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REVIEW

Structural atlas of dynein motors at atomic resolution

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Abstract

Dynein motors are biologically important bio-nanomachines, and many atomic resolution structures of cytoplasmic dynein components from different organisms have been analyzed by X-ray crystallography, cryo-EM, and NMR spectroscopy. This review provides a historical perspective of structural studies of cytoplasmic and axonemal dynein including accessory proteins. We describe representative structural studies of every component of dynein and summarize them as a structural atlas that classifies the cytoplasmic and axonemal dyneins. Based on our review of all dynein structures in the Protein Data Bank, we raise two important points for understanding the two types of dynein motor and discuss the potential prospects of future structural studies.

Keywords Dynein · Dynein subunits · Axonemal dynein light chain-1 · Molecular motor · Structural analysis

Introduction

Dyneins are microtubule (MT)-based molecular motors that perform diverse biological functions (Roberts et al. 2013). They work as large multiple bio-nanomachines (> 1 MDa) consisting of a heavy chain (HC), intermediate chain (IC), light intermediate chain (LIC), and light chain (LC). The HC possesses ATPase activity and provides the driving force for power generation to conduct a wide variety of cellular functions. The other chains are involved in regulating the HC and are therefore called accessary chains. Dyneins are classified as either cytoplasmic or axonemal on the basis of their physiological function and cellular localization, and there is a clear distinction between the two types. Cytoplasmic dyneins serve as power generators for migration and intracellular transport, whereas axonemal dyneins are located in the axoneme and are responsible for ciliary/flagellar beating. Complete genome analysis of several organisms has revealed that there are at least 15 HC genes present in most organisms; two of which encode cytoplasmic dyneins, while the others encode axonemal ones.

The structural analysis of dynein motors began with the LCs with relatively smaller molecular weights and then progressed to the larger chains, such as ICs and HCs (Table 1). The first HC structure to be published was that of dynein-c (Burgess et al. 2003), which is an isoform of an axonemal dynein purified from a green alga, *Chlamydomonas reinhardtii*. Although the resolution of the negatively stained electron microscopy (EM) images was not sufficient to build the atomic coordinates, the first model of the dynein power stroke was proposed from the two different EM structures with and without nucleotide. However high-resolution structural information on the HC, which is crucial to understand the molecular mechanism of the mechano-chemical coupling of dynein motors, was long awaited.

Since the establishment of a method for producing functional recombinant cytoplasmic dynein motor domains (Nishiura et al. 2004), X-ray crystallographic high-resolution structures have been reported that describe detailed structural elements, such as the N-terminal linker, AAA+ (ATPases associated with various cellular activities) ring, stalk/strut coiled coils with the microtubule binding domain (MTBD), and the C-terminal non-AAA structure named "C-sequence," as well as revealing the structural changes that occur upon ATP hydrolysis (Carter et al. 2008, 2011, Kon et al. 2011, 2012;

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Table 1 Structures of cytoplasmic dynein available in the PDB

PDB ID	Dynein component	Complex	Organism	Method	Res. (Å)	References
Heavy chain						
3ERR	Cytoplasmic dynein 1 heavy chain 1	MTBD–SRS chimera	*Mus musculus*	X-ray	2.27	Carter et al. 2008
3J67	Dynein motor domain		*Strongylocentrotus purpuratus*	EM	34.00	Lin et al. 2014
3J68	Dynein motor domain		*Strongylocentrotus purpuratus*	EM	30.00	Lin et al. 2014
3J1T	Cytoplasmic dynein 1 heavy chain 1	MTBD–SRS chimera–MT complex	*Mus musculus*	EM	9.70	Redwine et al. 2012
3J1U	Cytoplasmic dynein 1 heavy chain 1	MTBD–SRS chimera–MT complex	*Mus musculus*	EM	9.70	Redwine et al. 2012
3J6P	Dynein heavy chain, cytoplasmic	Dynein MTBD–MT complex	*Dictyostelium discoideum*	EM	8.20	Uchimura et al. 2015
3QMZ	Cytoplasmic dynein heavy chain		*Saccharomyces cerevisiae*	X-ray	6.00	Carter et al. 2011
3AY1	Dynein heavy chain, cytoplasmic	Dynein motor domain–ADP	*Dictyostelium discoideum*	X-ray	4.50	Kon et al. 2011
3VKG	Dynein heavy chain, cytoplasmic	Dynein motor domain–ADP	*Dictyostelium discoideum*	X-ray	2.81	Kon et al. 2012
3VKH	Dynein heavy chain, cytoplasmic	Dynein motor domain–ADP	*Dictyostelium discoideum*	X-ray	3.80	Kon et al. 2012
3W-UQ	Cytoplasmic dynein 1 heavy chain 1		*Mus musculus*	X-ray	3.50	Nishikawa et al. 2014
4AI6	Dynein heavy chain, cytoplasmic	Dynein motor domain–GST chimera–ADP	*Saccharomyces cerevisiae*	X-ray	3.40	Schmidt et al. 2012
4AKG	Dynein heavy chain, cytoplasmic	Dynein motor domain–GST chimera–ATP	*Saccharomyces cerevisiae*	X-ray	3.30	Schmidt et al. 2012
4AKH	Dynein heavy chain, cytoplasmic	Dynein motor domain–GST chimera–AMPPNP	*Saccharomyces cerevisiae*	X-ray	3.60	Schmidt et al. 2012
4AKI	Dynein heavy chain, cytoplasmic	Dynein motor domain–GST chimera–LuAc derivative	*Saccharomyces cerevisiae*	X-ray	3.70	Schmidt et al. 2012
4RH7	Cytoplasmic dynein 2 heavy chain 1	GFP–Cytoplasmic dynein 2 heavy chain 1 synthetic construct	*Homo sapiens*	X-ray	3.41	Schmidt et al. 2014
4W8F	Dynein heavy chain, cytoplasmic	Dynein heavy chain–lysozyme chimera	*Saccharomyces cerevisiae. Enterobacteria phase t4 sensu lato*	X-ray	3.54	Bhabha et al. 2014
5AFR	Dynein heavy chain, cytoplasmic		*Saccharomyces cerevisiae*	X-ray	5.00	Urnavicius et al. 2015
5AFU	Cytoplasmic dynein	Dynein tail–dynactin–BICD2 complex	*Sus scrofa*	EM		Urnavicius et al. 2015
5AYH	Cytoplasmic dynein 1 heavy chain 1		*Mus musculus*	X-ray	3.01	Nishikawa et al. 2016
5NUG	Cytoplasmic dynein 1 heavy chain 1		*Homo sapiens*	EM	3.80	Zhang et al. 2017
5NVS	Cytoplasmic dynein 1 heavy chain 1	Dynein tail–IC–RobI–LIC–LC8–Tc-Tex complex	*Homo sapiens*	EM	8.40	Zhang et al. 2017
5NVU	Cytoplasmic dynein 1 heavy chain 1	Phi-particle conformation	*Homo sapiens*	EM	15.00	Zhang et al. 2017
5NW4	Cytoplasmic dynein 1 heavy chain 1	Dynactin–BICD2 complex	*Homo sapiens*	EM	8.70	Zhang et al. 2017
5VH9	Dynein heavy chain, cytoplasmic			EM	7.70	

Table 1 (continued)

PDB ID	Dynein component	Complex	Organism	Method	Res. (Å)	References
		Dynein heavy chain–Lis1 complex	*Saccharomyces cerevisiae*			DeSantis et al. 2017
5VLJ	Dynein heavy chain, cytoplasmic	Dynein heavy chain–Lis1 complex	*Saccharomyces cerevisiae*	EM	10.50	DeSantis et al. 2017
Light intermediate chain						
4W7G	Dynein light intermediate chain		*Chaetomium thermophilum*	X-ray	2.10	Schroeder et al. 2014
Light chain						
LC8						
1CMI	Dynein light chain 1, cytoplasmic	LC8–PIN complex	*Homo sapiens*	X-ray	2.50	Liang et al. 1999
1F3C	Dynein light chain 1, cytoplasmic		*Rattus norvegicus*	NMR	–	Fan et al. 2001
1F95	Dynein light chain 1, cytoplasmic	DLC8–BIM peptide complex	*Rattus norvegicus*	NMR	–	Fan et al. 2001
1F96	Dynein light chain 1, cytoplasmic	DLC8–NNOS peptide complex	*Rattus norvegicus*	NMR	–	Fan et al. 2001
1RE6	Dynein light chain 2, cytoplasmic		*Mus musculus*	NMR	–	Day et al. 2004
1RHW	Dynein light chain 1, cytoplasmic		*Drosophila melanogaster*	NMR	–	Makokha et al. 2004
1PWJ	Dynein light chain 2, cytoplasmic		*Rattus norvegicus*	NMR	–	Wang et al. 2003
1PWK	Dynein light chain 2, cytoplasmic		*Rattus norvegicus*	NMR	–	Wang et al. 2003
1Y4O	Dynein light chain roadblock-type 1		*Mus musculus*	NMR	–	Song et al. 2005
1YO3	Dynein light chain 1, putative		*Plasmodium falciparum*	X-ray	1.65	Vedadi et al. 2007
1Z09	Dynein light chain roadblock-type 1		*Homo sapiens*	NMR	–	Ilangovan et al. 2005
2B95	Dynein light chain roadblock-type 1		*Homo sapiens*	NMR	–	
2E8J	Dynein light chain roadblock-type 1		*Homo sapiens*	NMR	–	
2HZ5	Dynein light chain roadblock-type 1		*Homo sapiens*	X-ray	2.10	Liu et al. 2006
2P2T	Dynein light chain 1, cytoplasmic	LC8–IC74 peptide complex	*Drosophila melanogaster*	X-ray	3.00	Benison et al. 2007
2XQQ	Dynein light chain 2, cytoplasmic	DYNLL2–peptide complex	*Homo sapiens*	X-ray	1.31	Rapali et al. 2011
3BRI	Dynein light chain 1, cytoplasmic		*Drosophila melanogaster*	X-ray	1.70	Benison et al. 2008
3BRL	Dynein light chain 1, cytoplasmic	LC8(S88E) –Swa peptide complex	*Drosophila melanogaster*	X-ray	1.90	
3DVH	Dynein light chain 1, cytoplasmic	K36P mutant	*Drosophila melanogaster*	X-ray	2.00	Lightcap et al. 2008
3DVP	Dynein light chain 1, cytoplasmic	LC8–Pak1 peptide complex	*Drosophila melanogaster*	X-ray	2.50	Lightcap et al. 2008
3DVT	Dynein light chain 1, cytoplasmic		*Drosophila melanogaster*	X-ray	2.30	Lightcap et al. 2008
3E2B	Dynein light chain 1, cytoplasmic	LC8–Swallow peptide complex	*Drosophila melanogaster*	X-ray	2.00	Benison et al. 2008
3GLW	Dynein light chain 1, cytoplasmic	IC–LC8 complex	*Drosophila melanogaster*	X-ray	3.15	Hall et al. 2009
3P8M	Dynein light chain 2, cytoplasmic	DYNLL2–GCN4 complex	*Homo sapiens*	X-ray	2.90	Rapali et al. 2011
3RJS	Dynein light chain motor protein		*Toxoplasma gondii*	X-ray	1.50	

Table 1 (continued)

PDB ID	Dynein component	Complex	Organism	Method	Res. (Å)	References
						Qureshi et al. 2013
3ZKE	Dynein light chain 1, cytoplasmic	LC8–Nek9 peptide complex	*Homo sapiens*	X-ray	2.20	Gallego et al. 2013
3ZKF	Dynein light chain 1, cytoplasmic	LC8–Nek9 peptide complex	*Homo sapiens*	X-ray	2.60	Gallego et al. 2013
4D07	Dynein light chain 2, cytoplasmic	Dynll2–Myosin 5A tail complex	*Homo sapiens*	X-ray	1.85	Bodor et al. 2014
4DS1	Dynein light chain 1, cytoplasmic	Dyn2–Nup159 complex	*Saccharomyces cerevisiae*	X-ray	1.85	Romes et al. 2012
4HT6	Dynein light chain 1, cytoplasmic	Dyn2–Pac11 complex	*Saccharomyces cerevisiae*	X-ray	1.90	Rao et al. 2013
4QH7	Dynein light chain 1, cytoplasmic	LC8-Ana2(159–168) complex	*Drosophila melanogaster*	X-ray	1.83	Slevin et al. 2014
4QH8	Dynein light chain 1, cytoplasmic	LC8–Ana2(237–246) complex	*Drosophila melanogaster*	X-ray	1.90	Slevin et al. 2014
5E0L	Dynein light chain 1, cytoplasmic	LC8 (DLC1, DYNLL)–Chica (415–424) complex	*Drosophila melanogaster*	X-ray	1.31	Clark et al. 2016
5E0M	Dynein light chain 1, cytoplasmic	LC8 (DLC1, DYNLL)–Chica (468–476) complex	*Drosophila melanogaster*	X-ray	1.65	Clark et al. 2016
5WOF	Dynein light chain 1, putative		*Plasmodium falciparum*	X-ray	1.65	Vedadi et al. 2007
TcTex-1						
1YGT	Dynein light chain TcTex-type		*Drosophila melanogaster*	X-ray	1.70	Williams et al. 2005
5HXL	dynein light chain TcTex-1		*Magnaporthe oryzae*	X-ray	1.97	
5JPW	Dynein light chain TcTex-type 1	TcTex1–IC2 complex	*Homo sapiens*	NMR	–	Merino-Gracia et al. 2016
LC7/RobI						
3L7H	Dynein light chain roadblock		*Drosophila melanogaster*	X-ray	1.95	
3L9K	Dynein light chain roadblock	RE64145p–IC complex	*Drosophila melanogaster*	X-ray	3.00	
Lis1						
1UUJ	Platelet-activating factor acetylhydrolase IB subunit alpha		*Mus musculus*	X-ray	1.75	Kim et al. 2004
1VYH	Platelet-activating factor acetylhydrolase IB subunit beta	Lis1–PAF–AH complex	*Mus musculus*	X-ray	3.40	Tarricone et al. 2004
Complex						
2PG1	(Dynein light chain 1, cytoplasmic, Dynein light chain TcTex-type, Cytoplasmic dynein 1 intermediate chain 2	LC8–TcTex1–IC	*Drosophila melanogaster*	X-ray	2.80	Williams et al. 2007
3FM7	Dynein light chain 1, cytoplasmic, Dynein light chain TcTex-type, Cytoplasmic dynein 1 intermediate chain	IC–TcTex-1–LC8 complex	*Drosophila melanogaster*	X-ray	3.50	Hall et al. 2009

IC intermediate, *LC* light chain, *MT* microtubule, *MTBD* MT-binding domain

Schmidt et al. 2012). Most recently, a single-particle cryogenic electron microscopy (cryo-EM) structure of a vast complex of cytoplasmic dynein 1 bound to dynactin and an N-terminal construct of BICD2 (total molecular mass, 1.4 MDa) has been reported (Zhang et al. 2017).

To date, many structures of cytoplasmic dynein components from different organisms have been analyzed by X-ray crystallography, cryo-EM, and NMR spectroscopy (Tables 1 and 2). As the number of solved structures of cytoplasmic dynein increases year by year, the information is becoming

Table 2 Structures of axonemal dynein available in the PDB

PDB ID	Dynein component	Complex	Organism	Method	Res. (Å)	References
Heavy chain						
2RR7	Dynein heavy chain 9		*Chlamydomonas reinhardtii*	NMR	–	Kato et al. 2014
Light chain						
LC1						
1DS9	Dynein light chain 1, axonemal		*Chlamydomonas reinhardtii*	NMR	–	Mullen et al. 2000
1M9L	Dynein light chain 1, axonemal		*Chlamydomonas reinhardtii*	NMR	–	Wu et al. 2003
5YXM	Dynein light chain 1, axonemal		*Chlamydomonas reinhardtii*	X-ray	1.55	this study
TcTex-1						
1XDX	Dynein light chain TcTex1		*Chlamydomonas reinhardtii*	NMR	–	Wu et al. 2005

too complex to assess the accumulated structural data at a glance (Table 1). By contrast, only four atomic structures of axonemal dynein are available in the Protein Data Bank (Table 2) (Mullen et al. 2000; Wu et al. 2003; Kato et al. 2014).

In this short article, we review representative structural studies of the components that classify cytoplasmic and axonemal dyneins and summarize them as a structural atlas (Fig. 1) with additional updated structural data on LC1 from *C. reinhardtii* obtained by ourselves (Fig. 2).

Light chain

Many structures of LCs from different organisms have been reported, including LC7, LC8, TcTex-1, and Lis1 (Table 1). It is thought that the LCs are important for dynein–cargo interactions. Several structures of LC8 (also called DYNLL or dynein light chain 1) have been determined as a complex with peptides derived from binding partners by X-ray crystallography and NMR (Table 1, Fig. 1). Details of the molecular function of LC8 remain unknown, but *Chlamydomonas* cells of a LC8 deletion mutant lack retrograde intraflagellar transport and display short deficient flagella (Pazour et al. 1998).

Axonemal dynein light chain-1 (LC1) in *C. reinhardtii* (DNAL1 in *Homo sapiens*), whose structure has been solved by NMR spectroscopy (Mullen et al. 2000; Wu et al. 2003) (Fig. 2), is a component of outer arm dynein (OAD) (Table 2). Knockdown of LC1 has been found to reduce beat frequency in the flatworm planarian (Rompolas et al. 2010), and the expression of an LC1 mutant shows dominant-negative effects on swimming velocity and beat frequency in *C. reinhardtii* (Patel-King and King 2009). These observations suggest that LC1 acts as a regulator to beat cilia/flagella. Originally, LC1 was thought to be directly bound to tubulins and to tether the OADγ HC to the microtubule (Patel-King and King 2009). Furthermore, it has been widely assumed that LC1 associates with AAA1 and AAA3 or AAA4 of the AAA+ ring in the gamma heavy chain of OAD (OADγ) in *C. reinhardtii*

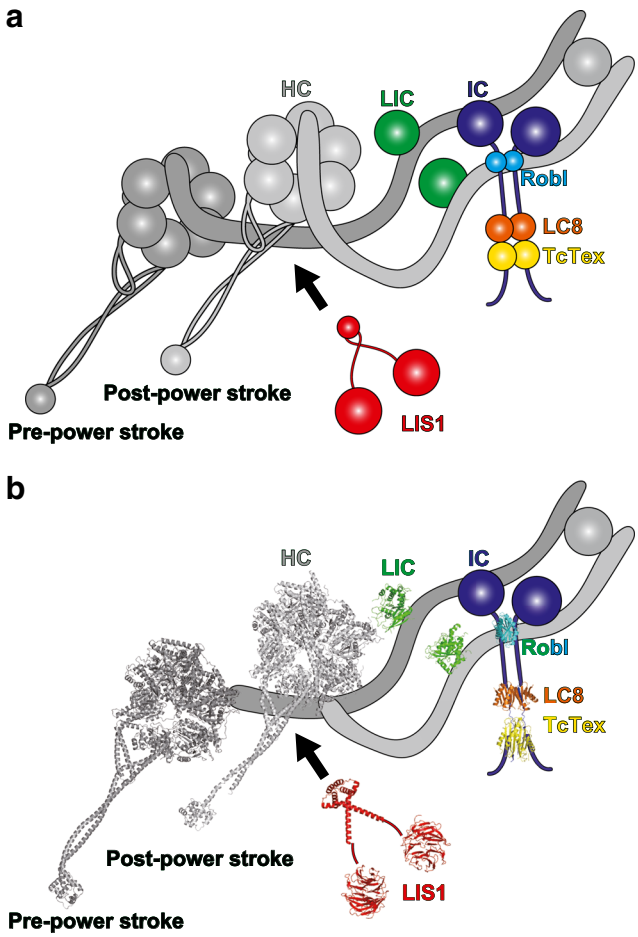


Fig. 1 Structural atlas of cytoplasmic dynein. **a** Schematic diagram of the cytoplasmic dynein complex. **b** Superposition of solved dynein structures on the schematic diagram. Atomic structures at a resolution of 4 Å or higher are shown. From left to right, pre-power stroke (dark gray) and post-power stroke (light gray) structures of the dynein motor domain, LIS1 (red), LIC (green), IC (navy) with Robl (cyan), LC8 (orange), and TcTex (yellow) are shown

(Benashski et al. 1999). However, it was recently reported that LC1 is tightly bound to the MTBD of OADγ, which is located at the tip of stalk region in the motor domain (Ichikawa et al. 2015). This was the first report of an LC interacting with the MTBD. Moreover, it was also discovered that the binding of LC1 to the MTBD decreases the MT-binding affinity of the HC (Ichikawa et al. 2015). Because it has been reported that the ATPase activity of the HC is increased in the presence of MTs (Kon et al. 2009), both results imply that LC1 indirectly changes the ATPase activity of OADγ and regulates ciliary/flagellar beating. However, the molecular mechanism that tunes ATPase activity through the MTBD still remains poorly understood.

Although NMR structures of LC1 are available, we determined the X-ray structure of LC1 at 1.55-Å resolution to enable a more detailed discussion (Fig. 2a). As expected from a comparison of the amino acid sequences and NMR structures of LC1 (Benashski et al. 1999; Mullen et al. 2000; Wu et al. 2003), the crystal structure of LC1 shows a leucine-rich repeat conformation. However, there are large conformational differences between the X-ray and NMR structures, especially in the N- and C- terminal regions and the crystal structure differs from the NMR structures at the secondary structure level (Fig. 2b, c). In particular, the differences in the secondary structure between the X-ray and NMR structures are surprisingly large at Ala22–Glu24 and Met182–Val184 in the N-terminal and C-terminal regions, respectively (Fig. 2c). These results suggest that these two terminal regions may play the role of flexible hinges and that large conformational differences may be induced when LC binds to its partner proteins.

We also analyzed the anisotropic temperature factors of the X-ray structure with reference to the main chain conformation of the NMR structures (Fig. 2d). There were significant correlations between the anisotropic directions of the temperature factors and the structural differences between the X-ray and NMR structures, which implies that the intrinsic flexibility of LC1 is manifested in the structural discrepancy between X-ray and NMR structures.

Light intermediate chain

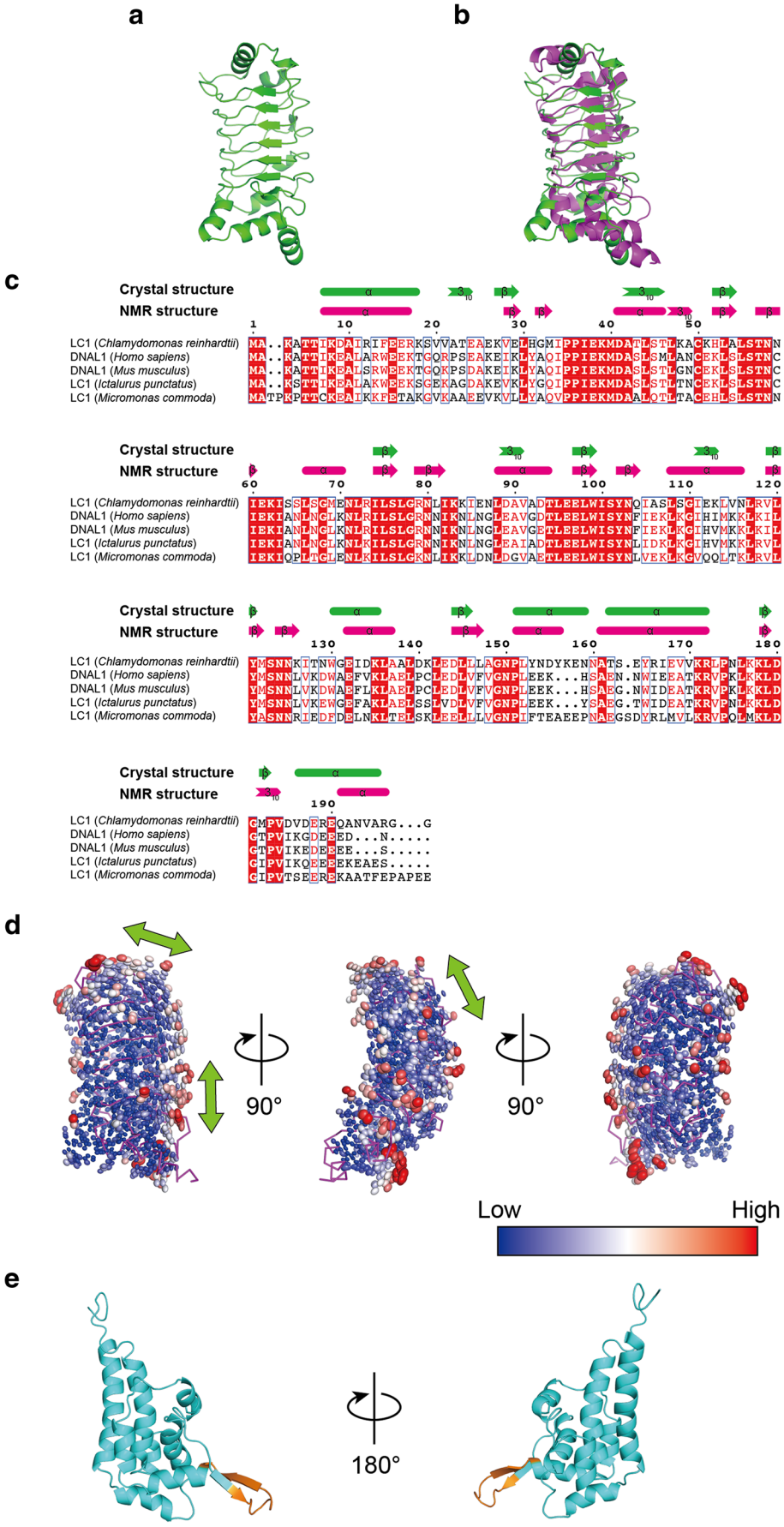
The LIC subunit is present in cytoplasmic dynein, but not in axonemal dynein (Inaba 2007). There are three LIC homologs in *H. sapiens*: LIC1, LIC2, and LIC3. On the one hand, LIC1 and LIC2 are associated with cytoplasmic dynein 1 (Hughes et al. 1995) and are thought to play important roles in cargo transport and stability of the HC (Trokter et al. 2012). On the other hand, LIC3 interacts with cytoplasmic dynein 2 (Grissom et al. 2002). Sequence analysis indicates that the LICs are divided into two domains: a conserved N-terminal domain and the other domain. The only known structure of LIC is the structure of the conserved N-terminal domain of LIC from a thermophilic hyphal fungus, *Chaetomium thermophilum* (Schroeder et al. 2014). Although the structure shows a Ras-like G-protein fold, the nucleotide pocket is empty. Biochemical experiments confirmed that this fungus LIC does not bind nucleotide, whereas human LIC1 does bind nucleotides (Schroeder et al. 2014). To clarify the differences in LICs by species and isoform, further structural studies and biochemical experiments will be needed.

Fig. 2 Currently available atomic structures of axonemal dynein. **a** Crystal structure of LC1 (PDB ID: 5YXM). LC1 crystals were grown at 4 °C via the sitting-drop vapor diffusion method by mixing 200 nL of LC1 (20 mg/mL protein) with an equal volume of reservoir solution (0.1 M ammonium phosphate monobasic, 10% (*w*/*v*) PEG3,350). LC1 crystals were soaked in cryo-protectant solution (0.1 M ammonium phosphate monobasic, 35% (*w*/*v*) PEG3,350, 10 mM Tris-HCl (pH 8.0), 100 mM NaCl) overnight, and then flash-cooled in liquid nitrogen. The X-ray diffraction experiment was performed on beamline BL44XU, SPring-8, Harima Japan. The collected images were processed by using HKL2000 software (Otwinowski and Minor 1997). Molecular replacement and refinement were performed by using Phenix (Adams et al. 2002) and COOT (Emsley and Cowtan 2004). TLS parameters were analyzed by using the TLSMD server (Painter and Merritt 2006), and 12 TLS groups were introduced in the subsequent refinement. The final structure was validated by using MolProbity (Lovell et al. 2003). The detailed crystallographic statistics information can be available in the PDB (https://pdbj.org/mine/summary/5yxm). The ribbon diagram of LC1 is shown in green. **b** Superposition of the X-ray (green) and NMR (magenta) structures of LC1. A representative NMR structure is shown (PDB ID: 1M9L). **c** Amino acid sequence alignment of LC1 with secondary structure assignments. **d** Superposition of the X-ray structure with anisotropic B factors and main chain conformation of the NMR structure (magenta). **e** NMR structure of the MTBD of dynein-c (PDB ID: 2RR7). The additional flap structure which is an insertion sequence in the MTBD of the axonemal dynein is shown in orange

Intermediate chain

There are four IC homologs in *H. sapiens*. DYNC1I1 (cytoplasmic IC1) and DYNC1I2 (cytoplasmic IC2) associate with cytoplasmic dynein HC, while DNAI1 (axonemal IC1) and DNAI2 (axonemal IC2) interact with axonemal HCs. All IC homologs possess a conserved WD40 domain in the C-terminus that interacts with HCs (Tynan et al. 2000). A secondary structure analysis using Jpred and RONN predicted that the N-terminal region of cytoplasmic IC1 and IC2 comprise coiled coil and highly disordered regions (Williams et al. 2012). However, the secondary structure prediction analysis also indicated that the N-terminal region of IC1 is highly disordered but that of IC2 possesses a folded structure in axonemal ICs (Williams et al. 2012).

In terms of atomic structure, so far, there is no high-resolution structure of either the whole IC or its WD40 domain (Tables 1 and 2). However, crystal structures of TcTex1


a
b
c
Crystal structure
NMR structure
LC1 (Chlamydomonas reinhardtii)
DNAL1 (Homo sapiens)
DNAL1 (Mus musculus)
LC1 (Ictalurus punctatus)
LC1 (Micromonas commoda)
d
90°
90°
Low
High
e
180°

and LC8 with IC peptides containing the interaction sites for LCs have been determined and explain how IC interacts with LCs (Williams et al. 2007; Hall et al. 2009). Moreover, the whole structure of a cytoplasmic dynein complex determined by cryo-EM has revealed the structural arrangement of IC within the cytoplasmic dynein complex (Zhang et al. 2017). However, the precise site of the IC–HC interaction remains unclear due to the low resolution. Thus, more work is needed to gain structural insights into the dynein ICs.

Heavy chain

The HC is the largest polypeptide of the dynein complex and contains the motor domain, which is the minimum component needed for ATP-dependent motor activity. In 2008, Carter and colleagues determined the first crystal structure of the MTBD of an HC fused to the seryl–tRNA synthetase (SRS) from *Thermus thermophilus* (Carter et al. 2008). Several years later, a more complete structure of the stalk coiled coil with the MTBD was reported by our group (Nishikawa et al. 2014, 2016). Since then, many crystal structures of the cytoplasmic dynein motor domain in different nucleotide states have been determined (Carter et al. 2011; Kon et al. 2011, 2012, Schmidt et al. 2012, 2014; Bhabha et al. 2014). According to these structures, the HC is composed of multiple functional units, including the tail, linker, AAA+ ring, stalk/strut, and C-sequence. Each unit possesses distinct functions to drive force generation in the motor. In addition to X-ray crystallography, cryo-EM has more recently revealed the structure of a cytoplasmic dynein complex including the HC, IC, LIC, and LC, both alone and together with dynactin–BICD2 (DDB) (Zhang et al. 2017). The cryo-EM structures have revealed the relative arrangement of the cytoplasmic dynein components in an inhibitory state and provide insights into how cytoplasmic dynein is inhibited and activated.

In contrast to the genes encoding cytoplasmic dynein HCs, those encoding axonemal dyneins are many and diverse. The arrangement of the HC and the characteristics of the motor activity along the MT differ completely between axonemal dynein and cytoplasmic dynein. Cytoplasmic dyneins work as a dimer, whereas the functional oligomeric states of axonemal dynein HCs include monomers, dimers, and trimers. Moreover, an MT gliding assay has revealed that some axonemal dyneins display clockwise translocation of MTs (Kikushima and Kamiya 2008; Yamaguchi et al. 2015). These findings indicate that axonemal dyneins are highly diverse proteins in terms of the functional properties of their HCs. Among the axonemal dynein HCs, only the NMR structure of the MTBD of dynein-c from *C. reinhardtii* has been determined so far (Kato et al. 2014) (Fig. 2e). As compared with cytoplasmic dynein, the molecular mechanism underlying the motor activities of axonemal dyneins remains relatively unclear. Clearly, structural and functional studies of axonemal dynein HCs need to be addressed as soon as possible.

Conclusions and future prospects

Dynein motors are biologically important bio-nanomachines. In parallel with recent developments in structural biology, such as single-particle cryo-EM and synchrotron-based X-ray nano-crystallography, more and more fascinating three-dimensional structures of dyneins have become available. Based on the survey of the structures available at atomic resolutions shown above, we would like to point out two important directions for future research. One is the imbalance in structural information between cytoplasmic and axonemal dynein. Atomic data are very much focused on cytoplasmic dyneins and remarkably less structural work on axonemal dyneins has been reported. The oligomeric states of axonemal dyneins are so diverse that each dynein is likely to have a specific structural role. The expansion of structural information on axonemal dyneins is greatly anticipated.

The second point is that structures of dynein on MTs are lacking. This point is important because the dynein that walks along the MT is really the functional molecule. For the other cytoskeletal motors, kinesin and myosin, not only structures in different nucleotide states but also structures in complex with the α, β-tubulin dimer or actin filament have been reported. Two structures of the dynein MTBD and MT complex have been solved by cryo-EM using a helical averaging technique (Table 1), one of which was done by a collaborative team including one of the authors. However, the resolutions of the two structures are 9.7 and 8.2 Å, and only flexible docking based on the available crystal structures is applicable at those resolutions. In 2014, Imai and his colleagues reported the structure of dynein walking on MTs using engineered chimeric dynein construct (Imai et al. 2015), but the resolution is not high enough to discuss the structure at the residue level. We await with impatience a high-resolution structure of dynein walking on MTs.

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Compliance with ethical standards

Conflict of interest Akiyuki Toda declares that he has no conflict of interest. Hideaki Tanaka declares that he has no conflict of interest. Genji Kurisu declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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