X-ray diffraction patterns from flagellar axonemes of *Chlamydomonas*

Cilia and flagella are motile organelles found in various types of eukaryotes. Their scaffold, called the axoneme, is composed of nine doublet microtubules (doublets) surrounding a pair of singlet microtubules and forms highly regular arrays of such axonemal components on doublets as dynein arms and radial spokes [1]. Within the axoneme, dynein arms generate shear between adjacent doublets, and this shear is then converted into complex three-dimensional waveforms. The mechanism of this conversion has remained a long-standing unresolved issue. The investigation of this mechanism requires structural information at near-atomic-level resolution.

Because X-rays have such short wavelengths (approximately 0.1 nm), X-ray diffraction techniques can potentially reveal the structures of biomolecules or their assemblies at an atomic resolution. X-ray diffraction is particularly useful when the target molecules are periodically arranged because X-rays scattered by periodic arrays interfere with each other and generate strong signals. In addition, the high penetration of X-rays allows the technique to be applied to functioning natural structures in an aqueous environment. An axoneme with its highly arranged components is therefore a potentially suitable target for X-ray diffraction.

A useful model system for experiments on the structure and function of eukaryotic cilia and flagella is provided by a genus of green algae, *Chlamydomonas*, because of the high homology of axonemal components with humans and a large repertoire of axoneme mutants [2]. Using *Chlamydomonas*, we explored the spatial arrangement of axonemal components under physiological conditions by small angle X-ray fiber diffraction. The axonemes were oriented in a physiological solution by continuous shear flow and were exposed to intense and stable X-rays generated in the synchrotron radiation facility SPring-8 at beamline **BL45XU** [3].

Flow alignment of flagellar axonemes

We used the apparatus designed for the spatial arrangement of fibrous biological materials [4] to apply shear flow to a suspension containing flagellar axonemes of *Chlamydomonas*. The apparatus for shear flow alignment consisted of two round coverslips (diameter 17 mm) that faced each other across a small gap (0.1–0.35 mm) filled with a suspension of axonemes (Fig. 1). The suspension was held in place by surface tension. One of the coverslips remained stationary while the other was rotated by a motor. X-ray

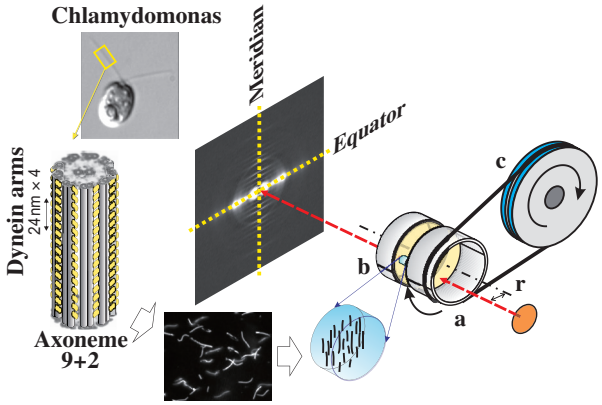

Fig. 1. Experimental system used for the shear flow alignment of axonemes of *Chlamydomonas*. The X-ray beam passes through an area 6 mm off the center (**r**) of a pair of tubes (**a** and **b**). The suspension of axonemes (2–5 mg/ml) is placed in the gap (0.1–0.35 mm) between the two parallel discs. The highlighted area schematically shows the alignment of axonemes under shear flow and in the beam. One of the discs **(a)** is rotated by a DC motor **(c)**.

beams were directed at a point close to the edge of the coverslips where the shear rate was nearly at its greatest. The continuous shear flow at this point caused the axonemes to align with the shear.

The diffraction pattern of the axonemes comprised two series of reflections: equatorial and meridional (Fig. 2). The very intense equatorial reflections occurred in the direction perpendicular to the longitudinal axes of the axonemes. The weaker meridional reflections were ladder-like reflections aligned along the meridian. When viewed along the meridian, the peaks of the meridional reflections were sharp and well-separated from each other and could be indexed to a basic axial repeat of 96 nm, which was the periodicity of the radial spokes and inner dynein arms. Although the first-order reflection at 1/96 nm^{-1} was too close to the beamstop and not clearly recognizable, reflections were observed up to the 12th (at 1/8 nm^{-1}, Fig. 2(a)) and 24th (at 1/4 nm^{-1}, Fig. 2(b)) orders. The diffusive layer-line reflections (4nm-LL arrow) were observed at 1/4 nm^{-1}; these were the first microtubule-based reflections.

Assignment of diffractions to axonemal components

The interpretation of signals in reciprocal space is not always straightforward and requires knowledge of the theory of diffraction, although the basic theory of diffraction from axonemes has been provided and the diffraction pattern of the axonemes has been calculated

[5]. We have taken an advantage of using axonemes of *Chlamydomonas* flagella, namely, the availability of a variety of mutants lacking specific axonemal components. The use of these mutants allows us to discuss directly the origin of observed reflections. We recorded diffraction patterns from axonemes of *oda1* (lacking the whole outer dynein arm) and *pf14* (lacking radial spokes). Diffraction patterns from mutant axonemes exhibited a systematic loss/attenuation of meridional/layer line reflections, making it possible to determine the origin of various reflections (Fig. 3).

The 1/24 and 1/12 nm^{-1} meridional reflections of *oda1* were much weaker than those of the wild-type (blue arrows in Fig. 3(a)), suggesting that the outer dynein arms were the main contributor to these reflections. The weaker 1/32 and 1/13.7 nm^{-1} meridional reflections from *pf14* (green arrows in Fig. 3(b)) compared with that from the wild-type suggested that these reflections mainly came from the radial spokes.

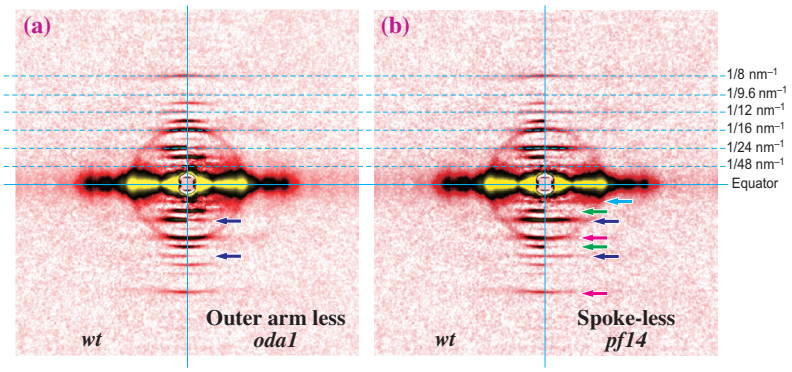

Fig. 3. Diffraction patterns from axonemes of wild-type and mutant strains of *Chlamydomonas* in the presence of 1 mM ATP and 100 μM vanadate. **(a)** Wild-type in the left half and *oda1* in the right half. Blue arrows: 4th and 8th (of 96 nm repeat) meridional reflections. **(b)** Wild-type in the left half and *pf14* in the right half. The meridional part of the second reflection (1/48 nm^{-1}, cyan arrow) becomes weaker in *pf14*, but its off-meridional part is clearly visible. Green arrows: 3rd and 7th meridional reflections. Magenta arrows: 6th and 12th meridional reflections.

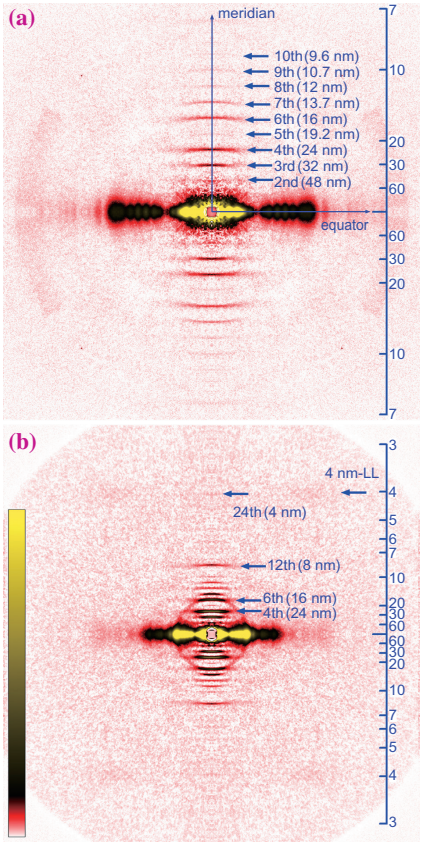

Fig. 2. Diffraction patterns from flow-oriented axonemes of wild-type *Chlamydomonas* flagella. **(a)** The pattern was recorded with a long specimen-to-detector distance (3.5 m) and a long X-ray wavelength (0.15 nm) (long-camera settings); sum of 50 frames (0.7 s exposure each). **(b)** The pattern was recorded with a short specimen-to-detector distance (2 m) and a short X-ray wavelength (0.09 nm) (short-camera settings); sum of 40 frames (0.8 s exposure each).

X-ray diffraction allows the extraction of structural information from unfixed biological materials that remain functional in an aqueous environment. Because of the noninvasive nature of the technique, dynamic or time-resolved measurements are possible, and the structures of a single axoneme sample can be compared before and after experimental intervention. This first detailed description of axoneme reflections presented here should serve as a basis for further X-ray diffraction studies to monitor the action of the constituent proteins in functional axonemes.

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