DNA REPAIR

Guanine glycation repair by DJ-1/Park7 and its bacterial homologs

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DNA damage induced by reactive carbonyls (mainly methylglyoxal and glyoxal), called DNA glycation, is quantitatively as important as oxidative damage. DNA glycation is associated with increased mutation frequency, DNA strand breaks, and cytotoxicity. However, in contrast to guanine oxidation repair, how glycated DNA is repaired remains undetermined. Here, we found that the parkinsonism-associated protein DJ-1 and its bacterial homologs Hsp31, YhbO, and YajL could repair methylglyoxal- and glyoxal-glycated nucleotides and nucleic acids. DJ-1–depleted cells displayed increased levels of glycated DNA, DNA strand breaks, and phosphorylated p53. Deglycase-deficient bacterial mutants displayed increased levels of glycated DNA and RNA and exhibited strong mutator phenotypes. Thus, DJ-1 and its prokaryotic homologs constitute a major nucleotide repair system that we name guanine glycation repair.

D NA repair systems undo DNA damage produced by reactive oxygen species, reactive carbonyls, alkylating agents, ultraviolet (UV) radiation, deoxyuracil incorporation, and replication errors. DNA repair mechanisms include nucleotide pool sanitization, direct repair, base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination repair, and nonhomologous end joining (1, 2). Nucleic acids undergo permanent glycation by glyoxal (GO) (CHO-CHO) and methylglyoxal (MGO) (CH_3-CO-CHO), which are ubiquitously present in cells as by-products of sugar metabolism and constitute their major glycating agents (3). The most susceptible nucleotides are guanosine (G) and deoxyguanosine (dG) (3). Cellular amounts of dG glycated by MGO (dG-MG) are similar to those of the major oxidized nucleotide, 8-oxo-dG (3). Thus, glycation is an important source of DNA damage in vivo and is associated with increased mutation frequency, DNA strand breaks, and cytotoxicity (3, 4). However, whereas oxidized nucleotides are repaired by the guanine oxidation repair system, no dedicated system is known for glycated nucleotide repair, although it may be mediated by NER and MMR (3).

We recently reported that DJ-1, generally described as an oxidative stress response protein (5), functions as a protein deglycase that repairs MGO- and GO-glycated cysteines, arginines, and lysines and releases repaired proteins with lactate or glycolate, respectively (6). The nonenzymatic reaction between carbonyl groups and amino acids was discovered by Louis Camille Maillard in 1912 (7) and involves the spontaneous reaction of reactive carbonyls with thiol and amino groups of proteins and nucleic acids (3). Defense against carbonyl stress involves carbonyl scavengers [glyoxalases, aldoketoreductases, and efflux pumps (3, 8)], which reduce their cellular concentration,

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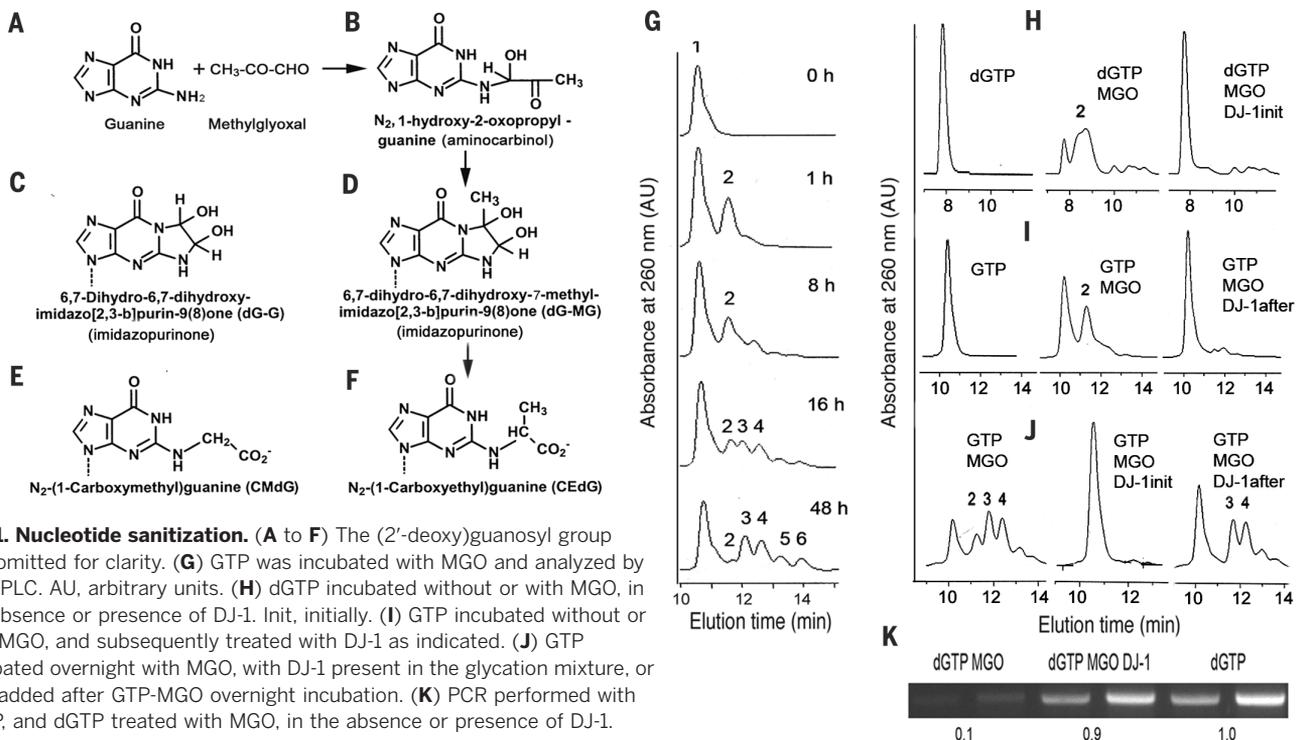†These authors contributed equally to this work.

Fig. 1. Nucleotide sanitization. (**A** to **F**) The (2′-deoxy)guanosyl group was omitted for clarity. (**G**) GTP was incubated with MGO and analyzed by RP-HPLC. AU, arbitrary units. (**H**) dGTP incubated without or with MGO, in the absence or presence of DJ-1. Init, initially. (**I**) GTP incubated without or with MGO, and subsequently treated with DJ-1 as indicated. (**J**) GTP incubated overnight with MGO, with DJ-1 present in the glycation mixture, or DJ-1 added after GTP-MGO overnight incubation. (**K**) PCR performed with dGTP, and dGTP treated with MGO, in the absence or presence of DJ-1. Samples of 5 and 10 µl were loaded in left and right wells, respectively. All figures are representative of three independent experiments.

and fructosamine-3-kinases (9) and DJ-1, which repair proteins having undergone glycation by glucose and GOs, respectively.

Guanine glycation by MGO (Fig. 1A) starts with rapid formation of an aminocarbinol (3, 4) (Fig. 1B) that slowly transforms itself into cyclic imidazopurinones dG-MG (3, 10–12) (Fig. 1D) and carboxyethyl-deoxyguanosine (CEdG) (Fig. 1F). Guanine glycation by GO leads to the formation of the imidazopurinone dG-G (Fig. 1C) and carboxymethyl-deoxyguanosine (CMdG) (Fig. 1E). We analyzed the glycation kinetics of guanosine 5′-triphosphate (GTP) by MGO (Fig. 1G). Addition of MGO produced a second peak after the GTP peak, and these changes reflected the rapid formation of aminocarbinol (peak 2). This peak then decreased, whereas more slowly migrating peaks increased, reflecting the conversion of the aminocarbinol into imidazopurinones (dG-MG) (peaks 3 and 4) and CEdG, as described previously (12).
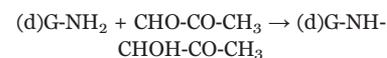
Nucleotide sanitizers are major contributors to DNA damage repair. Altered deoxyribonucleotides induce mutations after being incorporated into DNA and transcriptional mutagenesis by directing misincorporation of ribonucleotides into RNA (2). Altered ribonucleotides are re-sponsible for translational defects and unequal availability of nucleoside diphosphates for ribonucleotide reductase (2). Deoxyguanosine triphosphate (dGTP) and GTP each migrated as a single peak on a reversed-phase high-performance liquid chromatography (RP-HPLC) column (Fig. 1, H and I). After incubation with MGO, 80% of dGTP and 46% of GTP migrated as slower-eluting peaks containing glycated nucleotides (13). When DJ-1 was added to the initial glycation mixture (Fig. 1H) or at 2 hours after glycation onset (Fig. 1I), dGTP and GTP eluted as intact nucleotides, sug-gesting that they were deglycated by DJ-1. After overnight incubation of GTP with MGO, glycated GTP migrated as three peaks (peaks 2 to 4 in Fig. 1J). When added to the initial glycation mixture, DJ-1 prevented the formation of glycated GTP (Fig. 1J). However, when added after overnight incubation of the glycation mixture, it deglycated only molecular species contained in peak 2, where-as peaks 3 and 4 were minimally affected (Fig. 1J). These findings suggest that DJ-1 deglycates only aminocarbinols. Aminocarbinols and imidazo-purinones were characterized by nuclear magnetic resonance, mass spectrometry (MS), and reactivity with 2,4-dinitrophenylhydrazine and pe-riodate (fig. S1, A to G). DNA polymerase from *Pyrococcus furiosus* incorporated repaired dGTP into a polymerase chain reaction (PCR) prod-uct with an efficiency similar to intact dGTP's, whereas the dGTP-MGO sample produced little PCR product (Fig. 1K).

DJ-1 also deglycated guanosine diphosphate (GDP) and guanosine monophosphate (GMP) (fig. S2, A and B), as well as GTP glycated by GO (fig. S2C). DJ-1 deglycated dGTP and amino acids with similar kinetics [the observed rate constant (k_{obs}) = 0.25 s^{-1}] (6), suggesting that DJ-1 prin-cipally recognizes the glycated region of its sub-strates (fig. S2D). Hsp31 (the *hchA* gene product) and YhbO efficiently deglycated GTP, whereas YajL deglycated these compounds much less effi-ciently (fig. S2E).

Our experiments show that DJ-1 deglycates aminocarbinols but not imidazopurinones (Fig. 1J and fig. S1, C and D). Thus, the mechanism of nucleotide deglycation by DJ-1 is likely similar to that described for lysine and arginine (6). The following reactions are likely to occur.

$$(d)G\text{-}NH_2 + CHO\text{-}CO\text{-}CH_3 \rightarrow (d)G\text{-}NH\text{-}CHOH\text{-}CO\text{-}CH_3$$
(spontaneous aminocarbinol formation)

$$(d)G\text{-}NH\text{-}CHOH\text{-}CO\text{-}CH_3 \rightarrow (d)G\text{-}NH\text{-}CO\text{-}CHOH\text{-}CH_3$$
(H migration catalyzed by DJ-1, reminiscent of its glyoxalase 1 activity)

$$(d)G\text{-}NH\text{-}CO\text{-}CHOH\text{-}CH_3 \rightarrow (d)G\text{-}NH_2 + COOH\text{-}CHOH\text{-}CH_3$$
(amidolysis catalyzed by DJ-1, reminiscent of its amidase or peptidase activity)

Thus, DJ-1 repairs nucleotides before their con-version into the advanced glycation end products dG-MG, CEdG, dG-G, and CMdG. As reported for amino acid repair (6) and in accordance with the above reactions, nucleotide repair by DJ-1 from MGO damage was associated with lactate pro-duction (fig. S2F).

To test DNA repair by DJ-1, we incubated the forward PCR primer for the *Escherichia coli yeaG* gene with MGO, in the absence or presence of DJ-1, and assessed glycation by analyzing nucleo-tides after DNA hydrolysis. The intensity of the dGMP peak decreased, and several peaks con-taining the glycated dinucleotides dGA, dGC, and dGT (characterized by mass spectrometry) appeared (Fig. 2A, Table 1, and fig. S3), suggesting that glycated dG is (partially) resistant to nuclease treatment. The chromatogram of the DNA–MGO–DJ-1 sample was identical to that of intact DNA, suggesting that DJ-1 repairs glycated DNA. The repaired DNA was efficient as a PCR primer, in contrast to glycated DNA (Fig. 2B). DJ-1 also deglycated double-stranded DNA (fig. S4A) and ribosomal RNA (fig. S4B). The antiglycation ef-fect of DJ-1 resulted from direct nucleotide and DNA repair but not from MGO depletion, showing that DJ-1 functions as a deglycase but not as a glyoxalase (fig. S5, A to D) (6). Moreover, the DJ-1 C106S mutant (in which serine replaces
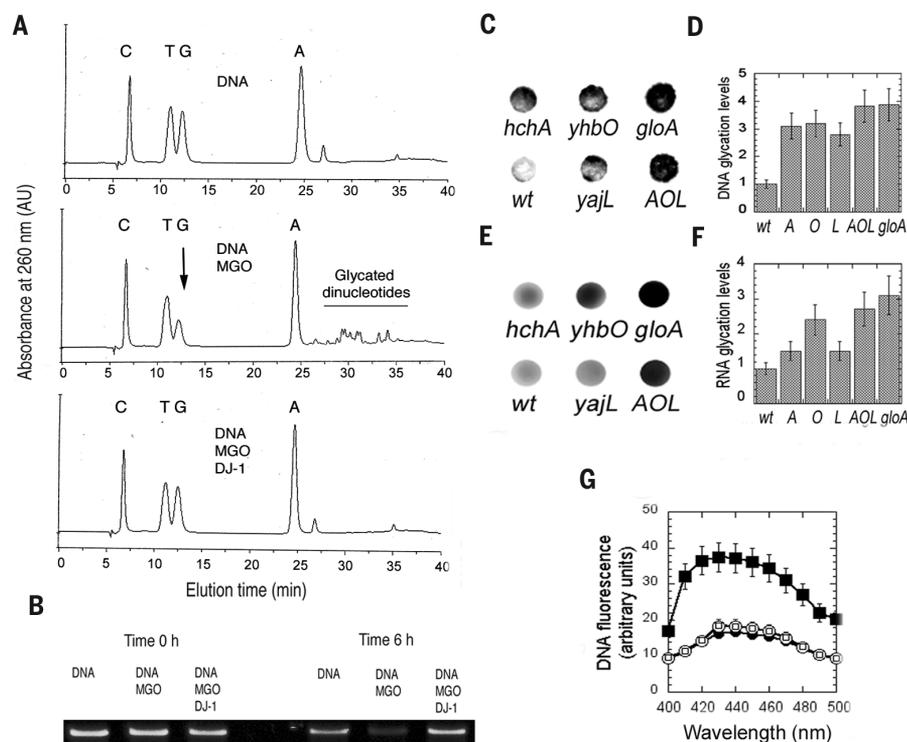

Fig. 2. DNA repair. (**A**) DNA was incubated with buffer alone, with MGO, or with MGO and DJ-1. DNAs were hydrolyzed, and nucleotides analyzed by RP-HPLC. AU, arbitrary units. (**B**) PCR was performed in the presence of the forward primer after pretreatment for 0 or 6 hours with buffer alone, with MGO, or with MGO and DJ-1. (**C**) Immunoreactivity of DNA to antibodies against AGE: immunodots of DNA from the wild-type (*wt*) strain, and from *hchA*, *yhbO*, *yajL*, *hchAyhbOyajL* (*AOL*), and *gloA* mutants. (**D**) Glycation levels of dot blots displayed in (C). (**E**) Immunoreactivity of RNA to antibodies against AGE. Immunodots of RNA from deglycase and *gloA* mutants. (**F**) Glycation levels of dot blots displayed in (E). (**G**) DNA glycation levels of the *wt* strain (filled circles), and *hchAyhbOyajL* (filled squares), *uvrA* (empty circles) and *mutL* (empty squares) mutants measured by fluorescence emission upon excitation at 360 nm. Identical DNA amounts were used. All figures are representative of three independent experiments.

Table 1. Characterization of nuclease-resistant dinucleotides. Fractions 27 to 35 from the RP-HPLC column loaded with the DNA-MGO sample after nuclease treatment (displayed in Fig. 2A) were analyzed by liquid chromatography–tandem MS (LC-MS/MS). We characterized mono- and diglycated dinucleotides dGA, dGT, and dGC. dG* and dG** stand for mono- and diglycated dG, respectively.

	Nuclease-resistant glycated dinucleotide masses obtained by LC-MS/MS analysis [in $(M + H)^+$]	
Oligonucleotide	Observed	Calculated
dG*A	733.149	733.148
dG*T	724.138	724.136
dG*C	709.139	709.137
dG**A	805.170	805.169
dG**T	796.160	796.157
dG**C	781.161	781.158
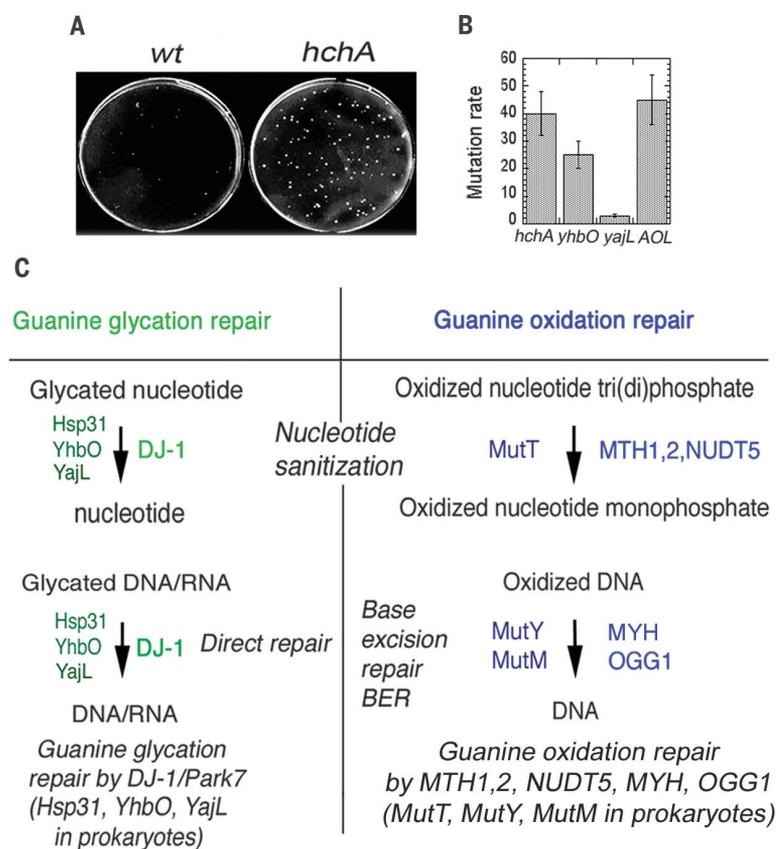
Fig. 3. Mutator phenotypes, guanine glycation repair versus guanine oxidation repair. (**A** and **B**) Mutator phenotypes of *hchA*, *yhbO*, *yajL* and *hchAyhbOyajL* mutants. Figures are representative of three independent experiments. (**C**) Parallel between guanine glycation repair by DJ-1/Park7, involving nucleotide sanitization and direct DNA repair, and guanine oxidation repair, involving nucleotide sanitization and BER.

cysteine-106), deficient in its active-site cysteine (5, 6), was unable to repair free nucleotide and DNA (fig. S6, A and B).

Escherichia coli DNA is glycated under normal physiological conditions (13). DNA and RNA from deglycase mutants *hchA*, *yhbO*, and *yajL* displayed higher glycation levels than DNA and RNA from the parental strain, suggesting that Hsp31, YhbO, and YajL prevent DNA and RNA glycation. The glycation level of the *hchAyhbOyajL* (AOL) mutant was higher than those of single mutants and similar to that of the *gloA* mutant deficient in glyoxalase 1 (Fig. 2, C to F). Free nucleotides and the chromosomal DNA from the

hchAyhbOyajL mutant displayed higher glycation levels than those from the parental strain (figs. S7 and S8).

Because NER and MMR are involved in glycated DNA repair (3, 11), we compared DNA glycation levels of the *hchAyhbOyajL* mutant with those of the *uvrA* (NER) and *mutL* (MMR) mutants, by measuring DNA fluorescence between 400 and 500 nm. The *uvrA* and *mutL* mutants displayed DNA glycation levels similar to those of the wild-type strain, whereas the *hchAyhbOyajL* mutant displayed 2.5 times the glycation levels (Fig. 2G), suggesting that deglycases constitute the primary repair system for glycated DNA, with NER and MMR functioning as backup systems.

DNA glycation increases mutation frequencies (3, 4). Mutation rates to rifampicin resistance in the *hchA*, *yhbO*, *yajL*, and *hchAyhbOyajL* mutants were 41, 23, 2.6, and 46 times as high, respectively, as those of the parental strain (Fig. 3, A and B). They fall in the same range as those of the *mutM*, *mutY*, and *mutT* mutants deficient in guanine oxidation repair (14). However, the *hchAyhbOyajL* mutant displayed increased levels of GC>AT and AT>GC transitions, in contrast with *mutT* mutants, which display AT>CG transversions, and *mutM* or *mutY* mutants, which display GC>TA transversions (14) (table S1). Thus, DNA damage and possibly damage to the DNA repair proteome in deglycase mutants result in impressive mutator phenotypes, suggesting that deglycases play important roles in repairing nucleotide glycation damage under normal physiological conditions.

DJ-1 depletion by small interfering RNA in HeLa cells resulted in increased DNA glycation (fig. S9A), increased p53 phosphorylation (fig. S9B), and DNA strand breaks (fig. S9D). Moreover, DJ-1 displayed partial nuclear localization (fig. S9C), as previously reported (5).

DJ-1 and its homologs constitute a nucleotide repair system dedicated to reversing MGO and GO damage. The accumulation of protein and nucleotide advanced glycation end products (AGEs) in deglycase mutants, their mutator phenotype, and loss of viability (6, 15) contradict the idea that deglycases would be inefficient in vivo (16). Several reports are consistent with an involvement of DJ-1 deglycases in nucleic acid metabolism. An RNA binding activity of DJ-1 has been reported (5), Hsp31 is overexpressed after DNA damage (17), YhbO protects against UV stress (18), and *pfpI* is an antimutator gene (19). Moreover, *yajL* mutants display translational defects (20, 21) and induce a DNA damage response (22, 23). Because DJ-1–depleted keratinocytes display increased protein glycation levels (24), both the protein and nucleic acid deglycase activities of DJ-1 prevent cellular glycation.

Here we have shown that, in addition to guanine oxidation repair (1, 2), cells perform guanine glycation repair. Whereas guanine oxidation repair comprises nucleotide sanitizers and BER glycosylases, guanine glycation repair involves nucleotide sanitization and direct nucleic acid repair (Fig. 3C). Oxidative and glycation damage occur at similar frequencies (3) and result in increased mutation frequencies, DNA strand breaks, cytotoxicity,

and tumorigenesis (*3, 4*). Thus, guanine glycation repair by DJ-1 may be as important to maintain DNA integrity as guanine oxidation repair. DJ-1 acts catalytically, in contrast to O^6-methylguanine-DNA methyltransferase, which repairs methylated nucleotides by suicidal transfer of the methyl group to its cysteine (*1*). The DJ-1 deglycases may represent the only enzymes that repair both proteins and nucleic acids. Because Maillard adducts are their substrates, we renamed them the DJ-1 family Maillard deglycases (*25, 26*).

REFERENCES AND NOTES

1. N. J. Curtin, *Nat. Rev. Cancer* **12**, 801–817 (2012).
2. P. Rai, *Mutat. Res.* **703**, 71–81 (2010).
3. P. J. Thornalley, *Drug Metabol. Drug Interact.* **23**, 125–150 (2008).
4. M. Pischetsrieder, W. Seidel, G. Münch, R. Schinzel, *Biochem. Biophys. Res. Commun.* **264**, 544–549 (1999).
5. M. A. Wilson, *Antioxid. Redox Signal.* **15**, 111–122 (2011).
6. G. Richarme *et al.*, *J. Biol. Chem.* **290**, 1885–1897 (2015).
7. L. C. Maillard, *C. R. Acad. Sci. Ser. 2* **154**, 66–68 (1912).
8. D. R. Sell, V. M. Monnier, *Gerontology* **58**, 227–237 (2012).
9. E. Van Schaftingen, F. Collard, E. Wiame, M. Veiga-da-Cunha, *Amino Acids* **42**, 1143–1150 (2012).
10. C. Bidmon, M. Frischmann, M. Pischetsrieder, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **855**, 51–58 (2007).
11. P. J. Thornalley, *Biochem. Soc. Trans.* **31**, 1372–1377 (2003).
12. P. J. Thornalley *et al.*, *Nucleic Acids Res.* **38**, 5432–5442 (2010).
13. R. Mironova, T. Niwa, Y. Handzhiyski, A. Sredovska, I. Ivanov, *Mol. Microbiol.* **55**, 1801–1811 (2005).
14. T. Tajiri, H. Maki, M. Sekiguchi, *Mutat. Res.* **336**, 257–267 (1995).
15. J. Abdallah, M. Mihoub, V. Gautier, G. Richarme, *Biochem. Biophys. Res. Commun.* **470**, 282–286 (2016).
16. N. Rabbani, P. J. Thornalley, *Biochem. Biophys. Res. Commun.* **458**, 221–226 (2015).
17. D. R. Kim, R. D. Gidvani, B. P. Ingalls, B. P. Duncker, B. J. McConkey, *Proteome Sci.* **9**, 62 (2011).
18. J. Abdallah, T. Caldas, F. Kthiri, R. Kern, G. Richarme, *J. Bacteriol.* **189**, 9140–9144 (2007).
19. A. Rodríguez-Rojas, J. Blázquez, *J. Bacteriol.* **191**, 844–850 (2009).
20. F. Kthiri *et al.*, *J. Biol. Chem.* **285**, 10328–10336 (2010).
21. F. Kthiri *et al.*, *J. Bacteriol.* **192**, 6302–6306 (2010).
22. N. Messaoudi *et al.*, *J. Bacteriol.* **195**, 1167–1178 (2013).
23. A. Giraud, M. Radman, I. Matic, F. Taddei, *Curr. Opin. Microbiol.* **4**, 582–585 (2001).
24. T. Advedissian, F. Deshayes, F. Poirier, M. Viguier, G. Richarme, *Biochem. Biophys. Res. Commun.* **473**, 87–91 (2016).
25. G. Richarme, J. Dairou, *Biochem. Biophys. Res. Commun.* **483**, 387–391 (2017).
26. G. Richarme, E. Marguet, P. Forterre, S. Ishino, Y. Ishino, *Biochem. Biophys. Res. Commun.* **478**, 1111–1116 (2016).

SUPPLEMENTARY MATERIALS

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Guanine glycation repair by DJ-1/Park7 and its bacterial homologs

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Not-so-sweet DNA damage repaired
Glyoxal and methylglyoxal, by-products of sugar metabolism that are present in all cells, can react with, and thus damage, DNA. Indeed, glycation of guanine (G) is as prevalent as the major product of oxidative damage in DNA, 8-oxo-dG. Richarme *et al.* show that both prokaryotes and eukaryotes have dedicated systems that specifically repair glycation damage (see the Perspective by Dingler and Patel). The parkinsonism-associated protein DJ-1/Park7 and its bacterial homologs Hsp31, YhbO, and YajL direct the enzymatic repair of damaged glycated bases in DNA. The proteins also clean up the more vulnerable pool of free nucleotides in the cell, which are more susceptible to glycation than the nucleotides within DNA.
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