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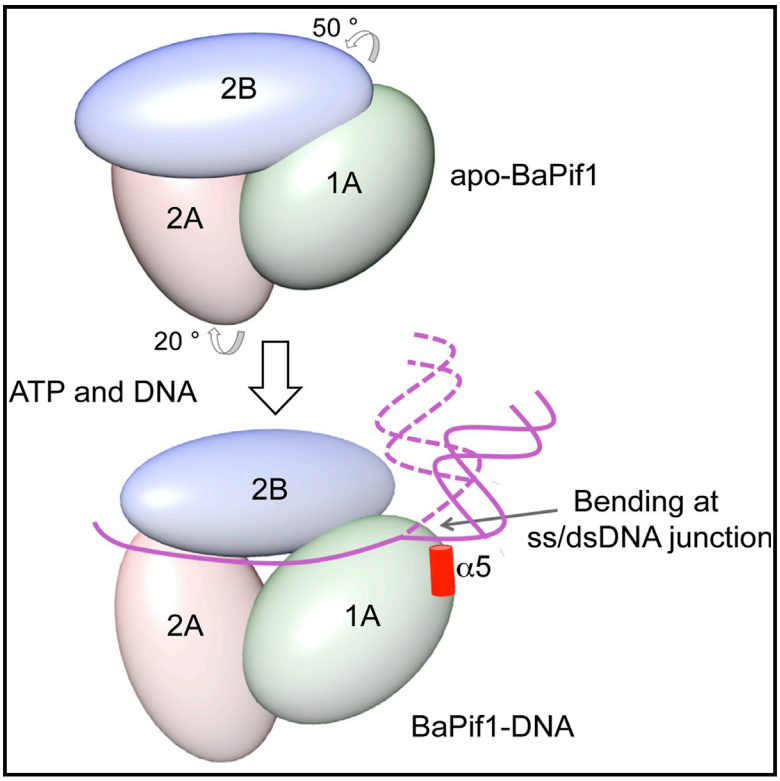
Structural and Functional Insights into the Unwinding Mechanism of *Bacteroides sp* Pif1

Graphical Abstract



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In Brief

Zhou et al. report the crystal structures of the helicase core domain of human Pif1 as well as *Bacteroides sp* Pif1 in different conformational states. The results reveal the functional role of the Pif1 signature motif and provide mechanistic insights into the unwinding activity of the Pif1 family helicases.

Highlights

- Structures of *Bacteroides sp* Pif1 in different conformational states are reported
- The wedge region folds into an extended loop followed by an α helix.
- The Pif1 signature motif indirectly exerts its functional role
- ATP and ssDNA synergistically promote a large conformational change of BaPif1

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Structural and Functional Insights into the Unwinding Mechanism of *Bacteroides sp* Pif1

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SUMMARY

Pif1 is a conserved SF1B DNA helicase involved in maintaining genome stability through unwinding double-stranded DNAs (dsDNAs), DNA/RNA hybrids, and G quadruplex (G4) structures. Here, we report the structures of the helicase domain of human Pif1 and *Bacteroides sp* Pif1 (BaPif1) in complex with ADP-AlF$_4^-$ and two different single-stranded DNAs (ssDNAs). The wedge region equivalent to the β hairpin in other SF1B DNA helicases folds into an extended loop followed by an α helix. The Pif1 signature motif of BaPif1 interacts with the wedge region and a short helix in order to stabilize these ssDNA binding elements, therefore indirectly exerting its functional role. Domain 2B of BaPif1 undergoes a large conformational change upon concomitant binding of ATP and ssDNA, which is critical for Pif1's activities. BaPif1 cocrystallized with a tailed dsDNA and ADP-AlF$_4^-$, resulting in a bound ssDNA bent nearly 90° at the ssDNA/dsDNA junction. The conformational snapshots of BaPif1 provide insights into the mechanism governing the helicase activity of Pif1.

INTRODUCTION

The prototypical member of Pif1 family helicases was first identified from mitochondria of *Saccharomyces cerevisiae* (ScPif1) (Lahaye et al., 1993). Pif1 plays multiple roles in maintaining genome stability in both nucleus and mitochondria. The nuclear functions of Pif1 include the inhibition of telomerase at both telomeres and double-strand breaks (DSBs) (Boulé et al., 2005; Myung et al., 2001; Schulz and Zakian, 1994; Zhou et al., 2000), processing Okazaki fragments (Bochman et al., 2010; Boulé and Zakian, 2006), promoting break-induced replication (Wilson et al., 2013), regulating rDNA replication (Ivessa et al., 2000), and preventing replication pausing and DSBs at G quadruplex (G4) structures (Lopes et al., 2011; Paeschke et al., 2011, 2013).

Pif1 belongs to superfamily 1 (SF1) helicases, which can be divided into two groups based on the directionality of translocation: 3′–5′ for SF1A helicases and 5′–3′ for SF1B helicases (Singleton et al., 2007). SF1B helicases include Upf1-like RNA helicases and Pif1-like DNA helicases (Raney et al., 2013). The Pif1-like helicases include baker's yeasts Pif1 and Rrm3, fission yeast Pfh1, human Pif1, and bacteriophage T4 Dda and RecD (Bochman et al., 2010). Pif1-like helicases share seven conserved motifs—I, Ia, II, III, IV, V, and VI—common to SF1 enzymes as well as three additional motifs, A, B, and C (Bochman et al., 2011) (Figure S1). A degenerate sequence located between motifs II and III, termed the Pif1 "signature motif," is only found in Pif1 helicases and not RecD or Dda (Bochman et al., 2011). However, the function of this signature motif is not known.

Biochemical studies showed that Pif1 in ScPif1 inhibits telomerase function (Zhou et al., 2000) by removing telomerase from telomeric DNA (Boulé et al., 2005), and the process involves unwinding of the DNA/RNA duplex formed in the telomerase-telomere stalled complex (Boulé and Zakian, 2007; Ramanagoudr-Bhojappa et al., 2013; Zhang et al., 2006). ScPif1 and Human Pif1 (hPif1) require single-stranded DNA (ssDNA) region for binding and prefers forked DNA structures to non-forked structures for unwinding (George et al., 2009; Gu et al., 2008; Lahaye et al., 1993). The N-terminal domain of hPif1 enhances interaction with ssDNA but is not essential to carry out its helicase functions (Gu et al., 2008). Kinetic analysis showed that ScPif1 exhibits a single-base-pair kinetic step for unwinding duplex DNA, powered by hydrolysis of one molecule of ATP (Ramanagoudr-Bhojappa et al., 2013). ScPif1 preferentially binds G4 (Paeschke et al., 2013), and the conserved helicase domain of ScPif1 and hPif1 possess both G4- and dsDNA-unwinding activities (George et al., 2009; Paeschke et al., 2011; Ribeyre et al., 2009; Sanders, 2010). A more recent kinetic analysis showed that ScPif1 binds to a parallel quadruplex DNA tightly but unfolds it slowly (Byrd and Raney, 2015).

It has been reported that DNA binding induces dimerization of ScPif1 (Barranco-Medina and Galletto, 2010) and that dimerization is not required for the helicase to translocate efficiently on ssDNA (Galletto and Tomko, 2013). Recently, single-molecule