nature structural & molecular biology

Structure of the SWI2/SNF2 chromatin-remodeling domain of eukaryotic Rad54

Nicolas H Thomä[1], Bryan K Czyzewski[1,2], Andrei A Alexeev[3], Alexander V Mazin[4], Stephen C Kowalczykowski[3] & Nikola P Pavletich[1,2]

SWI2/SNF2 chromatin-remodeling proteins mediate the mobilization of nucleosomes and other DNA-associated proteins. SWI2/SNF2 proteins contain sequence motifs characteristic of SF2 helicases but do not have helicase activity. Instead, they couple ATP hydrolysis with the generation of superhelical torsion in DNA. The structure of the nucleosome-remodeling domain of zebrafish Rad54, a protein involved in Rad51-mediated homologous recombination, reveals that the core of the SWI2/SNF2 enzymes consist of two α/β-lobes similar to SF2 helicases. The Rad54 helicase lobes contain insertions that form two helical domains, one within each lobe. These insertions contain SWI2/SNF2-specific sequence motifs likely to be central to SWI2/SNF2 function. A broad cleft formed by the two lobes and flanked by the helical insertions contains residues conserved in SWI2/SNF2 proteins and motifs implicated in DNA-binding by SF2 helicases. The Rad54 structure suggests that SWI2/SNF2 proteins use a mechanism analogous to helicases to translocate on dsDNA.

Cellular processes such as transcription, replication, DNA repair and recombination require direct access to DNA. This process is facilitated by the SWI2/SNF2 family of ATPases, which detach DNA from histones and other bound proteins[1,2]. The SWI2/SNF2 chromatin remodeling enzymes such as BRG1, Ino80, ISWI and MI-2 are components of large protein complexes with varying subunit compositions[3,4]. Eukaryotes contain a variety of additional SWI2/SNF2 enzymes that function outside the major chromatin-remodeling complexes. These include Rad54 involved in the Rad51-mediated recombination processes, the Cockayne syndrome B (CSB) DNA-repair protein implicated in the removal of stalled RNA polymerases, and the Mot1p protein implicated in the removal of the TATA box-binding protein[1].

The SWI2/SNF2 remodeling enzymes share sequence similarity with superfamily 2 (SF2) helicases[5,6]. The similarity is limited to the SF2 catalytic core of two RecA-like helicase domains and in particular to the seven SF2 helicase signature motifs[7,8]. SWI2/SNF2 family members share additional sequence similarity that is not present in helicases; three SWI2/SNF2-specific sequence motifs interspersed among helicase motifs have been described[9–11].

Like the helicases, the SWI2/SNF2 remodeling enzymes can translocate on dsDNA in a manner dependent on ATP hydrolysis but do not catalyze the separation of DNA strands[12]. Instead, SWI2/SNF2 enzymes are characterized by their ability to generate superhelical torsional strain in DNA[13], although it is not known to what extent supercoiling is required for the various remodeling activities.

Rad54 was initially identified as member of the Rad52 epistasis group in *Saccharomyces cerevisiae*[14]. It was subsequently shown to bind to the Rad51 recombinase and to be necessary for the repair of double-strand breaks by Rad51-mediated homologous recombination[15–20]. Like other SWI2/SNF2 remodeling enzymes, Rad54 can translocate on DNA, generate superhelical torsion and enhance the accessibility to nucleosomal DNA[18,19,21]. Rad54 has a primary Rad51-binding site within a ~90-residue N-terminal region that is unstructured and a second weaker site in the remainder of the protein[22–25]. The N-terminal region is expendable for the nucleosome-remodeling activity of Rad54 but is important for its Rad51-specific functions[25,26].

Here we describe the structure of the 647-residue nucleosome-remodeling domain of zebrafish Rad54. The structure reveals that SWI2/SNF2 remodeling enzymes closely resemble SF2 helicases but contain two α-helical domains that are characteristic of the SWI2/SNF2 family and likely play a central role in the remodeling activity.

RESULTS

Overall structure of Rad54

The construct used for crystallization was derived from zebrafish Rad54 (residues 91–738 dnRad54ΔN). *In vitro*, dnRad54ΔN has dsDNA-dependent ATPase activities similar to full-length human Rad54. It can generate supercoiling in closed circular DNA (**Supplementary Fig. 1** and **Supplementary Table 1** online), and the corresponding fragment of fly Rad54 is active in chromatin remodeling[26]. The structure of dnRad54ΔN consists of a 52-residue N-terminal domain (NTD), two RecA-like α/β-domains found in helicases[7,8], two α-helical domains (HD1 and HD2) inserted within each RecA-like domain, and a 75-residue C-terminal extension (CTD) (**Fig. 1**).

The individual RecA-like domains of Rad54 are topologically and structurally most similar to those of the SF2 superfamily of

[1]Structural Biology Program, and [2]Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA. [3]Division of Biological Sciences, Sections of Microbiology and of Molecular and Cellular Biology, University of California, Davis, California 95616-8665, USA. [4]Department of Biochemistry, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102-1192, USA. Correspondence should be address to N.P.P. (nikola@xray2.mskcc.org).

Published online April 3 2005; doi:10.1038/nsmb919

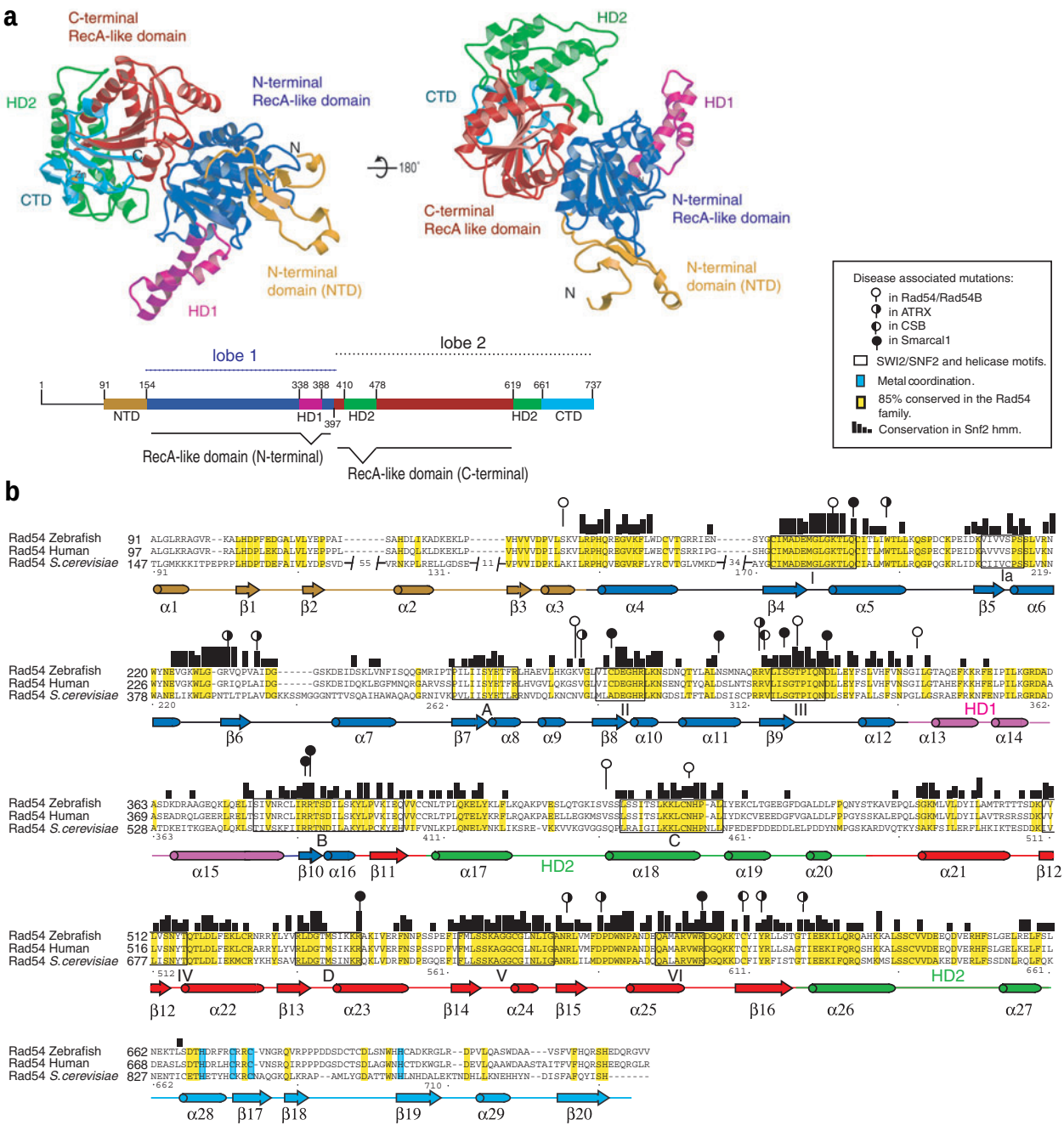

Figure 1 Overview of the structure of zebrafish Rad54 (dnRad54ΔN). (**a**) The structure of the core of Rad54. SWI2/SNF2 specific elements are depicted in magenta (HD1) and green (HD2), the RecA-like helicase domains are blue (lobe1) and red (lobe2), and NTD and CTD are yellow and cyan, respectively. The locations of the individual domains in the sequence are shown below the structure. (**b**) Sequence alignment for zebrafish Rad54 (NCBI: NP_957438), human Rad54 (NP_003570) and *S. cerevisiae* Rad54 (P32863). Conservation within the SWI2/SNF2 family (based on Snf2 hmm) is shown in bar graphs above the alignment; conserved residues in the Rad54 family are highlighted yellow. Helicase specific motifs are referred to with roman numerals[6]; SWI2/SNF2-specific elements are denoted with letters A, B, C[11] and D (this work). Mutations in ATRX (GeneCard: ATRX GC0XM075518), CSB (GeneCard: ERCC6 GC10M050011), Smarcal1 (ref. 38) and Rad54 (GeneCard: Rad54L GC01P046083) and Rad54B (GeneCard: Rad54BGC08M095340) are as indicated.

helicases[27,28], and we will refer to them as the Rad54 helicase lobes. The first helicase lobe of Rad54 is most similar to the replication-fork reversal helicase RecG[29], the RNA helicases eIF4A (eukaryotic translation initiation factor 4A)[30] and mjDEAD[31] (r.m.s. deviations of 2.5–3.1 Å for ~170 Cα positions). The second helicase lobe is most similar to the corresponding domains of RecG, the dsDNA helicase RecQ[32] and the nucleotide excision repair helicase UvrB[33] (r.m.s. deviations of ~2.5 Å for ~135 Cα positions). The relative arrangement of the helicase lobes is similar to those seen in several structures of SF2 and the related SF1 helicases, within ~5° and ~5 Å of the arrangements in RecQ[32] and the herpes simplex virus (HSV) RNA helicase NS3 (ref. 34).

HD1 has a three-helix structure formed by a single 55-residue insertion near the end of the first helicase lobe. HD2, which is formed by two insertions in the second helicase lobe, is larger. It contains 125 amino

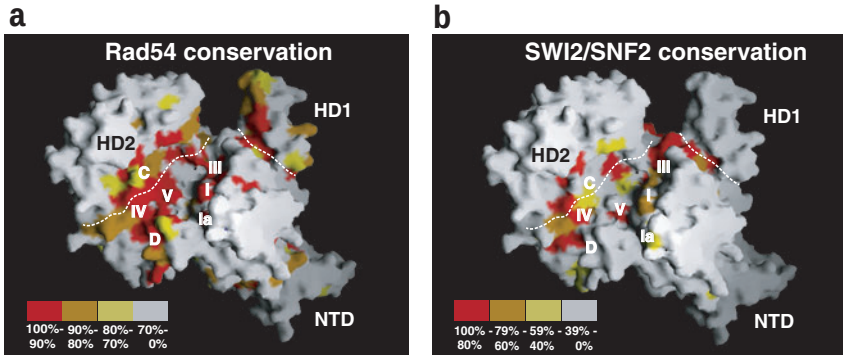

Figure 2 Surface conservation of Rad54. (**a**,**b**) Conservation among Rad54 orthologs (**a**) (see **Supplementary Fig. 2** online for alignment) and conservation within the SWI2/SNF2 family (**b**) (based on the Snf2 hmm; see **Supplementary Methods** online). Both profiles show a conserved patch that starts between HD1 and HD2 and extends over the two helicase lobes. The positions of SWI/SNF-specific motifs (SNF-C, and D) and the helicase motifs involved in DNA binding (I, Ia, III, IV and V) are indicated.

acids and folds into a six-helix structure. HD2 and, to a lesser extent, HD1 contain regions that are highly conserved in Rad54 orthologs and in most other SWI2/SNF2 family members (**Fig. 1**). The NTD structure consists of a three-stranded β-sheet stabilized by three small α-helices and packs extensively with the first helicase lobe (**Fig. 1a**). The CTD has a zinc-stabilized α/β-structure that extends the β-sheet of the second helicase lobe by an additional three β-strands. Both the NTD and CTD sequences are conserved among Rad54 orthologs and the Rad54B paralog but not in other SWI2/SNF2 proteins. The positions of the Rad54 HD1, HD2, NTD and CTD domains coincide with areas where helicases often have accessory domains. Although the precise functions of the accessory domains in helicases vary, they often impart specificity for unique DNA or RNA substrates and also contribute to the strand separating activity[7]. The Rad54 structure contains a cleft between the two helicase lobes, which partially overlaps with the DNA-binding sites of other helicases. This cleft is flanked by HD1 and HD2 and contains residues conserved in the SWI2/SNF2 family (**Fig. 2**).

SWI2/SNF2-specific sequence motifs

Sequence analyses of SWI2/SNF2 proteins initially identified three conserved regions that are distinct from the canonical helicase motifs[6,9,11]. We will refer to them as Snf-A, B and C, as their original designation conflicts with the updated helicase motif nomenclature (**Fig. 1b**). In the Rad54 structure, the position of the 11-residue Snf-A coincides with the helicase motif TxGx involved in DNA binding. The 25-residue Snf-B maps to the end of HD1 and the connection between the two helicase lobes; the 16-residue Snf-C is contained within the first portion of HD2 (**Fig. 1**). To facilitate the analysis of additional SWI2/SNF2-specific features, we constructed a Hidden Markov Model (hmm) profile based on the human proteome (referred to as Snf2 hmm; see **Supplementary Methods** online)[35]. The Snf2 hmm is a position-dependent statistical description of a multiple sequence alignment and clearly indicates that both Rad54 lobes are conserved in the SWI2/SNF2 family. In addition, residues 536–546 in the second helicase lobe are highlighted as a further SWI2/SNF2-specific motif (referred to as Snf-D; **Fig. 1b**).

The first helicase lobe

Analogous to SF1/SF2 helicases, the seven helicase motifs of Rad54 map to the broad interface between the two helicase lobes, typically to connections between the β-strands and α-helices of the α/β-fold[6,8] (**Figs. 1** and **2**). The amino acid sequences and structures of the motifs that bind ATP (motif I) and Mg^{2+} (motif II) are most similar between Rad54 and canonical helicases (see also **Supplementary Table 2** online for more details). In the dnRad54ΔN structure, motif I binds a sulfate group through the side chain of Lys183 and backbone amide groups of Gly180 and Gly182, and these interactions are equivalent to those between helicases and the γ-phosphate of ATP.

The sequences of the remaining motifs in the first helicase lobe (Ia, TxGx and III) are considerably different from those in the SF2/SF1 helicases. Nevertheless, the structure suggests that these motifs overall have similar functions in Rad54. For example, the Rad54 region corresponding to the helicase motifs Ia (Snf2 hmm PlSTl; conserved residues are indicated by uppercase letters) and TxGx (Snf2 hmm TSYEl)[8] preserves the serine/threonine side chains and exposed backbone amide groups that bind to DNA ribose and phosphate groups in the SF2/SF1 helicase structures[8]. Although structural differences exist, such as a one-residue truncation of motif Ia in Rad54 and most other SWI2/SNF2 proteins, they would not preclude the mode of DNA-binding seen in helicase structures. Based on their high degree of conservation among SWI2/SNF2 protein motifs, Ia and TxGx (which corresponds structurally to Snf-A) are also expected to interact with DNA. Consistent with this possibility, the Rad54 structure contains a sulfate ion bound by the backbone amide groups of Ser214 (motif Ia), Tyr269 (TxGx) and Glu270 (TxGx), and the side chain of Arg294 (motif II). The sulfate group is in a position similar to DNA phosphate groups in the DNA-bound structures of the SF2 helicase NS3 (ref. 34) and the SF1 helicase PcrA[36].

Motif III of Rad54 has sequence and structural similarity to SF2 helicases (**Fig. 1b**), but contains additional residues conserved in SWI2/SNF2 proteins. These include Gln323 and Asn324 (Snf2 hmm TGTPlQN), which interact with the SWI2/SNF2-specific HD1 and HD2 domains (see also **Supplementary Table 2**). In helicase structures, motif III interacts with the γ-phosphate group of ATP, DNA and the second helicase domain. This motif has been suggested to be a sensor of ATP hydrolysis[7,8,37].

Other conserved features of the first helicase lobe of Rad54 include the Snf-B motif, which begins near the end of the HD1 insertion and extends into the first strand of lobe2 (**Figs. 1** and **3a**). The Snf-B motif has two highly conserved arginine residues (Arg388 and Arg389 in Rad54)[10,11]. Mutation of the second arginine in the yeast SNF2 protein was detrimental for the *in vivo* function of SWI2/SNF2 (ref. 10), and mutations at the corresponding residue in human SMARCAL1 are associated with the genetic disorder Schimke immuno-osseous dysplasia[38]. The Rad54 structure shows that the Arg389 side chain makes a network of hydrogen bonds in a mostly buried environment, stabilizing the structure around the ATP-binding motif I. The other Snf-B arginine, Arg388, points toward the interlobe channel and binds one of the seven sulfate groups included in the refined model (not shown).

HD1 structure

The HD1 insertion (residues 337–385) folds into three α-helices (α13–α15) in a kinked 'V'-like arrangement (**Fig. 3**). Sequence conservation indicates that HD1 is a feature common to most SWI2/SNF2 proteins, and that the last turn of α15 is part of the Snf-B motif (**Figs. 1c** and **4a**). Overall, the conservation maps primarily to residues involved in interhelix packing and in the packing of HD1 with helicase lobe 1

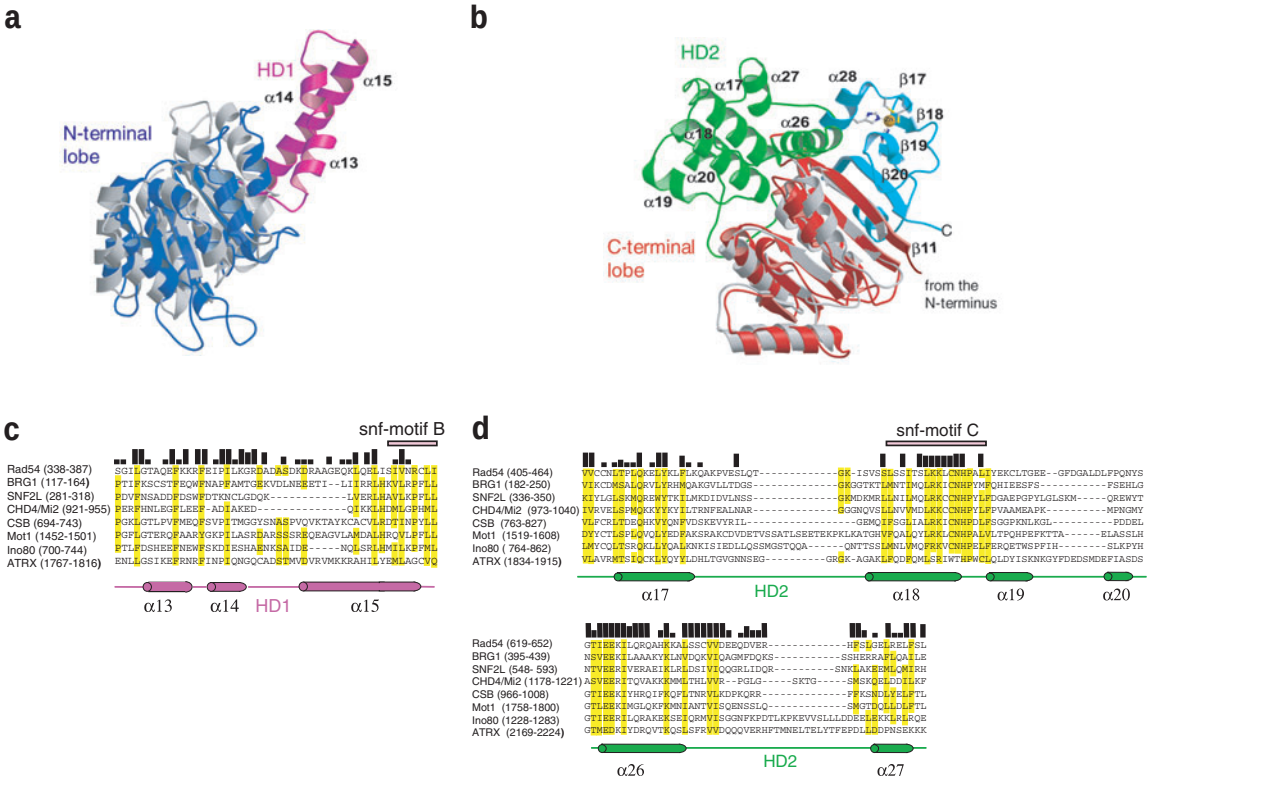

Figure 3 Overview of the SWI2/SNF2-specific domains HD1 and HD2. (**a**,**b**) Superposition of the first (**a**) and second lobe (**b**) of Rad54 with the corresponding domain of RecQ (gray). The dnRad54ΔN structure is color coded as in **Figure 2**, with the numbers referring to strands and α-helices in HD1, HD2 and the CTD. (**c**,**d**) Sequence alignments for the SWI2/SNF2-specific regions HD1 and HD2. The proteins used in the alignments are human BRG1 (AAC97986), SNF2 (P28370), Mi-2 (Q12873), CSB (Q03468), Mot1 (NP_0003963), Ino80 (NP_060023) and ATRX (NP_00480). The bar graph indicates Rad54 family sequence conservation (based on alignment in **Supplementary Fig. 2** online).

(Phe347, Phe351, Leu378 Leu341, Ile355 and Leu356). HD1 also displays subfamily-specific features. Specifically, Pro354, which creates a kink between α13 and α14, is conserved in BRG1, CSB, Mot1 and ATRX but not in ISWI, CHD and Ino80. These last three proteins seem to have shorter α14 and α15 helices (**Fig. 3a**).

The second helicase lobe

The transition from the first to the second helicase lobe occurs over α16, which packs in between the two lobes. The α16 helix, as well as the β-strands before and after (β10 and β11), are part of the Snf-B motif, indicating that other SWI2/SNF2 proteins have a similar structural coupling of the two lobes. This similarity is in contrast to the SF1/SF2 helicases, which have interlobe connections of varying lengths and structures.

The second helicase lobe contains a DNA-binding motif (IV) that is structurally conserved in SF1/SF2 helicases and Rad54 (**Fig. 4b**). In other helicase structures, motif IV interacts with DNA through one or two basic residues, as well as through backbone amide groups at the start of a helix. Although Rad54 contains the solvent-exposed backbone amide groups (Thr517 and Gln518), Rad54 and the remainder of the SWI2/SNF2 family do not contain the basic residues seen in helicases (Snf2 hmm: VLiFSQmt). We note, however, that the side chain of Lys449 from HD2 is at a position similar to the basic side chains of motifs IV seen in SF1/SF2 helicases (**Fig. 5**, inset). Thus, Lys449 not only provides a potential DNA contact that is otherwise absent in motif IV, but also provides a mechanism for coupling HD2 to DNA binding.

The Rad54 Snf-D motif (**Fig. 1c**) is located in a region that is divergent in SF1/SF2 helicases and has a structure most similar to the SF2 helicase NS3 (ref. 34). In NS3, this region contacts RNA through an arginine residue; in Rad54, the side chain of residue Arg546, which is conserved in the SWI2/SNF2 family, is in a similar position.

The helicase motifs V and VI have sequences, lengths and precise roles that seem to vary among the SF1 and SF2 helicases. In the SF1 helicases PcrA and Rep, motif V interacts with DNA[8]. In Rad54 and the remainder

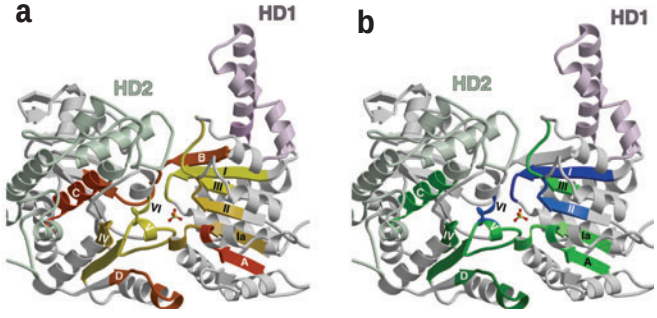

Figure 4 The position of canonical helicase motifs and SWI2/SNF2-specific helicase motifs in Rad54. (**a**) The canonical motifs Ia, II, III, IV, V and VI are yellow, while the SWI/SNF-specific motifs A,B,C and D are brown and red. (**b**) Motifs involved in DNA binding are green, while those mainly involved in ATP binding are blue. A sulfate ion bound to the ATP-binding site is shown in both panels in ball-and-stick.

of the SWI2/SNF2 proteins, motif V (Snf2 hmm: STRAG) has conserved lysine/arginine (Lys568), serine/threonine (Ser566 and Ser567) and glycine (Gly570) residues that could in principle interact with DNA in a manner analogous to PcrA and Rep.

Motif VI, thought to mediate interlobe communication, is characterized by an arginine that is conserved in SF1/SF2 helicase families. Rad54 has an equivalent arginine (Arg600) conserved in the SWI2/SNF2 family (Snf2 hmm: QaqdRaHR). In the crystal structure, Arg600 interacts with a sulfate ion bound in the ATP γ-phosphate-binding site of motif I in a manner analogous to that seen in PcrA.

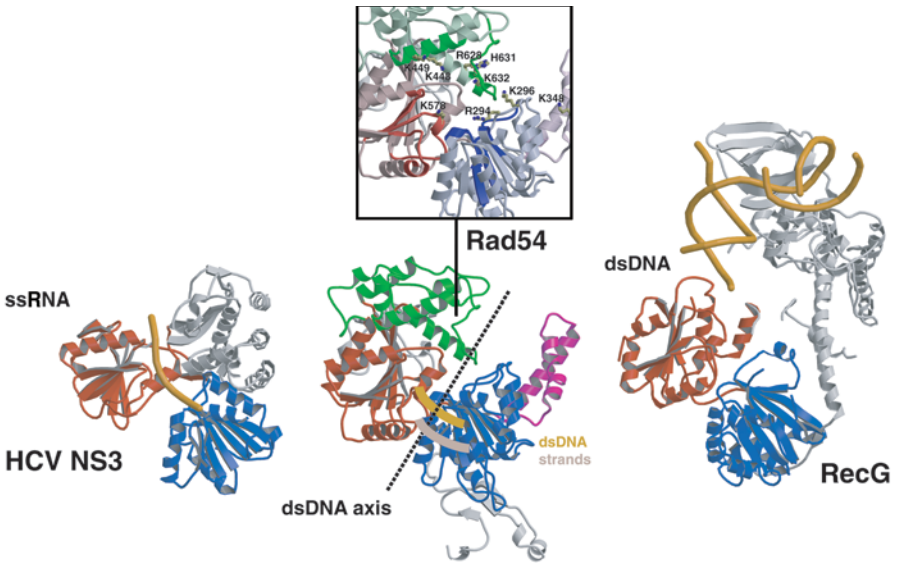

Figure 5 Juxtapositions of nucleic acid–bound RecG and HCV NS3 with the structure of Rad54. The first helicase lobe in RecG, Rad54 and HCV NS3 is blue, the second lobe is red. The double-stranded DNA in RecG and the single-stranded RNA in HCV NS3 are orange. The proposed dsDNA-binding site in Rad54 is shown with an orange line depicting the DNA strand contacting the motifs, while its complementary strand is shown in gray. The dsDNA axis is indicated with a dotted line. Inset, a close-up view of the DNA-binding site and the conserved arginine and lysine residues involved.

HD2 structure

HD2 has a globular structure that consists of six α-helices, four from the first insertion (α17–α20) and two from the second (α26 and α27) (**Fig. 3b**). The HD2 structure is stabilized by a hydrophobic core and by extensive packing interactions with the second helicase lobe. The α17, α18 and α26 helices are highly conserved in the SWI2/SNF2 family, with helix α18 and the start of α19 corresponding to the Snf-C motif (Snf2 hmm: LRKcCNHPyl). Sequence conservation in this area maps either to residues important for HD2 structural integrity and packing with the helicase lobe or to solvent-exposed residues facing the interlobe channel (**Fig. 5**, inset). Solvent-exposed HD2 residues that are conserved in the SWI2/SNF2 family include Lys448 and Lys449 from Snf-C, as discussed earlier for motif IV, and Arg628 and Lys632 from α26.

The packing of HD2 on the helicase lobe is primarily through hydrophobic interactions, except for the packing of α26, which involves buried charged residues. In addition, the loop connecting α26 and α27 (residues 631–646) is poorly ordered in the crystal, even though it is highly conserved among Rad54 orthologs (**Fig. 1c** and **Supplementary Fig. 2** online). These observations suggest that the α26-loop segment may adopt alternate conformations.

The conservation indicates that HD2 is a common accessory domain of the SWI2/SNF2 family and is likely to have a functional role. This notion is supported by mutations of HD2 residues implicated in human disease. For example, residues corresponding to Asn452 and Val438 of dnRad54 are mutated in human RAD54 and RAD54B in non-Hodgkin's lymphomas and adenocarcinomas[39–41], and the residue corresponding to Tyr418 of dnRad54 is mutated in ATRX in X-linked mental retardation[42].

Structures of the Rad54-specific NTD and CTD domains

The NTD (residues 91–144) consists of a curved, three-stranded β-sheet and three short α-helices (**Fig. 1a**). Its sequence is conserved among Rad54 orthologs and paralogs but not in other SWI2/SNF2 proteins (**Fig. 1c** and **Supplementary Fig. 2**). The NTD packs with the first helicase lobe through extensive hydrophobic interactions and seems to be an integral part of the Rad54 structure. Its importance for structural stability is supported by limited proteolysis experiments with human, fly and zebrafish Rad54, which failed to detect a soluble Rad54 fragment lacking the entire NTD. The NTD contains several solvent-exposed hydrophobic residues (Leu94, Phe107, Ala122, Ile126 and Leu133) and basic residues (Arg96, His123, Lys127 and Lys130) on the concave face or edges of the β-sheet. In the structure, the NTD participates in crystal packing via a subset of its exposed hydrophobic residues, raising the possibility that the second, weaker Rad51-binding site[25] is in the NTD.

The CTD (residues 662–738) extends the central β-sheet of the helicase lobe by two additional strands (**Fig. 3b** and **Supplementary Fig. 3** online). This β-sheet extension is flanked by two CTD helices and an α/β-hairpin structure. The entire assembly is stabilized by a metal ion, coordinated by His670, Cys675, Cys678 and His702. Based on the tetrahedral geometry and the nature of the ligands, we tentatively assign the bound metal as a zinc ion. The CTD provides a platform that extends the interlobe cleft and contains a positively charged surface patch formed by seven solvent-exposed basic residues (Arg729, Arg710, Arg707, Lys706, Arg685, Arg682, and Arg676). The CTD structure appears to be unique to Rad54. Several other SWI2/SNF2 proteins such as ATRX have C-terminal extensions that could in principle also serve in extending the helicase lobe β-sheet and the interlobe cleft, but others such as Rad16 lack any C-terminal extensions[9].

DISCUSSION

The Rad54 structure reveals that the SWI2/SNF2 chromatin-remodeling domain has an overall structure similar to SF2 helicases. The two conserved SWI2/SNF2-specific insertions are located where other helicases have accessory domains. There are no major differences that could indicate that SWI2/SNF2 proteins use their RecA-like α/β-domains in ways fundamentally different from SF2 helicases.

Recent structural and mechanistic studies with the RecG and PcrA helicases have shown that translocation on DNA and strand separation are separable activities. The translocation activity resides within the bi-lobal ATPase core, whereas strand separation requires wedge-like DNA-binding accessory domains specific for the particular helicase[7]. In this respect, the lack of helicase activity in SWI2/SNF2 ATPases can

Table 1 Summary of crystallographic analysis

	Native	$KAu(CN)_2$		
Data collection				
Space group	*H*32	*H*32		
Cell dimensions				
a, *b*, *c* (Å)	205, 205, 189	206, 206, 193		
α, β, γ (°)	90, 90, 120	90, 90, 120		
		Peak	*Inflection*	*Remote*
Wavelength (Å)	1.04	1.03909	1.04013	1.02635
Resolution (Å)	3.0	3.9	4.1	3.9
R_{sym} (last shell) (%)	7.8 (38)	8 (34)	8.9 (38)	9.3 (34)
$I / \sigma I$	12.6 (2.3)	32.1 (3.4)	24.1 (2.4)	28.1 (3.2)
Completeness (%)	93.5	92.3	91.1	92.9
Redundancy	3.2	8.7	6.7	8.5
Refinement				
Resolution (Å)	20.0–3.0			
No. reflections	29139			
R_{work} / R_{free}	19.8 / 23.5			
No. atoms				
Protein	5,098			
Ligand/ion	35			
B-factors (Å^2)				
Protein	71.58			
Ligand/ion	89.3			
R.m.s. deviations				
Bond lengths (Å)	0.008			
Bond angles (°)	1.237			

Values in parenthesis are for the highest-resolution shell.

be rationalized by the absence of equivalent wedge-like features in the Rad54 structure.

As expected from their sequences, the structures and interactions of the ATP and Mg^{2+}-binding motifs (I and II) of Rad54 are essentially identical to those in other helicases. In fact, the Rad54 motifs with divergent sequences still preserve the structural and chemical features important for DNA binding and translocation in helicases (motifs Ia, TxGx, IV, Snf-D and V). The similarities between helicases and Rad54 also extend to motifs III and VI, which are involved in interlobe communications and sensing of ATP hydrolysis[8]. These observations suggest that SWI2/SNF2 proteins use the interlobe cleft to interact with and translocate on DNA in a manner mechanistically similar to helicases.

In the structures of the SF2 helicase NS3 bound to ssRNA and of the SF1 helicases PcrA and Rep bound to the single-stranded tails of dsDNA, the single-stranded nucleic acid binds across the two lobes (**Fig. 5**). One end of the nucleic acid interacts with motifs Ia and TxGx of the first lobe, whereas the other end contacts motifs IV and V of the second lobe[8]. In Rad54, a segment of one of the dsDNA strands could readily follow a similar path, with its backbone first interacting with motifs Ia and Snf-A/TxGx and then crossing over to interact with motifs IV, Snf-D and V (**Fig. 5**). This path of the phosphodiester backbone would place the axis of the DNA double helix running approximately along the length of the interlobe cleft toward the other end where HD1 and HD2 are located. This model is in accord with experimental data determining the minimal dsDNA length required to bind Rad54 (**Supplementary Fig. 4** online).

Based on the comparison with the structure of the SF2 helicase RecG[7], we presume that the DNA enters the cleft at the end flanked by HD1 and HD2. In the RecG structure, the DNA is bound to an accessory domain and appears poised to enter the cleft approximately where the Rad54 cleft has a constriction between HD1 and HD2 (**Fig. 5**). The basic residues noted in the CTD would also be near the entry point of DNA between HD1 and HD2, and this domain may help bind DNA as do accessory domains in other helicases[7].

The opening and closing of the interlobe cleft during the ATPase cycle of the helicase is thought to cause the relative DNA-affinities of the two lobes to alternate, resulting in translocation[36]. Since the Rad54 structure preserves many of the helicase elements central to this process, the simplest model for how Rad54 translocates would be one whereby the cleft opening and closing translocates the dsDNA by acting on one of the strands[7]. If the DNA is not released from HD1-HD2 during the translocation cycle, then the DNA-HD1-HD2 interaction could serve as an anchor point and directly give rise to supercoiling. In this model, as Rad54 translocates by following one DNA strand, it will also be twisting around the DNA that is anchored on HD1-HD2, with the intervening DNA segment bulging out as a supercoiled loop whose length increases as Rad54 translocates.

This model has some experimental support in atomic force microscopy studies that have detected supercoiled DNA loops anchored on Rad54 (ref. 43). Although the anchoring of Rad54 on DNA could also result from an oligomeric Rad54 structure, this possibility is less likely because our equilibrium ultracentrifugation measurements show that Rad54 is a monomer in solution (**Supplementary Fig. 5** online) and the yeast SWI2/SNF2 complex contains only one SWI2/SNF2 family ATPase[44].

In principle, several other models can also explain the generation of supercoiling by SWI2/SNF2 proteins. For example, it is possible that the helicase DNA-binding motifs on the two lobes each bind one of the two dsDNA strands. This arrangement could cause DNA unwinding when the lobes open and close during the ATPase cycle, and, coupled to an anchoring mechanism, it could thus give rise to supercoiling. However, translocation in this model would require additional processes during the ATPase cycle that are distinct from those used by helicases.

In summary, the Rad54 structure indicates that SWI2/SNF2 proteins have a bi-lobal helicase domain structurally and functionally closely related to the SF2 helicases. The structure also suggests that the HD1-HD2 features have a central role in the generation of the supercoiling that is characteristic of the SWI2/SNF2 family of chromatin-remodeling enzymes.

METHODS

Protein expression and purification. Rad54 proteins were expressed in insect cells infected with recombinant baculovirus as described in the **Supplementary Methods** online.

Crystallization, data collection and structure determination. Crystals of zebrafish Rad54 were grown in hanging drops equilibrated against 25 mM Tris 8.0, 150 mM NaCl, 0.5–2 M ammonium sulfate, 200–500 mM sodium malonate, 2.5 mM DTT and 0.1 mM trimethyamine HCl. Crystals were harvested after 1 week, slowly transferred into 3 M ammonium sulfate, 230 mM sodium malonate and flash frozen in liquid nitrogen. The crystals belonged to space group *H*32. Native and derivative data sets were collected at the Advanced Photon Source at Argonne National Laboratories on beamlines 8-BM and ID-17. Derivatives were prepared by soaking crystals in 0.4 mM $KAu(CN)_2$ for 1 h before cryotransfer and freezing.

The structure was solved using a three-wavelength MAD experiment based on the gold derivative (**Table 1**). Reflection data were indexed, integrated, and scaled using DENZO and SCALEPACK[45]. Heavy atoms were located using ShelxD[46] and refined using SHARP[47]. Iterative model building and refinement cycles were carried out using O[48] and REFMAC 5.0 (ref. 49) with cross-validation using composite omit maps as implemented in CNS[50].

In the course of refinement, σA-weighted difference Fourier maps showed several strong peaks that could not be modeled as water molecules. Because our

crystals were equilibrated in saturated ammonium sulfate to improve their diffraction limits, we presume that many of these peaks represent well-ordered sulfate ions. The final refined model contains seven sulfate ions that were assigned based on their intensity in difference Fourier maps (18.5–8.6 σ), their proximity to arginine or lysine side chains or to backbone amide groups and the fact that their refined overall temperature factors were lower than 100 Å^2. Inclusion of these seven sulfate ions lowered the R_{free} by 0.7%.

Coordinates. The structure has been deposited in the Protein Data Bank (accession code 1Z3I).

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

ACKNOWLEDGMENTS

The authors thank C. Ogata at APS 8BM, and A. Mulichak and L. Keefe at APS IMCA-CAT ID17, for help during data collection; and M. Lu of the Weill Cornell Medical Center (New York) for help with the analytical ultracentrifugation experiments. N.H.T would like to acknowledge the Human Frontiers Science Program for a long-term fellowship.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 15 February; accepted 17 March 2005
Published online at http://www.nature.com/nsmb/

1. Flaus, A. & Owen-Hughes, T. Mechanisms for ATP-dependent chromatin remodelling: farewell to the tuna-can octamer? *Curr. Opin. Genet. Dev.* **14**, 165–173 (2004).
2. Langst, G. & Becker, P.B. Nucleosome remodeling: one mechanism, many phenomena? *Biochim. Biophys. Acta* **1677**, 58–63 (2004).
3. Flaus, A. & Owen-Hughes, T. Mechanisms for ATP-dependent chromatin remodelling. *Curr. Opin. Genet. Dev.* **11**, 148–154 (2001).
4. Becker, P.B. & Horz, W. ATP-dependent nucleosome remodeling. *Annu. Rev. Biochem.* **71**, 247–273 (2002).
5. Davis, J.L., Kunisawa, R. & Thorner, J. A presumptive helicase (MOT1 gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**, 1879–1892 (1992).
6. Gorbalenya, A.E. & Koonin, E.V. Helicases: amino acid sequence comparisons and structure-function relationships. *Curr. Opin. Struct. Biol.* **3**, 419–429 (1993).
7. Singleton, M.R. & Wigley, D.B. Modularity and specialization in superfamily 1 and 2 helicases. *J. Bacteriol.* **184**, 1819–1826 (2002).
8. Caruthers, J.M. & McKay, D.B. Helicase structure and mechanism. *Curr. Opin. Struct. Biol.* **12**, 123–133 (2002).
9. Eisen, J.A., Sweder, K.S. & Hanawalt, P.C. Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res.* **23**, 2715–2723 (1995).
10. Richmond, E. & Peterson, C.L. Functional analysis of the DNA-stimulated ATPase domain of yeast SWI2/SNF2. *Nucleic Acids Res.* **24**, 3685–3692 (1996).
11. Bork, P. & Koonin, E.V. An expanding family of helicases within the 'DEAD/H' superfamily. *Nucleic Acids Res.* **21**, 751–752 (1993).
12. Cote, J., Quinn, J., Workman, J.L. & Peterson, C.L. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**, 53–60 (1994).
13. Havas, K. *et al.* Generation of superhelical torsion by ATP-dependent chromatin remodeling activities. *Cell* **103**, 1133–1142 (2000).
14. Krogh, B.O. & Symington, L.S. Recombination proteins in yeast. *Annu. Rev. Genet.* **38**, 233–271 (2004).
15. Wolner, B., van Komen, S., Sung, P. & Peterson, C.L. Recruitment of the recombinational repair machinery to a DNA double-strand break in yeast. *Mol. Cell* **12**, 221–232 (2003).
16. Mazin, A.V., Alexeev, A.A. & Kowalczykowski, S.C. A novel function of Rad54 protein. Stabilization of the Rad51 nucleoprotein filament. *J. Biol. Chem.* **278**, 14029–14036 (2003).
17. Alexiadis, V. & Kadonaga, J.T. Strand pairing by Rad54 and Rad51 is enhanced by chromatin. *Genes Dev.* **16**, 2767–2771 (2002).
18. Alexeev, A., Mazin, A. & Kowalczykowski, S.C. Rad54 protein possesses chromatin-remodeling activity stimulated by the Rad51-ssDNA nucleoprotein filament. *Nat. Struct. Biol.* **10**, 182–186 (2003).
19. Jaskelioff, M., Van Komen, S., Krebs, J.E., Sung, P. & Peterson, C.L. Rad54p is a chromatin remodeling enzyme required for heteroduplex DNA joint formation with chromatin. *J. Biol. Chem.* **278**, 9212–9218 (2003).
20. Solinger, J.A., Kiianitsa, K. & Heyer, W.D. Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. *Mol. Cell* **10**, 1175–1188 (2002).
21. Van Komen, S., Petukhova, G., Sigurdsson, S., Stratton, S. & Sung, P. Superhelicity-driven homologous DNA pairing by yeast recombination factors Rad51 and Rad54. *Mol. Cell* **6**, 563–572 (2000).
22. Tan, T.L., Kanaar, R. & Wyman, C. Rad54, a Jack of all trades in homologous recombination. *DNA Repair (Amst.)* **2**, 787–794 (2003).
23. Petukhova, G., Stratton, S. & Sung, P. Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. *Nature* **393**, 91–94 (1998).
24. Golub, E.I., Kovalenko, O.V., Gupta, R.C., Ward, D.C. & Radding, C.M. Interaction of human recombination proteins Rad51 and Rad54. *Nucleic Acids Res.* **25**, 4106–4110 (1997).
25. Raschle, M., Van Komen, S., Chi, P., Ellenberger, T. & Sung, P. Multiple interactions with the Rad51 recombinase govern the homologous recombination function of Rad54. *J. Biol. Chem.* **279**, 51973–51980 (2004).
26. Alexiadis, V., Lusser, A. & Kadonaga, J.T. A conserved N-terminal motif in Rad54 is important for chromatin remodeling and homologous strand pairing. *J. Biol. Chem.* **279**, 27824–27829 (2004).
27. Holm, L. & Sander, C. Protein structure comparison by alignment of distance matrices. *J. Mol. Biol.* **233**, 123–138 (1993).
28. Krissinel, E. & Henrick, K. Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. *Acta Crystallogr. D* **60**, 2256–2268 (2004).
29. Singleton, M.R., Scaife, S. & Wigley, D.B. Structural analysis of DNA replication fork reversal by RecG. *Cell* **107**, 79–89 (2001).
30. Caruthers, J.M., Johnson, E.R. & McKay, D.B. Crystal structure of yeast initiation factor 4A, a DEAD-box RNA helicase. *Proc. Natl Acad. Sci. USA* **97**, 13080–13085 (2000).
31. Story, R.M., Li, H. & Abelson, J.N. Crystal structure of a DEAD box protein from the hyperthermophile *Methanococcus jannaschii*. *Proc. Natl. Acad. Sci. USA* **98**, 1465–1470 (2001).
32. Bernstein, D.A., Zittel, M.C. & Keck, J.L. High-resolution structure of the *E. coli* RecQ helicase catalytic core. *EMBO J.* **22**, 4910–4921 (2003).
33. Machius, M., Henry, L., Palnitkar, M. & Deisenhofer, J. Crystal structure of the DNA nucleotide excision repair enzyme UvrB from *Thermus thermophilus*. *Proc. Natl. Acad. Sci. USA* **96**, 11717–11722 (1999).
34. Kim, J.L. *et al.* 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 (1998).
35. Bateman, A. *et al.* The Pfam protein families database. *Nucleic Acids Res.* **30**, 276–280 (2002).
36. Velankar, S.S., Soultanas, P., Dillingham, M.S., Subramanya, H.S. & Wigley, D.B. Crystal structures of complexes of PcrA DNA helicase with a DNA substrate indicate an inchworm mechanism. *Cell* **97**, 75–84 (1999).
37. Tuteja, N. & Tuteja, R. Unraveling DNA helicases. Motif, structure, mechanism and function. *Eur. J. Biochem.* **271**, 1849–1863 (2004).
38. Boerkoel, C.F. *et al.* Mutant chromatin remodeling protein SMARCAL1 causes Schimke immuno-osseous dysplasia. *Nat. Genet.* **30**, 215–220 (2002).
39. Hiramoto, T. *et al.* Mutations of a novel human RAD54 homologue, RAD54B, in primary cancer. *Oncogene* **18**, 3422–3426 (1999).
40. Smirnova, M., Van Komen, S., Sung, P. & Klein, H.L. Effects of tumor-associated mutations on Rad54 functions. *J. Biol. Chem.* **279**, 24081–24088 (2004).
41. Matsuda, M. *et al.* Mutations in the RAD54 recombination gene in primary cancers. *Oncogene* **18**, 3427–3430 (1999).
42. Wada, T., Kubota, T., Fukushima, Y. & Saitoh, S. Molecular genetic study of Japanese patients with X-linked alpha-thalassemia/mental retardation syndrome (ATR-X). *Am. J. Med. Genet.* **94**, 242–248 (2000).
43. Ristic, D., Wyman, C., Paulusma, C. & Kanaar, R. The architecture of the human Rad54-DNA complex provides evidence for protein translocation along DNA. *Proc. Natl. Acad. Sci. USA* **98**, 8454–8460 (2001).
44. Smith, C.L., Horowitz-Scherer, R., Flanagan, J.F., Woodcock, C.L. & Peterson, C.L. Structural analysis of the yeast SWI/SNF chromatin remodeling complex. *Nat. Struct. Biol.* **10**, 141–145 (2003).
45. Otwinowski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **276**, 307–326 (1997).
46. Schneider, T.R. & Sheldrick, G.M. Substructure solution with SHELXD. *Acta Crystallogr. D* **58**, 1772–1779 (2002).
47. Bricogne, G., Vonrhein, C., Flensburg, C., Schiltz, M. & Paciorek, W. Generation, representation and flow of phase information in structure determination: recent developments in and around SHARP 2.0. *Acta Crystallogr. D* **59**, 2023–2030 (2003).
48. Jones, T.A., Zou, J.Y., Cowan, S.W. & Kjeldgaard, M. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr. A* **47**, 110–119 (1991).
49. Collaborative Computational Project, Number 4. The CCP4 suite: programs for protein crystallography. *Acta Crystallogr. D* **50**, 760–763 (1994).
50. Brunger, A.T. *et al.* Crystallography & NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr. D* **54**, 905–921 (1998).