extended state, how it is released during contraction and how the pyocin tube is optimized for dissipating proton motive force to kill bacteria, a task different from those of other contractile machines.

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Dynactin revealed

Samara L Reck-Peterson

Dynactin is an essential cofactor for the microtubule-based motor cytoplasmic dynein. Two recent papers report structures obtained by cryo-EM of dynactin, the dynein–dynactin complex and dynein–dynactin bound to its track, the microtubule.

Cytoplasmic dynein ('dynein' herein) is the cytoskeletal motor responsible for nearly all minus end–directed microtubule-based motility in eukaryotic cells. Dynein is a 1.4-MDa complex composed of dimers of six different proteins. Nearly all dynein functions require the cofactor dynactin[1], a 1.2-MDa complex composed of 23 polypeptides (11 different proteins). In addition, many dynein–dynactin functions require a cargo adaptor[2], such as BICD2, which provides a link between dynein–dynactin and its cargo and is required for maximal activation of motility[3–5]. Dynein–dynactin's cargos include organelles, proteins, mRNAs and viruses[1]. Mutations in dynein and dynactin subunits in humans result in neurodegenerative disease[6]. Despite dynactin's essential nature, very little was previously known about it structurally. This changed dramatically with two recent reports revealing detailed structural information about dynactin and the dynein–dynactin–BICD2 complex, both alone and bound to its microtubule track[7,8]. These structures provide important insights into how dynactin regulates dynein.

Dynactin was discovered as a factor capable of activating dynein-mediated vesicle motility *in vitro*[9,10]. Its architecture was first determined, at lower resolution, by EM and biochemistry[1,11–14]. Dynactin is built around a short actin-like filament composed of the actin-related protein Arp1. Like actin, the Arp1 filament is polar, with barbed and pointed ends. The actin-capping protein CapZ is found at the barbed end of the filament, whereas a divergent Arp, Arp11, is found at the pointed end, along with three additional proteins: p25, p27 and p62. β-actin has also been reported to be part of the dynactin complex[14]. The shoulder complex, which is made up of p50, p24 and p150, the largest subunit of the dynactin complex, is positioned toward the barbed end of the Arp1 filament. p150 is composed of multiple predicted coiled-coil domains, which have been proposed to be the structures that can be seen by EM as long extensions emerging from the rest of the dynactin complex[14]. Finally, the amino terminus of p150, located distal to the filament, contains a CAP-Gly domain, which has been shown to interact with microtubules[15]. The limited resolution of these earlier studies left several key questions unanswered, including those of what role the Arp1 filament has and what controls its length, how dynactin activates dynein and why BICD2 is important for this activation.

Both Chowdhury *et al.*[7] and Urnavicius *et al.*[8] used cryo-EM to solve structures of dynactin, taking advantage of recent advances in electron detector technology that have revolutionized the field of structural EM[16]. Chowdhury *et al.*[7], using native dynactin from bovine brain, reached a resolution of 6.5 Å in the dynactin filament. Urnavicius *et al.*[8] used native dynactin from porcine brain that was cross-linked with a small amount of glutaraldehyde; they were able to reach a resolution of 3.5 Å in the same region. Other portions of dynactin were resolved to lower resolutions in both cases.

The structures revealed that the dynactin filament is composed of two protofilaments: a 'top' one containing five subunits and a 'bottom' one containing four (**Fig. 1a**, dark and light purple). The higher resolution of the Urnavicius *et al.*[8] structure allowed the authors to determine that the subunit in the bottom protofilament at the pointed end was β-actin (**Fig. 1a**, dark purple). A CapZ α–β dimer caps the barbed end (**Fig. 1a**, green). Arp11 (**Fig. 1a**, yellow) binds to the interface created by β-actin and Arp1 at the pointed end. The pointed-end complex of p25, p27 and p62 (**Fig. 1a**, red and orange) binds to Arp11. The shoulder (**Fig. 1a**, blue) predominantly consists of two copies of p24 and four copies of p50 (ref. 8). Peptides (termed 'extended regions') that coat the dynactin filament likely come from p50 (ref. 8) (**Fig. 1a**, blue curved lines).

Urnavicius *et al.*[8] were able to resolve p150 (**Fig. 1a**, gray) to a resolution of 8.6 Å by averaging a subset of particles in which p150 docked along the side of dynactin. As predicted from the primary sequence, p150 consists of three separate coiled-coil domains (termed CC1a, CC1b and CC2) (**Fig. 1a**). A globular domain situated between CC1b and CC2, termed the intercoiled domain (ICD), can be seen in the structure; the length of the entire projection is 50 nm. In the undocked state, the p150 coiled-coil projection was not resolvable[7,8,11], thus suggesting that it adopts multiple conformations.

The Urnavicius *et al.*[8] structure suggests a model for how the length of the dynactin filament is set. In this model, the shoulder and the putative extended regions of p50 serve to scaffold the eight Arp1 subunits (**Fig. 1a**). The ninth position, the only one not reached by the p50 extensions, is occupied by β-actin, perhaps owing to its high concentration in the cell. β-actin and its neighboring Arp1 on the adjacent protofilament form a unique binding site for Arp11, which terminates the minus end of the filament. The capping proteins at the barbed end of the filament further stabilize it.

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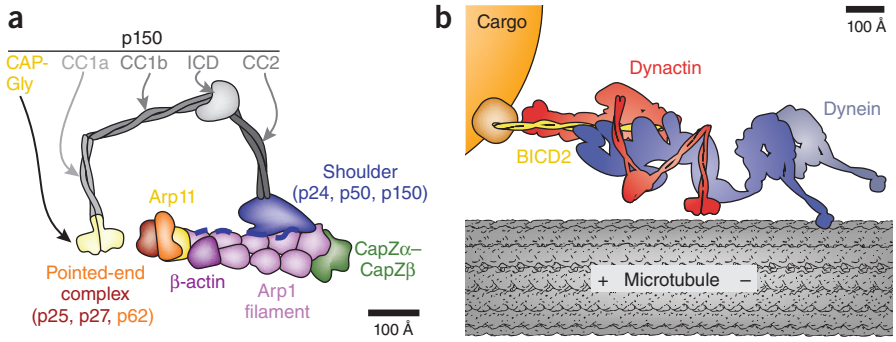

Figure 1 Structures of dynactin and dynein–dynactin–BICD2 bound to a microtubule. (**a**) Cartoon representation of the dynactin structure. The dynactin filament is shown by Arp1 (light purple) and β-actin (dark purple). It is capped by Arp11 (yellow) on the pointed end and by CapZ α–β (green) on the barbed end. The pointed-end complex (red and orange) binds to Arp11. p150 is found both in the shoulder complex (blue) and in the extended coiled coil (gray), at the end of which is its microtubule-binding CAP-Gly domain (pale yellow). The shoulder complex also contains p24 and p50 (blue). Extended regions (blue curved structures) along the dynactin filament (purple) are thought to come from p50. (**b**) Cartoon of the DDB complex bound to microtubules. The dynein motor domains (blue) point toward the minus end of the microtubule, with the rest of the dynein motor and dynactin (red) extending toward the microtubule plus end. BICD2 (yellow) stabilizes the interaction between dynein and dynactin and also provides a link to cargo (orange).

Dynein moves cargos over long distances in cells. Recent studies have suggested that robust processivity (the ability to take multiple steps) of mammalian dynein complexes *in vitro* requires the presence of both dynactin and a coiled coil–containing cargo adaptor, such as BICD2 (refs. 4,5). Previous biochemical studies have shown that human dynein and dynactin form a stable complex only in the presence of BICD2 (ref. 3). In addition to BICD2, three other known dynein-cargo adaptors have been shown to activate dynein–dynactin: Hook3, Spindly and Rab11-FIP3 (ref. 4). Urnavicius *et al.*[8] and Chowdhury *et al.*[7] took advantage of the stable dynein–dynactin–BICD2 (DDB) complex to solve structures of DDB and DDB bound to microtubules, respectively.

Urnavicius *et al.*[8] solved an 8.2-Å structure of the tail domain of dynein bound to dynactin and BICD2 (termed TDB). A key finding from this structure was that the tail of dynein binds directly to the dynactin filament and that this interaction is mediated in part by BICD2's N-terminal coiled coil (**Fig. 1b**). This dependence may be the physical basis for the requirement of a dynein-cargo adaptor to form a stable DDB complex. Interestingly, the dimeric dynein tail interacts with the Arp1 filament in the same cleft used by myosin to interact with F-actin. Another intriguing finding from comparison of the structures of dynactin alone with p150 in the docked conformation to the TDB complex was that p150 (CC1a) and BICD2 have overlapping binding sites near the pointed-end complex. This suggests that binding of p150 (CC1a) and BICD2 would be mutually exclusive and raises the possibility that the docked version of p150 could be an autoinhibited state that is relieved by the binding of a cargo adaptor.

Chowdhury *et al.*[7] report two-dimensional class averages of DDB bound to microtubules. These structures offer the first glimpse of the architecture of the entire DDB complex on microtubules, revealing an elongated structure with dynein's two motor domains arranged side by side, with their microtubule-binding domains both pointed toward the minus end of the microtubule (**Fig. 1b**). Dynein's tail and dynactin extend nearly parallel to the microtubule for ~50 nm (**Fig. 1b**). The dynactin shoulder is found on the side of the dynactin filament opposite to the microtubule. Although p150's CAP-Gly domain is not visible in these structures, the long coiled-coil domains of p150 would still be sufficient to reach the microtubule (as depicted in **Fig. 1b**).

The Chowdhury *et al.*[7] structures show, in the context of dynactin and BICD2, that the dynein motor domains are positioned in a manner that would favor unidirectional motility. The Urnavicius *et al.*[8] TDB structure suggests a mechanism by which dynactin may induce an active dynein conformation. In this structure, both tail fragments show a translational symmetry that matches that of the dynactin filament. However, the two tail fragments bind the filament in slightly different ways, thus breaking this symmetry. Given that dynein has been reported to be in an autoinhibited state when its two motor domains are arranged symmetrically[17], binding of dynactin to dynein may activate the motor by disrupting these arrangements.

The structures in the reports by Chowdhury *et al.*[7] and Urnavicius *et al.*[8] answer many questions about the overall structure of the dynactin complex and DDB bound to microtubules. Challenges for the future will be to determine higher-resolution structures of dynein–dynactin with different cargo adaptors, on and off microtubules. Such structures will yield further insights into how dynactin and dynein's cargo adaptors activate dynein for motility. In addition, single-molecule studies will allow a better understanding of the roles of dynactin and cargo adaptors in the stepping mechanism of the DDB complex. Although recombinant mammalian dynein can now be made[5,18], generating recombinant dynactin will be essential for further structure-function studies.

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