The 2.8 Å crystal structure of dynein motor domain

Dyneins are large motor complexes of 1–2 MDa that use ATP as an energy source to move toward the minus ends of microtubules [1,2]. This motor activity is crucial for a variety of cellular processes within eukaryotic cells, including the beating of cilia and flagella, cell division, cell migration, and the intracellular trafficking of various vesicles and organelles along microtubules. Dyneins power a wide range of cellular motility through the coordinated action of a number of subunits of the dynein complex together with various associated cellular components. Among them, the heavy chain (molecular mass ≥500 kDa), belonging to the AAA$^+$ superfamily of mechanochemical enzymes, is solely responsible for dynein's fundamental motor activity. Truncation studies have shown that the C-terminal 380 kDa portion of the heavy chain alone is sufficient to exert the motor activity, thus defining this portion as the dynein motor domain.

Here, we report the 2.8 Å crystal structure of the 380 kDa motor domain of *Dictyostelium discoideum* cytoplasmic dynein [3]. The X-ray diffraction data sets were collected at beamline **BL44XU**. The motor domain comprises the AAA$^+$ ring and three additional structural units: the linker, stalk/strut, and C-sequence (Fig. 1). The central ATP-hydrolyzing ring has six AAA$^+$ modules arranged around the ring central pore. Above the ring's front face, the linker exists as a rod-like structure extending from AAA1 above the ring to the periphery of AAA4. Between AAA4 and 5, two prominent coiled coils protrude and form a Y-shaped structure. The longer coiled coil extending from AAA4 is the stalk that includes the small microtubule-binding site (MTBD) at the tip, and the shorter one extending from AAA5 is the strut that appears to support the microtubule-binding stalk. On the ring's back face, the C-sequence exists as a complex structure comprising six α-helices and an incomplete six-strand antiparallel β-barrel, which spreads over AAA1, 5, and 6.

In the present structure, four ADP molecules are bound to the first four AAA$^+$ modules of the ring (Fig. 2, upper right). The results of detailed structural analyses suggest that dynein has three ATP hydrolysis sites—AAA1, 3, and 4, and one unique ATP and/or ADP binding site, AAA2. Our pre-steady-state kinetic analysis further supports this notion and highlights the importance of the AAA1 ATPase site in dynein motor function. In addition, the ring structure suggests the existence of an ATPase-dependent open/closed transition of the AAA1 and AAA4 ATPase sites, which may affect the ring structure during dynein's mechanochemical actions.

To understand the mechanism of dynein motility, it is critical to elucidate the structural details of interactions between the ATP-hydrolyzing ring and the linker whose swing-like motions have been proposed to be the major contributor to dynein's powerstroke. In the present structure, the linker position is close to that of the post-powerstroke state inferred from the results of EM analysis. When viewed from the ring's front face, the linker appears to bridge AAA1 and AAA4 by spanning the inner edges of AAA2, 3, and 4 (Fig. 2, left). However, as is evident from the side view, there is no direct contact between the linker and AAA3–AAA5, and two finger-like structures, the H2 insert β-hairpin

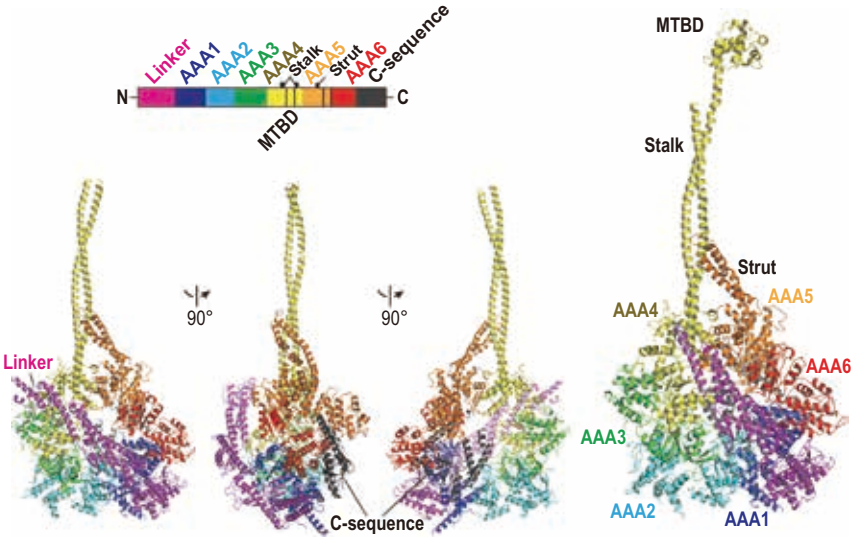

Fig. 1. Structure and sequence diagram of dynein motor domain, showing functional units: linker, six AAA$^+$ modules constituting ring, stalk/strut, and C-sequence.

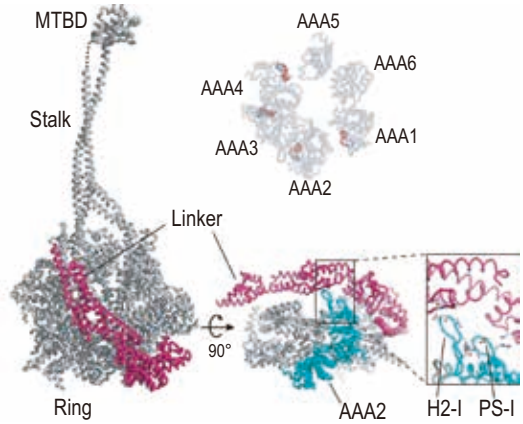

Fig. 2. Front and bottom views of the dynein motor domain showing the four ADP molecules bound to AAA1-AAA4 and the interaction interfaces between the linker and the ring.

and the PS-1 insert loop, protruding from AAA2 are identified as the major interaction interfaces between the linker and ring (Fig. 2, lower right). Our mutational analysis of the H2/PS-1 inserts suggests the critical involvement of the H2/PS-1 inserts in linker swing actions. Then, how do the H2/PS-1 inserts contribute to the force-generating linker swing? In some AAA+ proteins, the H2/PS-1 inserts serve as interaction interfaces with target proteins or DNA to catch and remodel the targets. Thus, our structural and functional analyses show that dynein also uses this remodeling apparatus for the linker swing. We thus hypothesize that during the mechanochemical cycle of dynein, the H2/PS-1 inserts could remodel the linker so that the linker bends around the interaction sites and swings against the ring.

Another key question regarding the dynein mechanism is how the motor coordinates MT binding at the MTBD of the stalk with the primary ATPase at AAA1, which must be communicated over a long distance through several structural units. A widely held assumption is that the information would be propagated through AAA1, 2, 3 and 4 in an ATPase-related manner analogous to other AAA+ proteins, because the first four modules, but not AAA5 or 6, have adenine nucleotide-binding or ATP-hydrolyzing activity. However, mutagenesis of the nucleotide binding sites in AAA2–4 does not completely block the communication between AAA1 and MTBD, and thus does not support this hypothesis. Instead, we propose that the back face sides of AAA5 and 6, that is, the C-sequence and strut, serve as a form of communication, based on the structural feature that these structures directly bridge AAA1 and the stalk (Fig. 3). In support of this model, we have shown that deletion of the C-sequence or the strut from the motor domain results in deregulation of both the microtubule-binding and ATPase activities.

In summary, the dynein motor domain structure at 2.8 Å resolution reveals the unique composite architecture of the AAA+ ATPase ring and dynein-specific force-generating units (linker, stalk/strut, and C-sequence). It also provides new insights into how dynein exploits the unique architecture to generate force and movements along MT. We propose that dynein uses the two distinct molecular machineries embedded on the different ring faces to execute the two fundamental motor actions required for dynein motility: driving of the linker swing for the powerstroke and allosteric regulation of MTBD for the modulation of MT-binding affinity. On the ring's front face, the linker swing could be driven by similar mechanisms as those of remodeling actions in other AAA+ ATPases through the critical participation of the remodeling apparatus, H2/PS-I inserts of AAA2 (Fig. 2). On the ring's back face, the long-range allosteric communication between AAA1 and MTBD required for coupling of the ATPase cycle with the switching of MT affinity could be relayed through dynein-specific structures, i.e., stalk, strut, and C-sequence (Fig. 3). The general AAA+ ATPase-type and dynein-specific actions coordinated by the primary ATPase cycle at AAA1 would drive dynein motility.

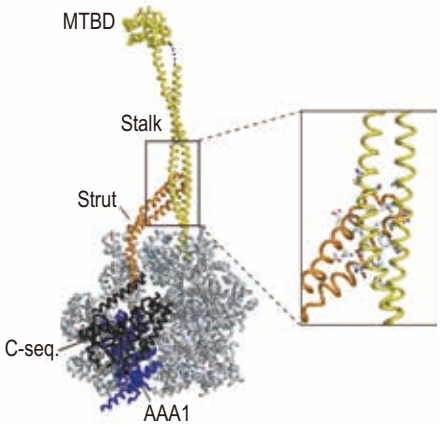

Fig. 3. Back view of the dynein motor domain showing the proposed allosteric communication pathway between the MTBD at the tip of the stalk and the AAA1 ATPase site in the ring. To facilitate visualization of the pathway, a portion of AAA5 (AAA5-extension) is not shown.

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