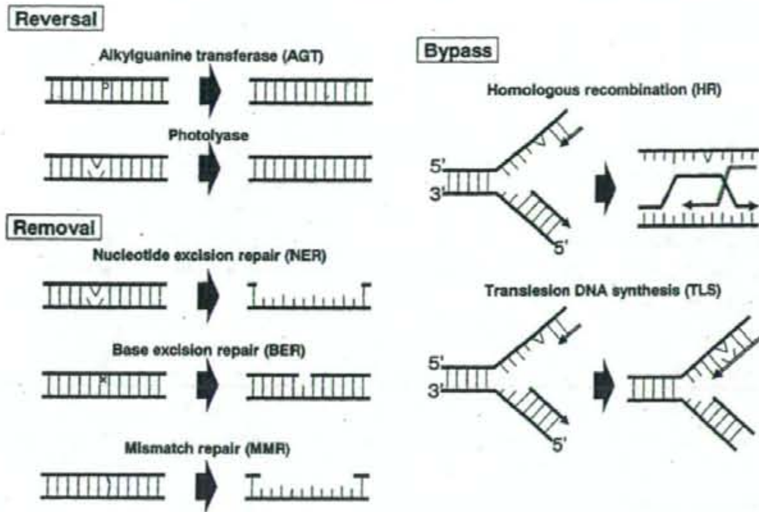


**Figure 7.1** Schematic representation of mechanisms of DNA repair. Alkylguanine transferase (AGT) and photolyase directly reverse the damaged bases to correct ones. Nucleotide excision repair (NER), DNA glycosylase involved in base excision repair (BER) and mismatch repair (MMR) remove damaged bases or DNA sequeces containing damaged bases, followed by DNA re-synthesis to fill in the gaps. Homologous recombination (HR) and TLS do not remove damaged bases but assist to resume stalled DNA replication at the damaged sites.

1998). The enzymes seem to be similar to a human AGT because they lack the alkylphosphotriester-DNA alkyltransferase activity seen in some prokaryotes. More over, *P. islandicum* and *Pyrococcus furiosus* have maximal AGT activity at approximately 100°C. These enzymes would thus appear to be able to act on ssDNA (Skorvaga *et al.*, 1998).

AGT in other Euryarchaeota were cloned from *Pyrococcus* sp. KOD1(Leclere *et al.*, 1998), *Archaeoglobus fulgidus* (*Af*-AGT) (Kanugula and Pegg, 2003) and *Ferroplasma acidarmanus* (*Fa*-AGT) (Kanugula *et al.*, 2005). The AGT from KOD1 displays the activity at 90°C for at least 30 min. The enzyme exhibits considerable similarity to the corresponding mammalian, yeast and bacterial enzymes. *Af*-AGT is active in repairing $O^6$-methylguanine and, at a lower rate, $O^4$-methylthymine in DNA, and is readily inactivated by the low-molecular-mass inhibitor $O^6$-benzylguanine. *Fa*-AGT consists of a fusion of the C-terminal active site domain of AGT with an endonuclease V (Endo V) domain. The purified protein repairs $O^6$-meG in DNA and also cleaves the deaminated bases, i.e. U, HX or xanthine, in a similar manner to *E. coli* Endo V. In this case, two distinct but functional DNA repair proteins co-exist in a single polypeptide chain.

## Photoreactivation

Photoreactivation is an efficient and direct repair mechanism for CPDs, which are the major DNA photoproducts induced by the UV component of solar radiation. This mechanism was discovered by the finding that survival of Bacteria irradiated with UV increased after exposure to visible light (Kelner, 1949). The DNA repair process is catalysed by a single enzyme, i.e. photolyase, which monomerizes UV-induced dimers by transfer of blue light energy absorbed by the chromophores (Sancar, 1990; Sancar, 1994). Most photolyases repair CPDs but some repair 6–4 photoproducts (6–4 PPs). Photoreactivation is widely distributed among species, ranging from Bacteria to plants and mammals. The ubiquity and efficient use of visible light emitted by the sun, the same source that induces DNA lesions, indicate that photolyase is an ancient protein that may have played an important role in evolution.

**Table 7.1** DNA repair pathways in Archaea

| | AGT | PHT | NER | UDG | MIG | AP | OGG | FEN-1 | MutS | HR |
|---|---|---|---|---|---|---|---|---|---|---|
| Crenarchaeota | | | | | | | | | | |
| *Aeropyrum pernix* | ++ | ++ | +++ | ++ | +++ | ++ | ++ | +++ | ++ | ++ |
| *Pyrobaculum aerophilum* | ++ | ++ | ++ | +++ | +++ | ++ | ++ | ++ | | ++ |
| *Pyrobaculum islandicum* | +++ | ++ | + | +++ | | ++ | | | | +++ |
| *Sulfolobus acidocaldarius* | +++ | +++ | ++ | +++ | | ++ | ++ | ++ | | ++ |
| *Sulfolobus solfataricus* | ++ | +++ | +++ | +++ | | +++ | ++ | ++ | | ++ |
| *Sulfolobus shibatae* | | | | +++ | | | | | | ++ |
| Euryarchaeota | | | | | | | | | | |
| *Archaoglobus fulgidus* | +++ | ++ | +++ | +++ | | ++ | +++ | +++ | | ++ |
| *Ferroplasma acidarmanus* | +++ | | + | + | | ++ | ++ | | ++ | + |
| *Halobacterium halobium* | | +++ | ++ | ++ | ++ | +++ | ++ | ++ | ++ | ++ |
| *Haloferax* | | + | ++ | | | | | | | +++ |
| *Methanococcus jannaschii* | ++ | | ++ | +++ | | ++ | +++ | +++ | | ++ |
| *Methanothermobacter thermautotrophicus* | ++ | +++ | +++ | | +++ | +++ | ++ | ++ | | ++ |
| *Pyrococcus furiosus* | +++ | ++ | ++ | +++ | + | ++ | ++ | +++ | ++ | +++ |
| *Pyrocuccus horikoshii* | ++ | ++ | ++ | ++ | | ++ | ++ | +++ | ++ | ++ |
| *Pyrocuccus sp. KOD1* | +++ | ++ | ++ | ++ | | ++ | ++ | ++ | ++ | ++ |
| *Thermococcus litoralis* | +++ | | | +++ | | | | | | |

The abbreviations are as follows: AGT – $O^6$-MeG methyltransferase, PHT – photolyase, NER – nucleotide excision repair (XPF, XPB, XPD), UDG – uracil DNA glycosylase, MIG – mismatch glycosylase, AP – AP endonuclease, OGG – 8-oxoG glycosylase, FEN-1 – flap endonuclease, MutS – mutS, HR – recA. The enzymes characterized biochemically are indicated as +++, enzymes with sequence homology as described in the NCBI GeneBank protein sequence database of March, 2007 are indicated as ++ while proteins with only limited homology are labelled as +.

Among Archaea, photolyases are not common, although the photoreactivation or photolyases are identified in several archaeal species. These include Crenarchaeota, i.e. *Sulfolobus acidocaldarius* (Wood *et al.*, 1997) and *Sulfolobus tokodaii* (Fujihashi *et al.*, 2007), and Euryarchaeota, i.e. *Halobacterium* sp. NRC-1 (McCready and Marcello, 2003) and *Methanothermobacter thermoautotrophicus* (Kiener *et al.*, 1989; Yasui *et al.*, 1994). In addition, several hypothetical genes encoding photolyases are identified in *Haloarcula marismortui* (Baliga *et al.*, 2004c), *Methanopyrus kandleri* (Slesarev *et al.*, 2002) and *Methanosarcina acetivorans* (Galagan *et al.*, 2002). Sso7d and Sac7d are two small (~7 kDa), but abundant, nucleoproteins in *Sulfolobus solfataricus* and *S. acidocaldarius*, respectively (Gao *et al.*, 1998; Robinson *et al.*, 1998). Recently it was demonstrated that Sso7d efficiently repairs the thymine dimer under irradiation conditions, suggesting that Sso7d and Sac7d may be involved in the photoreactivation in the organisms (Tashiro *et al.*, 2006). Two photolyase-like genes, *phr1* and *phr2*, are identified in *Halobacterium NRC-1*, and the *phr2* gene is shown to encode a CPD photolyase (McCready and Marcello, 2003). The photolyase from the *Methanothermobacter thermoautotrophicum* exhibits 37% homology to those of higher eukaryotes and only 15% to microbial photolyases(Yasui *et al.*, 1994)

### Nucleotide excision repair

NER is a highly conserved DNA repair pathway able to detect and remove a variety of bulky DNA lesions caused by UV light and environmental mutagens and thereby contributes to the genomic integrity of an organism (Sancar, 1996; Lindahl and Wood, 1999). NER is uni-

versal in Bacteria and Eukarya although other mechanisms, i.e. photoreactivation enzymes (see Photoreactivation), DNA glycosylase (see Base excision repair) and endonuclease known as UVDE (Yasui and McCready, 1998), also operate to counteract UV-induced DNA damage in a number of organisms. NER in higher eukaryotes involves the coordinated assembly of a large number of proteins, including the core NER factors XPC-HR23B, TFIIH, XPA, replication protein A (RPA) and two endonucleases XPG and ERCC1-XPF (Aboussekhra *et al.*, 1995). These proteins cooperate to recognize, unravel and excise a 24–32-mer oligonucleotide bearing the DNA lesion prior to filling in the missing gap (Araujo and Wood, 1999). Although Bacteria have a similar overall repair strategy, a much simpler and structurally unrelated multiprotein complex known as UvrABC carries out the same task (Petit and Sancar, 1999). The availability of fully sequenced archaeal genomes has revealed that many Archaea have proteins related to eukaryotic NER factors rather than to the UvrABC bacterial repair system (Aravind *et al.*, 1999; White, 2003; Grogan, 2000). Only certain Euryarchaeota, i.e. *Halobacterium sp.* NRC-1 (Ng *et al.*, 2000), *Methanothermobacter thermoautotrophicus* (Ogrunc *et al.*, 1998), *Haloarcula marismortui* (Baliga *et al.*, 2004a), *Methanosarcina acetivorans* (Galagan *et al.*, 2002) and *Methanosarcina mazei* (Deppenmeier *et al.*, 2002), have Bacteria-type repair proteins, although all of them have eukaryotic-type NER proteins too. It is interesting that Archaea lack damage recognition proteins such as XPA and XPC, which are essential for eukaryotic NER, while they have other eukaryotic-type components, such as XPF/ERCC1, Flap endonuclease, and XPB and XPD helicases (White, 2003). It is worth noting that Crenarchaea have a 'short' form of XPF that lacks the helicase-like domain while Euryarchaeota have a long form of XPF, which includes a helicase-like domain at the N-terminal (Roberts *et al.*, 2003).

In a few species, actual NER activities have been demonstrated. In Crenarchaeota, UV-induced CPDs are efficiently repaired in vivo in the dark in *Sulfolobus solfataricus* (Salerno *et al.*, 2003). In Euryarchaeota, *Methanobacterium thermoautotrophicum* is shown to remove UV-induced 6–4 PPs in the form of 10- to 11-mers by incising 3′- and 5′-sides of the DNA damage (Ogrunc *et al.*, 1998). In addition two structural analyses provide more precise information to this field; XPF homologue in *Aeropyrum pernix* (Newman *et al.*, 2005), and XPB homologue in *Archaeoglobus fulgidus* (AfXPB) (Fan *et al.*, 2006). Interestingly, the DNA damage recognition domain and the flexible thumb motif of AfXPB appear structurally as well as functionally analogous to the MutS mismatch recognition and DNA polymerase thumb domains.

### Base excision repair

BER pathway involves the initial recognition of specific types of base damage and/or damage associated basepairing mismatches by a BER DNA glycosylase. An estimated rate of $10^4$ damaging events/mammalian cell/day underscores the importance of the BER pathway (Nakamura *et al.*, 1998; Lindahl and Wood, 1999; Lindahl, 1993). After damage recognition, the glycosylase cleaves the damaged or misincorporated base from the DNA backbone, leaving an apurinic/apyrimidinic (AP) site (Lu *et al.*, 2001). These AP sites are cleaved by the major 5′ AP endonuclease, APE1, to leave a 5′ dRp terminus that is then removed by DNA Pol β producing a single nucleotide gap. DNA glycosylases/AP lyases or bifunctional glycosylases, such as endonuclease III (NTH1) and 8-oxoguanine DNA glycosylase (OGG1), besides base excision, cleave DNA at the resultant AP site by β elimination generating a 3′ terminal sugar phosphate that is then removed by APE1 yielding a gap with a 3′ OH (Demple and Harrison, 1994). Ligation will continue either via short-patch (SP) (for one nucleotide) (Dianov *et al.*, 1992) or long-patch (LP) (two or more nucleotides) repair (Frosina *et al.*, 1996; Klungland and Lindahl, 1997). Pol β is the repair-synthesis polymerase of the SP-repair. Pol β is also likely to incorporate the first nucleotide in the LP-repair (Podlutsky *et al.*, 2001) but the elongation step in this pathway is carried out by replicative DNA Pols. Proliferating cell nuclear antigen (PCNA), the sliding clamp for Pol β and flap endonuclease-1 (FEN-1), a structure specific nuclease that excises the displaced oligonucleotide (Pascucci *et al.*, 1999). The ligation step is then operated by the X-ray repair cross

complementing 1 (XRCC1)/DNA ligase IIIα complex in the SP-repair. The XRCC1/DNA ligase IIIα complex is dispensable for LP-repair implying that in this pathway the sealing step is performed by DNA ligase I (Cappelli *et al.*, 1997; Sleeth *et al.*, 2004).

Of various BER glycosylases, uracil DNA glycosylase (UDG) have been studied most extensively (Lindahl and Nyberg, 1974; Percival *et al.*, 1989). They are extremely efficient enzymes, which recognize U in ss DNA, in A/U pairs that arise when dUMP is incorporated opposite A during DNA replication, or in G/U mispairs arising through cytosine deamination. Thermostable UDG activities have been identified in Crenarchaeota, i.e. *Sulfolobus solfataricus*, *S. shibatae*, *S. acidocaldarius*, *Thermococcus litoralis*, *Pyrobaculum islandicum* and P. aerophilum, and Euryarcheota, i.e. *Pyrococcus furiosus*, *Methanococcus jannaschii* and *Archaeoglobus fulgidus* (Sandigursky and Franklin, 2000; Koulis *et al.*, 1996; Hinks *et al.*, 2002; Chung *et al.*, 2003b). In addition, novel types of UDGs have been identifed in hyperthermophilic Archaea (Pearl, 2000; Sartori *et al.*, 2002; Chung *et al.*, 2003b). One such example is *Pa*-UDGb in *Pyrobaculum aerophilum*, which lacks the polar amino acid at the active site (motif A) and removes HX as well as U in DNA. This enzyme is a functional component of in vitro BER system that consists of *Pa*-UDGb, AP endonuclease IV, DNA Pol B2 and DNA ligase (Sartori and Jiricny, 2003). Besides *Pa*-UDGb, *P. aerophilum* possesses two other UDGs, i.e. *Pa*-UDGa and *Pa*-MIG (mismatch specific DNA glycosylase). *Pa*-UDGa has glutamate at the active site and is more active than *Pa*-UDGb. *Pa*-UDGa appears to be the major UDG in this organism and *Pa*-UDGb is probably responsible for the removal of HX or hydroxymethylU in DNA. *Pa*-MIG can remove T as well as U paired with G so that it may contribute to mismatch correction (Sartori *et al.*, 2002; Yang *et al.*, 2000). MIG activites are also identified in *Aeropyrum pernix* and *Methanobacterium thermoautotrophicum* (Yang *et al.*, 2000; Horst and Fritz, 1996; Fondufe-Mittendorf *et al.*, 2002).

Hypothetical counterparts of OGG1, which removes 8-oxoguanine (8-oxo-G) in DNA, have been identified in the genome of Crenarchaeota, i.e. *Sulfolobus solfataricus* (She *et al.*, 2001) and Euryarchaeota, i.e. *Methanococcus jannaschii* (Gogos and Clarke, 1999), *Archaeoglobus fulgidus* (Chung *et al.*, 2001), *Thermoplasma acidophilum* (Ruepp *et al.*, 2000) and *Thermoplasma volcanicum* (Kawashima *et al.*, 2000). Among them, enzymatic activities against 8-oxo-G in DNA have been demonstrated in *M. jannaschii* and *A. fulgidus* (Gogos and Clarke, 1999; Chung *et al.*, 2001).

FEN-1 has important roles in DNA repair as well as DNA replication and recombination. The enzyme cleaves 5′ flap structure endonucleolytically and has double-strand-specific 5′–3′-exonuclease activity. When working in BER, FEN-1 removes damaged nucleotides after AP endonuclease has incised the 5′ side of the AP site in LP-repair (Klungland and Lindahl, 1997; Kim *et al.*, 1998; Gary *et al.*, 1999). It recognizes a specific DNA structure, independent of the DNA sequence. FEN-1 seems highly conserved in Archaea and Eukaryotes (Matsui *et al.*, 1999; Hosfield *et al.*, 1998; Kaiser *et al.*, 1999; Rao *et al.*, 1998) and the crystal structures have been solved in Euryarchaeota, i.e. *Methanococcus janaschii* (Hwang *et al.*, 1998; Sayers and Artymiuk, 1998; Bae *et al.*, 1999), *Pyrococcus furiosus* (Hosfield *et al.*, 1998), *Archaeoglobus fulgidus* (Chapados *et al.*, 2004) and *Pyrococcus horikoshii* (Matsui *et al.*, 2002) and in Crenarchaeota, i.e. *Aeropyrum pernix* (Collins *et al.*, 2004).

### Mismatch repair

The ability to recognize and repair mismatches in DNA after replication has been well documented in many species (Modrich and Lahue, 1996). The most extensively studied general MMR system is the one in the bacterium *E. coli*. It functions with the MutSLH, UvrD, and RecJ proteins through a d(GATC)-specific methylase (dam) methylation-directed pathway and is initiated by the mismatch-stimulated d(GATC) endonuclease activity of MutH, which takes advantage of transient undermethylation of the DNA during replication, and specifically nicks the newly synthesized, that is, unmethylated daughter strand. Subsequently, the RecJ exonuclease selectively degrades the nicked strand, and the mutations are corrected by the action of a DNA Pol, a helicase, and a ligase (Marti *et al.*, 2002).

Hypothetical MutS-like proteins were found in several species of Euryarchaeota, but not in ones of Crenarchaeota, such as *Sulfolobus solfataricus* (She *et al.*, 2001). The MutS-like proteins that have ATPase domain may be divided into two subfamilies, i.e. MutS-I lineage related to MMR and MutS-II lineage related to chromosome aberrations and meiotic crossing-over (Eisen, 1998). The MutS-I lineage includes bacterial MutS and eukaryotic MSH2/6, and the MutS-II includes eukaryotic MSH4 and MSH5. Phylogenetic analysis also shows that the proteins in the MutS-II subfamily are distant and distinct from those involved in MMR (Kawarabayasi *et al.*, 1998; Smith *et al.*, 1997; Robb *et al.*, 2001; Cohen *et al.*, 2003). *Halobacterium* NRC-1 and *Methanosarcina activorans* have both types of MutS homologues as well as a MutL-like protein (Ng *et al.*, 2000).

Unlike MutS and MutL, which are absolutely necessary for MMR, and therefore evolutionarily highly conserved, MutH is significantly less conserved. In fact, no MutH homologue has been identified in either the Eukaryotes or the Archaea. Since it has been revealed that Eukaryotic MMR protein complex, MutLα, is an endonuclease (Kadyrov *et al.*, 2006), it is not surprising that Eukaryote does not possess MutH endonuclease. Although *Halobacterium* NRC-1 does not have MutH endonuclease or a *dam* methylase, it has d(CTAG) methylase (Zim), MutS/L, four RecJ-like exonucleases and the UvrD repair helicase (Ng *et al.*, 2000). However, a d(CTAG)-specific endonuclease was not detected using either sequence- or structure-based annotation methods (Baliga *et al.*, 2004b). *P. furiosus* has detectable enzymatic activities of MutS2 protein, an ATPase activity and a non-mismatch specific DNA-binding activity (Vijayvargia and Biswas, 2002).

Spontaneous mutation frequency in *P. aerophilum* is high, especially in mononucleotide repeats (Fitz-Gibbon *et al.*, 2002). Although this is consistent with the absence of orthologues of MMR proteins, *P. aerophilum* may possess a distinct MMR system. Taken together, it has been, so far, unclear how mismatches generated during DNA replication are corrected in Archaea. It is possible that a novel system that manages mismatch errors operates in Archaea such as *S. solfataricus*, where no MutS/L homologues are identified.

### Recombinational repair

DNA double-strand breaks (DSBs) are highly cytotoxic and mutagenic, thus the repair of DSBs in DNA is an essential process in all organisms. DSBs are predominantly repaired by two pathways, i.e. non-homologous end joining and HR. HR conserves genetic identity by using an undamaged chromosome as a template to replace missing or damaged nucleotides. It also serves to underpin genome replication by providing the means to resume replication when the replication machinery is blocked (Seigneur *et al.*, 1998; McGlynn and Lloyd, 2000). Mre11/Rad50 complex plays a key role in DSB repair. The complex participates in the generation of the 3′ ssDNA tails possibly by bridging DNA ends or sister chromatids in the very early steps of HR. RecA family of recombinases entails the reciprocal exchange of the single strands between homologous DNA duplexes by utilizing conserved DNA-binding modules and a common core ATPase domain to form a four-way branched intermediate commonly referred to as the Holliday junction (HJ). In the late step of HR, the HJ is resolved by dual-strand incision across the branch point to release nicked duplexes, then whole procedure completes with sealing by DNA ligase. Enzymes of HR have been unearthed in all three domains of life, i.e. Bacteria, Archaea and Eukarya.

Hyperthermophilic Archaea are reported to be more resistant to ionizing radiation than hyperthermophilic Bacteria or *E. coli* (DiRuggiero *et al.*, 1997). A γ-ray dose allowing 75% survival of *Pyrococcus furiosus* was shown initially to fragment the chromosome into the lengths of about 30 kb, yet full-length chromosomes eventually reappeared *in vivo* after prolonged incubation at 95°C. There is evidence that RadA, the archaeal RecA protein, is induced by DNA damage in thermophilic and mesophilic Archaea (Reich *et al.*, 2001). Although the results imply that effective DSB repair mechanisms, which could be HR, exist in Archaea, very little is known about the enzymes for homologous exchange in Archaea, with the exception of RecA-like strand exchange enzymes (Seitz *et al.*, 1998) and en-

zymes that cleave HJs *in vitro* found in *P. furiosus* (Komori *et al.*, 1999), or *Sulfolobus solfataricus* (Kvaratskhelia and White, 2000a).

Structural analyses of a Rad50 homologue from *P. furiosus* illuminate the catalytic core of the enzyme, an ATP-binding domain related to the ABC transporter family of ATPases (Hopfner *et al.*, 2000; Hopfner *et al.*, 2001). Its S793R mutation prevents ATP binding and dimerization, and disrupts the communication among the other ATP-binding loops (Moncalian *et al.*, 2004). Crystal structure of Mre11 and Rad50 of *P. furiosus* reveal a dual functional complex consisting of $(Mre11)_2/(Rad50)_2$ heterotetrameric DNA (Hopfner *et al.*, 2001). Putative *recA* and *RAD51* homologues in three archaeans were identified by PCR and referred to as *radA* (Sandler *et al.*, 1996). The same research group amplified fragments of *radA* genes from 11 archaeal species and analysed the phylogenetic relationships (Sandler *et al.*, 1999). RadA proteins that are functional *in vivo* are found in Euryarchaeota, i.e. *Haloferax volcanii* (Woods and Dyall-Smith, 1997) and *P. furiosus* (Komori *et al.*, 2000b; Komori *et al.*, 2000a; Hayashi *et al.*, 1999), and Crenarchaeota, i.e. *Pyrobaculum islandicum* (Spies *et al.*, 2000). Crystal structure is presented for a RadA from *Methanococcus voltae* (Qian *et al.*, 2005). These enzymes share a higher level of sequence and more structural similarity with eukaryotic Rad51 than bacterial RecA (Uemori *et al.*, 1997; DiRuggiero *et al.*, 1997). RadB was also identified as a Rad51-like protein on the basis of conserved ATPase sequences (Rashid *et al.*, 1996). However, RadB from *P. furiosus* is not a recombinase. It does not catalyse strand exchange and does not turn over ATP efficiently (Komori *et al.*, 2000b). Despite the lack of ATPase activities, an ATP-binding activity seems required for RadB to promote survival of *Haloferax volcanii* after UV irradiation. ATP induces pronounced conformational change in RadB (Akiba *et al.*, 2005), which seems to be important for maintaining genome stability in Archaea. RPA is found in *P. furiosus* and is composed of three subunits, i.e. RPA41, RPA14, and RPA32, like the eukaryotic RPA (p70-p14-p32). RPA clearly stimulates a RadA-mediated strand exchange reaction (Komori and Ishino, 2001).

Regarding the late stage, in which the HJ intermediates are processed, the first archaeal HJ resolvase (Hjc, HJ cleavage) is discovered in *P. furiosus* (Komori *et al.*, 1999). Similar resolvases have been purified from *S. solfataricus* (Kvaratskhelia and White, 2000b) and *Methanobacterium thermoautotrophicum* (Bolt *et al.*, 2001). Although Hjc does not resemble any of the known HJ resolvases, it does share significant similarity to restriction endonucleases and exonuclease (Aravind *et al.*, 2000; Kvaratskhelia *et al.*, 2000). Thus, Hjc is an archaeal-specific HJ resolvase. Neither its sequence nor its three-dimensional structure is similar to other known HJ resolvases (Nishino *et al.*, 2001; Komori *et al.*, 2000c). HJ resolvases with distinct properties have been characterized from bacteriophages (T4 endo VII, T7 endo I, RusA and Rap), Bacteria (RuvC), Archaea (Hjc and Hje), yeast (CCE1) and poxviruses (A22R). Thus, the HJ resolvase is quite interesting, from an evolutional point of view (Lilley and White, 2000). The Hjc enzymes target junctions with high specificity but, unlike RuvC and RuvA, they show no sequence-preference for strand cleavage. Hjc from *P. furiosus* has been found to interact with the archaeal equivalent of PCNA and with RadB (Komori *et al.*, 1999; Komori *et al.*, 2000c). The Hje (HJ endonuclease) protein has similar properties to Hjc, including a preference for four-way junction DNA and the ability to resolve both static and mobile HJs (Kvaratskhelia and White, 2000a). Its crystal structure serves more precise information for understanding its function (Nishino *et al.*, 2005). The gene, *hjm* (HJ migration), encodes a protein composed of 720 amino acids. A homology search revealed that Hjm shares sequence similarity with the human PolQ, HEL308, and *Drosophila* Mus308 proteins, which are involved in a DNA repair, whereas no similar sequences were found in Bacteria and yeast (Fujikane *et al.*, 2005).

## DNA damage tolerance by archaeal DNA polymerases

Life represents a succession of biological information stored in DNA from ancestors to descendants. Thriving in harsh habitats such as high temperatures, therefore, requires organisms to evolve unique mechanisms to maintain the

genome integrity. Temperatures more than 80°C where thermophilic Bacteria optimally grow pose challenges to the genome to be denatured or decomposed. Although denaturation may not be a serious problem for DNA as long as it is covalently closed, heat-induced depurination, oxidation and deamination could have deleterious effects. Depurination leads to AP site, a major cytotoxic lesion, and oxidation generates a variety of DNA lesions such as 8-oxo-G in DNA (Lindahl, 1993). Hydrolytic deamination of exocyclic amino groups of cytosine leads to the formation of U in DNA, and G:U basepairs result in G:C to A:T transitions in a half of the progeny if not repaired (Lindahl and Nyberg, 1974; Pearl, 2000). Although most of the lesions can be removed by DNA repair mechanisms as has been reviewed above, some escape from the repair mechanisms and persist in the template DNA. These residual lesions can block DNA replication and induce mutations or chromosome breaks. To counteract the lesions, cells evolved the mechanisms called DNA damage tolerance to accomplish the chromosome replication. Unlike DNA repair, the damage tolerance mechanism does not remove DNA damage but instead allows DNA Pols proceed beyond the damage and permits the accomplishment of chromosome replication. The mechanisms may also include the behaviour of DNA Pols that stop replication when they encounter spontaneous DNA lesions, thereby giving a chance to cells to remove the lesions before Pols incorporate incorrect dNMPs opposite the lesions. Although DNA damage tolerance mechanisms may include recombination repair in theory, we focus on the mechanisms associated with DNA Pols of Archaea, i.e. read-ahead mechanisms and translesion bypass replication (Lasken *et al.*, 1996; Nohmi, 2006; Fogg *et al.*, 2002). The physiological significance of damage tolerance mechanisms in Archaea is still open to question, but they may be critical to survive in harsh environments and maintain the genome stability. Since Pols involved in damage tolerance are also involved in induction of mutations by incorporation of incorrect dNMP opposite template lesions, they could play a role in evolution or genetic flexibility of archaeal Bacteria living in variable harsh environments. In the following sections, we will see how B-family and Y-family DNA Pols contribute to DNA damage tolerance in Archaea.

### Read-ahead mechanism

Deamination of bases in DNA such as cytosine may be the most common genotoxic threat to all living organisms. Deamination of cytosine to U leads to C to T transitions unless repaired and disrupts the succession of genetic information stored in the genome. To counteract the mutagenic potential of U in DNA, Eukarya, Bacteria and plants have a BER pathway (see above) utilizing a UDG or a G:U/T mismatch specific DNA glycosylase. In addition, cells in Bacteria, Eukarya and Archaea possess dUTPase, which converts dUTP to dUMP and pyrophosphate (PPi), to minimize the levels of dUTP in the nucleotide pool (Hogrefe *et al.*, 2002). dUTP is a major source of U in DNA because of their ease of incorporation into DNA by Pols. In fact, it is estimated that $10^4$ dUTP is incorporated per DNA replication based on a genome size of $10^{10}$ basepairs (Savva *et al.*, 1995). In Bacteria and yeast, cells lacking dUTPase exhibit DNA fragmentation and cell death because of the excess excision of U in DNA by the action of UDGs and the following steps of BER (Gadsden *et al.*, 1993). Despite the presence of UDG and dUTPase, elevated temperatures should pose an increase risk of DNA damage in hyperthermophilic Archaea because hydrolytic deamination of U greatly accelerated at high temperatures. However, the spontaneous mutation rate in hyperthermophilic Archaea *Sulfolobus acidocardarium* is reported to be similar to that of *Escherichia coli* (Grogan *et al.*, 2001). Thus, hyperpethermophilic Archaea appear to possess additional mechanisms to protect a stability of the genome from the mutagenic threat of deaminated bases generated at high temperatures.

Interestingly, B-family DNA Pols from hyperthomophilic Archaea such as *Sulfolobus solfataricus* DNA Pol B1 (Pol B1), *Pyrococcus furiosus* DNA Pol (Pfu) and *Thermococcus litoralis* DNA Pol (Vent and Vent exo-), recognize the presence of U in DNA and tightly bind to U-containing oligonucleotides (Lasken *et al.*, 1996). These Pols stall DNA replication several basepairs before template U and thus the recognition and stalling mechanisms seem to contribute to counteract the

mutagenic potential of U in DNA because the stalling mechanisms may reduce the chance of misincorporation of adenine opposite template U (Greagg *et al.*, 1999). More interestingly, both Pol B1 and Pfu exo- bind not only to template U but also to template HX, a deamination product of adenine (Gruz *et al.*, 2003). Pfu exo- stalls three to four basepairs before HX as well as U in the template strand, and Pol B1 displays a similar stalling pattern. HX pairs with cytosine during DNA replication and induces A to G transitions if not corrected (Lindahl, 1993). Thus, the stalling behaviour seems to suppress the incorporation of cytosine opposite template HX, thereby reducing the mutagenic potential of HX.

The recognition and stalling mechanism seems unique to archaeal B family DNA Pols because viral B family Pols such as T4 Pol or Pols from hyperthermophilic eubacteria such as *Thermus aquaticus* (Taq) do not stall when the template DNA has U or HX (Greagg *et al.*, 1999). Eukaryotic B-family Pol, i.e. human Pol alpha, does not tightly bind to a U-containing oligonucleotide (Lasken *et al.*, 1996). Several lines of evidence suggest that single molecules of the archaeal Pols scan the template DNA and stall when they encounter U several basepairs ahead of the progress (Asami *et al.*, 2006; Shuttleworth *et al.*, 2004). Thus, this is called 'read-ahead mechanism'. Structural analysis of archaeal B-family Pols suggest that they comprise five domains, i.e. the N-terminal domain, the exonuclease domain and three polymerase active site domains, and also that a pocket in the N-terminal domain is responsible for the discrimination of U from normal DNA bases (Fogg *et al.*, 2002). The pocket seems highly conserved in the N-terminal domains of 15 archaeal B-family Pols including seven euryarchaeal and eight crenarchaeal Pols. It remains to be seen how this pocket accommodates not only U but also HX in template DNA (Savino *et al.*, 2004).

Since archaeal B-family DNA Pols such as *Pfu* or *Vent* are frequently used as PCR enzymes, the inhibitory effects of U are practical problems to achieve long and efficient PCR amplification of DNA. To circumvent the problems, dUTPase is included in the reaction mixture to reduce the levels of dUTP, which are generated during PCR through deamination of dCTP and limit the efficiency of PCR achieved by archaeal B-family DNA Pols (Hogrefe *et al.*, 2002). Pfu dUTPase, which converts dUTP to dUMP and PPi, improves the yield of products amplified by *Pfu* DNA Pol by preventing dUTP incorporation and subsequent inhibition of the Pol by U-containing DNA. It is suggested that archaeal dUTPase may play an important role in preventing dUTP incorporation and inhibition of DNA synthesis by B-family DNA Pols in vivo.

Although it seems plausible that the read-ahead mechanism contributes to the genome stability in hyperthermophilic Archaea, it is an open question of what will happen after the Pols bind to the template U or HX (Fig. 7.2). A hint is that the archaeal Pol binds to UDG (Connolly *et al.*, 2003). It is tempting to speculate, therefore, that HR occurs after the stalling and double-strand DNA is formed where one strand possesses U and the other possesses guanine. UDG excises U from one strand and the resulting single base gap is filled by Pol using a guanine base in the opposite strand as a template. If these events actually occurred, the correct base, i.e. cytosine, would be restored in the original site. Another hint is the interaction between Pol B1 from *S. solfataricus* and Pol Y1 (Dpo4) from the same species (De Felice *et al.*, 2007). Pol Y1 bypasses a variety of DNA lesions including U (Gruz *et al.*, 2001; Boudsocq *et al.*, 2004). It does not stall even when it encounters U or HX during in vitro DNA synthesis (Gruz *et al.*, 2003). Thus, translesion bypass across U or HX by Y-family Pols may occur to continue the DNA replication after removal of stalling Pols at the lesions. The deficit of the bypass pathway is that the translesion reactions induce mutations of C to T as an expense of continuation of chromosome replication. PCNA may be involved in these events because it is a molecular scaffold to recruit several proteins involving in the processing of DNA damage (Gruz *et al.*, 2001; Dionne *et al.*, 2003). In either case, further work is needed to clearly establish the molecular mechanisms after stalling Pols at the lesions in hyperthermophilic Archaea.

## DNA lesion tolerance

The need of high fidelity in chromosomal replication imposes significant constrains on the

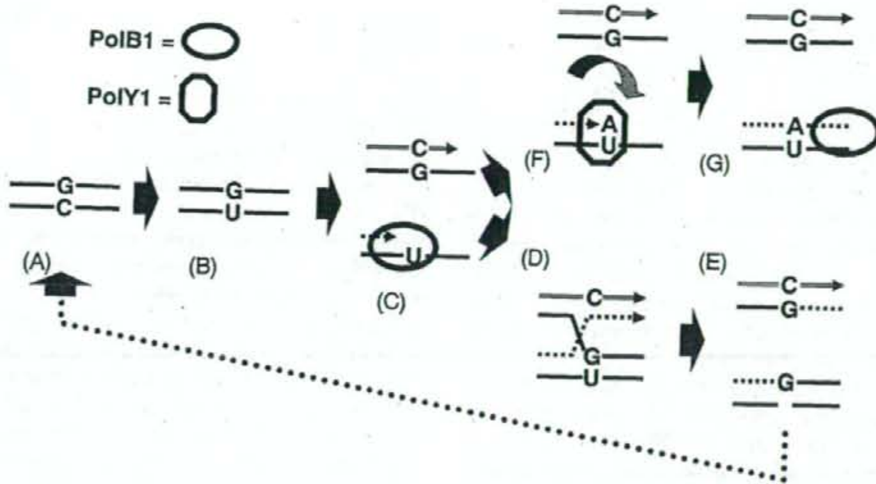


**Figure 7.2** Daughter strand gaps by stalling of Pol B1 at template U (or HX) are sealed by HR and/or translesion bypass by Pol Y1. Cytosine in DNA (A) is deaminated by heat, thereby generating G:U mismatch (B). When the strand containing U is copied by Pol B1 before the U is removed, Pol B1 may stop before U (C). ssDNA region downstream of the U can be sealed by HR with sister chromatid (D). UDG may remove the U (E) and provide a chance to regenerate normal G:C basepair (A). However, if Pol B1 is switched to Pol Y1, the Y-family DNA Pol may bypass U by incorporating adenine opposite template U (F). Pol B1 extends the primer strand containing adenine opposite U, thereby generating a mutagenic mispair in DNA (G).

conventional DNA replicases. Whenever they encounter an unrepaired lesion in the template DNA strand, the default replication apparatus would halt. However, most living organisms are equipped with alternative Pols that can tolerate or even correctly accommodate certain abnormalities at the primer-template junction and thus relieve replication blocks. This process termed TLS is faster and energetically less expensive than other DNA reparation mechanisms that do not leave damaged parental strand behind. Depending on DNA lesion context, TLS can result in progeny mutations and that is probably why it is predominantly used under adverse and stressful conditions when an adaptive change in the genotype may be desirable.

According to their primary amino acid sequence similarities signifying common evolutionary origin, Pols are currently classified into seven families, namely A, B, C, D, E, X and Y (Ito and Braithwaite, 1991; Lipps *et al.*, 2003; Ohmori *et al.*, 2001). The enzymes from Y-family are exclusively engaged in the TLS process. They specialize in copying DNA at stalled replication forks, which arise *in vivo* from mismatched or misaligned primer ends usually due to the presence of damage on DNA template. They lack 3′–5′ exonuclease (proofreading) activity and are poorly processive and *error-prone* when copying undamaged DNA resulting often in untargeted mutagenesis (Kim *et al.*, 2001; Kim *et al.*, 1997). The archaeal members of the Y-family Pols have served as great models for solving the three-dimensional structure of these unique enzymes (Ling *et al.*, 2001; Silvian *et al.*, 2001). Their catalytic core consists of finger, palm, thumb and little finger (or wriest or PAD) domains, but the finger and thumb domains are much smaller than in high-fidelity polymerases. An active site cleft consisted of five sequence motifs common to all known Y-family Pols can accommodate DNA and nucleotide triphosphates with relaxed geometric constraints, which result in higher rates of misincorporation and low processivity (Fiala *et al.*, 2007; Ling *et al.*, 2003; Ling *et al.*, 2004b; Zang *et al.*, 2006). Some of these enzymes, however, accurately bypass certain DNA lesions. Examples include accurate bypass across cis-syn thymine-dimer by human Pol eta (Trincao *et al.*, 2001; Washington *et al.*, 2003) and correct

incorporation of cytosine opposite template $N^2$-guanine minor groove adducts (Choi *et al.*, 2006; Jarosz *et al.*, 2006) such as the steroid hormone derived DNA adduct by human Pol kappa (Suzuki *et al.*, 2004). REV1 and Pol zeta can bypass an AP site efficiently *in vitro* (Haracska *et al.*, 2002; Masuda *et al.*, 2002). Although not essential for 'short-term' survival, the Y-family Pols may play important roles in 'long-term' survival (or evolution) by counteracting DNA adducts resulting from radiation and genotoxic byproducts of metabolism in an energetically efficient way (Yeiser *et al.*, 2002).

The archaeal Y-family Pols have been studied mostly as models of their eukaryotic counterparts due to the availability of the three-dimensional structures and feasibility to do co-crystallization studies. The complexes of Pol Y1 (Dpo4) from *S. solfataricus* with benzo[*a*]pyrene (Chandani and Loechler, 2007; Ling *et al.*, 2004b), *cis-syn* thymine dimer (Ling *et al.*, 2003), 8-oxo-G (Zang *et al.*, 2006) and an AP site (Ling *et al.*, 2004a) have been described in great details. The crystal structures also serve as models for the prediction and manipulation of structural properties of other homologous enzymes from low temperature organisms such as the genetic model *E. coli* (Lee *et al.*, 2006). The three dimensional structure of Pol Y1 (Dpo4) was even used in molecular dynamics simulation study to elucidate the catalytic mechanism and kinetics of DNA lesion bypass at atomic resolution (Chandani and Loechler, 2007; Wang *et al.*, 2006). However, it remains to be determined by *in vivo* genetic experiments what the physiological relevance of the Y-family Pols is in Archaea. A pioneering work related to this topic is the disruption of the *dpo4* gene of *S. solfataricus* (P. Blum, unpublished results). The resulting disruptants display enhanced expression of genes involved in protection of oxidative stress, suggesting the roles of the Y-family DNA Pol in the tolerance against oxidative DNA damage.

Apart from the recently heavily studied *Sulfolobus* enzymes, the Y-family Pol homologues are present in numerous but interestingly not all archaeal species. This contrasts with the other two kingdoms of life, i.e. Eukarya and Bacteria, where at least one Y-family member is present. Of the completely sequenced 38 archaeal genomes, there are only 15 having a classified Y-family Pol homologue as described in the current version of the NCBI GeneBank Protein Sequence Database. We have used the program ClustalX (Thompson *et al.*, 1997) to construct an alignment of all the 18 archaeal protein sequences and generated their phylogenetic tree (Fig. 7.3). From this tree we can distinguish four distinct evolutionary branches representing the homologues found in euryarchaeal halobacteria and methanomicrobia as well as Crenarchaeota. Interestingly, the methanomicrobia group splits into two subgroups. One group including *Methanosarcina acetivorans* and *hungate* possesses only one Y-family homologue while another group including *Methanosarcina burtoni*, *mazei* and *barkeri* harbour two. Although *Picrophilus torridus* is an Euryarchaeota, its Y-family homologue resembles those of Crenarchaeota probably because it originated by a horizontal gene transfer, which is known to occur between this species and Crenarchaea (Futterer *et al.*, 2004). Because the temperate deepwater planktonic Crenarchaeote 4B7 homologue (Beja *et al.*, 2002) is standing apart from all the branches, it may represent a common evolutionary predecessor. It is likely that at least one of the *Methanosarcina mazei* representatives originates by a lateral gene transfer since this species is known to harbour numerous ORFs whose closest homologues are present in the bacterial domain (Deppenmeier *et al.*, 2002) and its natural habitat is rice paddies, a niche shared by other bacterial species such as *Bacillus subtillis* known to harbour numerous Y-family Pol genes (Sung *et al.*, 2003). One of the related species *Methanosarcina acetivorans* with the largest known archaeal genome of 5.8 MB (Galagan *et al.*, 2002) has only one Y-family Pol.

It still remains an open question of how old the Y-family Pols are on evolutionary scale and whether they precede the chromosomal replicases belonging to the B-family. Given the absence of the homologous genes or pseudogenes in about half of the sequenced archaeal genomes, it is tempting to speculate that Y-family Pols are late comers to compensate for genotoxic effects of an aggressive energy metabolism or life under higher radiation exposures. It is conceivable that the tolerated DNA lesions would become gradu-

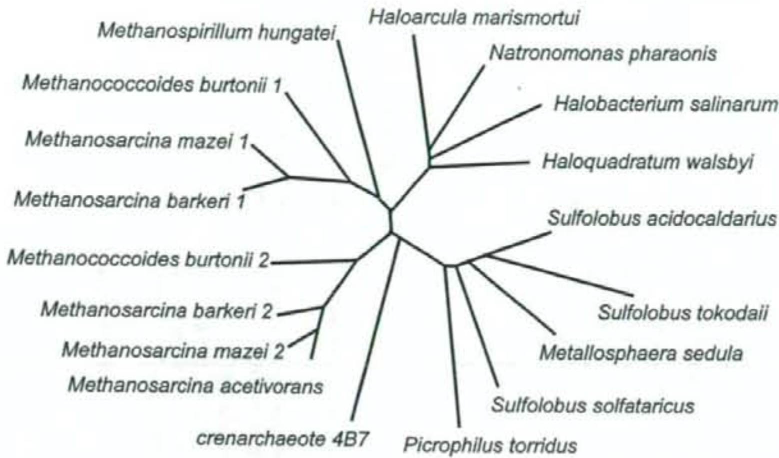


**Figure 7.3** Tree showing the phylogeny of Y-family of DNA Pols in archeal species listed in Table 7.2. The tree was drawn using the drawtree program of the PHYLIP phylogeny package based on multiple amino acid sequence alignment generated by the ClustalX program (Thompson *et al.*, 1997). The amino acid sequence alignment is available upon request.

ally diluted under fast growing conditions when symmetric cell division prevails, rather than they would accumulate like in unrepaired genomes of non-dividing cells. In fact, evidence is presented recently that a mechanism may be in place to reduce mutagenesis in non-dividing resting cells by restricting TLS Pol expression to the G2/M phase of cell cycle (Waters and Walker, 2006). Such strategy, i.e. replication first and correctness second, may represent a selective advantage over the opposite strategy, i.e. correctness first and replication second, given that the living environment for Archaea may change rapidly and the most common lesions are bypassed in an *error-free* manner. The obligate symbiont *Nanoarchaeum equitans*, which is deeply branched on an evolutionary tree and probably represents a novel archaeal kingdom, lacks a genuine Y-family homologue despite having an extensive repertoire of other DNA repair enzymes (Waters *et al.*, 2003). Surprisingly, a RecA/RadA recombinase homologue in this species contains a short 53 amino acids segment sharing homology to human Pol iota, a representative of human Y-family Pols (GeneBank accession # AAR39271). The deep water archaeal species like *Pyrococcus* are also lacking Y-family Pols probably because they are well shielded from the surface UV radiation and oxygen. As the major components of archaeal cell membranes are isoprenoid ether-linked glyceroldiethers or tetraethers (De Rosa and Gambacorta, 1988; Itoh *et al.*, 2001), these organisms may be exposed to lower amounts of genotoxic lipid peroxides, which are generated from fatty acid-derived membranes of Bacteria and Eukarya by oxidative metabolism (Chung *et al.*, 2003a). One of the major lipid peroxidation products, trans-4-hydroxy-2-nonenal (Feng *et al.*, 2003; Feng *et al.*, 2004), forms a $N^2$-dG adduct which is known to be efficiently bypassed in an *error-free* manner by the TLS Y-family Pols (Wolfle *et al.*, 2006; Lone *et al.*, 2007). In fact, at least the DinB homologues, which are the most widespread among all Y-family members, are likely to function during an *error-free* bypass of the $N^2$-dG minor groove bulky DNA adducts (Jarosz *et al.*, 2006). All the 18 currently sequenced Y-family DNA Pols from Archaea seem to be enzymatically active because they harbour the important catalytic acidic residues in motifs I and III, which bind to the metal $Mg^{2+}$ cofactor. Therefore TLS seems to play an important role in the physiology of certain archaeal species probably by letting them tolerate and become 'immune' against the genotoxic effects of specific DNA lesions resulting either from constant exposure to external radiation or internal reactive by-products of metabolism.

**Table 7.2** The archaeal species containing Y-family DNA Pol homologues

| Species | Classification | # | Habitat | Metabolism | Genome ref. |
|---|---|---|---|---|---|
| *Sulfolobus acidocaldarius* | *Crenarchaeota Thermoprotei* | 1 | Acid hot spring, USA Yellowstone Natl. Park, pH 2–3, 80°C | Aerobic, oxidation of elemental sulphur | (Chen *et al.*, 2005) |
| *Sulfolobus tokodaii* | *Crenarchaeota Thermoprotei* | 1 | Beppu hotsprings, Kyushu, pH 2–3, 80°C | Aerobic thermoacidophilic | (Kawarabayasi *et al.*, 2001) |
| *Sulfolobus solfataricus* | *Crenarchaeota Thermoprotei* | 1 | Continental solfatara, Naples, 87°C | Strictly aerobic, produces sulfuric acid | (She *et al.*, 2001) |
| *Crenarchaeote 4B7* | *Crenarchaeota Thermoprotei* | 1 | Temperate water plankton, 200 m deep near the coast of Oregon | | (Beja *et al.*, 2002) |
| *Metallosphaera sedula* | *Crenarchaeota; Thermoprotei* | 1 | | | Unpublished |
| *Picrophilus torridus* | *Euryarchaeota; Thermoplasmata* | 1 | Dry solfataric field in northern Japan, 65°C | Most thermoacidophilic organism, growing on organic substrates, extracellular pH 0, intracellular pH 4.6 | (Futterer *et al.*, 2004) |
| *Methanosarcina mazei* | *Euryarchaeota; Methanomicrobia* | 2 | Rice paddies | Fermenting acetate, methylamines and methanol to methane, carbon dioxide and ammonia, metabolically versatile methanogen | (Deppenmeier *et al.*, 2002) |
| *Methanosarcina barkeri* | *Euryarchaeota; Methanomicrobia* | 2 | Freshwater coastal lagoon west of Naples, Italy | Obligate $CO_2$ reduction with $H_2$, methyl reduction with $H_2$, aceticlastic fermentation of acetate or methylotrophic catabolism of methanol, methylated amines and dimethylsulphide, also grows nonmethanogenically with CO, fixes molecular nitrogen, metabolic diversity | (Maeder *et al.*, 2006) |
| *Methanococcoides burtonii* | *Euryarchaeota; Methanomicrobia* | 2 | anoxic and methanesaturated hypolimnion of Ace Lake (Antarctica), 1.7–29.5°C | strictly anaerobic, slightly halophilic, psychrophilic and methylotrophic | Unpublished |

| | | | | | |
|---|---|---|---|---|---|
| *Methanosarcina acetivorans* | *Euryarchaeota; Methanomicrobia* | 1 | | acetate-utilizing methanogen | (Galagan *et al.*, 2002) |
| *Methanospirillum hungatei* | *Euryarchaeota; Methanomicrobia* | 1 | Anaerobic, freeze-dried granular sludge obtained from an upflow anaerobic sludge bed reactor treating sugar beet wastewater | | Unpublished |
| *Halobacterium salinarum* | *Euryarchaeota; Halobacteria* | 1 | High salinity (10 times that of sea water) | Aerobic halophilic chemoorganotroph growing on the degradation products of less halophilic organisms as the salinity reaches near saturation | (Ng *et al.*, 2000) |
| *Haloquadratum walsbyi* | *Euryarchaeota; Halobacteria* | 1 | Spanish solar saltern, thalassic NaCl-saturated environments, sub-lethal conditions of an extremely high $MgCl_2$ concentration and high solar irradiance | Pigmented, synthesize sialic acid and poly-gamma-glutamate capsule, lives in near anoxic conditions due to the extremely high salinity | (Bolhuis *et al.*, 2006) |
| *Natronomonas pharaonis* | *Euryarchaeota; Halobacteria* | 1 | Salt-saturated lakes of pH 11 | Grows optimally at pH 8.5 in 3.5 M NaCl, lacks key enzymes of glycolytic pathways but has functional respiration and oxidative phosphorylation, lipoproteins make up one-third of the predicted secretome, harbours blue-light photoreceptor | (Falb *et al.*, 2005) |
| *Haloarcula marismortui* | *Euryarchaeota; Halobacteria* | 1 | Dead Sea | physiologically capable of exploiting diverse environments, large number of environmental response regulators | (Baliga *et al.*, 2004c) |

The results are based on the NCBI GeneBank protein sequence database as of March 2007. The number of homologues in each species is indidicated under # and references are shown for each corresponding genome where available. The information about habitat and metabolism is taken from the EMBL-EBI Integr8 database or directly from the specified literature.

## Future perspectives

Hyperthermophilic Archaea are interesting model organisms in terms of finding novel molecular strategies of how cells survive in harsh environments. As has been reviewed here, the replicative DNA Pols have unique molecular features that halt DNA synthesis when they encounter deaminated bases, i.e. U or HX, in template DNA. We expect that more unique mechanisms in DNA repair and damage tolerance will be revealed in Archeaa given the wealth of genome sequence information and ease of crystallization of proteins involved in the processing of DNA damage. However, there are several technical limitations that may hamper the progress of research in this area. The first problem may be the difficulty of cultivation. Many hyperthermophilic Archaea should be cultivated at extremely high temperatures and often require strictly anaerobic conditions and inorganic energy sources. Therefore, the actual genome stabilities such as mutation rates of most of hyperthermophilic Archaea are not experimentally determined. In addition, because of the difficulty to culture, information about chemical analysis of DNA lesions is very rare. We suppose that hyperthermophilic Archaea should have massively damaged bases in DNA such as U, 8-OH-G and AP sites. However, quantitative data concerning the DNA damage are scare and thus it is not sure that hyperthermophilic Archaea protect their genome stability mainly via chemical prevention such as using antioxidants to prevent oxidation of DNA or via efficient DNA repair and damage avoidance mechanisms discussed in this chapter. Collaboration with analytical chemists with experts of Archaea is essential to solve the problem. The second problem may be the lack of genetic approaches in particular gene disruption methods. Only a limited number of Archaea, such as *Halobacterium* species, *Sulfolobus solfataricus*, *Pyrococcus Kodakaraensis*, can be utilized for genetic studies. It is hard to evaluate the physiological roles of the mechanisms even if the proteins involved are crystallized and the fine three-dimensional structures are solved. In this regard, it is desirable to establish the gene knockout and knockin approaches to identify the roles of critical amino acids predicted by structural studies. Nevertheless, recent developments, such as measurement of mutation rates based on DNA instead of phenotypes of living organisms (Bielas and Loeb, 2005), high resolution mass spectrometry equipped with high performance liquid chromatography (Zhu *et al.*, 2003) and RNA-based gene knockdown (Collins and Cheng, 2005), will enable hyperthermophilic Archaea to be better model organisms to establish the relationships between genome integrity and a variety of environmental stresses.

### Acknowledgements

This work was supported by grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, the Ministry of Health, Labour and Welfare, Japan and the Japan Health Science Foundation. Part of the wok was financially supported by the Budget for Nuclear Research of the Ministry of Education, Culture, Sports, Science and Technology, based on the screening and counselling by the Atomic Energy Commission.

### References

Aboussekhra, A., Biggerstaff, M., Shivji, M. K., Vilpo, J. A., Moncollin, V., Podust, V. N., Protic, M., Hubscher, U., Egly, J. M., and Wood, R. D. (1995). Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Cell *80*, 859–868.

Akiba, T., Ishii, N., Rashid, N., Morikawa, M., Imanaka, T., and Harata, K. (2005). Structure of RadB recombinase from a hyperthermophilic archaeon, Thermococcus kodakaraensis KOD1: an implication for the formation of a near-7-fold helical assembly. Nucleic Acids Res. 33, 3412–3423.

Araujo, S. J. and Wood, R. D. (1999). Protein complexes in nucleotide excision repair. Mutat. Res. *435*, 23–33.

Aravind, L., Makarova, K. S., and Koonin, E. V. (2000). SURVEY AND SUMMARY: holliday junction resolvases and related nucleases: identification of new families, phyletic distribution and evolutionary trajectories. Nucleic Acids Res. *28*, 3417–3432.

Aravind, L., Walker, D. R., and Koonin, E. V. (1999). Conserved domains in DNA repair proteins and evolution of repair systems. Nucleic Acids Res. *27*, 1223–1242.

Asami, Y., Murakami, M., Shimizu, M., Pisani, F. M., Hayata, I., and Nohmi, T. (2006). Visualization of the interaction between archaeal DNA polymerase and uracil-containing DNA by atomic force microscopy. Genes Cells *11*, 3–11.

Bae, K. W., Baek, K. W., Cho, C. S., Hwang, K. Y., Kim, H. R., Sung, H. C., and Cho, Y. (1999). Expression, purification, characterization and crystallization of flap endonuclease-1 from *Methanococcus jannaschii*. Mol. Cells 9, 45–48.

Baliga, N. S., Bjork, S. J., Bonneau, R., Pan, M., Iloanusi, C., Kottemann, M. C., Hood, L., and DiRuggiero, J. (2004a). Systems level insights into the stress response to UV radiation in the halophilic archaeon Halobacterium NRC-1. Genome Res. *14*, 1025–1035.

Baliga, N. S., Bjork, S. J., Bonneau, R., Pan, M., Iloanusi, C., Kottemann, M. C., Hood, L., and DiRuggiero, J. (2004b). Systems level insights into the stress response to UV radiation in the halophilic archaeon Halobacterium NRC-1. Genome Res. *14*, 1025–1035.

Baliga, N. S., Bonneau, R., Facciotti, M. T., Pan, M., Glusman, G., Deutsch, E. W., Shannon, P., Chiu, Y., Weng, R. S., Gan, R. R., Hung, P., Date, S. V., Marcotte, E., Hood, L., and Ng, W. V. (2004c). Genome sequence of Haloarcula marismortui: a halophilic archaeon from the Dead Sea. Genome Res. *14*, 2221–2234.

Beja, O., Koonin, E. V., Aravind, L., Taylor, L. T., Seitz, H., Stein, J. L., Bensen, D. C., Feldman, R. A., Swanson, R. V., and DeLong, E. F. (2002). Comparative genomic analysis of archaeal genotypic variants in a single population and in two different oceanic provinces. Appl. Environ. Microbiol. *68*, 335–345.

Bielas, J. H. and Loeb, L. A. (2005). Quantification of random genomic mutations. Nat. Methods *2*, 285–290.

Bolhuis, H., Palm, P., Wende, A., Falb, M., Rampp, M., Rodriguez-Valera, F., Pfeiffer, F., and Oesterhelt, D. (2006). The genome of the square archaeon Haloquadratum walsbyi : life at the limits of water activity. BMC. Genomics 7, 169.

Bolt, E. L., Lloyd, R. G., and Sharples, G. J. (2001). Genetic analysis of an archaeal Holliday junction resolvase in Escherichia coli. J. Mol. Biol. *310*, 577–589.

Boudsocq, F., Kokoska, R. J., Plosky, B. S., Vaisman, A., Ling, H., Kunkel, T. A., Yang, W., and Woodgate, R. (2004). Investigating the role of the little finger domain of Y-family DNA polymerases in low fidelity synthesis and translesion replication. J. Biol. Chem. *279*, 32932–32940.

Cappelli, E., Taylor, R., Cevasco, M., Abbondandolo, A., Caldecott, K., and Frosina, G. (1997). Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. J. Biol. Chem. *272*, 23970–23975.

Chandani, S. and Loechler, E. L. (2007). Molecular modeling benzo[a]pyrene N2-dG adducts in the two overlapping active sites of the Y-family DNA polymerase Dpo4. J. Mol. Graph. Model. *25*, 658–670.

Chapados, B. R., Hosfield, D. J., Han, S., Qiu, J., Yelent, B., Shen, B., and Tainer, J. A. (2004). Structural basis for FEN-1 substrate specificity and PCNA-mediated activation in DNA replication and repair. Cell *116*, 39–50.

Chen, L., Brugger, K., Skovgaard, M., Redder, P., She, Q., Torarinsson, E., Greve, B., Awayez, M., Zibat, A., Klenk, H. P., and Garrett, R. A. (2005). The genome of Sulfolobus acidocaldarius, a model organism of the Crenarchaeota. J. Bacteriol. *187*, 4992–4999.

Choi, J. Y., Angel, K. C., and Guengerich, F. P. (2006). Translesion synthesis across bulky N2-alkyl guanine DNA adducts by human DNA polymerase kappa. J. Biol. Chem. *281*, 21062–21072.

Chung, F. L., Pan, J., Choudhury, S., Roy, R., Hu, W., and Tang, M. S. (2003a). Formation of trans-4-hydroxy-2-nonenal- and other enal-derived cyclic DNA adducts from omega-3 and omega-6 polyunsaturated fatty acids and their roles in DNA repair and human p53 gene mutation. Mutat. Res. *531*, 25–36.

Chung, J. H., Im, E. K., Park, H. Y., Kwon, J. H., Lee, S., Oh, J., Hwang, K. C., Lee, J. H., and Jang, Y. (2003b). A novel uracil-DNA glycosylase family related to the helix-hairpin-helix DNA glycosylase superfamily. Nucleic Acids Res. *31*, 2045–2055.

Chung, J. H., Suh, M. J., Park, Y. I., Tainer, J. A., and Han, Y. S. (2001). Repair activities of 8-oxoguanine DNA glycosylase from *Archaeoglobus fulgidus*, a hyperthermophilic archaeon. Mutat. Res. *486*, 99–111.

Cohen, G. N., Barbe, V., Flament, D., Galperin, M., Heilig, R., Lecompte, O., Poch, O., Prieur, D., Querellou, J., Ripp, R., Thierry, J. C., Van der, O. J., Weissenbach, J., Zivanovic, Y., and Forterre, P. (2003). An integrated analysis of the genome of the hyperthermophilic archaeon *Pyrococcus abyssi*. Mol. Microbiol. *47*, 1495–1512.

Collins, B. K., Tomanicek, S. J., Lyamicheva, N., Kaiser, M. W., and Mueser, T. C. (2004). A preliminary solubility screen used to improve crystallization trials: crystallization and preliminary X-ray structure determination of Aeropyrum pernix flap endonuclease-1. Acta Crystallogr. D. Biol. Crystallogr. *60*, 1674–1678.

Collins, R. E. and Cheng, X. (2005). Structural domains in RNAi. FEBS Lett. *579*, 5841–5849.

Connolly, B. A., Fogg, M. J., Shuttleworth, G., and Wilson, B. T. (2003). Uracil recognition by archaeal family B DNA polymerases. Biochem. Soc. Trans. *31*, 699–702.

De Felice, M., Medagli, B., Esposito, L., De Falco, M., Pucci, B., Rossi, M., Gruz, P., Nohmi, T., and Pisani, F. M. (2007). Biochemical evidence of a physical interaction between *Sulfolobus solfataricus* B-family and Y-family DNA polymerases. Extremophiles. *11*, 277–282.

De Rosa, M. and Gambacorta, A. (1988). The lipids of archaebacteria. Prog. Lipid Res. 27, 153–175.

Demple, B. and Harrison, L. (1994). Repair of oxidative damage to DNA: enzymology and biology. Annu. Rev. Biochem. *63*, 915–948.

Deppenmeier, U., Johann, A., Hartsch, T., Merkl, R., Schmitz, R. A., Martinez-Arias, R., Henne, A., Wiezer, A., Baumer, S., Jacobi, C., Bruggemann, H., Lienard, T., Christmann, A., Bomeke, M., Steckel, S., Bhattacharyya, A., Lykidis, A., Overbeek, R., Klenk, H. P., Gunsalus, R. P., Fritz, H. J., and Gottschalk, G. (2002). The genome of Methanosarcina mazei: evidence for lateral gene transfer between Bacteria and Archaea. J. Mol. Microbiol. Biotechnol. *4*, 453–461.

Dianov, G., Price, A., and Lindahl, T. (1992). Generation of single-nucleotide repair patches following excision of uracil residues from DNA. Mol. Cell Biol. *12*, 1605–1612.

Dionne, I., Nookala, R. K., Jackson, S. P., Doherty, A. J., and Bell, S. D. (2003). A heterotrimeric PCNA in the hyperthermophilic archaeon *Sulfolobus solfataricus*. Mol. Cell *11*, 275–282.

DiRuggiero, J., Santangelo, N., Nackerdien, Z., Ravel, J., and Robb, F. T. (1997). Repair of extensive ionizing-radiation DNA damage at 95 degrees C in the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Bacteriol. *179*, 4643–4645.

Eisen, J. A. (1998). A phylogenomic study of the MutS family of proteins. Nucleic Acids Res. *26*, 4291–4300.

Falb, M., Pfeiffer, F., Palm, P., Rodewald, K., Hickmann, V., Tittor, J., and Oesterhelt, D. (2005). Living with two extremes: conclusions from the genome sequence of Natronomonas pharaonis. Genome Res. *15*, 1336-1343.

Fan, L., Arvai, A. S., Cooper, P. K., Iwai, S., Hanaoka, F., and Tainer, J. A. (2006). Conserved XPB core structure and motifs for DNA unwinding: implications for pathway selection of transcription or excision repair. Mol. Cell 22, 27–37.

Feng, Z., Hu, W., Amin, S., and Tang, M. S. (2003). Mutational spectrum and genotoxicity of the major lipid peroxidation product, trans-4-hydroxy-2-nonenal, induced DNA adducts in nucleotide excision repair-proficient and -deficient human cells. Biochemistry *42*, 7848–7854.

Feng, Z., Hu, W., and Tang, M. S. (2004). Trans-4-hydroxy-2-nonenal inhibits nucleotide excision repair in human cells: a possible mechanism for lipid peroxidation-induced carcinogenesis. Proc. Natl. Acad. Sci. U.S. A *101*, 8598–8602.

Fiala, K. A., Brown, J. A., Ling, H., Kshetry, A. K., Zhang, J., Taylor, J. S., Yang, W., and Suo, Z. (2007). Mechanism of template-independent nucleotide incorporation catalyzed by a template-dependent DNA polymerase. J. Mol. Biol. *365*, 590–602.

Fitz-Gibbon, S. T., Ladner, H., Kim, U. J., Stetter, K. O., Simon, M. I., and Miller, J. H. (2002). Genome sequence of the hyperthermophilic crenarchaeon Pyrobaculum aerophilum. Proc. Natl. Acad. Sci. U.S. A 99, 984–989.

Fogg, M. J., Pearl, L. H., and Connolly, B. A. (2002). Structural basis for uracil recognition by archaeal family B DNA polymerases. Nat. Struct. Biol. *9*, 922–927.

Fondufe-Mittendorf, Y. N., Harer, C., Kramer, W., and Fritz, H. J. (2002). Two amino acid replacements change the substrate preference of DNA mismatch glycosylase Mig.MthI from T/G to A/G. Nucleic Acids Res. *30*, 614–621.

Frosina, G., Fortini, P., Rossi, O., Carrozzino, F., Raspaglio, G., Cox, L. S., Lane, D. P., Abbondandolo, A., and Dogliotti, E. (1996). Two pathways for base excision repair in mammalian cells. J. Biol. Chem. *271*, 9573–9578.

Fujihashi, M., Numoto, N., Kobayashi, Y., Mizushima, A., Tsujimura, M., Nakamura, A., Kawarabayasi, Y., and Miki, K. (2007). Crystal structure of archaeal photolyase from *Sulfolobus tokodaii* with two FAD molecules: implication of a novel light-harvesting cofactor. J. Mol. Biol. *365*, 903–910.

Fujikane, R., Komori, K., Shinagawa, H., and Ishino, Y. (2005). Identification of a novel helicase activity unwinding branched DNAs from the hyperthermophilic archaeon, *Pyrococcus furiosus*. J. Biol. Chem. *280*, 12351–12358.

Futterer, O., Angelov, A., Liesegang, H., Gottschalk, G., Schleper, C., Schepers, B., Dock, C., Antranikian, G., and Liebl, W. (2004). Genome sequence of Picrophilus torridus and its implications for life around pH 0. Proc. Natl. Acad. Sci. U.S. A *101*, 9091–9096.

Gadsden, M. H., McIntosh, E. M., Game, J. C., Wilson, P. J., and Haynes, R. H. (1993). dUTP pyrophosphatase is an essential enzyme in Saccharomyces cerevisiae. EMBO J. *12*, 4425–4431.

Galagan, J. E., Nusbaum, C., Roy, A., Endrizzi, M. G., Macdonald, P., FitzHugh, W., Calvo, S., Engels, R., Smirnov, S., Atnoor, D., Brown, A., Allen, N., Naylor, J., Stange-Thomann, N., DeArellano, K., Johnson, R., Linton, L., McEwan, P., McKernan, K., Talamas, J., Tirrell, A., Ye, W., Zimmer, A., Barber, R. D., Cann, I., Graham, D. E., Grahame, D. A., Guss, A. M., Hedderich, R., Ingram-Smith, C., Kuettner, H. C., Krzycki, J. A., Leigh, J. A., Li, W., Liu, J., Mukhopadhyay, B., Reeve, J. N., Smith, K., Springer, T. A., Umayam, L. A., White, O., White, R. H., Conway, d. M., Ferry, J. G., Jarrell, K. F., Jing, H., Macario, A. J., Paulsen, I., Pritchett, M., Sowers, K. R., Swanson, R. V., Zinder, S. H., Lander, E., Metcalf, W. W., and Birren, B. (2002). The genome of M. acetivorans reveals extensive metabolic and physiological diversity. Genome Res. *12*, 532–542.

Gao, Y. G., Su, S. Y., Robinson, H., Padmanabhan, S., Lim, L., McCrary, B. S., Edmondson, S. P., Shriver, J. W., and Wang, A. H. (1998). The crystal structure of the hyperthermophile chromosomal protein Sso7d bound to DNA. Nat. Struct. Biol. 5, 782–786.

Gary, R., Kim, K., Cornelius, H. L., Park, M. S., and Matsumoto, Y. (1999). Proliferating cell nuclear antigen facilitates excision in long-patch base excision repair. J. Biol. Chem. *274*, 4354–4363.

Gogos, A. and Clarke, N. D. (1999). Characterization of an 8-oxoguanine DNA glycosylase from *Methanococcus jannaschii*. J. Biol. Chem. *274*, 30447–30450.

Greagg, M. A., Fogg, M. J., Panayotou, G., Evans, S. J., Connolly, B. A., and Pearl, L. H. (1999). A read-ahead function in archaeal DNA polymerases detects promutagenic template-strand uracil. Proc. Natl. Acad. Sci. U.S. A *96*, 9045–9050.

Grogan, D. W. (2000). The question of DNA repair in hyperthermophilic Archaea. Trends Microbiol. *8*, 180–185.

Grogan, D. W., Carver, G. T., and Drake, J. W. (2001). Genetic fidelity under harsh conditions: analysis of spontaneous mutation in the thermoacidophilic archaeon Sulfolobus acidocaldarius. Proc. Natl. Acad. Sci. U.S. A *98*, 7928–7933.

Gruz, P., Pisani, F. M., Shimizu, M., Yamada, M., Hayashi, I., Morikawa, K., and Nohmi, T. (2001). Synthetic activity of Sso DNA polymerase Y1, an archaeal DinB-like DNA polymerase, is stimulated by processivity factors proliferating cell nuclear an-

tigen and replication factor C.J. Biol. Chem. *276*, 47394–47401.

Gruz, P., Shimizu, M., Pisani, F. M., De Felice, M., Kanke, Y., and Nohmi, T. (2003). Processing of DNA lesions by archaeal DNA polymerases from *Sulfolobus solfataricus*. Nucleic Acids Res. *31*, 4024–4030.

Haracska, L., Prakash, S., and Prakash, L. (2002). Yeast Rev1 protein is a G template-specific DNA polymerase. J. Biol. Chem. *277*, 15546-15551.

Hayashi, I., Morikawa, K., and Ishino, Y. (1999). Specific interaction between DNA polymerase II (PolD) and RadB, a Rad51/Dmc1 homolog, in *Pyrococcus furiosus*. Nucleic Acids Res. 27, 4695–4702.

Hinks, J. A., Evans, M. C., De Miguel, Y., Sartori, A. A., Jiricny, J., and Pearl, L. H. (2002). An iron–sulfur cluster in the family 4 uracil-DNA glycosylases. J. Biol. Chem. *277*, 16936-16940.

Hogrefe, H. H., Hansen, C. J., Scott, B. R., and Nielson, K. B. (2002). Archaeal dUTPase enhances PCR amplifications with archaeal DNA polymerases by preventing dUTP incorporation. Proc. Natl. Acad. Sci. U.S. A *99*, 596–601.

Hopfner, K. P., Karcher, A., Craig, L., Woo, T. T., Carney, J. P., and Tainer, J. A. (2001). Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. Cell *105*, 473–485.

Hopfner, K. P., Karcher, A., Shin, D. S., Craig, L., Arthur, L. M., Carney, J. P., and Tainer, J. A. (2000). Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. Cell *101*, 789–800.

Horst, J. P. and Fritz, H. J. (1996). Counteracting the mutagenic effect of hydrolytic deamination of DNA 5-methylcytosine residues at high temperature: DNA mismatch N-glycosylase Mig.Mth of the thermophilic archaeon *Methanobacterium thermoautotrophicum* THF. EMBO J. *15*, 5459–5469.

Hosfield, D. J., Mol, C. D., Shen, B., and Tainer, J. A. (1998). Structure of the DNA repair and replication endonuclease and exonuclease FEN-1: coupling DNA and PCNA binding to FEN-1 activity. Cell *95*, 135–146.

Hwang, K. Y., Baek, K., Kim, H. Y., and Cho, Y. (1998). The crystal structure of flap endonuclease-1 from *Methanococcus jannaschii*. Nat. Struct. Biol. *5*, 707–713.

Ito, J. and Braithwaite, D. K. (1991). Compilation and alignment of DNA polymerase sequences. Nucleic Acids Res. *19*, 4045–4057.

Itoh, Y. H., Sugai, A., Uda, I., and Itoh, T. (2001). The evolution of lipids. Adv. Space Res. *28*, 719–724.

Jarosz, D. F., Godoy, V. G., Delaney, J. C., Essigmann, J. M., and Walker, G. C. (2006). A single amino acid governs enhanced activity of DinB DNA polymerases on damaged templates. Nature *439*, 225–228.

Kadyrov, F. A., Dzantiev, L., Constantin, N., and Modrich, P. (2006). Endonucleolytic function of MutLalpha in human mismatch repair. Cell *126*, 297–308.

Kaiser, M. W., Lyamicheva, N., Ma, W., Miller, C., Neri, B., Fors, L., and Lyamichev, V. I. (1999). A comparison of eubacterial and archaeal structure-specific 5′-exonucleases. J. Biol. Chem. *274*, 21387–21394.

Kanugula, S., Pauly, G. T., Moschel, R. C., and Pegg, A. E. (2005). A bifunctional DNA repair protein from *Ferroplasma acidarmanus* exhibits O6-alkylguanine-DNA alkyltransferase and endonuclease V activities. Proc. Natl. Acad. Sci. U.S. A *102*, 3617–3622.

Kanugula, S. and Pegg, A. E. (2003). Alkylation damage repair protein O6-alkylguanine-DNA alkyltransferase from the hyperthermophiles Aquifex aeolicus and *Archaeoglobus fulgidus*. Biochem. J. 375, 449–455.

Kawarabayasi, Y., Hino, Y., Horikawa, H., Jin-no, K., Takahashi, M., Sekine, M., Baba, S., Ankai, A., Kosugi, H., Hosoyama, A., Fukui, S., Nagai, Y., Nishijima, K., Otsuka, R., Nakazawa, H., Takamiya, M., Kato, Y., Yoshizawa, T., Tanaka, T., Kudoh, Y., Yamazaki, J., Kushida, N., Oguchi, A., Aoki, K., Masuda, S., Yanagii, M., Nishimura, M., Yamagishi, A., Oshima, T., and Kikuchi, H. (2001). Complete genome sequence of an aerobic thermoacidophilic crenarchaeon, *Sulfolobus tokodaii* strain7. DNA Res. *8*, 123–140.

Kawarabayasi, Y., Sawada, M., Horikawa, H., Haikawa, Y., Hino, Y., Yamamoto, S., Sekine, M., Baba, S., Kosugi, H., Hosoyama, A., Nagai, Y., Sakai, M., Ogura, K., Otsuka, R., Nakazawa, H., Takamiya, M., Ohfuku, Y., Funahashi, T., Tanaka, T., Kudoh, Y., Yamazaki, J., Kushida, N., Oguchi, A., Aoki, K., and Kikuchi, H. (1998). Complete sequence and gene organization of the genome of a hyper-thermophilic archaebacterium, *Pyrococcus horikoshii* OT3. DNA Res. *5*, 55–76.

Kawashima, T., Amano, N., Koike, H., Makino, S., Higuchi, S., Kawashima-Ohya, Y., Watanabe, K., Yamazaki, M., Kanehori, K., Kawamoto, T., Nunoshiba, T., Yamamoto, Y., Aramaki, H., Makino, K., and Suzuki, M. (2000). Archaeal adaptation to higher temperatures revealed by genomic sequence of Thermoplasma volcanium. Proc. Natl. Acad. Sci. USA *97*, 14257–14262.

Kelner, A. (1949). Effect of Visible Light on the Recovery of Streptomyces Griseus Conidia from Ultra-violet Irradiation Injury. Proc. Natl. Acad. Sci. U.S. A *35*, 73–79.

Kiener, A., Husain, I., Sancar, A., and Walsh, C. (1989). Purification and properties of *Methanobacterium thermoautotrophicum* DNA photolyase. J. Biol. Chem. *264*, 13880–13887.

Kim, K., Biade, S., and Matsumoto, Y. (1998). Involvement of flap endonuclease 1 in base excision DNA repair. J. Biol. Chem. *273*, 8842–8848.

Kim, S. R., Maenhaut-Michel, G., Yamada, M., Yamamoto, Y., Matsui, K., Sofuni, T., Nohmi, T., and Ohmori, H. (1997). Multiple pathways for SOS-induced mutagenesis in Escherichia coli: an overexpression of dinB/dinP results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA. Proc. Natl. Acad. Sci. U.S. A *94*, 13792–13797.

Kim, S. R., Matsui, K., Yamada, M., Gruz, P., and Nohmi, T. (2001). Roles of chromosomal and episomal dinB genes encoding DNA pol IV in targeted and untar-

geted mutagenesis in Escherichia coli. Mol. Genet. Genomics 266, 207–215.

Klungland, A. and Lindahl, T. (1997). Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). EMBO J. 16, 3341–3348.

Komori, K. and Ishino, Y. (2001). Replication protein A in *Pyrococcus furiosus* is involved in homologous DNA recombination. J. Biol. Chem. 276, 25654–25660.

Komori, K., Miyata, T., Daiyasu, H., Toh, H., Shinagawa, H., and Ishino, Y. (2000a). Domain analysis of an archaeal RadA protein for the strand exchange activity. J. Biol. Chem. 275, 33791–33797.

Komori, K., Miyata, T., DiRuggiero, J., Holley-Shanks, R., Hayashi, I., Cann, I. K., Mayanagi, K., Shinagawa, H., and Ishino, Y. (2000b). Both RadA and RadB are involved in homologous recombination in *Pyrococcus furiosus*. J. Biol. Chem. 275, 33782–33790.

Komori, K., Sakae, S., Daiyasu, H., Toh, H., Morikawa, K., Shinagawa, H., and Ishino, Y. (2000c). Mutational analysis of the *Pyrococcus furiosus* holliday junction resolvase hjc revealed functionally important residues for dimer formation, junction DNA binding, and cleavage activities. J. Biol. Chem. 275, 40385–40391.

Komori, K., Sakae, S., Shinagawa, H., Morikawa, K., and Ishino, Y. (1999). A Holliday junction resolvase from *Pyrococcus furiosus*: functional similarity to Escherichia coli RuvC provides evidence for conserved mechanism of homologous recombination in Bacteria, Eukarya, and Archaea. Proc. Natl. Acad. Sci. U.S. A 96, 8873–8878.

Koulis, A., Cowan, D. A., Pearl, L. H., and Savva, R. (1996). Uracil-DNA glycosylase activities in hyperthermophilic micro-organisms. FEMS Microbiol. Lett. 143, 267–271.

Kvaratskhelia, M., Wardleworth, B. N., Norman, D. G., and White, M. F. (2000). A conserved nuclease domain in the archaeal Holliday junction resolving enzyme Hjc. J. Biol. Chem. 275, 25540–25546.

Kvaratskhelia, M. and White, M. F. (2000a). An archaeal Holliday junction resolving enzyme from *Sulfolobus solfataricus* exhibits unique properties. J. Mol. Biol. 295, 193–202.

Kvaratskhelia, M. and White, M. F. (2000b). Two Holliday junction resolving enzymes in *Sulfolobus solfataricus*. J. Mol. Biol. 297, 923–932.

Lasken, R. S., Schuster, D. M., and Rashtchian, A. (1996). Archaebacterial DNA polymerases tightly bind uracil-containing DNA. J. Biol. Chem. 271, 17692–17696.

Leclere, M. M., Nishioka, M., Yuasa, T., Fujiwara, S., Takagi, M., and Imanaka, T. (1998). The O6-methylguanine-DNA methyltransferase from the hyperthermophilic archaeon Pyrococcus sp. KOD1: a thermostable repair enzyme. Mol. Gen. Genet. 258, 69–77.

Lee, C. H., Chandani, S., and Loechler, E. L. (2006). Homology modeling of four Y-family, lesion-bypass DNA polymerases: the case that E. coli Pol IV and human Pol kappa are orthologs, and E. coli Pol V and human Pol eta are orthologs. J. Mol. Graph. Model. 25, 87–102.

Lilley, D. M. and White, M. F. (2000). Resolving the relationships of resolving enzymes. Proc. Natl. Acad. Sci. U.S. A 97, 9351–9353.

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. Nature 362, 709–715.

Lindahl, T. and Nyberg, B. (1974). Heat-induced deamination of cytosine residues in deoxyribonucleic acid. Biochemistry 13, 3405–3410.

Lindahl, T., Sedgwick, B., Sekiguchi, M., and Nakabeppu, Y. (1988). Regulation and expression of the adaptive response to alkylating agents. Annu. Rev. Biochem. 57, 133–157.

Lindahl, T. and Wood, R. D. (1999). Quality control by DNA repair. Science 286, 1897–1905.

Ling, H., Boudsocq, F., Plosky, B. S., Woodgate, R., and Yang, W. (2003). Replication of a cis-syn thymine dimer at atomic resolution. Nature 424, 1083–1087.

Ling, H., Boudsocq, F., Woodgate, R., and Yang, W. (2001). Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. Cell 107, 91–102.

Ling, H., Boudsocq, F., Woodgate, R., and Yang, W. (2004a). Snapshots of replication through an abasic lesion; structural basis for base substitutions and frameshifts. Mol. Cell 13, 751–762.

Ling, H., Sayer, J. M., Plosky, B. S., Yagi, H., Boudsocq, F., Woodgate, R., Jerina, D. M., and Yang, W. (2004b). Crystal structure of a benzo[a]pyrene diol epoxide adduct in a ternary complex with a DNA polymerase. Proc. Natl. Acad. Sci. U.S. A 101, 2265–2269.

Lipps, G., Rother, S., Hart, C., and Krauss, G. (2003). A novel type of replicative enzyme harbouring ATPase, primase and DNA polymerase activity. EMBO J. 22, 2516-2525.

Lone, S., Townson, S. A., Uljon, S. N., Johnson, R. E., Brahma, A., Nair, D. T., Prakash, S., Prakash, L., and Aggarwal, A. K. (2007). Human DNA polymerase kappa encircles DNA: implications for mismatch extension and lesion bypass. Mol. Cell 25, 601–614.

Lu, A. L., Li, X., Gu, Y., Wright, P. M., and Chang, D. Y. (2001). Repair of oxidative DNA damage: mechanisms and functions. Cell Biochem. Biophys. 35, 141–170.

Maeder, D. L., Anderson, I., Brettin, T. S., Bruce, D. C., Gilna, P., Han, C. S., Lapidus, A., Metcalf, W. W., Saunders, E., Tapia, R., and Sowers, K. R. (2006). The Methanosarcina barkeri genome: comparative analysis with *Methanosarcina acetivorans* and Methanosarcina mazei reveals extensive rearrangement within methanosarcinal genomes. J. Bacteriol. 188, 7922–7931.

Margison, G. P., Povey, A. C., Kaina, B., and Santibanez Koref, M. F. (2003). Variability and regulation of O6-alkylguanine-DNA alkyltransferase. Carcinogenesis 24, 625–635.

Marti, T. M., Kunz, C., and Fleck, O. (2002). DNA mismatch repair and mutation avoidance pathways. J. Cell Physiol 191, 28–41.

Masuda, Y., Takahashi, M., Fukuda, S., Sumii, M., and Kamiya, K. (2002). Mechanisms of dCMP transferase reactions catalyzed by mouse Rev1 protein. J. Biol. Chem. 277, 3040–3046.

Matsui, E., Kawasaki, S., Ishida, H., Ishikawa, K., Kosugi, Y., Kikuchi, H., Kawarabayashi, Y., and Matsui, I.

(1999). Thermostable flap endonuclease from the archaeon, *Pyrococcus horikoshii*, cleaves the replication fork-like structure endo/exonucleolytically. J. Biol. Chem. *274*, 18297–18309.

Matsui, E., Musti, K. V., Abe, J., Yamasaki, K., Matsui, I., and Harata, K. (2002). Molecular structure and novel DNA binding sites located in loops of flap endonuclease-1 from *Pyrococcus horikoshii*. J. Biol. Chem. *277*, 37840–37847.

McCready, S. and Marcello, L. (2003). Repair of UV damage in Halobacterium salinarum. Biochem. Soc. Trans. *31*, 694–698.

McGlynn, P. and Lloyd, R. G. (2000). Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. Cell *101*, 35–45.

Modrich, P. and Lahue, R. (1996). Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. *65*, 101–133.

Moncalian, G., Lengsfeld, B., Bhaskara, V., Hopfner, K. P., Karcher, A., Alden, E., Tainer, J. A., and Paull, T. T. (2004). The rad50 signature motif: essential to ATP binding and biological function. J. Mol. Biol. *335*, 937–951.

Nakamura, J., Walker, V. E., Upton, P. B., Chiang, S. Y., Kow, Y. W., and Swenberg, J. A. (1998). Highly sensitive apurinic/apyrimidinic site assay can detect spontaneous and chemically induced depurination under physiological conditions. Cancer Res. *58*, 222–225.

Newman, M., Murray-Rust, J., Lally, J., Rudolf, J., Fadden, A., Knowles, P. P., White, M. F., and McDonald, N. Q. (2005). Structure of an XPF endonuclease with and without DNA suggests a model for substrate recognition. EMBO J. *24*, 895–905.

Ng, W. V., Kennedy, S. P., Mahairas, G. G., Berquist, B., Pan, M., Shukla, H. D., Lasky, S. R., Baliga, N. S., Thorsson, V., Sbrogna, J., Swartzell, S., Weir, D., Hall, J., Dahl, T. A., Welti, R., Goo, Y. A., Leithauser, B., Keller, K., Cruz, R., Danson, M. J., Hough, D. W., Maddocks, D. G., Jablonski, P. E., Krebs, M. P., Angevine, C. M., Dale, H., Isenbarger, T. A., Peck, R. F., Pohlschroder, M., Spudich, J. L., Jung, K. W., Alam, M., Freitas, T., Hou, S., Daniels, C. J., Dennis, P. P., Omer, A. D., Ebhardt, H., Lowe, T. M., Liang, P., Riley, M., Hood, L., and DasSarma, S. (2000). Genome sequence of Halobacterium species NRC-1. Proc. Natl. Acad. Sci. U.S. A *97*, 12176-12181.

Nishino, T., Komori, K., Tsuchiya, D., Ishino, Y., and Morikawa, K. (2001). Crystal structure of the archaeal holliday junction resolvase Hjc and implications for DNA recognition. Structure. *9*, 197–204.

Nishino, T., Komori, K., Tsuchiya, D., Ishino, Y., and Morikawa, K. (2005). Crystal structure and functional implications of *Pyrococcus furiosus* hef helicase domain involved in branched DNA processing. Structure. *13*, 143–153.

Nohmi, T. (2006). Environmental stress and lesion-bypass DNA polymerases. Annu. Rev. Microbiol. *60*, 231–253.

Ogrunc, M., Becker, D. F., Ragsdale, S. W., and Sancar, A. (1998). Nucleotide excision repair in the third kingdom. J. Bacteriol. *180*, 5796–5798.

Ohmori, H., Friedberg, E. C., Fuchs, R. P., Goodman, M. F., Hanaoka, F., Hinkle, D., Kunkel, T. A., Lawrence, C. W., Livneh, Z., Nohmi, T., Prakash, L., Prakash, S., Todo, T., Walker, G. C., Wang, Z., and Woodgate, R. (2001). The Y-family of DNA polymerases. Mol. Cell *8*, 7–8.

Olsen, G. J. and Woese, C. R. (1996). Lessons from an Archaeal genome: what are we learning from *Methanococcus jannaschii*? Trends Genet. *12*, 377–379.

Pascucci, B., Stucki, M., Jonsson, Z. O., Dogliotti, E., and Hubscher, U. (1999). Long patch base excision repair with purified human proteins. DNA ligase I as patch size mediator for DNA polymerases delta and epsilon. J. Biol. Chem. *274*, 33696-33702.

Pearl, L. H. (2000). Structure and function in the uracil-DNA glycosylase superfamily. Mutat. Res. *460*, 165–181.

Percival, K. J., Klein, M. B., and Burgers, P. M. (1989). Molecular cloning and primary structure of the uracil-DNA-glycosylase gene from Saccharomyces cerevisiae. J. Biol. Chem. *264*, 2593–2598.

Petit, C. and Sancar, A. (1999). Nucleotide excision repair: from E. coli to man. Biochimie *81*, 15–25.

Podlutsky, A. J., Dianova, I. I., Podust, V. N., Bohr, V. A., and Dianov, G. L. (2001). Human DNA polymerase beta initiates DNA synthesis during long-patch repair of reduced AP sites in DNA. EMBO J. *20*, 1477–1482.

Prakash, S., Johnson, R. E., and Prakash, L. (2005). Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. Annu. Rev. Biochem. *74*, 317–353.

Qian, X., Wu, Y., He, Y., and Luo, Y. (2005). Crystal structure of Methanococcus voltae RadA in complex with ADP: hydrolysis-induced conformational change. Biochemistry *44*, 13753–13761.

Rao, H. G., Rosenfeld, A., and Wetmur, J. G. (1998). *Methanococcus jannaschii* flap endonuclease: expression, purification, and substrate requirements. J. Bacteriol. *180*, 5406–5412.

Rashid, N., Morikawa, M., and Imanaka, T. (1996). A RecA/RAD51 homologue from a hyperthermophilic archaeon retains the major RecA domain only. Mol. Gen. Genet. *253*, 397–400.

Reich, C. I., McNeil, L. K., Brace, J. L., Brucker, J. K., and Olsen, G. J. (2001). Archaeal RecA homologues: different response to DNA-damaging agents in mesophilic and thermophilic Archaea. Extremophiles. *5*, 265–275.

Robb, F. T., Maeder, D. L., Brown, J. R., DiRuggiero, J., Stump, M. D., Yeh, R. K., Weiss, R. B., and Dunn, D. M. (2001). Genomic sequence of hyperthermophile, *Pyrococcus furiosus*: implications for physiology and enzymology. Methods Enzymol. *330*, 134–157.

Roberts, J. A., Bell, S. D., and White, M. F. (2003). An archaeal XPF repair endonuclease dependent on a heterotrimeric PCNA. Mol. Microbiol. *48*, 361–371.

Robinson, H., Gao, Y. G., McCrary, B. S., Edmondson, S. P., Shriver, J. W., and Wang, A. H. (1998). The hyperthermophile chromosomal protein Sac7d sharply kinks DNA. Nature *392*, 202–205.

Ruepp, A., Graml, W., Santos-Martinez, M. L., Koretke, K. K., Volker, C., Mewes, H. W., Frishman, D., Stocker, S., Lupas, A. N., and Baumeister, W. (2000). The genome sequence of the thermoacidophilic scavenger Thermoplasma acidophilum. Nature *407*, 508–513.

Salerno, V., Napoli, A., White, M. F., Rossi, M., and Ciaramella, M. (2003). Transcriptional response to DNA damage in the archaeon *Sulfolobus solfataricus*. Nucleic Acids Res. *31*, 6127–6138.

Sancar, A. (1994). Structure and function of DNA photolyase. Biochemistry *33*, 2–9.

Sancar, A. (1996). DNA excision repair. Annu. Rev. Biochem. *65*, 43–81.

Sancar, G. B. (1990). DNA photolyases: physical properties, action mechanism, and roles in dark repair. Mutat. Res. *236*, 147–160.

Sandigursky, M. and Franklin, W. A. (2000). Uracil-DNA glycosylase in the extreme thermophile *Archaeoglobus fulgidus*. J. Biol. Chem. *275*, 19146-19149.

Sandler, S. J., Hugenholtz, P., Schleper, C., DeLong, E. F., Pace, N. R., and Clark, A. J. (1999). Diversity of radA genes from cultured and uncultured Archaea: comparative analysis of putative RadA proteins and their use as a phylogenetic marker. J. Bacteriol. *181*, 907–915.

Sandler, S. J., Satin, L. H., Samra, H. S., and Clark, A. J. (1996). recA-like genes from three archaean species with putative protein products similar to Rad51 and Dmc1 proteins of the yeast Saccharomyces cerevisiae. Nucleic Acids Res. *24*, 2125–2132.

Sartori, A. A., Fitz-Gibbon, S., Yang, H., Miller, J. H., and Jiricny, J. (2002). A novel uracil-DNA glycosylase with broad substrate specificity and an unusual active site. EMBO J. *21*, 3182–3191.

Sartori, A. A. and Jiricny, J. (2003). Enzymology of base excision repair in the hyperthermophilic archaeon Pyrobaculum aerophilum. J. Biol. Chem. *278*, 24563–24576.

Savino, C., Federici, L., Johnson, K. A., Vallone, B., Nastopoulos, V., Rossi, M., Pisani, F. M., and Tsernoglou, D. (2004). Insights into DNA replication: the crystal structure of DNA polymerase B1 from the archaeon *Sulfolobus solfataricus*. Structure. *12*, 2001–2008.

Savva, R., McAuley-Hecht, K., Brown, T., and Pearl, L. (1995). The structural basis of specific base-excision repair by uracil-DNA glycosylase. Nature *373*, 487–493.

Sayers, J. R. and Artymiuk, P. J. (1998). Flexible loops and helical arches. Nat. Struct. Biol. *5*, 668–670.

Sedgwick, B. (2004). Repairing DNA-methylation damage. Nat. Rev. Mol. Cell Biol. *5*, 148–157.

Seigneur, M., Bidnenko, V., Ehrlich, S. D., and Michel, B. (1998). RuvAB acts at arrested replication forks. Cell *95*, 419–430.

Seitz, E. M., Brockman, J. P., Sandler, S. J., Clark, A. J., and Kowalczykowski, S. C. (1998). RadA protein is an archaeal RecA protein homolog that catalyzes DNA strand exchange. Genes Dev. *12*, 1248–1253.

She, Q., Singh, R. K., Confalonieri, F., Zivanovic, Y., Allard, G., Awayez, M. J., Chan-Weiher, C. C., Clausen, I. G., Curtis, B. A., De Moors, A., Erauso, G., Fletcher, C., Gordon, P. M., Heikamp-de Jong, I., Jeffries, A. C., Kozera, C. J., Medina, N., Peng, X., Thi-Ngoc, H. P., Redder, P., Schenk, M. E., Theriault, C., Tolstrup, N., Charlebois, R. L., Doolittle, W. F., Duguet, M., Gaasterland, T., Garrett, R. A., Ragan, M. A., Sensen, C. W., and Van der, O. J. (2001). The complete genome of the crenarchaeon *Sulfolobus solfataricus* P2. Proc. Natl. Acad. Sci. U.S. A *98*, 7835–7840.

Shuttleworth, G., Fogg, M. J., Kurpiewski, M. R., Jen-Jacobson, L., and Connolly, B. A. (2004). Recognition of the pro-mutagenic base uracil by family B DNA polymerases from Archaea. J. Mol. Biol. *337*, 621–634.

Silvian, L. F., Toth, E. A., Pham, P., Goodman, M. F., and Ellenberger, T. (2001). Crystal structure of a DinB family error-prone DNA polymerase from *Sulfolobus solfataricus*. Nat. Struct. Biol. *8*, 984–989.

Skorvaga, M., Raven, N. D., and Margison, G. P. (1998). Thermostable archaeal O6-alkylguanine-DNA alkyltransferases. Proc. Natl. Acad. Sci. U.S. A *95*, 6711–6715.

Sleeth, K. M., Robson, R. L., and Dianov, G. L. (2004). Exchangeability of mammalian DNA ligases between base excision repair pathways. Biochemistry *43*, 12924–12930.

Slesarev, A. I., Mezhevaya, K. V., Makarova, K. S., Polushin, N. N., Shcherbinina, O. V., Shakhova, V. V., Belova, G. I., Aravind, L., Natale, D. A., Rogozin, I. B., Tatusov, R. L., Wolf, Y. I., Stetter, K. O., Malykh, A. G., Koonin, E. V., and Kozyavkin, S. A. (2002). The complete genome of hyperthermophile Methanopyrus kandleri AV19 and monophyly of archaeal methanogens. Proc. Natl. Acad. Sci. U.S. A *99*, 4644–4649.

Smith, D. R., Doucette-Stamm, L. A., Deloughery, C., Lee, H., Dubois, J., Aldredge, T., Bashirzadeh, R., Blakely, D., Cook, R., Gilbert, K., Harrison, D., Hoang, L., Keagle, P., Lumm, W., Pothier, B., Qiu, D., Spadafora, R., Vicaire, R., Wang, Y., Wierzbowski, J., Gibson, R., Jiwani, N., Caruso, A., Bush, D., Reeve, J. N., and. (1997). Complete genome sequence of *Methanobacterium thermoautotrophicum* deltaH: functional analysis and comparative genomics. J. Bacteriol. *179*, 7135–7155.

Spies, M., Kil, Y., Masui, R., Kato, R., Kujo, C., Ohshima, T., Kuramitsu, S., and Lanzov, V. (2000). The RadA protein from a hyperthermophilic archaeon *Pyrobaculum islandicum* is a DNA-dependent ATPase that exhibits two disparate catalytic modes, with a transition temperature at 75 degrees C. Eur. J. Biochem. *267*, 1125–1137.

Sung, H. M., Yeamans, G., Ross, C. A., and Yasbin, R. E. (2003). Roles of YqjH and YqjW, homologs of the Escherichia coli UmuC/DinB or Y superfamily of DNA polymerases, in stationary-phase mutagenesis and UV-induced mutagenesis of Bacillus subtilis. J. Bacteriol. *185*, 2153–2160.

Suzuki, N., Itoh, S., Poon, K., Masutani, C., Hanaoka, F., Ohmori, H., Yoshizawa, I., and Shibutani, S. (2004). Translesion synthesis past estrogen-derived DNA adducts by human DNA polymerases eta and kappa. Biochemistry *43*, 6304–6311.