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Structure of the motor subunit of type I restriction-modification complex EcoR124I

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Type I restriction-modification enzymes act as conventional adenine methylases on hemimethylated DNAs, but unmethylated recognition targets induce them to translocate thousands of base pairs before cleaving distant sites nonspecifically. The first crystal structure of a type I motor subunit responsible for translocation and cleavage suggests how the pentameric translocating complex is assembled and provides a structural framework for translocation of duplex DNA by RecA-like ATPase motors.

Type I restriction-modification enzymes are molecular machines that recognize specific, typically asymmetric, short DNA sequences[1]. Three subunits either act together as a typical methyltransferase or recruit two endonuclease motor subunits that translocate duplex DNA through the stationary enzyme, driven by helicase-like motors that consume about one molecule of ATP per base pair[2–5]. We report the crystal structure of motor subunit HsdR of the *Escherichia coli* pR124 plasmid-borne type I restriction-modification enzyme EcoR124I, revealing structural constraints on assembly and the mechanism of the translocation and cleavage complex.

EcoR124I HsdR with selenomethionine labeling was expressed in *E. coli*, and its structure was solved in complex with Mg^{2+}-ATP at 2.6-Å resolution (**Supplementary Table 1** and **Supplementary Methods** online). HsdR presents four globular domains in a square-planar arrangement, generating prominent grooves between adjacent domain pairs (**Fig. 1a** and **Supplementary Fig. 1** online). Similarity (SSM, http://www.ebi.ac.uk) identifies its endonuclease domain (residues 13–260) and RecA-like helicase domains 1 and 2 (261–461 and 470–731); a helical domain (732–892) has no apparent structural relatives, and residues 893–1038 were confirmed present but disordered.

The endonuclease domain presents the typical αβα core of type II restriction enzymes[6]. Asp151, Glu165 and Lys167 clustered opposite the helical domain are proposed to be involved in catalyzing DNA cleavage[1]. Lys220 on α8 is 3.1 Å from N3 on the exposed edge of ATP bound at the helicase domains (**Fig. 1b**), potentially coupling endonuclease and helicase functions. Adenine stacks on Arg273 and is surrounded by other residues of motif Q. A variant DEAD box (408-DECHR-412) makes the closest approach to ATP, 4.85 Å from Glu409 O1γ to ATP O1ε. Unlike helicases that present a bridging water molecule[7], the position of the Glu409 carboxylate is altered by interaction with Lys313, allowing a small change in the Glu409 Cβ-Cγ dihedral angle to bring O1γ to within ∼3.4 Å of ATP O1ε.

A uniformly positive surface groove with a clear match to the size and shape of duplex DNA (**Fig. 2a**,**b**) proceeds from a canonical helicase cleft in a continuous path down the 'front' of the motor subunit between the helical and endonuclease domains, where the cleavage site is recessed slightly from the surface (**Fig. 2c**). The continuity of exonuclease III footprints of EcoKI–DNA complexes[8] indicates that DNA follows the protein surface without looping; footprinting of EcoR124I used DNAs too short to accommodate HsdR[9]. DNA following the surface groove would be deflected ∼80° from linear, a degree found in other protein-DNA complexes. The helicase cleft superimposes with cocrystal structures of

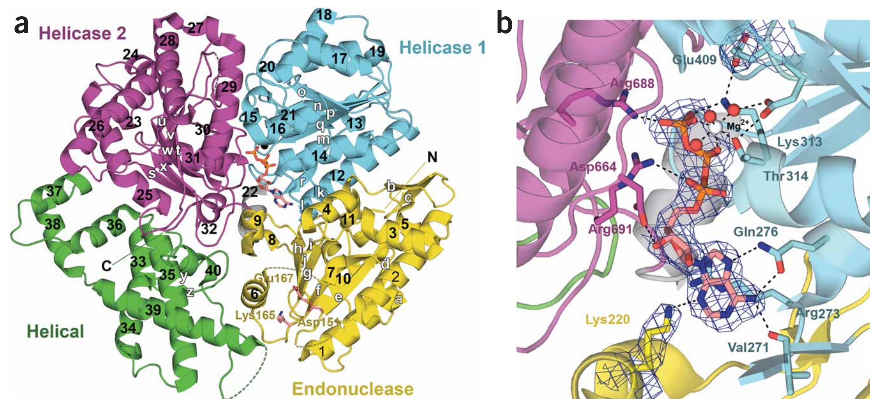

Figure 1 Structure of the motor subunit. (**a**) Planar domain assembly. 'Front' view with helices numbered and strands lettered sequentially from the N to the C terminus. Dashed lines indicate short regions of disorder; unresolved residues 893 to 1038 are not shown. Endonuclease active-site residues and ATP are shown as skeletal models; the black sphere represents Mg^{2+}. (**b**) ATP binding. ATP, Lys220 and Glu409 electron density are shown in blue mesh; the white sphere represents Mg^{2+}; red spheres represent water oxygens. Dashed lines connect atoms that are close enough for bonding.

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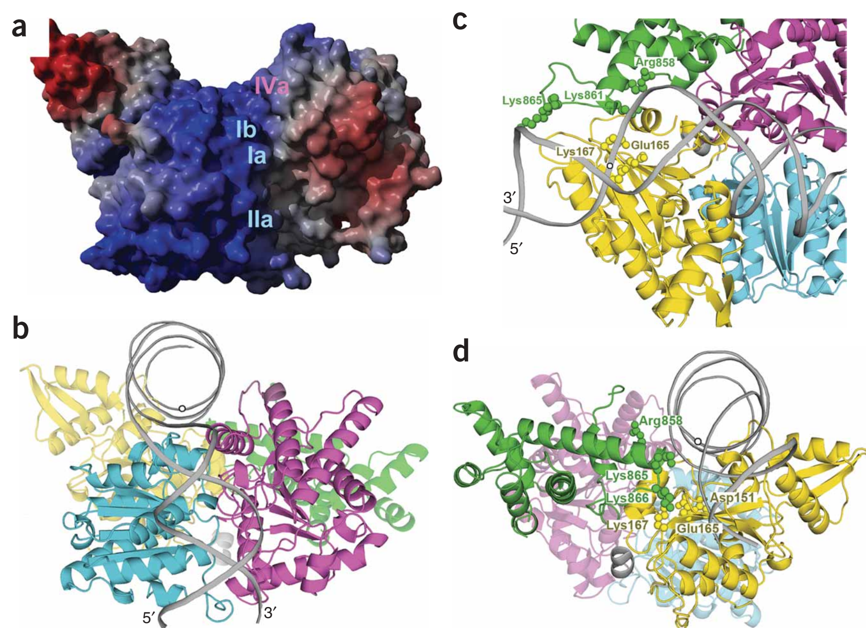

Figure 2 Proposed DNA path. (**a**) Electrostatic potential surface. Blue, positive; red, negative. Shown is the top view onto the helicase cleft with the front of the subunit facing upward; canonical sequence motifs are numbered in helicase domains 1 and 2 (light blue and pink, respectively). (**b**) DNA path. Ribbon model of view in **a**. The DNA backbone model is shown in gray ribbon; the open circle shows the closest approach to the active site. (**c**) Active site. Front view shown, with the top of the subunit at the right. Spheres represent residue side chain atoms: yellow, catalytic (Asp151 unlabeled); green, exit. (**d**) DNA exit. Bottom view with the front of the subunit facing upward. The positions of unresolved Lys865 and 866 between helices 39 and 40 were determined by loop modeling as described in **Supplementary Methods**.

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Rad54–dsDNA[10] and helicases complexed with single-stranded nucleic acids (helicase–ss)[11] and would similarly contact the strand defined in those cocrystals as the 3′-to-5′ strand, which would also contact helix 29 in domain 2 via motif IVa; the 5′-to-3′ strand would contact domain 1 via motif IIa, as in Rad54–dsDNA. Superposition of the HsdR endonuclease core with type II restriction enzymes[6] predicts that the major groove of the 5′-to-3′ strand faces the active site.

Although HsdR belongs to helicase superfamily 2 with RecA-like sequence motifs common to all helicases[1], EcoR124I tracks mainly on one strand of the duplex without strand separation[2–4] and thus acts as a true translocase. The Rad54 translocase structure with dsDNA bound in the helicase cleft showed that one strand superimposes with the single strand of the helicase–ss cocrystal structure, suggesting that transport by Rad54 might use a helicase-like, ATP-driven screw mechanism[10], but the extremely rotated state of crystalline Rad54 helicase domain 2 raised doubt about its similarity to helicases[11]. Transport direction in the helicases is known to occur from the point of strand separation toward the helicase cleft[12], consistent with fluorescence resonance energy transfer (FRET) analysis of Rad54 translocation stages[13]. The path of dsDNA down the front of the HsdR motor subunit supports a common mechanism for helicases and translocases because it predicts transport toward the endonuclease active site, that is, in the same direction as proposed for Rad54.

Given the transport direction, DNA translocation toward the stationary enzyme[2,3] requires that motor subunits flank methyltransferase with their helicase domains facing outward, bringing the HsdR helical domain near methyltransferase as in a cartoon model[14] for EcoKI–DNA based on biochemical and genetic[15] evidence. The axial orientation should align the HsdR surface groove with DNA emerging from methyltransferase, leading to the pentamer model shown in **Supplementary Figure 2** online. Atomic force microscopy (AFM) images of EcoR124I initiation complexes show shortening of the DNA contour length by ~8 nm with one motor subunit and by ~11 nm with two[16], representing contributions from structural alterations and two-dimensional projection. The observed shortening would be in agreement with a pentamer in which HsdR and HsdM abut with little or no overlap, bringing the two DNA bends to ~87 bp apart and thus ~90° out of the helical phase on average B-form DNA.

DNA exits the bottom of the motor subunit where clustered positive residues (**Fig. 2d**) could favor maintenance of the duplex and confine accumulating negative supercoils to the extruding loop[2–4]. Supercoil extrusion is likely to occur between the motor subunit and methyltransferase, where DNA topology can couple methylation status to translocation and cleavage[2].

Accession codes. Protein Data Bank: Coordinates for EcoR124I HsdR have been deposited with accession code 2W00.

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

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