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Motor domain-based motility system and motile properties of alpha heavy chain in *Tetrahymena* outer arm dynein

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Axonemal dynein plays an essential role in ciliary motility, and impaired ciliary motility causes human diseases such as primary ciliary dyskinesia (PCD). The motor domain of axonemal dynein powers ciliary motility and its function is regulated by several accessary proteins bound to the tail region. Therefore, to understand the essential properties of dynein motility, examining the motile properties of the motor domain without the tail is necessary. In this study, the functional motor domain of the alpha heavy chain in *Tetrahymena* outer arm dynein was purified, and its motile properties were examined using an *in vitro* motility system. The purified protein caused microtubules to glide at a velocity of 5.0 μm/s with their minus-end trailing, and motility was inhibited in an ATP concentration-dependent manner, which is in contrast with kinesin1. This method could be applicable to other axonemal dyneins and will enable further molecular studies on diverse axonemal dyneins and ciliary motility.

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1. Introduction

In motile cilia and flagella, nine peripheral doublet microtubules surround the central pair of singlet microtubules (9 + 2 structure). The peripheral doublets are associated with outer and inner arm dyneins that produce the force required for ciliary and flagellar motilities. Dysfunction of axonemal dynein causes the most prominent ciliopathy, primary ciliary dyskinesia (PCD), which is associated with respiratory symptoms, male infertility and *situs inversus* [1–3].

Dynein is a large AAA+ ATPase and is divided into a catalytic motor domain and a tail region [4,5]. The motor domain contains four P-loops implicated in ATP hydrolysis, and the tail binds to peripheral doublet microtubules and several accessary proteins such as intermediate chains (IC) and light chains (LC) that regulate dynein function.

Axonemal dyneins have unique properties that are not found in other motor proteins. In ciliary beating, the dyneins on doublet microtubules must be locally activated in a coordinated manner to generate a ciliary waveform. In addition, the motility of axonemal dyneins is affected by the concentration of ATP and/or ADP [6,7]. In *Tetrahymena*, permeabilized cells were shown to swim in a wide range of ATP concentrations but were immotile in concentrations higher than 7 mM ATP, and somatic axonemes trembled in an uncoordinated fashion at high ATP concentrations [6]. ATP concentration-dependent inhibition has also been observed in other organisms [6,7]. Axonemal dyneins have been implicated in this phenomenon, but the molecular mechanism underlying this process remains to be elucidated.

Recently, a functional expression and *in vitro* motility system of axonemal dynein heavy chain (HC) has been developed in *Tetrahymena* [8,9]. Although this expression system is useful for functional and structural studies of axonemal dyneins, another motility system is still needed to investigate the essential properties of dynein motility because the purified protein contains a second microtubule-binding site in the tail and is associated with several accessary proteins, such as ICs and LCs, that regulate dynein function.

In this study, the functional motor domain of axonemal dynein was purified, and an *in vitro* motility system for the motor domain was established and revealed the unique motile properties of the alpha HC of *Tetrahymena* outer arm dynein.

2. Materials and methods

2.1. Strains and culture conditions

Wild-type *Tetrahymena thermophila* strains B2086.1 and CU428.1 were obtained from the Stock Center for *T. thermophila* at Cornell University (Ithaca, NY, USA). *Tetrahymena* cells were

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grown in SPP medium (1% protease peptone, 0.1% yeast extract, 0.2% glucose and 0.003% Fe–EDTA) at 30 °C.

2.2. Construction of the expression cassette and transformation

The genomic sequence of the DYH3 gene (gene ID: TTHERM_01276420) was obtained from the *Tetrahymena* Genome Database (TGD) Wiki (http://ciliate.org/index.php/home/welcome). The expression cassette was constructed as previously described [9]. The F1 (1.8-kb untranslated region of DYH3), neo (neomycin resistance gene), BTU1P (beta-tubulin 1 promoter) and F2 (6.8-kb tail region of DYH3, HRV-FLAG-*Tt*BCCP and 2.2-kb fragment of DYH3 motor domain) regions were sequenced and sequentially introduced into the pGBKT7 vector (Clontech; Santa Clara, CA, USA). The PCR primers used in this study are listed in Supplemental Table S1. Transformation was performed as previously described [8].

2.3. PCR analysis

Genomic DNA was isolated as previously described [8]. The primers for PCR analysis are listed in Supplemental Table S1. The PCR products were analyzed using agarose gel electrophoresis and visualized by GR Green nucleic acid stain (LabSupply; Dunedin, New Zealand). Homologous recombination was also confirmed by sequencing the corresponding region.

2.4. Fluorescence microscopy

Permeabilized *Tetrahymena* cells were prepared as previously described [6]. The permeabilized cells were incubated with 30 nM Cy3-streptavidin (Life Technologies; Tokyo, Japan) for 30 min on ice and observed by fluorescence microscopy (BX60, Olympus; Tokyo, Japan).

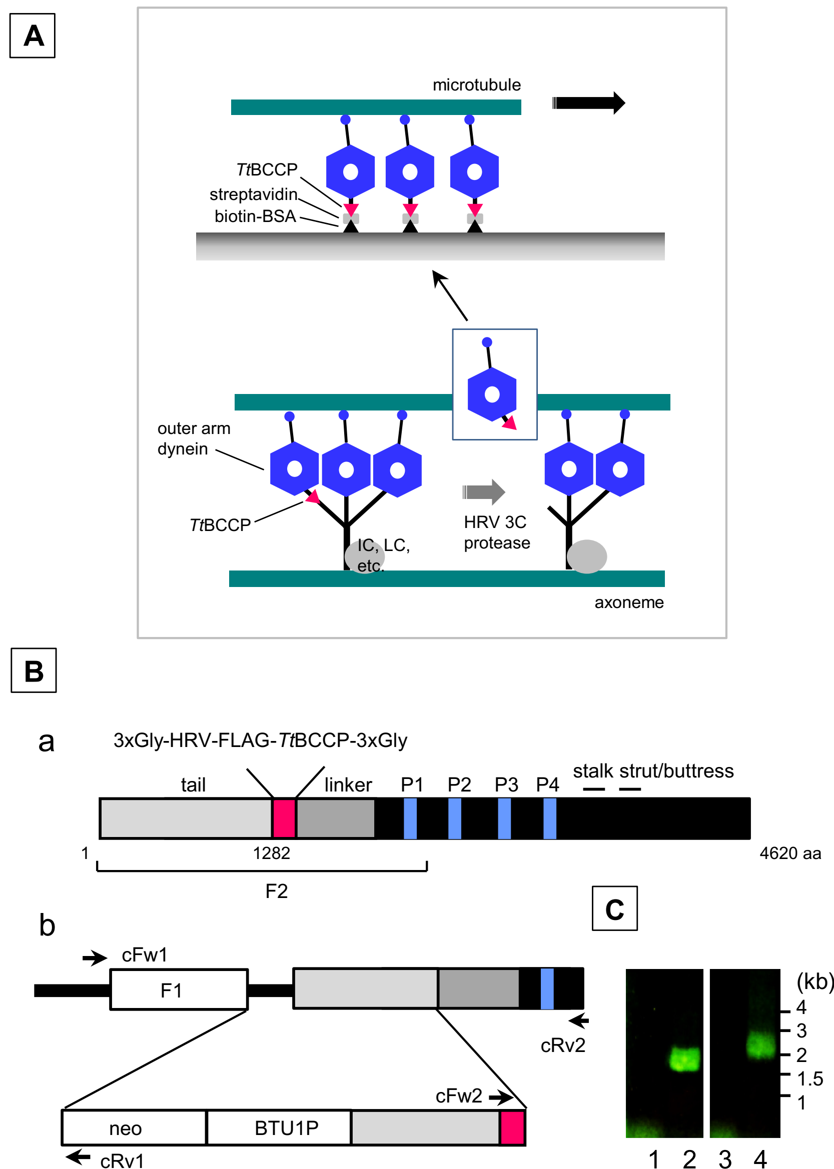

Fig. 1. Motor domain-based motility system. (A) Schematic representation of the purification and motility system. (B) Construction of the expression cassette and PCR analysis. (a) Domain structure of DYH3. The *bracket* shows the region used for the expression cassette. (b) Homologous recombination of the expression cassette into the DYH3 locus. The arrows represent the positions of the primers used in the PCR analysis. (C) PCR analysis. Lanes 1 and 2: PCR using cFw1 and cRv1 primers; lanes 3 and 4: PCR using cFw2 and cRv2 primers. Lanes 1 and 3: wild-type; lanes 2 and 4: transformant.

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