Crystal structure of UvrB, a DNA helicase adapted for nucleotide excision repair

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Nucleotide excision repair (NER) is a highly conserved DNA repair mechanism. NER systems recognize the damaged DNA strand, cleave it on both sides of the lesion, remove and newly synthesize the fragment. UvrB is a central component of the bacterial NER system participating in damage recognition, strand excision and repair synthesis. We have solved the crystal structure of UvrB in the apo and the ATP-bound forms. UvrB contains two domains related in structure to helicases, and two additional domains unique to repair proteins. The structure contains all elements of an intact helicase, and is evidence that UvrB utilizes ATP hydrolysis to move along the DNA to probe for damage. The location of conserved residues and structural comparisons allow us to predict the path of the DNA and suggest that the tight pre-incision complex of UvrB and the damaged DNA is formed by insertion of a flexible β -hairpin between the two DNA strands.

Keywords: crystallography/DNA damage/helicase/nucleotide excision repair/UvrB

Introduction

Maintenance of the correct genetic information is crucial for all living organisms. DNA damage, if not repaired, can cause mutations leading to cancer and may also be involved in aging. A variety of DNA repair mechanisms have been identified such as direct damage reversal, base excision repair, nucleotide excision repair (NER), mismatch repair and recombinational repair (Friedberg *et al.*, 1995). NER is a major DNA repair mechanism, which is highly conserved among all biological systems. Its importance is reflected by the broad substrate range recognized by this system, which includes UV-induced photoproducts, alkylated bases and anti-cancer drug–DNA adducts. In humans, three severe diseases are associated with defects in NER: xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy (Sancar, 1996).

In *Escherichia coli* the three proteins UvrA, UvrB

and UvrC carry out the crucial steps of NER: damage recognition and incision of the damaged template (Van Houten, 1990; Lloyd and Van Houten, 1995; Goosen *et al.*, 1998; Grossman *et al.*, 1998). UvrA forms a heterotrimeric complex with UvrB, the UvrA₂B complex, which has helicase-like properties and specifically binds to damaged DNA. After damage recognition, UvrA dissociates, while UvrB remains bound to DNA in a stable pre-incision complex (Orren and Sancar, 1990). UvrC binds to this complex and triggers the incision four nucleotides 3' from the damaged site, followed by the 5' incision seven nucleotides from the site of damage (Sancar and Rupp, 1983; Lin and Sancar, 1992; Lin *et al.*, 1992). The repair process is completed by UvrD, DNA polymerase and DNA ligase. This multi-step process of DNA recognition and repair ensures the discrimination between damaged and non-damaged DNA.

Within this reaction cascade UvrB plays a central role since it interacts with all the components of excision repair, namely UvrA, UvrC, UvrD (helicase II), DNA polymerase I and DNA (Sancar and Sancar, 1988; Orren *et al.*, 1992). Sequence analysis of UvrB revealed an ATPase site with a Walker type A and B consensus sequence, and ATPase activity is observed in the presence of UvrA and DNA. UvrB's central role in DNA damage processing demands both UvrA and UvrC interacting domains. Residues 114–251 share high homology with the transcription repair coupling factor (TRCF). Since both TRCF and UvrB interact with UvrA, it was proposed that these homologous regions are involved in interactions with UvrA (Selby and Sancar, 1993). Truncated UvrB mutants lacking the C-terminal 43 amino acids are not able to form the UvrB–UvrC–DNA complex and are also strongly impaired in the 3' incision reaction (Moolenaar *et al.*, 1995, 1997).

Sequence comparisons identified six helicase motifs throughout the sequence of UvrB (Gorbalenya *et al.*, 1989) indicating that UvrB is a member of the helicase II superfamily, like the helicases Rad3 and XPD involved in eukaryotic NER (Sung *et al.*, 1987, 1993). In complex with UvrA, UvrB has been shown to have helicase-like activity in a reaction requiring the hydrolysis of ATP (Oh and Grossman, 1987, 1989). It has been proposed that the UvrA₂B complex uses this helicase activity as it tracks along the DNA in search of damage (Koo *et al.*, 1991; Thiagalingam and Grossman, 1993). In contrast, other studies have suggested that the limited strand-separating activity is a direct result of local DNA denaturation in the pre-incision complex (Gordienko and Rupp, 1997). In addition to its possible role of tracking along the DNA, UvrB alters the affinity of the UvrA₂B complex towards more bulky adducts compared with UvrA alone (Snowden and Van Houten, 1991; Visse *et al.*, 1991, 1994b,c). The UvrA dimer is sufficient in recognizing damaged DNA,

Fig. 1. Sequence and structure of UvrB from *B. caldodenax*. (A) Alignment of UvrB sequences from different species (from top to bottom: UvrB from *B. caldodenax*, *Thermus thermophilus* and *E. coli*). The alignment was generated with the programs ClustalW and ALSCRIPT (Barton, 1993; Thompson *et al.*, 1994). The sequence identity between UvrB from *B. caldodenax* and *E. coli* (*T. thermophilus*) is 59% (60%). Secondary structure elements as determined for the apo structure with the program PROMOTIF (Hutchinson and Thornton, 1994) are color coded as in (B). Residues strictly conserved in 16 UvrB sequences are shaded, and helicase motifs (Gorbalenya *et al.*, 1989) are boxed and labeled. (B) Structure of UvrB. Domains 1a, 1b, 2 and 3 are shown in yellow, green, purple and red, respectively. The β -hairpin bridging the gap between domains 1a and 1b is shown in cyan. The ATP molecule bound at the interface between domains 1 and 3 is indicated. This figure as well as Figures 2 and 4 were made with Molscript (Kraulis, 1991) and Raster3D (Merritt and Murphy, 1994).

but it is the UvrA₂B complex that binds to damaged sites with increased specificity and allows efficient DNA damage recognition *in vivo*. Furthermore, this damage processing, which involves bending and unwinding of the DNA (Lin *et al.*, 1992; Visse *et al.*, 1994a; Zou and Van Houten, 1999), leads to a stable UvrB–DNA pre-incision complex serving as a scaffold for binding of UvrC.

We have determined the three-dimensional structure of UvrB from the thermophilic organism *Bacillus caldodenax* at 2.6 Å resolution as a first step in understanding the structural details of NER. UvrB can be divided into four domains, termed 1a, 1b, 2 and 3, with the ATP binding site being located between domains 1a and 3. Two of the domains of UvrB (1a and 3) are structurally related to helicases belonging to superfamilies I and II, and all residues implicated in coupling ATP hydrolysis to strand

translocation in these helicases are present in UvrB as well. The UvrB structure is thus evidence that UvrB functions as a helicase adapted to the unique requirements of DNA repair. One of these requirements is the ability to form a tight pre-incision complex with damaged DNA. Based on the crystal structure we propose that in the pre-incision complex UvrB uses a padlock-like binding mode to wrap around one DNA strand by inserting a β -hairpin between the two strands of DNA.

Results and discussion

The fold of UvrB

We have cloned UvrB from the thermophilic organism *B. caldodenax*. The protein shares high sequence similarity with the *E. coli* protein (Figure 1A) and is able to substitute

Table I. Data collection and MIR statistics

	Nat-Ref	Nat-MIR	Au	Pb	EMTS	PIP	ATP
Maximum resolution (Å)	2.6	2.9	3.0	3.7	3.5	3.5	3.15
Completeness	1 (1)	0.993 (0.99)	1 (1)	1 (1)	0.994 (0.993)	1 (1)	1 (1)
Mean redundancy	10 (7)	7 (7)	7 (8)	8 (8)	6 (6)	6 (6)	6 (7)
R_{sym}	0.09 (0.55)	0.10 (0.60)	0.10 (0.63)	0.20 (0.62)	0.12 (0.42)	0.16 (0.53)	0.14 (0.52)
$\langle I/\sigma I \rangle$	29.4 (3.1)	18.0 (2.9)	16.8 (2.8)	11.4 (3.6)	9.4 (3.1)	13.7 (4.0)	17.7 (3.6)
No. of sites	—	—	2	2	1	4	1
Phasing power iso (ano)	—	—	0.57 (1.00)	1.23 (0.64)	0.44 (0.50)	0.96 (1.11)	—
Mean Figure of merit	—	—	0.454 (to 3.5 Å resolution)				—

$R_{\text{sym}} = \sum_{hkl} \sum_i |I_i - \langle I \rangle| / \sum_i \langle I \rangle$ where I_i is the i th measurement and $\langle I \rangle$ is the weighted mean of all measurements of I . $\langle I/\sigma I \rangle$ indicates the average of the intensity divided by its standard deviation. Numbers in parentheses refer to the respective highest resolution data shell in each data set. Phasing power is the mean value of the heavy atom structure factor amplitude divided by the lack of closure. Au refers to K[Au(CN)₂], Pb to trimethylleadacetate, EMTS to sodium ethylmercurythiosalicylate, PIP to di-μ-iodo-bis-(ethylenediamine) di-platinum II nitrate.

Table II. Refinement statistics

	Apo	ATP complex
Resolution limits (Å)	20.0–2.6	20.0–3.15
No. of reflections	31 926	19 075
No. of protein/	4636	4636
solvent atoms	83 H ₂ O, 2 Zn ²⁺	1 ATP, 1 Mg ²⁺ , 2 Zn ²⁺
R_{cryst} (R_{free})	25.6 (32.4)	26.2 (33.5)
Deviations from ideal values in:		
bond distances	0.016	0.009
angle distances	0.030	0.031
Ramachandran statistics	77.0/18.0/3.1/1.1	70.8/24.5/3.6/1.1

$R_{\text{cryst}} = \|F_o\| - \|F_c\| / \|F_o\|$ where F_o and F_c are the observed and calculated structure factor amplitudes. R_{free} same as R_{cryst} for 5% of the data randomly omitted from refinement. Ramachandran statistics indicate the fraction of residues in the most favored, additionally allowed, generously allowed and disallowed regions, respectively, of the Ramachandran diagram as defined by PROCHECK (Laskowski *et al.*, 1993).

for it in an *in vitro* excision assay (data not shown). The structure of UvrB was solved by multiple isomorphous replacement (MIR) with four heavy atom derivatives (Table I) and subsequent solvent flattening. The current crystallographic model consists of residues 2–186, 189–223 and 225–595 (Table II). Thirty-five residues in domain 2 were modeled as alanines due to lack of side chain density, and the C-terminal 63 residues are missing from the model due to disorder. The R -factor (free R -factor) at the current stage of refinement is 25.6% (32.4%).

The structure consists of four domains named 1a, 1b, 2 and 3 (Figure 1B). Surrounded by domains 1b, 2 and 3, domain 1a is located at the center of the molecule and folds as an $\alpha/\beta/\alpha$ -sandwich. The central β -sheet contains seven parallel strands, in the order 7, 1, 6, 5, 2, 4, 3. Helicase motif I (the ATP binding motif) is located at the C-terminal end of strand 1, and motifs II and III are at the C-terminal ends of strands 5 and 6, respectively. Domain 2 (residues 151–251) contains two anti-parallel β -sheets of four and two strands, respectively, which form a β/β -sandwich. According to sequence similarity, the TRCF (mfd protein) contains a similar domain. Domain 2 and one part of domain 1b (residues 252–323) are inserted between strands 4 and 5 of the central β -sheet. The other part of domain 1b (residues 347–378) is inserted between strands 5 and 6 of the central β -sheet; both sequence stretches are mainly α -helical. Domains 1a and 1b form

a large cleft that is bridged by a β -hairpin (residues 90–115) inserted between strand 3 and an α -helix of domain 1a. Similarly to domain 1a, domain 3 (residues 412–595) folds into an $\alpha/\beta/\alpha$ -sandwich. The parallel β -sheet contains six strands in the order 1, 6, 5, 2, 3, 4, connected by helices or loops on both sides of the sheet. Helicase motifs IV, V and VI are located in this domain, at β -strand 2, β -strand 4 and at the C-terminal end of the helix connecting strands 5 and 6, respectively. A large α -helix and a loop wrap around the domain such that the C-terminus of the model is located close to domain 1a.

The ATP binding site

UvrB binds specifically to ATP or dATP, and ATP hydrolysis is a requirement for NER (Oh and Grossman, 1987). Mutation of Lys45 in the ATP binding motif (helicase motif I) of UvrB results in failure to form the pre-incision complex between UvrB and the damaged DNA (Seeley and Grossman, 1989). To study the structural basis for this ATPase requirement, we have soaked UvrB crystals in Mg-ATP-containing solutions and have solved the structure of the resulting complex (Tables I and II). The cofactor was clearly visible in the difference electron density map, including its triphosphate group. An additional difference density peak close to the β - and γ -phosphates was interpreted as a Mg²⁺ ion. Apparently, the UvrB crystals have not hydrolyzed the ATP during the 24 h of soaking. This is not surprising because full ATPase activity of UvrB requires the presence of both UvrA and DNA (Caron and Grossman, 1988). In addition, if ATP hydrolysis in UvrB were associated with domain movements as observed for related helicases (Kim *et al.*, 1998; Velankar *et al.*, 1999), residual hydrolytic activity would be further inhibited by crystal packing constraints, which prevent these movements.

The ATP molecule is bound to UvrB at the adenine and phosphate moieties (Figure 2). N6 and N7 of the adenine form hydrogen bonds to the side chain of the conserved Gln17 and the carbonyl oxygen of Glu12. The observed pattern of hydrogen bond donors and acceptors explains the specificity for adenine. The hydrophobic residues Pro414 and Tyr11 on either face of the base position N6 and N7 for hydrogen bonding. The phosphate moiety is mainly bound by hydrogen bonds donated from backbone nitrogens of helicase motif I residues Thr41, Gly42, Thr43 and Lys45. Side chains of the conserved residues Glu338

Fig. 2. The ATP binding site. Residues in the vicinity of the ATP are shown in an all-bonds representation. The ATP molecule is shown as a ball-and-stick model and the Mg^{2+} ion is indicated by a sphere; hydrogen bonds are shown as dotted lines.

and Asp339 of helicase motif II point toward the Mg^{2+} ion, but are too distant for direct interactions.

Random mutagenesis of UvrB from *E.coli* has demonstrated the importance of not only motif I, but also motifs V and VI for DNA repair (Moolenaar *et al.*, 1994). For example, the mutant R544H is deficient in DNA repair, shows no helicase activity and its ATPase activity is not activated by DNA in the presence of UvrA. The crystal structure reveals that Arg544 (corresponding to Arg543 in *E.coli*) is located in domain 3 at the interface to domain 1, close to the β - and γ -phosphates of ATP. With the ATP conformation and the domain orientation observed in the crystal, however, Arg543 and also the conserved charged residues Arg540 and Glu510 are too distant from the ATP molecule for direct interactions. The interface between domains 1a and 3 is highly conserved, with most of the helicase motifs and additional conserved residues unique to UvrB located in this region (Figure 3A).

The structural differences between UvrB in the apo and cofactor-bound forms are small, with root mean square (r.m.s.) differences between corresponding C_{α} positions in the two structures of 0.55 Å. Substantial local differences are observed in the backbone around residue Thr41. To make room for the γ -phosphate of ATP, the side chain of Thr41 is displaced; the distance between corresponding C_{β} and C_{α} atoms after superimposing domains 1a of the two structures is 1.7 and 1.1 Å, respectively. A small (2.3°) rotation of domain 3 relative to domain 1a is observed. Calculations of the electrostatic potential show that the interacting surfaces of domains 1a and 3 have opposite charges (Figure 3B). Cycling between apo, ATP- and ADP-bound forms will modulate the electrostatic interactions, which might contribute to domain motions.

The role of the helicase motifs in Mg-ATP binding and ATPase activity has been studied in detail for the helicase PcrA (Soultanas *et al.*, 1999). Structural comparisons of the ATPase site of PcrA in the presence and absence of DNA substrate and cofactor analogs showed that in addition to inter-domain movements, intra-domain movements and changes in side chain conformations are observed. Significantly, the conformation of the cofactor analog was different in the presence and absence of a DNA substrate. The structure of UvrB in complex with ATP clearly shows why the nucleotide can not be hydrolyzed, but it can only suggest which residues are

Fig. 3. Conserved residues and electrostatic potential on the surface of UvrB. For a better view into the ATP binding site, domain 3 has been rotated by 120° away from the remainder of UvrB to show the interface between domains 1a and 3. The ATP molecule has been duplicated in the figure, shown in its orientation with respect to domains 1a and 3. Residues 96–98 and 109–113 were omitted from the surface calculation and are shown as a cyan backbone worm for a better view into the cleft between domains 1a and 1b. (A) Side chains on the surface of UvrB that are conserved throughout 16 UvrB sequences are colored according to their location in helicase motifs I–VI. Magenta, motifs I and IV; green, motifs II and V; blue, motifs III and VI; yellow, conserved residues not belonging to any helicase motif. (B) Electrostatic potential calculated separately for domain 3 and for the remainder of the molecule including the bound ATP, at an ionic strength of 0.1 M contoured at $\pm 10 k_B T$ (k_B is the Boltzmann constant and T the absolute temperature). Blue, positively charged; red, negatively charged. Figures 3 and 5 were made using GRASP (Nicholls *et al.*, 1991).

Fig. 4. DNA binding. (A) Superposition of UvrB with the helicase domain of NS3 from hepatitis C virus complexed with single-stranded DNA. Domain 3 has been superimposed separately to allow for domain movements in UvrB. The r.m.s. deviations between UvrB and NS3 are 1.4 Å for 82 C_{α} atoms in domain 3 and 2.0 Å for 100 C_{α} atoms in domain 1a. The C_{α} atoms of UvrB and NS3 are shown in cyan and red traces, with the β -strands of domains 1a and 3 emphasized as arrows. Domain 2 of UvrB has been omitted for clarity. The DNA bound to NS3 is shown in an all-bonds representation. The β -hairpin loop of UvrB is highlighted. Residues Val432 and Trp501 of NS3 (red) and residues Phe527 and Tyr146 of UvrB (blue) are shown in a ball-and-stick representation. (B) The β -hairpin loop. C_{α} trace of the β -hairpin (cyan) and the neighboring α -helix, which together form the proposed binding site for single-stranded DNA. Selected residues are shown in an all-bonds representation. Salt bridges are indicated by gray dotted lines.

involved in Mg-ATP binding and hydrolysis in the active complex with UvrA and DNA.

Structural similarity to helicases

We searched the known protein structures for similarity to UvrB using the program Dali (Holm and Sander, 1995). The two proteins with highest similarity (Z-scores of 14.8 and 8.8) are the helicases NS3 (Protein Data Bank code 1HEI) and PcrA (Protein Data Bank code 1PJR), which share two structurally related domains with UvrB (Kim *et al.*, 1998; Velankar *et al.*, 1999). Domains 1a and 3 in UvrB correspond to domains 1 and 3 in NS3 and domains 1A and 2A in PcrA. Interestingly, no structural similarities to domain 1b or 2 of UvrB were detected, and no similarities of UvrB to nucleases were found.

The structural similarity of UvrB to helicases is greater than predicted from sequence alignments, which detect homologies in the helicase motifs only. PcrA and NS3 both show domain motion driven by ATP hydrolysis. From the high structural similarity of domains 1a and 3 to helicases, and the high sequence conservation of the domain interface, we conclude that UvrB undergoes domain motions driven by ATP hydrolysis in the presence of UvrA and DNA. The helicase activity of NS3 and PcrA

is attributed to alternate binding and release of the single strand by the two moving domains (Kim *et al.*, 1998; Velankar *et al.*, 1999). If UvrB has a similar mechanism for its helicase-like activity, one would expect to find DNA binding sites in or near domains 1a and 3.

Location of DNA binding sites

We calculated the electrostatic surface potential of UvrB to locate possible DNA binding sites (Figure 3B). While the net charge of the protein is negative and domain 1 has no extended surface area with a positive charge, the surface on domain 3 around helicase motif IV residues is charged positively and might interact with the phosphate backbone of DNA. The DNA-interacting surfaces of UvrB are expected to be conserved. Apart from the conserved residues at the ATP binding site, UvrB has two other surface patches with conserved residues (Figure 3A). One is located in domain 3 at the possible DNA binding site, and the other at the entrance to the opening formed by the β -hairpin and residues of domains 1a and 1b. Because the latter surface patch contains no residues from the helicase motifs, it probably conveys a function that is unique to UvrB.

To analyze whether the DNA binding sites suggested

Fig. 5. Hypothetical model of the UvrB-DNA pre-incision complex. Surface representation of UvrB with the domain orientation as in Figure 4A. Domains 1b and 3 are indicated. The proposed conformation of DNA is shown as phosphate backbone in red with the undisrupted base pairs shown as spokes. Residues Tyr145 and Phe527 are shown in purple and the β -hairpin is depicted in cyan.

for UvrB above correspond to those revealed by the structure of NS3 in complex with DNA (Kim *et al.*, 1998) (Protein Data Bank code 1A1V), we have superimposed the two structures (Figure 4A). To account for domain motions, domain 3 and domain 1a of UvrB were superimposed separately. This results in a domain rotation of 17° from that observed in the crystal structure. The C-terminal end of domain 1 and the N-terminal end of domain 3 do not move far away from each other as indicated by an increase of 4.9 Å in the C_α distance between residues 412 and 413, demonstrating that this reorientation could be accomplished by a hinge motion.

In the superposition, domain 3 of UvrB contacts the backbone of the DNA through conserved residues in helicase motifs IV and V. In the NS3 DNA complex, hydrophobic side chains near domains 1 and 3 (Trp501 and Val432) intercalate between consecutive bases of the DNA, presumably translocating the DNA. Val432 is part of an inter-domain stretch leading into domain 2, and thus has no direct counterpart in UvrB. However, there is a solvent-exposed side chain, Phe527, in close proximity that could act as an intercalator. The second DNA binding site in NS3 is located between domains 1 and 2. In the superposition with UvrB, the cleft between domains 1 and 2 of NS3 aligns with the cleft between domains 1a and 1b of UvrB, and the DNA passes underneath the β -hairpin (residues 90–115) of UvrB. The conserved Tyr146 of UvrB is in close proximity to Trp501 of NS3 and thus might also act as an intercalator. A superposition with PcrA in complex with DNA results in a similar path of DNA with respect to UvrB (not shown).

Structural comparisons and the location of charged and conserved residues thus suggest the same path for the translocated strand during UvrB helicase action. The extent of the proposed DNA binding sites in UvrB differs from that in NS3. In domain 1a/b, the proposed binding site of UvrB would surround the DNA single strand, effectively capturing it, whereas there are fewer possible interactions

in domain 3 of UvrB because of the missing inter-domain stretches. However, it is likely that UvrA strengthens the UvrB-DNA interaction by binding to both UvrB and DNA.

The β -hairpin

The structure of the β -hairpin is shown in detail in Figure 4B. Its tip forms non-bonded contacts with residues of domain 1b. There are two salt bridges, between Glu99 and Arg367, and between Lys111 and Glu307. In addition, the side chains of Tyr101, Tyr108, Leu361 and Phe366 form a small hydrophobic core. These residues are all strictly conserved or, in the case of Tyr108, type-conserved in UvrB. Spanning the gap between the domains, residues Tyr92–Glu99 and Asp112–Asn116 are solvent exposed and have high temperature factors indicating mobility. The content of conserved hydrophobic residues in this region is unusually high and suggests that the hairpin interacts with a hydrophobic binding partner. If single-stranded DNA binds to UvrB between the β -hairpin and domain 1b as suggested above, complex formation or dissociation requires either free DNA ends or a conformational change in UvrB such that the strand can pass between the β -hairpin and domain 1b. The natural substrate for UvrA₂B is damaged double-stranded DNA, which is partially unwound in the complex. Artificial substrates containing unpaired DNA bubble structures are also bound by UvrB, even in the absence of UvrA (Zou and Van Houten, 1999). In both cases, the single-stranded parts of the DNA have no free ends. The suggested binding mode would therefore lock the single strand between the β -hairpin and domain 1b of UvrB. Complex formation and dissociation would require that the β -hairpin acting as a lock is flexible and can open and close. The limited interactions of the β -hairpin with domain 1b and the lack of rigid secondary structure are consistent with this suggested mechanism of UvrB-DNA interaction.

Structural model of the pre-incision complex

UvrB is unable to bind double-stranded DNA, and binds single-stranded DNA only weakly. The pre-incision complex between UvrB and damaged double-stranded DNA formed with the help of UvrA, however, is extremely stable, even at high ionic strength (Orren and Sancar, 1989). It was therefore suggested that in the pre-incision complex UvrB is bound to DNA by intercalation or hydrophobic interactions. On the basis of structural comparisons between UvrB and NS3, the location of conserved residues and the flexibility of the hairpin, we propose that in the pre-incision complex UvrB locks a DNA single strand in the gap between domains 1a and 1b with the β -hairpin acting as a clamp. Opening and closing of the clamp would be slow unless catalyzed by a third component like UvrA. This would explain the low affinity of UvrB for DNA and the high stability of the pre-incision complex once it has formed.

Based on the assumption that one strand of DNA is clamped by the β -hairpin of UvrB we have constructed a model for the pre-incision complex between UvrB and DNA (Figure 5). Studies with double-stranded DNA containing mismatches indicate that UvrB binds if 3–6 bp are disrupted (Zou and Van Houten, 1999). As a starting model, we used coordinates of partially unwound DNA containing a cyclobutane T-T dimer as observed in the

endonuclease V–DNA complex (Protein Data Bank code 1VAS) (Vassilyev *et al.*, 1995). However, to insert the β -hairpin between the DNA strands, the duplex had to be further unwound to open up a total of 5 bp. The orientation of the DNA with respect to domain 3 was modeled based on the interaction of NS3 with single-stranded DNA. The other end of the DNA was modeled pointing away from the surface of UvrB because the electrostatic potential is negative at the exit of the opening. The resulting bend in the DNA is consistent with results from electron microscopy studies, which estimate a bending angle of 130° (Shi *et al.*, 1992). In our model, the conformation of the β -hairpin was kept constant during the docking procedure, but due to the mobility of the hairpin in the crystal structure it seems likely that it will change its conformation upon DNA binding. The model depicted in Figure 5 does not indicate whether UvrB locks the damaged or the undamaged strand, and both cases will be discussed in terms of damage recognition and excision below.

Recognition of DNA damage and dual incision

Recognition of the DNA lesion is accomplished by both UvrA and UvrB. The formation of the pre-incision complex proposed above requires that the DNA is unwound and the β -hairpin moves away from domain 1b for insertion between the DNA strands. Both processes require free energy, which is available either through ATP hydrolysis by UvrA₂B or as a result of complex formation. Two mechanisms of damage recognition leading to the proposed stable pre-incision complex of UvrB with DNA at the site of damage are possible. In the first mechanism, UvrA opens the double strand and UvrB's β -hairpin locks the damaged strand close to but not directly at the site of damage. UvrA₂B then translocates along the locked strand until it stalls upon encountering the lesion, thereby triggering the release of UvrA. In the second mechanism, UvrA opens the double-stranded DNA and moves the β -hairpin of UvrB away from domain 1b. The UvrA₂B complex translocates along the undamaged strand in this open conformation until it dissociates from the DNA after a limited time or encounters the lesion. In the latter case, UvrA would release both the DNA and the β -hairpin, which would resume interactions with domain 1b and thus lock the undamaged strand. The damage recognition in the first mechanism is indirect, recognizing all lesions that interfere with helicase activity because of size or chemical nature. In contrast, the second mechanism requires a more direct interaction of either UvrA or UvrB with the lesion not directly linked to the helicase activity.

The helicase-like activity leading to the proposed pre-incision complex will differ from the inchworm mechanism of NS3 and PcrA (Kim *et al.*, 1998; Velankar *et al.*, 1999) in several respects. While the latter proteins require a single strand–double strand junction as substrate, the substrate of UvrB is double-stranded DNA. In contrast to other helicases, UvrB does not separate long stretches of DNA. Our model of the pre-incision complex demonstrates that it is structurally feasible for the single-stranded DNA to re-anneal after it passes underneath the β -hairpin, allowing strand translocation without strand separation. While PcrA and NS3 each have helicase activity by themselves, UvrB's activity is present only in complex with UvrA. Owing to the lack of structural data on UvrA,

it is not clear how the UvrA dimer binds to the UvrB monomer and where the DNA binding domains of UvrA are located. Biochemical data suggest that UvrA interacts with domain 2 and the disordered C-terminus of UvrB. These binding sites would position UvrA on either side of the β -hairpin such that UvrA could assist UvrB in DNA binding. After dissociation of UvrA, the double-stranded regions on both sides of the unwound DNA fix UvrB in its position without the requirement for strong binding to the single strand. Thus, the proposed pre-incision complex is kinetically trapped rather than thermodynamically stable. In contrast to double-stranded DNA, single-stranded DNA with free ends would be able to escape, in agreement with the observed low binding constants of UvrB for single-stranded DNA (Hsu *et al.*, 1995).

Dual incision takes place after UvrC binds to the pre-incision complex. Biochemical data indicate that the active site for the 5' incision resides in UvrC (Lin and Sancar, 1992); the data concerning the location of the 3' incision are ambiguous (Lin *et al.*, 1992; Moolenaar *et al.*, 1995). If UvrB locks the damaged strand close to the lesion, the 3' incision would have to occur near the cleft between domains 1a and 1b, but there is no indication of a nuclease active site close to the hairpin. More importantly, the incised strand would be free to escape from its locked position without movement of the β -hairpin. In contrast, if UvrB locks the undamaged strand, it would remain bound even after dual incision. Removal of the oligonucleotide and UvrC by UvrD does not require processing of the undamaged strand; UvrB could remain locked to the undamaged strand until DNA polymerase I uses it as a template for resynthesis, displacing UvrB. Thus, we favor a model of the pre-incision complex in which UvrB locks the undamaged strand because it is more consistent with the biochemical data on events following dual incision.

We have solved the crystal structure of the UvrB protein as a first step in understanding the structural basis of damage recognition and processing during NER. UvrB has all the structural properties of a helicase, with a unique binding site for the translocated strand. The pre-incision complex between UvrB and damaged DNA is a key intermediate in excision repair, which links damage recognition to the location of dual incision. Once this complex is formed, UvrB has to remain bound to the DNA without translocating, ensuring precise removal of the damaged fragment. We propose that UvrB wraps a flexible β -hairpin around the undamaged strand and thus locks the DNA in the pre-incision complex.

Materials and methods

Cloning, expression and purification of the *B.caldotenus* uvrB gene product

During sequencing of a 4.4 kb *B.caldotenus* genomic DNA fragment containing the *uvrA* gene (Skorvaga *et al.*, manuscript submitted), we found that the insert also contained 370 bp of the 3' terminus of the *uvrB* gene. A 15 kb DNA fragment was generated using inverse PCR, which carried the complete *uvrB* gene. A more complete description of the cloning will be published elsewhere. In order to achieve high expression and rapid purification of the *B.caldotenus* UvrB protein in *E.coli*, the *uvrB* gene was subcloned into the pTYB1 vector of the T7 IMPACT™ system (New England Biolabs).

The UvrB protein was purified following the T7 IMPACT™ system

manual (New England Biolabs). Cell extracts were prepared by resuspending the cells in 1/50 volume of column/wash buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1 mM EDTA, 0.5% Triton X-100) containing 5 µg/ml leupeptin and 10 µg/ml pepstatin followed by sonication. Cell debris was removed by centrifugation and the supernatant was loaded onto a chitin column. The column was washed with at least 30 vols of column/wash buffer. The on-column cleavage of the fusion protein (UvrB-intein-CBD) was initiated by flushing the column quickly with two column volumes of freshly prepared cleavage buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1 mM EDTA, 30 mM DTT). A third column volume of cleavage buffer was added and cleavage was continued at 4°C overnight. UvrB (>98% pure) was eluted in three column volumes using additional cleavage buffer (without DTT). The appropriate fractions were pooled and dialyzed against storage buffer (50 mM Tris-HCl pH 7.5, 100 mM KCl, 0.1 mM EDTA, 50% glycerol) and concentrated to 1 mg/ml. The average yield was ~2 mg of purified protein per liter.

Crystallization and structure determination

UvrB crystals were grown by hanging drop vapor diffusion. Equal volumes of a solution containing 8 mg/ml UvrB in 500 mM NaCl, 20 mM Tris-HCl pH 8.2, 1 mM DTT, 0.1 mM EDTA, 0.03% dodecyl-maltoside were mixed with a precipitant solution containing 14–18% PEG 6000 or PEG 20 000, 10 mM ZnCl₂ and 100 mM Bicine at pH 9 and equilibrated against a reservoir solution containing 20% PEG 6000, 500 mM NaCl, 100 mM Tris-HCl pH 8.5. Diffraction data of crystals, cryocooled in liquid nitrogen, were collected at beamlines X26C and X25 at the National Synchrotron Light Source in Brookhaven. The crystals belong to space group *P*3₁21 with *a* = *b* = 150.4 Å, *c* = 79.5 Å and contain one molecule per asymmetric unit. The structure of UvrB was solved by MIR. Derivatives were prepared by soaking crystals in solutions containing 500 mM NaCl, 14–18% PEG 6000 or PEG 20 000, 10 mM ZnCl₂, 100 mM Bicine pH 9 and 1–2 mM of the different heavy atom compounds for 24 h (Table I). All data were indexed, integrated and scaled with the HKL software (Otwinowski and Minor, 1997). With exceptions as indicated, the CCP4 suite was used for all further crystallographic computations (Bailey, 1994). The gold derivative was solved by Patterson methods and direct methods using SHELX (Sheldrick, 1990). All other derivatives were solved by difference Fourier calculations. The ambiguity of enantiomorphic space groups and heavy atom handedness was resolved using the anomalous signal of the PIP derivative.

Phase refinement was performed with SHARP (De La Fortelle and Bricogne, 1997) to a resolution of 3.0 Å. Only the gold derivative provided experimental phases up to 3.0 Å resolution, but due to the high solvent content of 68% the quality of the maps was greatly improved after solvent flattening with SOLOMON (Abrahams and Leslie, 1996). The resulting electron density map was of sufficient quality to trace all domains with the exception of domain 2, and to assign side chains with the program O (Jones *et al.*, 1991). This assignment was checked against the results of the secondary structure prediction program PHD (Rost and Sander, 1993) and the known location of the ATP binding motif. The preliminary model was subjected to torsion angle dynamics refinement with X-PLOR (Brünger, 1992) at 2.9 Å resolution.

Because the electron density in the region of domain 2 remained unclear even after combination of MIR and model phases, we performed multi-crystal averaging between the native and the Au derivative data set. This derivative showed differences in cell constants (0.7% in *a* and *b*) and high non-isomorphism to the native data set. A refinement of the model against the derivative data showed that the non-isomorphism was caused by small domain movements. The density modification clearly improved the quality of the map in those regions, and it was possible to trace domain 2. The side chain density of residues 189–223 was weak and this part of UvrB has been modeled as poly-alanine.

Refinement against the 2.6 Å resolution data set was performed using a combination of the programs X-PLOR and REFMAC (Murshudov *et al.*, 1997). All data (no σ -cutoff) between 20 and 2.6 Å resolution were included in the refinement, and partial structure factors for the bulk solvent contribution were calculated in X-PLOR. The model contains residues 2–186, 189–223 and 225–595, two zinc ions and 83 water molecules. The average *B*-factor of all atoms is 70 Å², comparable to the Wilson *B*-factor of 68 Å². The C-terminal residues 596–658 were not visible in the electron density and are thus missing in the model. A mass spectrum of the protein sample and SDS gel electrophoresis of dissolved crystals indicated that the protein is expressed with full length and stays intact in the crystal.

The UvrB-ATP complex was prepared by soaking crystals in a

solution containing 5 mM ATP, 5 mM MgCl₂, 500 mM NaCl, 16% PEG 6000, 10 mM ZnCl₂, 100 mM Bicine pH 9 for 24 h. A difference Fourier map showed clear electron density for an ATP molecule and a Mg²⁺ ion. The coordinates from the apo structure were subjected to rigid body refinement and torsion angle dynamics refinement against the UvrB-ATP diffraction data. The ATP and the Mg²⁺ ion were then included in the model, which was refined with REFMAC and X-PLOR as described for the apo form.

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References

- Abrahams, J.P. and Leslie, A.G.W. (1996) Methods used in the structure determination of bovine mitochondrial F₁ ATPase. *Acta Crystallogr. D*, **52**, 30–42.
- Bailey, S. (1994) The CCP4 suite—programs for protein crystallography. *Acta Crystallogr. D*, **50**, 760–763.
- Barton, G.J. (1993) ALSCRIPT: a tool to format multiple sequence alignments. *Protein Eng.*, **6**, 37–40.
- Brünger, A.T. (1992) *X-PLOR Version 3.1—A System for X-ray Crystallography and NMR*. Yale University Press, New Haven, CT.
- Caron, P.R. and Grossman, L. (1988) Involvement of a cryptic ATPase activity of UvrB and its proteolysis product, UvrB* in DNA repair. *Nucleic Acids Res.*, **16**, 9651–9662.
- De La Fortelle, E. and Bricogne, G. (1997) Maximum-likelihood heavy-atom parameter refinement for multiple isomorphous replacement and multiwavelength anomalous diffraction methods. *Methods Enzymol.*, **276**, 472–494.
- Friedberg, E.C., Walker, G.C. and Siede, W. (1995) *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.
- Goosen, N., Moolenaar, G.F., Visse, R. and van de Putte, P. (1998) Functional domains of the *E. coli* UvrABC proteins in nucleotide excision repair. In Eckstein, F. and Lilley, D.M.J. (eds), *Nucleic Acids and Molecular Biology*. Springer-Verlag, Berlin, Germany, pp. 103–123.
- Gorbalenya, A.E., Koonin, E.V., Donchenko, A.P. and Blinov, V.M. (1989) Two related superfamilies of putative helicases involved in replication, recombination, repair and expression of DNA and RNA genomes. *Nucleic Acids Res.*, **17**, 4713–4730.
- Gordienko, I. and Rupp, W.D. (1997) The limited strand-separating activity of the UvrAB protein complex and its role in the recognition of DNA damage. *EMBO J.*, **16**, 889–895.
- Grossman, L., Lin, C.I. and Ahn, Y. (1998) Nucleotide excision repair in *Escherichia coli*. In Nickoloff, J.A. and Hoekstra, M.F. (eds), *DNA Damage and Repair*. Humana Press, Totowa, NJ, pp. 11–27.
- Holm, L. and Sander, C. (1995) Dali: a network tool for protein structure comparison. *Trends Biochem. Sci.*, **20**, 478–480.
- Hsu, D.S., Kim, S.-T., Sun, Q. and Sancar, A. (1995) Structure and function of the UvrB protein. *J. Biol. Chem.*, **270**, 8319–8327.
- Hutchinson, E.G. and Thornton, J.M. (1994) A revised set of potentials for β turn formation in proteins. *Protein Sci.*, **3**, 2207–2216.
- Jones, T.A., Zou, J.Y., Cowan, S.W. and Kjeldgaard, M. (1991) Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr. A*, **47**, 110–119.
- Kim, J.L., Morgenstern, K.A., Griffith, J.P., Dwyer, M.D., Thomson, J.A., Murcko, M.A., Lin, C. and Caron, P.R. (1998) Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding. *Structure*, **6**, 89–100.
- Koo, H.S., Claassen, L., Grossman, L. and Liu, L.F. (1991) ATP-dependent partitioning of the DNA template into supercoiled domains by *Escherichia coli* UvrAB. *Proc. Natl Acad. Sci. USA*, **88**, 1212–1216.
- Kraulis, P.J. (1991) MOLSCRIPT—a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.*, **24**, 946–950.
- Laskowski, R.A., McArthur, M.W., Moss, D.S. and Thornton, J.M. (1993) PROCHECK—a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.*, **26**, 283–291.
- Lin, J.J. and Sancar, A. (1992) Active site of (A)BC excinuclease: I.

- Evidence for 5' incision by UvrC through a catalytic site involving Asp³⁹⁹, Asp⁴³⁸ and His⁵³⁸ residues. *J. Biol. Chem.*, **267**, 17688–17692.
- Lin, J.J., Phillips, A.M., Hearst, J.E. and Sancar, A. (1992) Active site of (A)BC excinuclease: II. Binding, bending and catalysis mutants of UvrB reveal a direct role in 3' and an indirect role in 5' incision. *J. Biol. Chem.*, **267**, 17693–17700.
- Lloyd, R.S. and Van Houten, B. (1995) DNA damage recognition. In Vos, J.-M. (ed.), *DNA Repair Mechanisms: Impact on Human Diseases and Cancer*. R.G. Landes Biomedical Publishers, Austin, TX, pp. 25–66.
- Merritt, E.A. and Murphy, M.E.P. (1994) Raster3D version 2.0—a program for photorealistic molecular graphics. *Acta Crystallogr. D*, **50**, 869–873.
- Moolenaar, G.F., Visse, R., Ortiz-Buysse, M., Goosen, N. and van de Putte, P. (1994) Helicase motifs V and VI of the *Escherichia coli* UvrB protein of the UvrABC endonuclease are essential for the formation of the preincision complex. *J. Mol. Biol.*, **240**, 294–307.
- Moolenaar, G.F., Franken, K.L.M.C., Dijkstra, D.M., Thomas-Oates, J.E., Visse, R., van de Putte, P. and Goosen, N. (1995) The C-terminal region of the UvrB protein of *Escherichia coli* contains an important determinant for UvrC binding to the preincision complex but not the catalytic site for 3'-incision. *J. Biol. Chem.*, **270**, 30508–30515.
- Moolenaar, G.F., Franken, K.L.M.C., van de Putte, P. and Goosen, N. (1997) Function of the homologous regions of the *Escherichia coli* DNA excision repair proteins UvrB and UvrC in stabilization of the UvrBC–DNA complex and in 3'-incision. *Mutat. Res.*, **385**, 195–203.
- Murshudov, G., Vagin, A. and Dodson, E. (1997) Refinement of macromolecular structures by the maximum likelihood method. *Acta Crystallogr. D*, **53**, 240–255.
- Nicholls, A., Sharp, K.A. and Honig, B. (1991) Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins*, **11**, 281–296.
- Oh, E.Y. and Grossman, L. (1987) Helicase properties of the *Escherichia coli* UvrAB protein complex. *Proc. Natl Acad. Sci. USA*, **84**, 3638–3642.
- Oh, E.Y. and Grossman, L. (1989) Characterization of the helicase activity of the *Escherichia coli* UvrAB protein complex. *J. Biol. Chem.*, **264**, 1336–1343.
- Orren, D.K. and Sancar, A. (1989) The (A)BC excinuclease of *Escherichia coli* has only the UvrB and UvrC subunits in the incision complex. *Proc. Natl Acad. Sci. USA*, **86**, 5237–5241.
- Orren, D.K. and Sancar, A. (1990) Formation and enzymatic properties of the UvrB–DNA complex. *J. Biol. Chem.*, **265**, 15796–15803.
- Orren, D.K., Selby, C.P., Hearst, J.E. and Sancar, A. (1992) Post-incision steps of nucleotide excision repair in *Escherichia coli*. Disassembly of the UvrBC–DNA complex by helicase II and DNA polymerase I. *J. Biol. Chem.*, **267**, 780–788.
- Otwinowski, Z. and Minor, W. (1997) Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.*, **276**, 307–326.
- Rost, B. and Sander, C. (1993) Prediction of protein structure at better than 70% accuracy. *J. Mol. Biol.*, **232**, 584–599.
- Sancar, A. (1996) DNA excision repair. *Annu. Rev. Biochem.*, **65**, 43–81.
- Sancar, A. and Rupp, W.D. (1983) A novel repair enzyme: UvrABC excision nuclease of *Escherichia coli* cuts a DNA strand on both sides of the damaged region. *Cell*, **33**, 249–260.
- Sancar, A. and Sancar, G.B. (1988) DNA repair enzymes. *Annu. Rev. Biochem.*, **57**, 29–67.
- Seeley, T.W. and Grossman, L. (1989) Mutations in the *Escherichia coli* UvrB ATPase motif compromise excision repair capacity. *Proc. Natl Acad. Sci. USA*, **86**, 6577–6581.
- Selby, C.P. and Sancar, A. (1993) Molecular mechanism of transcription–repair coupling. *Science*, **260**, 53–58.
- Sheldrick, G.M. (1990) Phase annealing in SHELX-90—direct methods for larger structures. *Acta Crystallogr. A*, **46**, 467–473.
- Shi, Q., Thresher, R., Sancar, A. and Griffith, J. (1992) Electron microscopic study of (A)BC excinuclease—DNA is sharply bent in the UvrB–DNA complex. *J. Mol. Biol.*, **226**, 425–432.
- Snowden, A. and Van Houten, B. (1991) Initiation of the UvrABC nuclease reaction: extent of incisions is not correlated with UvrA binding affinity. *J. Mol. Biol.*, **220**, 19–33.
- Soultanas, P., Dillingham, M.S., Velankar, S.S. and Wigley, D.B. (1999) DNA binding mediates conformational changes and metal ion coordination in the active site of PcrA helicase. *J. Mol. Biol.*, **290**, 137–148.
- Sung, P., Prakash, L., Matson, S.W. and Prakash, S. (1987) RAD3 protein of *Saccharomyces cerevisiae* is a DNA helicase. *Proc. Natl Acad. Sci. USA*, **84**, 8951–8955.
- Sung, P., Bailly, V., Weber, C., Thompson, L.H., Prakash, L. and Prakash, S. (1993) Human xeroderma pigmentosum group D gene encodes a DNA helicase. *Nature*, **365**, 852–855.
- Thiagalingam, S. and Grossman, L. (1993) The multiple roles for ATP in the *Escherichia coli* UvrABC endonuclease-catalyzed incision reaction. *J. Biol. Chem.*, **268**, 18382–18389.
- Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.
- Van Houten, B. (1990) Nucleotide excision repair in *Escherichia coli*. *Microbiol. Rev.*, **54**, 18–51.
- Vassilyev, D.G., Kashiwagi, T., Mikami, Y., Ariyoshi, M., Iwai, S., Ohtsuka, E. and Morikawa, K. (1995) Atomic model of a pyrimidine dimer excision repair enzyme complexed with a DNA substrate: structural basis for damaged DNA recognition. *Cell*, **83**, 773–782.
- Velankar, S.S., Soultanas, P., Dillingham, M.S., Subramanya, H.S. and Wigley, D.B. (1999) Crystal structures of complexes of PcrA DNA helicase with a DNA substrate indicate an inchworm mechanism. *Cell*, **97**, 75–84.
- Visse, R., de Ruijter, M., Brouwer, J., Brandsma, J.A. and van de Putte, P. (1991) Uvr excision repair protein complex of *Escherichia coli* binds to the convex side of a cisplatin-induced kink in the DNA. *J. Biol. Chem.*, **266**, 7609–7617.
- Visse, R., King, A., Moolenaar, G.F., Goosen, N. and van de Putte, P. (1994a) Protein–DNA interactions and alterations in the DNA structure upon UvrB–DNA preincision complex formation during nucleotide excision repair in *Escherichia coli*. *Biochemistry*, **33**, 9881–9888.
- Visse, R., Moolenaar, G.F., Ruijter, M.D. and van de Putte, P. (1994b) DNA repair by UvrABC *in vitro* analysis of the preincision complex. In Wallace, S.S., Van Houten, B. and Kow, Y.W. (eds), *DNA Damage Effects on DNA Structure and Protein Recognition*. The New York Academy of Sciences, New York, NY, pp. 327–332.
- Visse, R., van Gool, A.J., Moolenaar, G.F., de Ruijter, M. and van de Putte, P. (1994c) The actual incision determines the efficiency of repair of cisplatin-damaged DNA by the *Escherichia coli* UvrABC endonuclease. *Biochemistry*, **33**, 1804–1811.
- Zou, Y. and Van Houten, B. (1999) Strand opening by the UvrA₂B complex allows dynamic recognition of DNA damage. *EMBO J.*, **18**, 4889–4901.

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In another study, the crystal structure of UvrB from *Thermus thermophilus* has been determined. This work has been published while the present paper was under review [Machius, M., Henry, L., Palnitkar, M. and Deisenhofer, J. (1999) Crystal structure of the DNA nucleotide excision repair enzyme UvrB from *Thermus thermophilus*. *Proc. Natl Acad. Sci. USA*, **96**, 11717–11722].