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Note

Identification of biotin carboxyl carrier protein in *Tetrahymena* and its application in *in vitro* motility systems of outer arm dynein

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ABSTRACT

Axonemal dynein plays a central role in ciliary beating. Recently, a functional expression system of axonemal dynein was established in the ciliated protozoan *Tetrahymena*. This study identifies biotin carboxyl carrier protein (BCCP) in *Tetrahymena* and demonstrates its application in *in vitro* motility systems of outer arm dynein.

Ciliary movement is driven by axonemal dyneins and the impaired motility of the dyneins causes primary ciliary dyskinesia (PCD) (Badano et al., 2006; Marshall, 2008; Satir and Christensen, 2008). Axonemal dyneins are large AAA$^+$ (*A*TPases *a*ssociated with diverse cellular *a*ctivities)-type motors (500–1500 kDa) and are divided into inner arm and outer arm dyneins (Gibbons, 1995; Vale, 2000; Kamiya, 2002). In *Tetrahymena*, there are a total of 23 axonemal dynein heavy chain genes (Wilkes et al., 2008). Axonemal dyneins are activated in a coordinated manner during ciliary beating, and this coordinated activation is a specific property of axonemal dyneins. These motors could be utilized as unique motile elements for nanoscale devices in the field of nanobiotechnology (Bachand et al., 2014; van den Heuvel and Dekker, 2007; Hess, 2011).

An expression system for the genetic engineering of axonemal dynein has recently been developed in *Tetrahymena* (Edamatsu, 2014). Although this expression system is useful for functional and structural studies of axonemal dyneins and ciliary movement, a versatile system for tagging axonemal dyneins is needed to further advance molecular studies of these proteins. In this study, a versatile BCCP tag was identified in *Tetrahymena* and applied to *in vitro* motility systems of axonemal dynein.

BCCP is one part of a biotin-dependent enzyme and is found in most prokaryotes and eukaryotes (Chen et al., 2012). BCCP consists of approximately 70 amino acids and is biotinylated by biotin protein ligase (BPL) in culture. The high affinity and specificity of BCCP for avidin offers an advantage in the binding of biotinylated motors to avidin-conjugated or coated materials.

It has been reported that intrinsic BCCP is efficiently biotinylated in host cells (Healy et al., 2010; Polyak et al., 2001); therefore, a search for the BCCP sequence was performed in the *Tetrahymena* Genome Database (TGD) Wiki (http://ciliate.org/index.php/home/welcome). The BCCP family is classified into 4 subfamilies, and 15 representative species from each subfamily [listed in (Chen et al., 2012)] were used for the homology search. One uncharacterized protein (gene ID: TTHERM_00502240) homologous to carbamoyl phosphate synthase (CPSase) was identified in the database. The domain structure of this protein is shown in Fig. 1A. The C-terminus of the protein is a BCCP-like domain containing the biotin-attachment consensus sequence (Healy et al., 2010) (Fig. 1B and D). As described below, this domain was biotinylated in *Tetrahymena* cells and was thus identified as *Tetrahymena* BCCP (*Tt*BCCP). *Tt*BCCP was close to subfamily C members in the phylogenetic analysis (Fig. 1E). Fig. 1C shows a homology model of *Tt*BCCP based on the structure of *Escherichia coli* BCCP. *Tt*BCCP was found to lack the "thumb loop", which contributes to substrate specificity in the BPL enzyme (Healy et al., 2010) (Fig. 1C and D).

Next, a *Tt*BCCP-fused outer arm dynein was generated. The *Tetrahymena* outer arm dynein forms a three-headed structure comprising alpha (DYH3), beta (DYH4) and gamma (DYH5) heavy chains (Wilkes et al., 2008). The expression cassette targeting the N-terminus of DYH3 is shown in (Fig. 2A and B). The PCR primers used in this study are shown in Supplemental Table S1. Transformations were performed according to (Edamatsu, 2014), and homologous recombination was confirmed by PCR analysis (Fig. 2C and D). Cilia were labeled with

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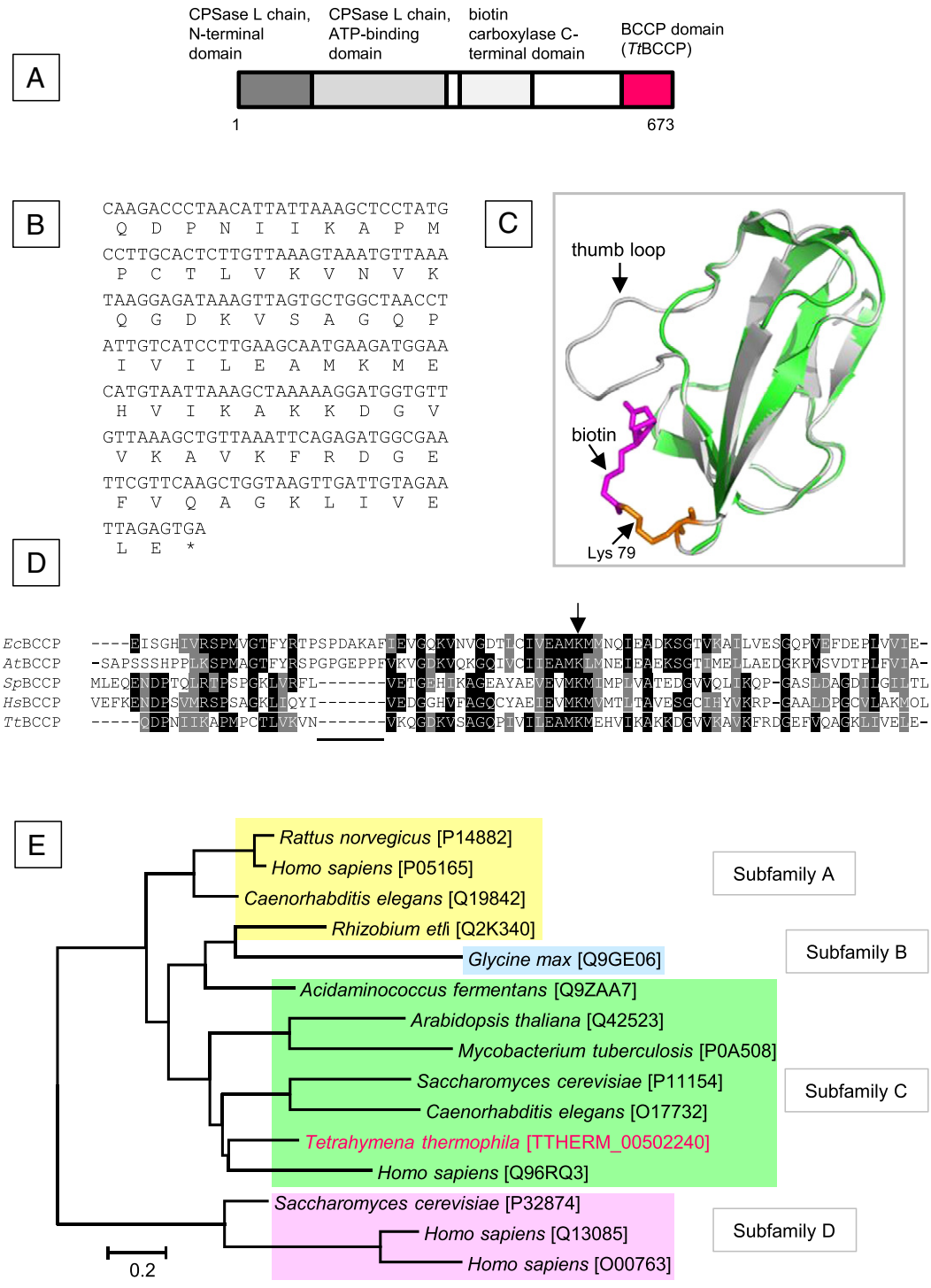

Fig. 1. Identification and characterization of *Tt*BCCP. (A) Domain structure of the CPSase-like protein identified in *Tetrahymena*. (B) Nucleotide and deduced amino acid sequences of *Tt*BCCP. (C) Homology modeling of *Tt*BCCP using SWISS-MODEL tools (Guex and Peitsch, 1997; Schwede et al., 2003; Arnold et al., 2006). *Tt*BCCP (green) and *Escherichia coli* BCCP (gray, PDB code 1 BDO) were superimposed using PyMOL software (http://www.pymol.org). The biotinylated lysine in *E. coli* BCCP is also shown. (D) Alignment of BCCP domains. The aligned sequences are *E. coli* BCCP (*Ec*BCCP) [P0ABE0], *Arabidopsis thaliana* BCCP (*At*BCCP) [Q9LLC1], *Schizosaccharomyces pombe* BCCP (*Sp*BCCP) [P78820], *Homo sapiens* BCCP (*Hs*BCCP) [Q13085], and *Tetrahymena thermophila* (*Tt*BCCP) [TTHERM_00502240]. The arrow represents the biotin attachment site. The underline represents the thumb loop. (E) Phylogenic tree of BCCPs constructed using MEGA 6 software (http://www.megasoftware.net).

Cy3-streptavidin (Cy3-SA) in permeabilized cells (Fig. 2E). The *Tetrahymena* oral apparatus was also labeled with Cy3-SA because it contains a large number of cilia. In control experiments, fluorescence in the cilia and in the oral apparatus was quenched by preincubation of Cy3-SA with 5 mM biotin (Fig. 2E).

Biotinylation of *Tt*BCCP-dynein was performed in proteose peptone medium (containing 74 nM biotin, as estimated from the manufacturer's manual), and the addition of 0–100 μM biotin to the medium did not significantly enhance biotinylation of the recombinant dynein (Fig. 2F). *Tt*BCCP-dynein was purified from ciliary extracts