The Role of Bicaudal D in motor protein mediated transport

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Preface

This thesis is written in the context of the Utrecht University master program Cancer Genomics and Developmental Biology. The main topic is: the role of Bicaudal D in motor protein mediated transport. In chapter 1 I will introduce the different cytoskeletal filaments and how they are regulated. In chapter 2 the properties and functions of motor proteins will be explained. Chapter 3 will focus on Bicaudal D and its functions in motor protein transport. Finally, chapter 4 includes a short summary and an overview of some future prospects.

I would like to thank Prof. Dr. Anna Akhmanova for the supervision during the writing of this thesis.

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1 The cytoskeleton

Eukaryotic cells contain a network of fibers extending throughout the cytoplasm, the cytoskeleton. It provides the cells with structural support to maintain their shape and adjust to their environment. The cytoskeleton is involved in processes like organelle anchorage, cell motility, intracellular transport and chromosome segregation. A cytoskeletal network is composed of three main types of molecular structures: microtubules, actin filaments and intermediate filaments. These three types of fibers are different in mechanical properties, dynamics, and biological roles (Campbell & Reece, 2005; Alberts, 2008).

1.1 Microtubules

Microtubules are hollow tubes measuring about 25 nm in diameter. The microtubule wall is constructed from a globular protein called tubulin. Each tubulin molecule is a dimer which consist of two slightly different polypeptide subunits, α-tubulin and ß-tubulin. Other tubulin family members are γ, δ and ε-tubulin. γ-tubulin is found at the centrosome and spindle pole bodies. δ and ε-tubulin are located at the centrioles and are proposed to be involved in mitotic spindle formation (Chang & Stearns, 2000). Post-translational modifications of tubulin family members further increases the diversity of tubulin isoforms (Verhey & Gaertig, 2007). Microtubules provide a network for anchoring and positioning of cellular structures like the Golgi, endoplasmatic reticulum and mitochondria. They shape and support the cell and serve as tracks along which cargo equipped with motor proteins can move (fig. 1).

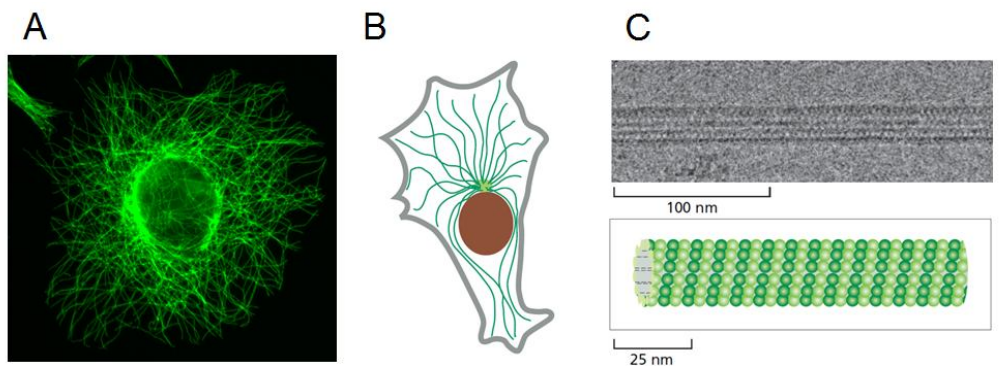

Figure 1: *Microtubules.* ***(A)*** *Immunofluorescent staining of microtubules (www.jefz.com).* ***(B)*** *Schematic illustration of microtubule distribution in a cell.* ***(C)*** *Electron micrograph and schematic illustration of a microtubule fiber with in green the protofilaments (Alberts et al., 2008).*

Microtubules are polarized protein structures which have a plus-end and a minus-end. *In vivo*, microtubule growth primarily initiates at a centrosome, a region often located near the nucleus that is considered to be the Microtubule Organizing Centre (MTOC) (Osborn & Weber, 1976). Within the centrosome of mammalian cells are a pair of centrioles, each composed of nine sets of triplet γ-tubulin containing microtubules arranged in a ring surrounded by pericentriolar material (Fuller *et al.*, 1995). Microtubules are anchored with the minus-end at the MTOC, whereas the microtubule plus-ends are growing towards the cell periphery (Alberts *et al.*, 2008).

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1.2 Microtubule dynamics

Tubulin dimers interact with one another, forming a long chain which twists around in a helical lattice. This way they form a hollow tube of 13 protofilaments which associate with one another laterally. The ß-tubulin of the dimer is bound to a nucleotide which can be either GTP or GDP. The hydrolysis of this GTP to GDP has an important effect on microtubule dynamics. Microtubule polymerization occurs at the dynamic plus-end. At this side, free GTP-bound tubulin dimers are incorporated (Tian *et al.*, 1997). After incorporation the ß-tubulin GTP is hydrolysed to GDP. In a growing microtubule, the addition of GTP bound tubulin dimers is faster than the hydrolysis from GTP to GDP. This results in a so-called GTP-cap which favors microtubule growth. However, if nucleotide hydrolysis proceeds more rapidly than subunit addition, the GTP-cap is lost. GDP bound tubulin dimers induce a bent conformation and the protofilaments try to curve outwards, resulting in shrinkage of the microtubule (Desai & Mitchison, 1997). Transition from a growing to a shrinking stage is called catastrophe. When a microtubule regains a GTP-cap and starts growing again it is called rescue. Treadmilling of microtubules is observed when the disassembly at the minus-end is as high as the assembly at the plus-end. In this situation the microtubule maintains a constant length. The dynamic properties of microtubules enable a cell to quickly respond to environmental cues (Alberts *et al.*, 2008).

1.3 Actin filaments

Actin filaments are solid rods about 7 nm in diameter. They consist of two-stranded helical polymers of the protein actin. Actin filaments appear as flexible structures which are organized into a variety of linear bundles, two-dimensional networks and three-dimensional gels. As a result of nucleating at the plasma membrane, most actin filaments are found at the *cortex* just beneath the plasma membrane, but they are also present in the remaining part of the cell (fig. 2).

In vertebrates, three main groups of actin isoforms have been identified: α, ß, and γ-actin (Vandekerckhove & Weber, 1978; Furukawa & Fechheimer, 1997). In muscle cells α-actin is the main component. It is a major constituent of the contractile apparatus. ß and γ-actin are mainly found in most cell types as components of the cytoskeleton and as mediators of internal cell motility.

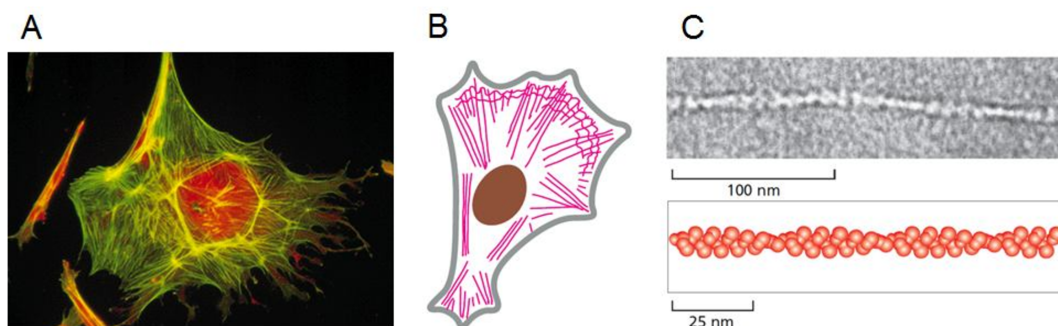

Figure 2: *Actin filaments.* ***(A)*** *Immunofluorescent staining of actin filaments (www.gwu.edu).* ***(B)*** *Schematic illustration of actin filament distribution in a cell.* ***(C)*** *Electron micrograph and schematic illustration of an actin filament with in red the protofilaments (Alberts et al., 2008).*

Actin polymerization starts with the formation of new nucleation sites. This nucleation can be catalyzed by three different types of regulated factors, the ARP2/3 complex, formin proteins and the protein Spire (Kerkhoff, 2006; Pollard, 2007). The ARP2/3 complex nucleates actin filament growth from the minus-end and allows rapid elongation at the plus-end. It is able to nucleate new filaments from the side of an existing filament, thereby building individual filaments into a treelike web. Formin and Spire are proteins which are able to nucleate the growth of straight, unbranched filaments that can be crosslinked by other proteins to form

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parallel bundles. When an actin filament extends, a formin dimer remains on the growing plus end, while still allowing the binding of new actin dimers. Spire nucleates a new filament by stabilizing an actin tetramer (Wang, 1985).

Like microtubules also actin filaments are polarized structures. The polarity of an actin filament can be determined by decorating the filaments with myosin (Wegner, 1976). Based on the myosin orientation, the two ends of an actin filament are called the barbed end and the pointed end. The barbed end is the fast growing plus-end whereas the pointed end is the minus-end. Non muscular eukaryotic cells contain a large pool of ATP-bound globular actin monomers (G-actin). By fast remodeling of the G-actin pool, polarized actin filaments (F-actin) which consist of two helical polymers of G-actin are formed. After being incorporated in F-actin, ATP bound to G-actin is hydrolyzed to ADP. Like in microtubule formation, an energy rich nucleotide cap is formed at the plus-end of an actin filament when enough ATP-bound G-actin is present. A shortage of ATP-bound G-actin results in the breakdown of actin filaments (Alberts *et al.*, 2008).

1.4 Intermediate filaments

The last of the three types of fibers are the intermediate filaments. Intermediate filaments are composed of rod-shaped proteins that can self-assemble into 8-12 nm non-polarised structures in the absence of both ATP and GTP (Strelkov *et al.*, 2003). They owe their name to their diameter which is smaller than the diameter of microtubules but larger than that of actin filaments. In contrast to microtubules and actin filaments, intermediate filaments are constructed of different molecular subunits belonging to a family of proteins (fig. 3). Five different intermediate filament classes are recognized, of which four are located in the cytoplasm. Only class V intermediate filaments, known as the lamins, are found in the nucleus.

Expression patterns of different intermediate filaments appear to be cell type- and tissue-specific (Parry *et al.*, 2007; Goldman *et al.*, 2008). Microtubules and actin filaments are often disassembled and reassembled in various parts of a cell. However, intermediate filaments appear to be more permanent fixtures. The outer layer of the skin consists of dead cells full of class I intermediate filaments known as epithelial keratins (Coulombe *et al.*, 2002). This shows that even after cells die, intermediate filament networks can persist. Removal of microtubules and actin filaments from the cytoplasm of living cells by chemical treatment leaves a web of intermediate filaments that maintains the original cell shape, suggesting that intermediate filaments are especially important in reinforcing the shape of the cell (Campbell & Reece, 2005).

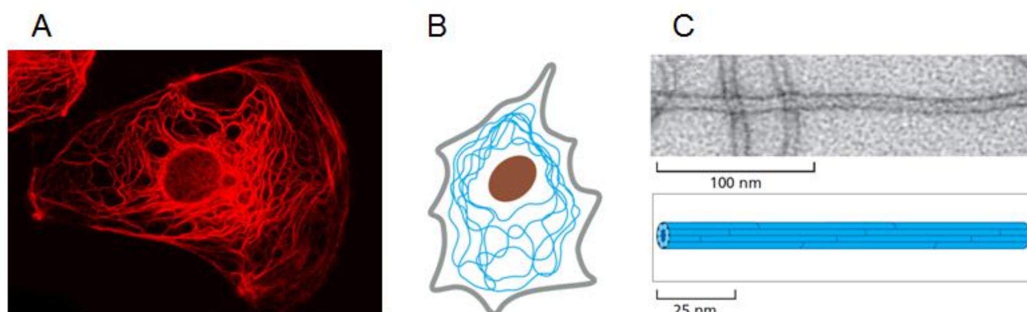

Figure 3: *Intermediate filaments.* ***(A)*** *Immunofluorescent staining of intermediate filaments (www.microscopyu.com).* ***(B)*** *Schematic illustration of intermediate filament distribution in a cell.* ***(C)*** *Electron micrograph and schematic illustration of an intermediate filament with in blue the individual polypeptides (Alberts et al., 2008).*

The individual polypeptides of intermediate filaments are elongated molecules with an extended central α-helical domain. Via this domain, individual polypeptides form a coiled coil with other monomers. A pair of parallel dimers then associates in an antiparallel fashion to form a staggered tetramer with two identical ends. The tetramers pack together to form the

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filament which includes eight parallel protofilaments made up of tetramers. This results in a rope-like structure composed of 32 individual α-helical coils which can easily bend but is extremely difficult to break (Kirmse *et al.*, 2007). There are some variations in the formation of dimers. Vimentin (a type III intermediate filament) is known to form homodimers, whereas keratins (type I and II intermediate filaments) assemble into heterodimers (Parry *et al.*, 2007).

Intermediate filaments are shown to have an important function in the positioning of cell organelles such as mitochondria and the Golgi apparatus (Tzur *et al.*, 2006; Toivola *et al.*, 2005). They make use of interactions with both microtubules and actin filaments via a variety of linker proteins like plectin and filaggrin (Capetanaki *et al.*, 2007; Alberts *et al.*, 2008).

1.5 Regulation of cytoskeletal filaments

Cytoskeletal filaments are highly dynamic structures. Cells are able to regulate their length, stability, number and geometry. Direct covalent modification of filament subunits regulates some filament properties, but most of the regulation is performed by a large array of accessory proteins that bind to either the filaments or their free subunits (Alberts *et al.*, 2008).

1.5.1 Sequestering proteins

In nonmuscle vertebrate cells, approximately 50% of the actin is present as a monomeric free subunit. This is due to a protein called thymosin which binds free actin subunits and thereby inhibits filament formation (Hannappel, 2007). Profilin is a protein which competes with thymosin to bind actin monomers and transports subunits to the growing plus end of an actin filament. Upon binding of the monomer to the filament, conformational change in the actin reduces its affinity for profilin, so it falls off (Carlsson *et al.*, 1977).

Like actin monomer sequestering, also tubulin subunits can be prevented from binding to a growing microtubule. A protein called stathmin was found to bind two tubulin heterodimers and prevents their addition onto the ends of microtubules. Phosphorylation of stathmin inhibits its binding to tubulin and therefore increases the rate of microtubule elongation (Steinmetz, 2007).

1.5.2 Severing proteins

As a result of filament degradation, many smaller filaments are created. These smaller filaments are in some cellular conditions able to nucleate and elongate, thereby forming many new long filaments. In this case severing accelerates the assembly of new filament structures. In contrast, when the cellular conditions do not encourage nucleation and elongation of the new filaments, severing promotes the filament depolymerization (Alberts *et al.*, 2008). A protein known by its ability to sever microtubules is katanin. This protein was found to release microtubules from the MTOC and it is thought to play an important role in microtubule depolymerization at the poles of spindles during meiosis and mitosis. Katanin is composed of a small and a large subunit. The smaller subunit hydrolizes ATP and is responsible for the severing of microtubules. The larger subunit directs katanin to the centrosome (McNally & Vale, 1993).

Severing of actin filaments is mainly performed by proteins of the gelsolin superfamily. The severing activity of gelsolins is activated by high levels of Ca^{2+} in the cytosol. In contrast to katanin, gelsolin acts in an ATP independent manner. Subdomains of gelsolin bind two different sites on the actin subunit. After binding, gelsolin 'waits' until a thermal fluctuation happens to create a small gap between two actin subunits. Gelsolin then insinuates a subdomain into the gap which severs the subunit (Sun *et al.*, 1999).

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1.5.3 Lattice-binding proteins

Once a cytoskeletal filament is formed, its stability and mechanical properties are altered by a set of proteins that bind along the sides of the polymer. Microtubule associated proteins (MAPs) are known to influence the dynamic behavior of microtubules. Their phosphorylation by several protein kinases can have a primary role in controlling both its activity and localization inside cells. MAP2 and Tau are MAPs which are known to decorate microtubules, stabilize them and protect them against microtubule-severing proteins (Tucker, 1990; Weingarten *et al.*, 1975). Both proteins also contribute to filament cross-linking. MAP2 and Tau are composed of two domains. One binds along the microtubule and thereby stabilizes the filament and one projects outward to contact other MAP-coated microtubules.

Also actin filaments are affected by the binding of accessory proteins. Tropomyosin binds simultaneously to seven adjacent actin subunits in one protofilament, thereby stabilizing the actin polymers. In addition, tropomyosin prevents other proteins from binding to the actin filaments (Pittenger *et al.*, 1994). Another important actin filament binding protein is cofilin. Cofilin is able to destabilize and depolymerize actin polymers. It forces the filaments to twist more tightly which weakens the contact between actin subunits. Because cofilin preferentially binds ADP-bound actin, older actin filaments are more likely to be depolymerized (Lappalainen & Drubin, 1997).

1.5.4 End-binding proteins

Since subunit addition and loss occur primarily at filament ends, a protein that binds preferentially to the ends of filaments can have dramatic effects on filament dynamics even when they are present at very low levels. The most rapid changes in actin filaments occur at the plus-end. To restrain the actin filament from growth and depolymerization, capping proteins can bind the filament plus ends. A well characterized example of an actin plus end capping protein is CapZ, which has an important role in muscle cells (Casella *et al.*, 1989).

Microtubule properties make the microtubule ends much more complex than actin filament ends. This provides many more possibilities for accessory proteins to regulate microtubule dynamics. The minus-ends of microtubules are stabilized by association with the centrosome or serve as microtubule depolymerization sites. The plus ends, in contrast, are associated by an interesting group of MAPs, the plus-end tracking proteins (+TIPs). +TIPs are known to remain associated with growing plus ends and can link them to other cellular structures such as the cell cortex or kinetochores. In addition, they have an important function in stabilizing the microtubules (Akhmanova & Hoogenraad, 2005). Within the +TIPs protein family the End Binding (EB) proteins can be found. EB proteins are described as a platform on which other plus-end binding proteins, which affect the microtubule dynamics, can bind (Lansbergen & Akhmanova, 2006).

1.5.5 Large scale structure organizing proteins

To form a useful intracellular structure that gives the cell mechanical integrity and determines its shape, individual filaments must be organized and attached to one another in larger-scale structures. The centrosome is one example which holds the microtubule minus-ends together at the cell centre. As described above, MAPs play an important role in microtubule filament cross-linking (Albert *et al.*, 2008).

Actin filaments are organized in either a parallel bundle or a gel-like network of filaments. These different filaments are initiated and maintained by different proteins. The small monomeric bundling protein fimbrin packs actin filaments in very tight parallel bundles (Arruda *et al.*, 1995). Also the larger dimeric bundling protein α-actinin organizes actin filaments in bundles. In contrast to fimbrin packed bundles, α-actinin packed bundles are contractile due to oppositely oriented actin filaments and the presence of the motor protein myosin (Youssoufian *et al.*, 1990). To organize actin filaments into a gel-like network of filaments, linker proteins have to connect the filaments in a non-parallel fashion. An example

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of such a protein is filamin. Filamin has two actin-binding sites with a V-shaped linkage in between them, so that it cross-links actin filaments into a network with the filaments oriented almost at right angles to one another (Feng *et al.*, 2004). Another well studied example is spectrin. Spectrin is a tetramer composed of two α and two β subunits. It is arranged in a way that two actin binding sites are separated by about 200 nm. Spectrin was first identified in red blood cells where it forms an actin-web which is linked to the plasma membrane. The actin web enables red blood cells to regain their original shape after going trough very small capillaries (Matteis & Morrow, 2000). Responsible for the linkage between actin and the plasma membrane are members of the protein ERM family. The C-terminus of these proteins binds actin filaments whereas the N-terminus is responsible for binding to integral proteins of the plasma membrane or scaffolding proteins localized beneath the plasma membrane (Tsukita *et al.*, 1997).

Besides microtubules and actin filamens also intermediate filaments are cross-linked and bundled into strong arrays. Many intermediate filaments bundle themselves by self-association but also accessory proteins play a role in intermediate filament bundling. Filaggrin is an intermediate filament associated protein that binds to keratin fibers in epithelial cells. This gives the outermost layers of the skin their toughness (Sandilands *et al.*, 2007).

A protein which acts as a linker between all the three main components of the cytoskeleton is plectin (Svitkina *et al.*, 1996). Besides binding to microtubules, actin filaments and intermediate filaments, plectin is also found to bind junctions in the plasma membrane that structurally connect different cells (Wiche, 1998).

2. Molecular motors

In order to function properly, cells transport proteins, lipids, mRNA's and cell organelles to various destinations in the cytoplasm. Molecular motors are responsible for most forms of movement encountered in the cell. These proteins use polarized cytoskeletal filaments as rails on which they convey their cargoes. Molecular motors differ in the filament track they bind (actin filaments or microtubules), in the direction they move and in the cargo they transport. All motor proteins use the energy derived from repeated cycles of ATP hydrolysis. This leads to conformational changes in the motor domains and the movement of the proteins along their filament track. Next to the motor domain, motor proteins contain a tail domain which is responsible for dimerizatoin, regulation and interactions with other molecules (Schliwa & Woehlke, 2003).

2.1 Myosin

The first motor protein identified was skeletal muscle myosin (Pollard & Korn, 1973). This myosin, called myosin II, generates the force for muscle contraction. Myosin II appeared to be a member of a myosin superfamily the members of which have very specialized functions in certain cell types, while others are ubiquitous (Sellers, 1999; Mermall *et al.*, 1998). Sequence comparisons among diverse eukaryotes indicate that there are at least 37 distinct myosins types (Alberts *et al.*, 2008). Myosins use actin filaments as a rail to transport different cargoes. Except myosin VI (Sweeney & Houdusse, 2010) all myosins move towards the plus end of the actin filaments. Myosins are constructed of three functional subdomains. A globular motor or head domain at the N-terminus which interacts with actin and binds ATP, a neck domain which functions as a lever arm and a tail domain. The tail domains vary widely in sequence and length between different myosins. This enables the myosins to bind different cargoes very specifically. Besides cargo binding, the tail domains also allow myosins to dimerize due to coiled-coil forming sequences.

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The motor domain of the myosin is the force-generating part of the protein which contains an ATP binding site (Sellers, 2000). The hydrolysis of ATP to ADP results in a small conformational change of the ATP binding site and leads to a rotation of another protein subunit. This rotation is relayed to the actin binding interface. The plus-end movement of the myosins is generated by the swinging of the α-helix lever arm. In myosin II, the α-helix lever arm is structurally stabilized by the binding of light chains. At the basis of this α-helix, another piston like helix connects the movements of the ATP binding site to a small rotation of a converter domain resulting in the swing of the lever arm. These changes in the conformation of the myosin are coupled to changes in the actin binding affinity, allowing the myosins to move towards the plus ends of actin filaments (fig. 4) (Alberts *et al.*, 2008).

As decribed above, myosin II plays a very important role in muscle contraction. A muscle sarcomere is composed of thin actin filaments and thick myosin filaments. The myosin heads in the thick myosin filaments are oppositely oriented which makes them efficient at sliding pairs of actin filaments past each other. If no stimulus is present, the myosin-binding sites of actin are blocked by tropomyosin. In the presence of an activating signal, calcium ions bind to troponin which inhibits the blocking action of tropomyosin and enables the myosin heads to bind the actin filaments. Due to alternate attachment and detachment of the myosin heads the actin filaments are pulled towards the centre of the sarcomere resulting in muscle contraction (Campbell & Reece, 2005).

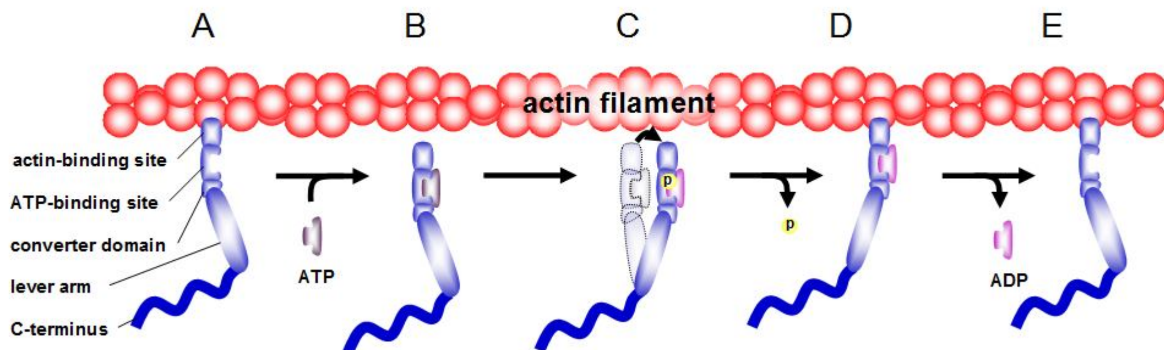

Figure 4: *The working mechanism of myosin.* ***(A)*** *Myosin lacking ATP is tightly bound to the actin filament via the actin-binding site.* ***(B)*** *An ATP molecule binds to the ATP-binding site and causes a slight conformational change in the actin binding site. Myosin comes of the actin filament.* ***(C)*** *Hydrolysis of ATP to ADP results in a relayed structural change of the converter domain and the lever arm. Myosin is displaced along the actin filament by a distance of about 5 nm.* ***(D)*** *Weak binding of the the actin-binding site to the actin filament causes release of the inorganic phosphate. This results in tight binding of the actin-binding site to the actin filament.* ***(E)*** *The release of ADP triggers the myosin power stroke and brings the myosin back to the start conformation.*

2.2 Kinesin

Like myosins, kinesins are member of a large protein superfamiliy, for which the motor domain is the only common element. Instead of using actin filaments for transport, the motor protein kinesin uses microtubules to transport cargo (Alberts *et al*, 2008). A kinesin monomer consists of a motor head, a neck linker, a long coiled-coil dimerization region and a globular tail domain (Kozielski *et al.*, 1997). The active dimeric form of conventional kinesin has a similar structure to myosin II in having two globular head motor domains and an elongated coiled coil tail responsible for heavy chain dimerization (Bloom *et al.*, 1988). *In vitro*, kinesin-1 dimers are capable of making up to hundred steps along microtubules before dissociation. Also *in vivo* cargoes can be transported over very long distances without losing their track, most likely because several motors are simultaneously attached to a cargo.

In the majority of the 45 kinesins known in humans, the motor domain is localized on the N-terminal part of the protein. These kinesins move towards the microtubule plus ends (Miki *et al.*, 2001). An exception are the members of the kinesin-13 family, such as mitotic centromere-associated kinesin (MCAK). In these proteins the motor domains are located in the middle. MCAK binds the plus-ends of the microtubules and functions as a microtubule depolymerase which is particularly important during mitosis (Hunter *et al.*, 2003; Ogawa *et al.*,

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2004). A small group of kinesins, like KIFC2, has their motor domains located at the C-terminus. These proteins are able to transport their cargo towards the minus-ends of microtubules (Hanlon *et al.*, 1997; Saito *et al.*, 1997; Yang *et al.*, 2001).

In all kinesins, the motor domain is responsible for microtubule and ATP binding (Scholey *et al.*, 1989; Hirokawa *et al.*, 1989). Hydrolysis of ATP results in the conformational change of a mechanical element which is called the neck linker. This conformational change enables kinesin to move along microtubules (Rice *et al.*, 1999) (fig. 5). Different mechanisms have been proposed to explain the moving ability of kinesin dimers. The inchworm model implies a stepping behavior without rotation of the motor. After attachment of the leading motor head to the microtubule, the rear head will step towards the leading head and binds the microtubule. Subsequently the leading head makes a new step forward and the process will be repeated (Block & Svoboda, 1995; Kozielski *et al.*, 1997) (fig. 6A). In the hand-over-hand mechanisms, the heads exchange their positions every step. Two hand-over-hand models have been proposed. According to the symmetric hand-over-hand model the moving head passes the attached head on the same side every step. This results in a 360° rotation of the motor every two steps (fig. 6B). According to the asymmetric hand-over-hand model the moving heads of kinesin pass each other on different sides. With every step the motor rotates 180 degrees and the rotation reverses with every subsequent step. The netto rotation according to this mechanism after two steps is zero (Hua *et al.*, 2002; Asbury *et al.*, 2003) (fig. 6C).

Next to kinesin dimers, also kinesin monomers are able to move along microtubules. Initially it was though that neither the inchworm model nor the hand-over-hand models could explain the motility of kinesin monomers, as they do not have a second head to support the moving head while searching for the next binding site. Their moving ability was explained by electrostatic interactions between the positively charged neck of the motor (K-loop) and the negatively charged C-terminus of tubulin (E-hook). This should enable kinesin monomers to move to the next binding site without fully detaching from the microtubule (Okada & Hirokawa, 1999; Lakämper & Meyhöfer, 2005). However, subsequent research showed that kinesin monomers are able to dimerize. This suggests that they operate by a mechanism similar to conventional kinesin. The regulation of motor dimerization could therefore by a mechanism which controls transport by this class of kinesins (Tomishige et al., 2002).

The neck linkers of kinesin monomers are connected to a common stalk (Vale & Fletterick, 1997). At the end of the stalk, there is a kinesin light chain which modulates the cargo-binding affinity of the protein and enables the kinesin to bind different cargoes very specifically (Hirokawa *et al.*, 1989). When there is no cargo bound, the kinesin stalk is bent in such a way that the kinesin light chain blocks the movement of the motor domain. This prevents the hydrolysis of ATP and thus the movement of kinesis without transporting cargo (Hackney *et al,*. 1992; Friedman & Vale, 1999).

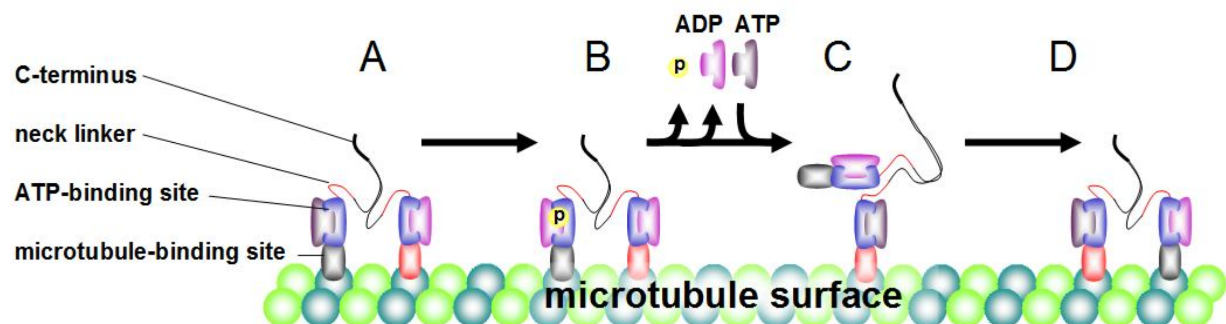

Figure 5: *The hand-over-hand working mechanism of kinesin.* ***(A)*** *The rear head is tighly bound to ATP and the microtubule surface while the front head is loosely bound to the microtubule surface with ADP in its binding site.* ***(B, C)*** *Due to ATP exchange with ADP in the front head, the front head neck linker is shifted from a rearward-pointing to a forward-pointing conformation. This together with the release of an inorganic phosphate pulls the rear head forward.* ***(D)*** *The rear head loosely binds to the microtubule surface and the kinesin is ready to repeat the process.*

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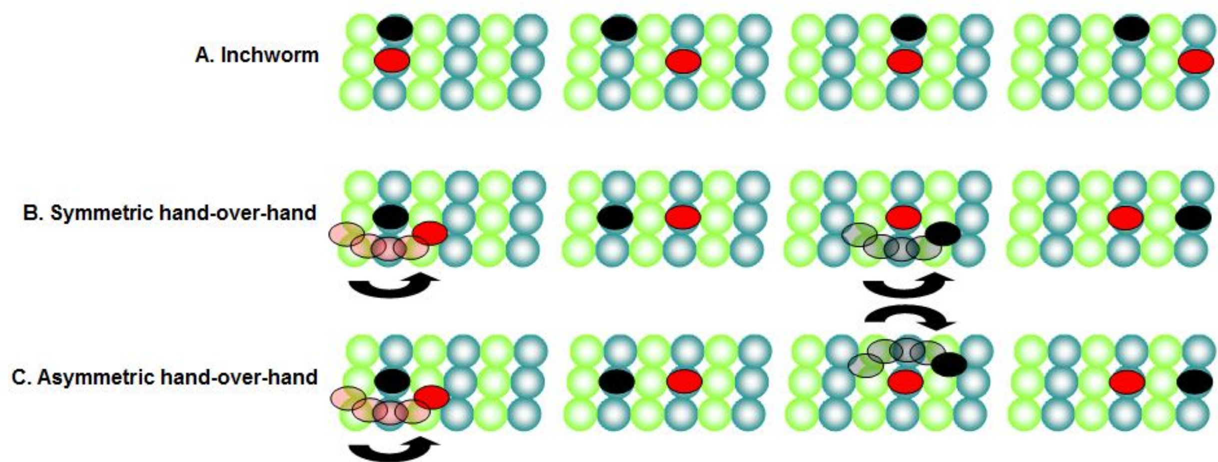

Figure 6: *Models of kinesin walking patterns.* ***(A)*** *Inchworm model.* ***(B)*** *Symmetric hand-over-hand model. The netto rotation of the motor after two steps is 360°.* ***(C)*** *Asymmetric hand-over-hand model. The netto rotation of the motor after two steps is zero. Arrows indicate direction of rotation.*

2.3 Dynein

The largest and the fastest among the molecular motor proteins is dynein. Dyneins are a family of minus-end directed microtubule motors unrelated to the kinesin superfamily. More then 15 dynein species are discoved which can be divided into two dynein subclasses; axonemal and cytoplasmic dynein. Axonemal dynein is an immobile motor involved in waving of cilia and beating of flagella. Most cilia and flagella have a core of two microtubules surrounded by nine fused pairs of microtubule doublets. The core is connected to the outer doublets via cross-linking proteins (Summers & Gibbons, 1971). The microtubule assembly of a cilium or flagellum is anchored in the cell by a basal body, which is structurally identical to a centriole. In many multicellular organisms the basal body of the fertilizing sperm's flagellum enters the egg and becomes a centriole. The axonemal dyneins are stably attached to the outer microtubule of the paired microtubules and connect the separated doublets. On the outside of the outer microtubule, the outer arm dyneins are located, whereas the inner arm dyneins are located on the inside of this microtubule. Because the microtubule doublets are physically restrained by cross-linking proteins, movement of the dyneins forces the cilia or the flagella to bend. This causes their beating and waving (Campbell & Reece, 2005).

Almost all minus-end directed transport within the cytoplasm, as well as several mitotic functions, are carried out by cytoplasmic dynein. Cytoplasmic dynein consist of two heavy chains which comprise a well conserved C-terminal motor domain and a N-terminal tail domain (Alberts *et al.*, 2008). Its N-terminal part forms a tail that binds a set of intermediate chains and light intermediate chains and connects to the other heavy chain in a dynein molecule. The smaller light chain 7 (LC7), light chain 8 (LC8) and T-complex testis-specific protein 1 (TCTEX1) assemble on the intermediate chain and facilitate interactions with dynein adaptor proteins (Kardon & Vale, 2009).

The C-terminal motor domain contains six tandem ATPase units arrayed in a ring and is related in sequence en structural organization to the AAA superfamily (Burgess *et al.*, 2003; Roberts *et al.*, 2009). AAA1 is the main site for ATP hydrolysis (Gee *et al.*, 1997; Gibbons *et al.,* 1987) but also AAA2, AAA3 and AAA4 have been implicated in motor function (Cho *et al.*, 2008; Reck-Peterson & Vale, 2004; Kon *et al.*, 2004). A linker domain, extending N-terminally from the AAA ring, connects the motor with the N-terminal tail domain. This linker is thought to be the mechanical element of the protein and therefore structurally changed upon ATP binding and hydrolysis. In the high-affinity microtubule binding state of the protein the linker arches and makes contact with AAA1 and AAA4 or AAA5. In response to ATP binding the linker position changes. The restorative movement of the linker after ATP hydrolysis is thought to be the force-producing power stroke step which enables dynein to move along microtubules (Roberts *et al.*, 2009; Reck-Peterson *et al.*, 2006). Projecting from

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the AAA ring is the stalk which is an antiparallel coiled-coil of two α-helices with a small microtubule binding domain at the distal tip (Gee *et al.*, 1997; Carter *et al.*, 2008). Microtubule binding affinity of the microtubule binding domain depends on ATP-hydrolysis. Stalk behavior is inter alia regulated by an additional coiled-coil buttress which extends from AAA5 and interacts directly with the stalk coiled coil (Carter *et al.*, 2011; Kon *et al.*, 2011). Following AAA6 a C-terminal domain is located at the C-terminal end of the protein (fig. 7). The specific function of this domain is not clear but removal of this domain in *Distyostelium discoideum* cytoplasmic dynein reduces the productivity suggesting that the C-terminal domain helps coordinating dimeric motor function (Numata *et al.*, 2011).

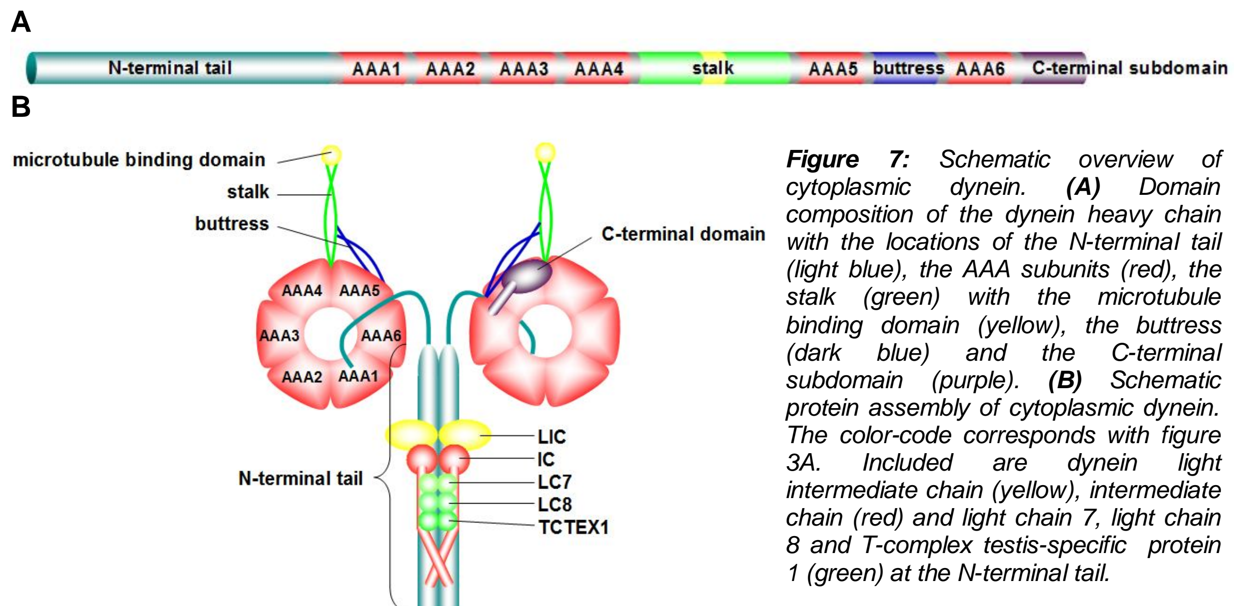

Figure 7: *Schematic overview of cytoplasmic dynein.* ***(A)*** *Domain composition of the dynein heavy chain with the locations of the N-terminal tail (light blue), the AAA subunits (red), the stalk (green) with the microtubule binding domain (yellow), the buttress (dark blue) and the C-terminal subdomain (purple).* ***(B)*** *Schematic protein assembly of cytoplasmic dynein. The color-code corresponds with figure 3A. Included are dynein light intermediate chain (yellow), intermediate chain (red) and light chain 7, light chain 8 and T-complex testis-specific protein 1 (green) at the N-terminal tail.*

2.4 Dynactin

Kinesins and myosins display a wide variety of tail domains, which mediate highly specific cargo interactions. Although cytoplasmic dynein does not have such a wide variety of tail domains, it is coupled to a wide range of cargoes. This is regulated by interactions with several factors that do not belong to the dynein itself but are crucial for attaching the motor to its cellular function. One of these factors which helps to target dynein to specific locations, links dynein to cargoes and increases the dynein processivity is dynactin.

Dynactin is a protein complex consisting of several different polypeptide subunits. The central scaffold of dynactin is formed by a filament-like structure called actin related protein 1 (ARP1). ARP1 was found to bind spectrin which coats the face of several organelles (Holleran *et al.*, 2001). This may be a general mechanism of linking cargoes to cytoplasmic dynein. The barbed end of ARP1 terminates with the actin-capping protein CapZ (Schafer *et al.*, 1994a). The pointed end of ARP1 is capped with a second actin like protein ARP11 (Eckley et al., 1999) and accessory subunits p25, p27 and p62 (Schafer *et al.*, 1994b). p25 and p27 are the smallest subunits of dynactin and are the only subunits which exist in a free soluble pool in the cell. Because they also act apart form dynactin, they could function as adaptor proteins for dynactin targeting (Eckley and Schroer, 2003).

Extending at the barbed end of ARP11 is a flexible and extendable subunit, composed of a dimer of $p150^{glued}$, a tetramer of dynamitin and p24/22. $p150^{glued}$ is the largest subunit of dynactin and consists of two coiled-coil domains which are separated by unstructured regions and form an elongated dimer. At the N-terminal end of $p150^{glued}$ two globular cytoskeleton-associated protein, glycine rich (CAP-Gly) motifs are located which are known to bind microtubule plus-end associated protein end binding 1 (EB1) and CAP-Gly domain-containing linker protein 170 (CLIP-170), two microtubule plus-end binding proteins (Berrueta *et al.*, 1999; Askham *et al.*, 2002). It was found that microtubule binding by

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p150^{glued} is required for the ability of dynactin to enhance the processivity of cytoplasmic dynein (King & Schroer, 2000). The middle region of p150^{glued} is able to bind dynein intermediate chain and thus enables the dynein-dynactin interaction (King *et al.*, 2003). The C-terminal part of p150^{glued} is capable of binding to ARP1 via the second coiled-coil domain of p150^{glued} (Waterman-Storer *et al.*, 1995). Dynamitin functions as a linker between the two dynactin structural domains. Free dynamitin causes p150^{glued}-p24/22 displacement from the rest of the dynactin subunits (Echeverri *et al.*, 1996). p24/22 has been found to bind p150^{glued} and dynamitin, thereby forming a stable complex (Karki *et al.*, 1998; Eckley *et al.*, 1999) (fig. 8).

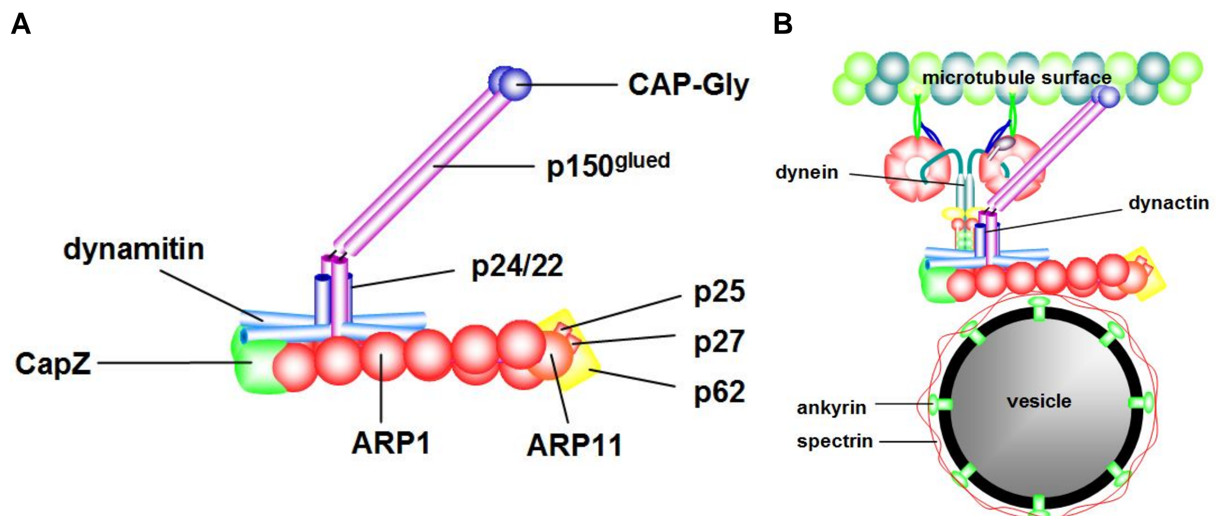

Figure 8: *Schematic overview of dynactin.* ***(A)*** *Schematic protein assembly of dynactin.* ***(B)*** *Proposed mechanism of vesicle transport via a dynein-dynactin complex.*

3 Bicaudal D

Dynactin has been shown to bind directly to vesicles via acidic phospholipids and spectrin (Holleran *et al.*, 2001). This appeared to be a general mechanism of dynein/dynactin cargo binding. In addition, multiple factors are found to contribute to the recruitment of dynein and dynactin to specific cargoes. Lysosomes use the small GTPase Rab7 to recruit the dynein/dynactin motor complex via p150^{glued} through interaction with Rab7-interacting lysosomal protein (RILP) (Jordens *et al.*, 2001; Johansson *et al.*, 2007).

Another cargo linking factor is the multi-purpose adaptor protein Bicaudal-D (BICD). BICD (bicaudal means two tails) was first identified in *Drosophila* and named after its heterozygous female mutants which produce double-abdomen embryos (Mohler & Wieschaus, 1985). BICD is a cytoplasmic coiled-coil protein which is highly conserved throughout evolution. It is composed of three coiled coil domains which show a high similarity among BICD family members (Terenzio & Schiavo, 2010). In *Drosophila* and *C. elegans* only one gene encoding BICD is present. In mammalians two *BICD* genes are present known as *BICD1* (Baens & Marynen, 1997) and *BICD2*, which are able to partially compensate for each other's function (Fumoto *et al.*, 2006) (fig. 9).

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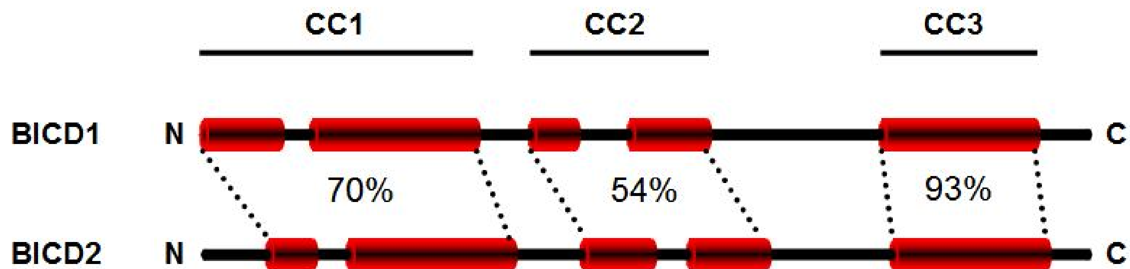

Figure 9: *Domain composition of the mammalian BICD proteins with the percentages of similarity between the different coiled-coil regions (red).*

3.1 Motor protein binding of Bicaudal D

To be able to function properly, cargo linking proteins require both a cargo binding, as well as a motor protein binding domain. BICD linkage to a motor protein was first established in mammalian tissue culture cells. It was shown that BICD2 interacts with the dynamitin subunit of dynactin and associates with cytoplasmic dynein (Hoogenraad *et al.*, 2001). By fusing the N-terminal portion of BICD to mitochondria and peroxisome-anchoring sequences, both mitochondria as well as peroxisomes were rapidly transported towards the perinuclear area where the minus-ends of microtubules are tethered. This suggests that the N-terminal portion of BICD is responsible for the dynein-dynactin binding (fig. 12). Full-length BICD reduces the dynein-recruiting activity compared to BICD lacking the C-terminus, indicating that the BICD C-terminus regulates the interaction between BICD and the motor protein complex (Hoogenraad *et al.*, 2003).

Next to the involvement of BICD in dynein mediated minus-end transport, the middle part of BICD was found to directly bind the plus-end directed motor kinesin-1 (Grigoriev *et al.*, 2007) (fig. 12). This suggests a function of BICD in the plus-end directed transport of cargo.

3.2 Bicaudal D in mRNA localization

In order to concentrate proteins at their site of function, cells often restrict the synthesis of a particular protein by localizing their mRNA molecules. This is for example important when the two daughter cells of a dividing cell have to perform different functions and therefore need a different protein content. Also in many neurons, mRNAs encoding proteins involved in synapse functions are localized close to the synapse. Such localization of mRNAs is carried out by motor proteins.

Also in *Drosophila* oogenesis and development the correct localization of mRNA is of vital importance. BICD plays a crucial role in these processes. After fertilization of the *Drosophila* oocyte, the nucleus starts to divide. This results in a multinucleated mass of cytoplasm that is separated into 16 individual cells which are interconnected via so-called ring channels (Kinderman, 1973). These ring channels are actin-rich structures that form cytoplasmic bridges. One of the cells becomes the oocyte and will develop into the embryo. The other cells become nurse cells, which supply the oocyte with required components that drive its development (fig. 10A). It was shown that BICD is required for the transport of mRNAs from the nurse cells to the oocyte anterior part. This indicates the involvement of BICD in dynein mediated minus-end transport (Swan and Suter, 1996; Clark *et al.*, 2007).

After fertilization, the developmental processes in the oocyte are largely regulated by localized gradients of mRNA molecules produced by follicle cells. These gradients determine the anatomical orientation of the embryo. For example, the future anterior part of the embryo contains mRNA coding for the protein Bicoid, whereas the posterior part of the embryo contains mRNA coding for the protein Nanos (fig. 10B). To localize the mRNA molecules to the intended sites, the oocyte takes advantage of its polarized microtubule skeleton. Where most microtubule minus-ends are clustered in the anterior part of the cell, the plus ends are located at the posterior part. BICD has been shown to play an important role in this mRNA

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distribution. For example, the anterior localization of *Bicoid* appeared to be regulated by BICD. In addition, also the anterior localization of *K10* mRNA, the dorso-anterior localization of *gurken* mRNA as well as the posterior localization of *oscar* mRNA were shown to be dependent on BICD activity (Swan & Suter, 1996; Ephrussi *et al.*, 1991).

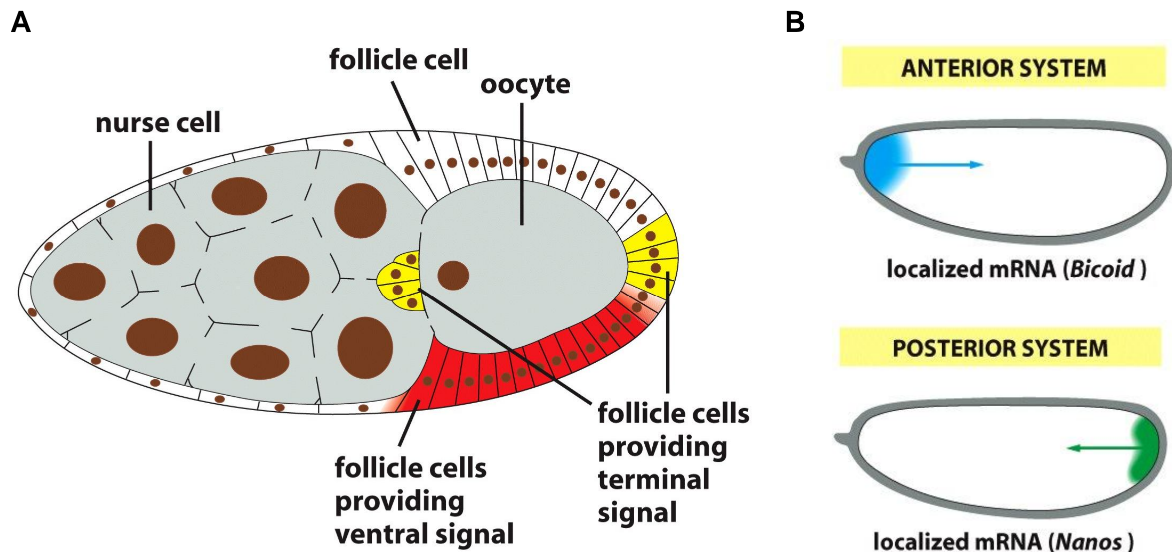

Figure 10: *Drosophila oogenesis.* ***(A)*** *Schematic illustration of a Drosophila oocyte attached by nurse cells and surrounded by follicle cells.* ***(B)*** *Localized Bicoid (bleu) and Nanos (green) mRNA gradients which determine the anatomical orientation of the embryo (Alberts et al., 2008).*

To fulfil its role in dynein-mediated mRNA transport during oogenesis, BICD binding to Egalitarian (Egl) was shown to be necessary (Bullock & Ish-Horowicz, 2001). Egl is a RNA-binding protein (Dienstbier *et al.*, 2009) and co-localizes with BICD in oocytes at all stages of oogenenis (Navarro *et al.*, 2009) (fig. 11). Both proteins co-immunoprecipitate, indicating that they are in the same protein-complex. In addition, Egl and BICD null mutants show very similar phenotypes (Mach & Lehmann, 1997). The involvement of BICD-Egl complexes in dynein mediated mRNA transport was investigated by the injection of fluorescent mRNA molecules. BICD-Egl complexes were recruited to the injected mRNA transcripts and were transported, together with the mRNA, to microtubule minus-ends in a dynein-dependent manner (Wilkie & Davis, 2001). In addition, it was found that the transport efficiency of mRNA correlates with the cytoplasmic levels of BICD, Egl and a dynein light chain (Bullock *et al.*, 2006)

Besides playing a role in oogenesis, BICD-Egl complexes also are important in several distinct mechanisms during *Drosophila* embryogenesis. During *Drosophila* embryogenesis, pair rule genes are expressed which divide the syncytial blastoderm into series of parasegments (Alberts, 2008). The BICD/Egl-dependent mRNA transport machinery plays an essential role in the dynein-mediated apical localization of the pair rule transcripts (Bullock & Ish-Horowicz, 2001; Wilkie & Davis, 2001).

The protein Inscuteable functions as part of a localized complex which coordinates the orientation of the mitotic spindle and the basal sorting of the anterior and posterior required mRNAs in asymmetric divisions along the apicobasal axis of neuroblasts in *Drosophila*. *Inscuteable* mRNAs are therefore apically localized. It was found that also this process is mediated by the BICD/Egl-dependent mRNA transport machinery (Hughes *et al.,* 2004).

Next to processive side-directed transport, mRNAs show short bi-directional movements in the cytoplasm. These movements are strongly reduced by interfering with dynein activity, showing that dynein is linked to these mRNAs. Surprisingly, inhibition of BICD or Egl function only prevents the processive transport of mRNA to the minus-end of microtubules. This suggests that dynein is still bound to the bi-directionally moving mRNAs without being bound to BICD or Egl (Bullock *et al.*, 2006). It is therefore possible that dynein

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is recruited to all the transcribed mRNA constructs, and that the binding of BICD and Egl induces the transport to the microtubule minus-ends.

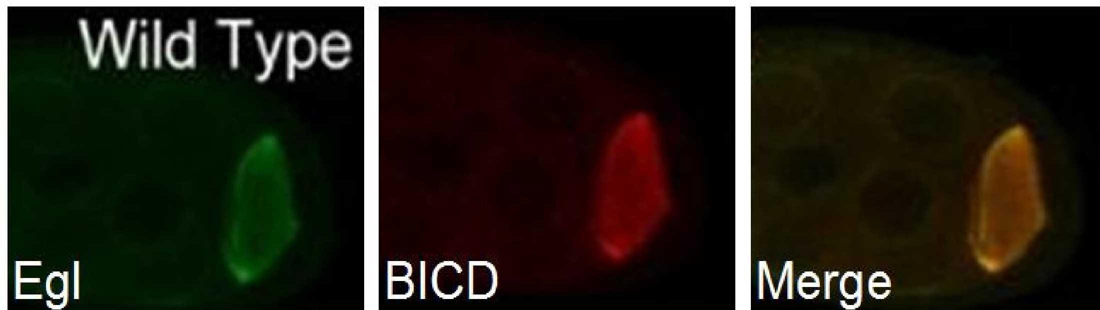

Figure 11: *Drosophila stage 8 egg chambers stained with anti-Egl (green) anti-BICD (red) and merge (yellow) (Navarro et al., 2009). Egl co-localizes with BICD.*

3.3 Motor proteins in vesicle transport

A major function of cytoskeletal motor proteins is the transport of membrane-enclosed organelles, such as mitochondria, Golgi stacks, or secretory vesicles, to their appropriate location in the cell. The microtubule array in cultured mammalian cells is oriented with the minus-ends near the centre of the cell at the centrosome and the plus-end extending to the cell periphery. This means that transport towards the cell centre is performed by minus-end directed motor proteins like dynein, while transport towards the cell periphery is carried out by plus-end directed motor proteins like kinesin. An example of the involvement of motor proteins on the behavior of intracellular membranes is their role in endoplasmatic reticulum (ER) and Golgi organization. *In vitro* kinesin is able to tether ER-derived membranes to preformed microtubule tracks, thereby pulling the membranes to the microtubule plus-ends. This results in the formation of a ER-like membranous structure. Conversely, dynein is required to position the Golgi close to the centrosome at the cell centre (Alberts *et al.*, 2008).

3.4 Bicaudal D in vesicle transport

Rab GTPases play a central role in many steps of membrane traffic, including vesicle formation, vesicle movement along actin and tubulin networks and membrane fusion. With approximately 70 known members with numerous and sometimes tissue specific isoforms, they are the largest subfamily of GTPases (Pereira-Leal & Seabra, 2000; Schultz *et al.*, 2000). Due to a highly selective distribution between vesicles, Rab GTPases serve as specific markers for the identification of membrane vesicles. Rab proteins exist in two states, a GTP- and GDP-bound state, and function as a switch. Inactive GDP-bound Rab proteins are bound to GDI (Rab-GDP dissociation inhibitor) which keeps them soluble in the cytosol. Active GTP-bound Rab proteins are tightly associated with membrane vesicles. The switching of Rab proteins from inactive Rab-GDP to active Rab-GTP is regulated by guanine nucleotide exchange factors (GEFs). Once active Rab proteins are bound to a membrane, they will recruit and bind Rab effectors like motor proteins. These effectors facilitate transport, tethering and fusion of the vesicles. After fulfillment of their task, specific GTPase activating proteins (GAPs) inactivate the Rab-GTP complexes and recycle the Rab proteins back to the inactive GDP-bound state (Pfeffer, 2001; Segev, 2001).

As mentioned above, Rab GTPases are found as vesicle specific markers which regulate vesicle formation, movement and fusion. For example, Rab27A tethers melanosomes to the actin cytoskeleton (Hume *et al.*, 2001; Wu *et al.*, 2001) and Rab7 controls lysosomal transport (Jordens *et al.*, 2001). Also Rab6 has been implicated in microtubule-dependent organelle motility (Martinez *et al.*, 1997; Echard *et al.*, 1998; White *et al.*, 1999; Monier *et al.*, 2002; Mallard *et al.*, 2002). In a screen for Rab6 interacting partners dynactin subunit p150^{glued} and both mammalian BICD proteins, BICD1 and BICD2 were

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identified. In addition, it was shown that the recruitment of dynactin to Golgi membranes is Rab6 dependent (Short *et al.* 2002). Microscopy analysis of BICD and Rab6 revealed co-localization of both proteins at the Golgi on vesicle like structures. BICD also co-localizes with Shiga toxin subunit B, a Rab6-dependent cargo in retrograde transport (Matanis *et al.*, 2002). These results suggest that the dynein/dynactin motor complex is linked via BICD to Rab6 coated vesicles (Fuchs *et al.*, 2005) (fig. 12).

The C-terminus of BICD was also found to interact with Rab6 in *Drosophila*. However, mRNA transport in blastoderm was not found to be altered in hypomorphic *Rab6* mutants (Coutelis & Ephrussi, 2007; Januschke *et al.*, 2007). This suggests that Rab6 is not involved in the mRNA transport mediated by BIDC/Egl complexes.

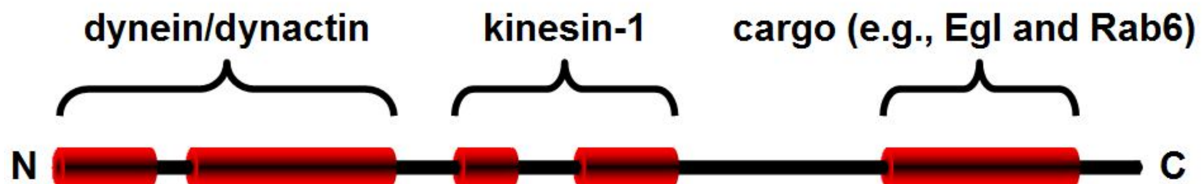

Figure 12: *Protein binding regions of BICD.*

3.5 Bicaudal D binding of other cargoes

Besides Egl and Rab6, also other proteins are labelled as potential cargoes for BICD. Ninein is an important protein involved in the positioning and anchoring of microtubule minus-ends at the centrosome (Hong *et al.*, 2000; Dammermann & Merdes, 2002). In mammalian cells BICD1 association with Ninein has been found. This association appeared to be required for dynein/dynactin-mediated localization of Ninein to the centrosome (Fumoto *et al.*, 2006).

In a yeast 2-hybrid assay, BICD interacts with the *Drosophila* protein Polo. Polo encodes a protein kinase homologue required for mitosis (Llamazares *et al.*, 1991). For proper localization of Polo, both BICD, Egl as well as the dynactin component dynamitin are necessary. In addition, Polo is required in BICD dependent transport from the nurse cells to the oocyte during *Drosophila* oogenesis (Mirouse *et al.*, 2006).

Class V intermediate filaments, known as lamins, are found in the nucleus where they play significant roles in its spatial organization. B-type Lamin DM_0, the precursor form of lamin, was found to interact with the *Drosophila* BICD C-terminal coiled-coil domain in a yeast 2-hybrid assay. Specific point mutations in *BICD* inhibit the interaction between the proteins (Moir *et al.*, 1995). However, a biological role of this interaction is hard to imagine because of the nuclear membrane, which forms a physical barrier between the proteins in a cell.

One of the largest known transport cargoes is the cell nucleus. Migration of the nucleus is for example found during mitoses in budding yeast. The nucleus must migrate into the daughter cell before the actual cleavage takes place, to ensure that both the mother and the daughter cell receive a complete set of genetic material. It was also found in *C. elegans* in which the male and female haploid pronuclei migrate towards the centre of the oocyte after fertilization. There they fuse resulting in a nucleus with a diploid genome. Both dynein as well as kinesin-1 are shown to function in nucleus migration (Tanembaum *et al.*, 2011). It was found that BICD2 functions as a linker between dynein and the nuclear core complex protein RanBP2 (Splinter *et al.*, 2010). Also Kinesin-1 is able to bind RanBP2 and BICD2. However it is not known whether Kinesin-1 interacts directly or through BICD2 with RanBP2 (Cai *et al.*, 2001; Grigoriev *et al.*, 2007).

Similar events were discovered in *C. elegans*. Here the KASH-domain containing protein Unc-83 is responsible for the recruitment of two dynein-regulating complexes to the cytoplasmic face of the nucleus. One consists of the NudE homologue NUD-2 and the Lis1 homologue LIS-1. The other includes dynein light chain, the Bicaudal D homologue BICD-1 and the Egalitarian homologue EGAL-1. Both complexes were found to act in a parallel way (Fridolfsson *et al.*, 2010).

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3.6 Bicaudal D in motor coordination

Although BICD preferentially binds to minus-end directed dynein/dynactin complexes, in many microscopy experiments plus-end directed movement of Rab6 vesicles is observed. In fact, the transport of vesicles along microtubules does not seem to go in a smooth motion but switches continuously between anterograde en retrograde movement (Matanis *et al.*, 2002; Grigoriev *et al.*, 2007). The plus-end directed movement of Rab6 vesicles was shown to be dependent on kinesin-1. This suggests cooperation between dynein/dynactin and kinesin motors.

Similar findings have been made in *Drosophila* oogenesis. mRNAs which determine the anatomical orientation of the developing embryo are transported towards different sides of the oocyte. Although it is well established that this bidirectional transport is based on both dynein/dynactin and kinesin motors, how this transport is regulated remains a question.

Bidirectional movement of cargo was also observed in the transport of lipid droplets via microtubules in *Drosophila*. This bidirectional movement is driven by dynein and kinesin-1. By lowering the levels of BICD both plus-end and minus-end directed movements are effected, suggesting that BICD also effects kinesin dependent transport (Larsen *et al.*, 2008; Shubeita *et al.*, 2008).

In all the above described examples, the minus-end directed motor protein dynein and the plus-end directed motor protein kinesin were simultaneously bound to one cargo. Besides being a linker between motor proteins and cargo, BICD might be part of a coordination complex by binding both dynein/dynactin and kinesin.

3.7 Bicaudal D related proteins

In search of proteins with sequences homologous to BICD two novel BICD related proteins were identified, Bicaudal D related protein 1 and 2 (BICDR-1 and BICDR-2). BICDR-1 is conserved in vertebrates and its expression is restricted to the developing brain, eye, dorsal root ganglia and kidney. It was identified as an effector of the small GTPase Rab6 and a key component of the molecular machinery that controls secretory vesicle transport in developing neurons. During early neuronal development, BICDR-1 expression is high, which results in the accumulation of Rab6-positive secretory vesicles around the centrosome. Anterograde secretory transport is therefore blocked resulting in the inhibition of neuritogenesis. BICDR-1 expression strongly declines during neurite outgrowth which permits anterograde secretory transport required for neurite extension. It was shown that BICDR-1 interacts with both the dynein/dynactin motor complex and the kinesin-3 motor protein Kif1C. These findings elucidate an important role of BICDR-1 as temporal regulator of secretory trafficking during the early fase of neuronal differentiation (Schlager *et al.*, 2010). As mentioned BICD2 mediated transport of Rab6-positive vesicles to the cell periphery. It might therefore be that BICDR-1 and BICD compete with one another in maintaining the balance between anterograde and retrograde transport of specific cargoes.

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4 Summary and future prospects

A highly important and comprehensive mechanism within the cell is the proper distribution of proteins, lipids, mRNA's and cell organelles to various destinations in the cellular matrix. In order to fulfill this task, cells make use of their network of fibers extending throughout the cytoplasm. Two types of fibers involved in cellular transport are microtubules and actin filaments which differ in mechanical properties, dynamics and biological roles (Campbell & Reece., 2005). Many proteins are known to regulate these cytoskeletal filaments by either support their generation or degradation (Alberts *et al.*, 2008).

Molecular motors use the polarized cytoskeletal filaments as rails on which they convey different cargoes. They differ in the filament track they bind, in the direction they move and in the cargo they transport. Three major classes of motor proteins have been identified: myosins, kinesins and dyneins. Myosins are plus-end directed actin binding motor proteins. They are, inter alia, responsible for muscle contraction. Also kinesins are plus-end directed motor proteins. However, they use microtubules as a rail to transport their cargo. Dyneins are a group of motor proteins which transports their cargo towards the minus-ends of microtubules. They are known to be the largest and the fastest among the molecular motors (Schliwa & Woelkhe, 2003). Dyneins are devided into two subclasses; axonemal dynein which is involved in the motion of cilia and flagella (Campbell & Reece, 2005), and cytoplasmic dynein which is responsible for almost all minus-end directed transport in the cytoplasm (Alberts *et al.*, 2008). Cytoplasmic dynein often functions together with dynactin. Dynactin is a protein complex that modulates binding of dynein to cargoes which have to be transported along microtubules. In addition, dynactin also enhances the processivity of cytoplasmic dynein (Alberts *et al.*, 2008).

Multiple factors are found to contribute to the recruitment of dynein and dynactin to specific cargoes. A well studied cargo linking factor is Bicaudal D (BICD). BICD is a cytoplasmic coiled-coil protein which is found in *Drosophila*, *C. elegans* and mammals (BICD1 and BICD2) (Baens & Marynen, 1997; Fridolfsson *et al.*, 2010; Fumoto *et al.*, 2006). In *Drosophila*, BICD and its binding partner Egalitarian (Egl) play important roles in oogenesis and embryogenesis. They are shown to be critical in mRNA distribution during several stages of development. In mammalian cells BICD2 acts as a linker protein between dynein/dynactin complexes and membrane vesicles. The BICD2 N-terminus is able to bind the motor protein whereas the C-terminus recognizes and binds Rab6 coated vesicles (Dienstbier & Li, 2009). In addition, several studies show that BICD acts as a regulator of bi-directional transport of the nucleus by dynein and kinesin-1 (Tanenbaum *et al.*, 2011).

Although BICD shows great affinity for dynein/dynactin and Rab6 coated vesicles, also other BICD binding proteins, including the motor protein kinesin-1, were identified (Grigoriev *et al.*, 2007). This suggests that BICD not only functions as a linker protein, but also coordinates the transport of cargoes via different motor proteins. It will be interesting to find whether different cargoes associate either directly or via adaptors to the C-terminus of BICD. If more adaptors are involved, they could be part of a transport coordination complex. The same is true for BICD N-terminal binding proteins. They might regulate the binding of BICD and thus the cargo to the motor proteins.

Some proteins were shown to posttranslational modify BICD. Both GSK3ß (glycogen synthase kinase 3 beta) as well as Nek8 are able to phosphorylate BICD1 and BICD2 *in vitro* (Fumoto *et al.*, 2006; Holland *et al.*, 2002). Polo and Misshapen can do the same for the *Drosophila* orthologue (Mirouse *et al.*, 2006; Houalla *et al.*, 2005). In addition, kinase activity of GSK3ß appeared to be required for complex formation of BICD1 and dynein in mammalian cells (Fumoto *et al.*, 2006). These findings indicate the BICD acitivity is dependent on its phosphorylation state. To confirm this, functional studies with BICD in different phosphorylation states should be done.

Altogether, many different factors, including dynein/dynactin, Egl, Rab6 and BICD are known which are involved in cellular transport. Next step is to find out how they work together and how they are controlled by signalling pathways. By investigating various regulation steps, many mechanisms will be clarified in the future.

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