Characterization of class II apurinic/apyrimidinic endonuclease activities in the human malaria parasite, *Plasmodium falciparum*

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We have reported that the human malaria parasite, *Plasmodium falciparum*, repairs apurinic/apyrimidinic (AP) sites on DNA by a long-patch base excision repair (BER) pathway. This biology is different from that in mammalian cells, which predominantly repair AP sites by a DNA-polymerase- β -dependent, one-nucleotide patch BER pathway. As a starting point for the identification and biochemical characterization of the enzymes involved in the parasite DNA BER pathway, we chose characterization of the AP endonuclease activity in a *P. falciparum* cell-free lysate. Evidence is provided for the presence of class II,

Mg²⁺-dependent and independent AP endonucleases in the parasite lysate. The investigation of the processing of AP sites in *Plasmodium* will provide new information about long-patch BER pathways; if they are different from those in the human host they might provide a new target for anti-malarial chemotherapy.

Key words: β -elimination, base excision repair, DNA, glycosylase.

INTRODUCTION

Base excision repair (BER) is employed on DNA lesions that require precise and rapid attention [1,2]. Substrates for BER include oxidative base damage, multiple forms of alkylation damage, apurinic/apyrimidinic (AP) sites formed by DNA glycosylases, spontaneous loss of bases, and uracil residues in DNA [1,2]. Studies in *Escherichia coli*, *Xenopus laevis* and human cells demonstrated that BER of DNA is initiated by DNA glycosylases, which catalyse the hydrolysis of the N-glycosyl bond linking particular damaged bases to the sugar-phosphate backbone [3]. Excision of the damaged bases generates AP sites in the DNA. The phosphodiester bonds 5' of these sites are incised by an AP endonuclease, generating 3'-OH and 5'-deoxyribose phosphate moieties. The latter are removed by a DNA deoxyribophosphodiesterase activity, DNA polymerase fills in the resulting single nucleotide gaps, and the covalent integrity of the DNA strands is restored by a DNA ligase [4,5].

DNA polymerase β is the major enzyme that operates during the repair synthesis of BER in higher organisms [6,7], generating a single nucleotide repair patch [8,9]. We investigated the repair of AP sites in DNA by using a cell-free lysate from the human malaria parasite, *Plasmodium falciparum*, and provided the first evidence for DNA repair activities in that organism [10]. The parasites repaired AP sites on DNA by a long-patch BER pathway. This biology was distinct from that of mammalian cells and *Xenopus laevis*, which predominantly repair AP sites by a DNA-polymerase- β -dependent, one-nucleotide patch BER pathway [4,6–9]. The apparent absence of DNA polymerase β and its associated activities from *P. falciparum* suggests major differences in DNA repair pathways between mammalian cells and malaria parasites.

As a starting point for the identification and biochemical characterization of the enzymes involved in the parasite DNA BER pathway, we chose to focus on the processing of the AP site

created by DNA glycosylases. Two classes of AP endonuclease have been described, on the basis of the mechanism of incision. Class I AP endonucleases, such as endonuclease III (EndoIII) [11] and EndoVIII [12] of *Escherichia coli*, or the human homologue, hNTH1 [13–15], are AP lyases that cleave the DNA on the 3' side of the AP site by β -elimination. The cleavage of the AP site by this mechanism results in the 5' fragment's possessing a 3'-terminal deoxyribose phosphate and the 3' fragment's bearing a 5'-terminal phosphate. In addition, these enzymes also have a DNA glycosylase activity that functions to remove lesions resulting from oxidative stress such as thymine glycol and urea [16]. Yeast were also shown to possess an enzyme, redox-endonuclease, encoded by two genes *SCR1* and *SCR2*, that was found to be a homologue of EndoIII [17,18].

Class II AP endonucleases, such as EndoIV and exonuclease III (ExoIII) of *E. coli*, cleave the DNA 5' of the AP site hydrolytically. Cleavage by this mechanism produces 3'-OH and 5'-deoxyribose phosphate moieties [1,16]. This class of AP endonucleases is specific for AP sites and does not possess a DNA glycosylase activity. Class II AP endonucleases are further subdivided into two families on the basis of their Mg²⁺ requirement. Mg²⁺-dependent AP endonucleases, such as ExoIII of *E. coli* and the human homologue HAP1 (also called APE, APEX and REF-1), comprise most (approx. 90%) of the AP endonuclease activity in these cells [1,19–21]. In *E. coli* the Mg²⁺-independent EndoIV comprises approx. 10% of the total activity and was found to be induced after DNA damage [1,16,22,23]. However, in yeast, the Mg²⁺-independent AP endonuclease Apn1 (EndoIV of *E. coli*) comprised the bulk of the AP endonuclease activity [22,24]. There are no reports of an ExoIII homologue in yeast, although the enzyme Pde1 possesses similar enzymic properties [24–26].

Here we report the presence of AP endonuclease activities in a *P. falciparum* cell-free lysate. Evidence is provided for both Mg²⁺-dependent and Mg²⁺-independent class II parasite AP endonucleases.

Abbreviations used: AP, apurinic/apyrimidinic; BER, base excision repair; Endo, endonuclease; Exo, exonuclease; THF, tetrahydrofuran.

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MATERIALS AND METHODS

Materials

EndoIII was a gift from Richard Cunningham [15]. EndoIV was purchased from Trevigen (Gaithersburg, MD, U.S.A.); uracil DNA glycosylase was from Epicentre Technologies (Madison, WI, U.S.A.); T4 polynucleotide kinase was from Promega (Madison, WI, U.S.A.); [γ - 32 P]ATP (3000 Ci/mmol) was from Amersham (Indianapolis, IN, U.S.A.); 28-mer oligonucleotides with a uracil (U28; 5'-TGG CGA AAG GGG GUT GTG CTG CAA GGC G-3') or a tetrahydrofuran (THF28; 5'-TGG CGA AAG GGG GTHFT GTG CTG CAA GGC G-3') at position 14 from the 5' end of the complementary strands (U28, G opposite U; THF28, A opposite THF) were obtained from Midland Scientific (Midland, TX, U.S.A.). These substrates were chosen because a natural AP site can be cleaved by a β -elimination mechanism, whereas the THF (synthetic) AP site is refractory to β -elimination. Polyacrylamide mix was purchased from Boehringer Mannheim (Indianapolis, IN, U.S.A.).

Preparation of oligonucleotides

U28 or THF28 (10 pmol) was labelled on the 5' end with [γ - 32 P]ATP and T4 polynucleotide kinase. The labelled strand was annealed to the complementary strand by being boiled in a water bath for 2 min, followed by slow cooling to 4 °C. The unincorporated nucleotides were removed by Sephadex G25 spin columns (Boehringer Mannheim), which were equilibrated with 10 mM Hepes, pH 7.9. The specific radioactivity was determined by liquid scintillation counting and was generally approx. 500 000 c.p.m./0.1 pmol. To generate a natural AP site (AP28), the duplex oligonucleotide containing U (U28) was pretreated with uracil DNA glycosylase (0.1 unit of uracil DNA glycosylase/0.1 pmol U28) for 30 min at 37 °C immediately before use.

Preparation of the *P. falciparum* lysate

The 3D7 clone of *P. falciparum* was cultured as described previously [27,28]. The erythrocytic cycle was synchronized to ± 6 h and the parasitized erythrocytes were harvested when the parasitaemia was 10–20%. The parasites were freed from the erythrocytes with 0.15% saponin and washed four times with PBS containing protease inhibitors [3 μ g/ml pepstatin, leupeptin, tosyl-lysylchloromethane ('TLCK') and 1 mM PMSF] [29]. The parasite pellet was resuspended in sonication buffer containing protease inhibitors and lysed ultrasonically [10]. The lysate was dialysed against 1 litre of buffer [25 mM Hepes/KOH (pH 7.9)/100 mM KCl/12 mM MgCl₂/1 mM EDTA/2 mM dithiothreitol/17% (v/v) glycerol] at 4 °C for 16 h and separated into 30 μ l aliquots for storage at –80 °C.

AP endonuclease assay

5'-end-labelled duplex AP28 or THF28 (0.1 pmol) was incubated at 37 °C in 20 μ l containing 200 mM KCl, 5 mM Hepes, pH 7.9, and 5 ng of EndoIII, 5 units of EndoIV or 20 μ g of parasite lysate protein. Reactions were for 30 min at 37 °C and were terminated by the addition of 100 μ l of formamide loading buffer (47.5% formamide/10 mM EDTA/0.025% Bromophenol Blue/0.025% Xylene Cyanol FF). Samples were heated for 2 min at 75 °C and 10 000 c.p.m. was loaded on each lane of a 42 cm, 15% (w/v) denaturing (8.3 M urea) polyacrylamide gel. Electrophoresis was in TBE [89 mM Tris/89 mM boric acid/2.0 mM EDTA (pH 8.4)] for 2 h at 55 W constant power. Products were detected by autoradiography of wet gels.

3'-Phosphodiesterase activity assay

The 5'-end-labelled duplex oligonucleotide AP28 (0.1 pmol) was pretreated with 5 ng of EndoIII for 30 min at 37 °C, generating a preincised oligonucleotide with a 3'-deoxyribose phosphate moiety. The preincised oligonucleotide (0.1 pmol) was incubated for 30 min at 37 °C in 20 μ l containing 200 mM KCl, 5 mM Hepes, pH 7.5, and either 5 units of EndoIV or 20 μ g of parasite lysate protein. Reactions were terminated by the addition of 100 μ l of formamide loading buffer; the mixtures were heated at 75 °C for 2 min and 10 000 c.p.m. was loaded on each lane of a 15% (w/v) denaturing (8.3 M urea) polyacrylamide gel. Products were detected by autoradiography of the wet gels.

Mg²⁺ dependence of the AP endonuclease activity

To determine the optimal conditions for investigating the Mg²⁺ dependence of the AP endonuclease activity, 0.1 pmol of THF28 and 20 μ g of lysate protein were incubated in 20 μ l of 100 mM KCl/5 mM Hepes/1.5 mM EDTA (pH 7.5) for 0–60 min at 37 °C. To investigate the Mg²⁺ dependence of the AP endonuclease activity, 0.1 pmol of THF28 and 20 μ g of lysate protein were incubated in 20 μ l of 100 mM KCl/5 mM Hepes/1.5 mM EDTA (pH 7.5) and 0–10 mM MgCl₂ (final concentration). Reactions were performed for 5 min at 37 °C and terminated by the addition of 100 μ l of formamide loading buffer. Samples were heated for 2 min at 75 °C and 10 000 c.p.m. was loaded on each lane of a 42 cm, 15% (w/v) denaturing (8.3 M urea) polyacrylamide gel. Products were quantified by phosphorimaging with a Molecular Dynamics PhosphorImager.

Illustrations

Images were scanned into Adobe Photoshop version 5 with a Hewlett Packard ScanJet 5100 scanner.

RESULTS

Identification of *P. falciparum* class II AP endonuclease activity

Class I AP endonucleases are AP lyases that cleave on the 3' side of the AP site by β -elimination. The products of this reaction differ from those resulting from the hydrolytic cleavage on the 5' side of the AP site by a class II AP endonuclease. When an oligonucleotide containing a natural AP site (AP28) was incubated with EndoIII (Figure 1, lane 2), the site was cleaved by β -elimination, generating a product with a 3'-deoxyribose phosphate. In contrast, EndoIV cleaved the oligonucleotide hydrolytically, generating a 3'-OH moiety (Figure 1, lane 3). Additionally, whereas EndoIV cleaved THF28 (Figure 1, lane 7), EndoIII was unable to cleave this synthetic AP site, which is refractory to β -elimination (Figure 1, lane 6). Incubation of the parasite lysate with AP28 caused hydrolytic cleavage at the AP site and the formation of a product that migrated at the same position as the EndoIV control (Figure 1; compare lanes 4 and 3). When THF28 was used as the substrate, the lysate caused incision at the AP site similarly to EndoIV (Figure 1; compare lanes 8 and 7). These results revealed that the parasite lysate contained a class II AP endonuclease activity.

P. falciparum AP endonuclease(s) possess a 3'-phosphodiesterase activity

Class II AP endonucleases are involved in the direct incision of AP sites, generating 3'-OH and 5'-deoxyribose phosphate moieties. In addition to this direct involvement in the processing of AP sites, class II AP endonucleases possess a 3'-phosphodiesterase activity that can remove phosphates and deoxyribose

Figure 1 Identification of class II AP endonuclease activity in the *P. falciparum* cell-free lysate

5'-end-labelled oligonucleotide (0.1 pmol) with a natural AP site, AP28 (lanes 1–4) or a synthetic AP site, THF28 (lanes 5–8) was either untreated (lanes 1 and 5) or treated with 5 ng EndoIII (lanes 2 and 6), 5 units of EndoIV (lanes 3 and 7) or 20 μ g of parasite lysate protein (lanes 4 and 8; P.f lysate) for 30 min at 37 °C. Reactions were analysed directly on 15% (w/v) denaturing (8.3 M urea) polyacrylamide gels. The positions of the substrate and products are indicated at the left. 13-OH marks the position of the product of the hydrolytic cleavage of the oligonucleotide by EndoIV, which generates a 13-mer with a 3'-OH moiety (lane 3).

phosphates from the 3' end of an AP site, after cleavage by the lyase activity of a class I AP endonuclease. Figure 2 shows that the parasite lysate (Figure 2, lane 4), similarly to EndoIV (Figure 2, lane 3), removed the 3'-deoxyribose phosphate generated after the pretreatment of an AP site with EndoIII (Figure 2, lane 2). This suggested that the parasite AP endonuclease(s) possessed a 3'-phosphodiesterase activity similar to that of other class II AP endonucleases [30,31].

P. falciparum lysate contains Mg^{2+} -independent and Mg^{2+} -dependent AP endonuclease activities

Because the parasite lysate was prepared in a buffer that contained 1.2 mM $MgCl_2$, it was unclear whether the class II AP endonuclease activity was due to a Mg^{2+} -dependent or Mg^{2+} -independent AP endonuclease (or both). To address this issue, it was necessary to characterize the activity of the parasite AP endonuclease in the absence and presence of Mg^{2+} ions. To investigate the activity of the parasite AP endonuclease in the absence of Mg^{2+} , reactions were run in the presence of up to 5 mM EDTA. Under these conditions, the incision of the AP site in THF28 was complete by 60 min. A representative time course of the Mg^{2+} -independent AP endonuclease activity in the presence of 1.5 mM EDTA is shown in Figure 3. A 5 min time point was chosen for investigation for the presence of a Mg^{2+} -dependent AP endonuclease, because at this time only approx. 30% of the AP sites in THF28 were incised, permitting the detection of stimulation of DNA incision by a Mg^{2+} -dependent AP endo-

Figure 2 Identification of *P. falciparum* 3'-phosphodiesterase activity

5'-end-labelled AP28 was pretreated with EndoIII to generate a preincised site containing a 3'-deoxyribose phosphate. Preincised AP28 (0.1 pmol) was either untreated (lane 2) or treated with 5 units of EndoIV (lane 3) or 20 μ g of parasite lysate protein (lane 4; P.f lysate) for 30 min at 37 °C. Unincised AP28 (lane 1) was run as a control.

nuclease. The addition of up to 2 mM Mg^{2+} (1.2 mM Mg^{2+} in the lysate buffer, 2.3 mM additional $MgCl_2$ and 1.5 mM EDTA) had little effect on the incision of the AP site in THF28 (Figure 4). However, a doubling of the activity was achieved with the addition of 4 mM Mg^{2+} from approx. 40% (at 2 mM Mg^{2+}) to 85% DNA incision, and 10 mM Mg^{2+} increased the incision

Figure 3 Time course of the incision of AP sites in DNA by a Mg^{2+} -independent AP endonuclease activity

5'-end-labelled THF28 (0.1 pmol) was incubated with 20 μ g of the lysate protein for 0–60 min at 37 °C in 100 mM KCl/5 mM Hepes/1.5 mM EDTA (pH 7.5). Reactions were analysed on 15% (w/v) denaturing (8.3 M urea) polyacrylamide gels. The percentage of substrate converted to product was determined by phosphorimaging of the radioactivity in the gels.

Figure 4 Identification of a Mg^{2+} -dependent AP endonuclease activity in the parasite lysate

5'-end-labelled THF28 (0.1 pmol) was incubated with 20 μg of lysate protein in 20 μl of 100 mM KCl/5 mM HEPES/1.5 mM EDTA (pH 7.5) and a final concentration of 0–10 mM MgCl_2 for 5 min at 37 °C. Reactions were analysed on 15% (w/v) denaturing (8.3 M urea) polyacrylamide gels. The percentage of substrate converted to product was determined by phosphorimaging of the radioactivity in the gels. The error bars show the S.E.M. for four independent experiments.

to 100% (Figure 4). Maximal activity of human apurinic endonuclease ('Ape1') was reported to require 4–10 mM MgCl_2 [32]. These results indicated that the parasite lysate contains both Mg^{2+} -independent and Mg^{2+} -dependent class II AP endonuclease activities.

DISCUSSION

We have recently investigated the presence of a long-patch DNA BER pathway in *P. falciparum* [10]. Although there was evidence for AP endonuclease activity in the parasite lysate, the classification of this activity required further inquiry. The current investigation revealed that *P. falciparum* possesses multiple class II AP endonuclease activities. Similarly to *E. coli*, *P. falciparum* contained both Mg^{2+} -dependent and Mg^{2+} -independent AP endonuclease activities. Mammalian cells contain only a Mg^{2+} -dependent AP endonuclease activity, which, however, suggests that the *P. falciparum* Mg^{2+} -independent AP endonuclease might be a chemotherapeutic target. Analysis of the malaria genome sequences that have so far been identified revealed a gene on chromosome 3 with sequence similarity to highly conserved active site residues in the class II Mg^{2+} -dependent AP endonucleases HAP-1 and ExoIII [33]. A gene sequence on chromosome 13 in the *P. falciparum* genome with sequence similarity to highly conserved active-site residues in Ape1 from *Saccharomyces cerevisiae* and *S. pombe*, CeApe1 from *Caenorhabditis elegans* [34], and <http://www.vetmed.ufl.edu/path/pfsgstp>, expressed sequence tag 0228C3; BLAST accession no. T02585) and EndoIV [35] has also been reported. Further investigations to identify and biochemically characterize the class II AP endonucleases of *P. falciparum* will require cloning of the genes and the expression of recombinant proteins.

A recent investigation of the AP endonucleases in the related Trypanosomatidae *Leishmania major* and *Trypanosoma cruzi* identified the gene for a class II AP endonuclease [36,37]. The *Trypanosoma* and *Leishmania* sequences had unique N-termini-containing sequences corresponding to nuclear import signals, whereas the C-terminal domains exhibited pronounced similarity to ExoIII. The expression *in vivo* of the AP endonuclease from *T.*

cruzi conferred viability by complementation to *E. coli* ExoIII deletion mutants [36]. The presence of a Mg^{2+} -dependent class II AP endonuclease activity in the *P. falciparum* lysate suggests that this might be an ExoIII (functional) homologue. It will be interesting to investigate which of the class II AP endonucleases is the dominant activity in *P. falciparum*. Mg^{2+} -dependent AP endonucleases (HAP1, ExoIII) comprise most (approx. 90%) of the AP endonuclease activity in human cells and *E. coli* respectively, [1,19–21]. However, in yeast the situation is reversed, with Ape1 comprising the bulk of the AP endonuclease activity [22,24,26].

We found no evidence of a class I AP endonuclease activity in the parasite lysate. However, it should be noted we would only have been able to detect a class I endonuclease activity in the lysate if there were no class II AP endonuclease activity. This is because modification of the AP site in DNA by a class I AP endonuclease would probably be acted on by the class II AP endonucleases in the parasite lysate. It is not possible at present to inhibit the class II AP endonucleases in the lysate to investigate for the presence of a class I AP endonuclease activity.

The identification of AP endonucleases in the malaria parasite will be greatly aided by the expected completion of the malaria genome project in the next 18–24 months [38]. The investigation of the processing of AP sites in *Plasmodium* will provide new information about the DNA BER process; if it is different from that in the human host it might provide a new target for anti-malarial chemotherapy.

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REFERENCES

- Doetsch, P. W. and Cunningham, R. P. (1990) *Mutat. Res.* **236**, 173–201
- Lindahl, T., Karran, P. and Wood, R. D. (1997) *Curr. Opin. Genet. Devel.* **7**, 158–169
- Krokan, H. E., Standal, R. and Slupphaug, G. (1997) *Biochem. J.* **325**, 1–16
- Wilson, III, D. M. and Thompson, L. H. (1997) *Proc. Natl. Acad. Sci. U.S.A.* **94**, 12745–12757
- Matsumoto, Y. and Bogenhagen, D. F. (1991) *Mol. Cell. Biol.* **11**, 4441–4447
- Sobol, R. W., Horton, J. K., Kühn, R., Gu, H., Singhal, R. K., Prasad, R., Rajewsky, K. and Wilson, S. H. (1996) *Nature (London)* **379**, 183–186
- Wilson, S. H. (1998) *Mutat. Res.* **407**, 203–215
- Bennett, R. A. O., Wilson, III, D. M., Wong, D. and Demple, B. (1997) *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7166–7169
- Singhal, R. K., Prasad, R. and Wilson, S. H. (1995) *J. Biol. Chem.* **270**, 949–957
- Haltiwanger, B. M., Matsumoto, Y., Nicolas, E., Dianov, G. L., Bohr, V. A. and Taraschi, T. F. (1999) *Biochemistry*, in the press
- Harrison, L. W., Hatahet, Z., Purmal, A. A. and Wallace, S. S. (1998) *Nucleic Acids Res.* **26**, 932–941
- Jiang, D., Hatahet, Z., Melamede, R. J., Kow, Y. K. and Wallace, S. S. (1997) *J. Biol. Chem.* **272**, 32230–32239
- Hilbert, T. P., Boorstein, R. J., Kung, H. C., Bolton, P. H., Xing, D., Cunningham, R. P. and Teebor, G. W. (1996) *Biochemistry* **35**, 2505–2511
- Ikeda, S., Biswas, T., Roy, R., Izumi, T., Boldogh, I., Kurosky, A., Sarker, A. H., Seki, S. and Mitra, S. (1998) *J. Biol. Chem.* **273**, 21585–21593
- Hilbert, T. P., Chaung, W., Boorstein, R. J., Cunningham, R. P. and Teebor, G. W. (1997) *J. Biol. Chem.* **272**, 6733–6740
- David, S. S. and Williams, S. D. (1998) *Chem. Rev.* **98**, 1221–1261
- Aguri, L., Lee, Y.-M., Barton, A. B. and Doetsch, P. W. (1997) *Biochemistry* **36**, 721–729
- You, H. J., Swanson, R. L. and Doetsch, P. W. (1998) *Biochemistry* **37**, 6033–6040
- Wilson, III, D. M., Bennett, R. A. O., Marquis, J. C., Ansari, P. and Demple, B. (1995) *Nucleic Acids Res.* **23**, 5027–5033

- 20 Strauss, P. R., Beard, W. A., Patterson, T. A. and Wilson, S. H. (1997) *J. Biol. Chem.* **272**, 1302–1307
- 21 Wilson, III, D. H., Takeshita, M., Grollman, A. P. and Demple, B. (1995) *J. Biol. Chem.* **270**, 16002–16007
- 22 Popoff, S. C., Spira, A. I., Johnson, A. W. and Demple, B. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4193–4197
- 23 Chan, E. and Weiss, B. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3189–3193
- 24 Ramotar, D., Vadnail, J., Masson, J.-Y. and Tremblay, S. (1998) *Biochim. Biophys. Acta* **1396**, 15–20
- 25 Sander, M. and Ramotar, D. (1997) *Biochemistry* **36**, 6100–6106
- 26 Ramotar, D. (1997) *Biochem. Cell Biol.* **75**, 327–336
- 27 Nicolas, E., Goodyer, I. and Taraschi, T. F. (1997) *Biochem. J.* **262**, 5908–5912
- 28 Nicolas, E., Beggs, J. M., Haltiwanger, B. M. and Taraschi, T. F. (1998) *J. Biol. Chem.* **273**, 17216–17220
- 29 Abu-Elheiga, L., Spira, D. and Bachrach, U. (1990) *Exp. Parasitol.* **71**, 21–26
- 30 Demple, B., Johnson, A. and Fung, D. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7731–7735
- 31 Sarker, A. H., Watanabe, S., Akiyama, K., Nakagawa, Y., Wakabayashi, H., Tan, Y. and Seki, S. (1995) *Biochim. Biophys. Acta* **1245**, 299–304
- 32 Masuda, Y., Bennett, R. A. O. and Demple, B. (1998) *J. Biol. Chem.* **273**, 30360–30365
- 33 Gorman, M. A., Morera, S., Rothwell, D. G., de La Fortelle, E., Mol, C. D., Tainer, J. A., Hickson, I. D. and Freemont, P. S. (1997) *EMBO J.* **16**, 6548–6558
- 34 Masson, J.-Y., Tremblay, S. and Ramotar, D. (1996) *Gene* **179**, 291–293
- 35 Hosfield, D. J., Guan, Y., Haas, B. J., Cunningham, R. P. and Tainer, J. A. (1999) *Cell* **98**, 397–408
- 36 Pérez, J., Gallego, C., Bernier-Villamor, V., Camacho, A., González-Pacanowska, D. and Riuz-Pérez, L. M. (1999) *Nucleic Acids Res.* **27**, 771–777
- 37 Olivares, M., Alonso, C. and López, M. C. (1997) *J. Biol. Chem.* **272**, 25224–25228
- 38 Craig, A. G., Waters, A. P. and Ridley, R. (1999) *Parasitol. Today* **15**, 211–214

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