

Non-centrosomal Microtubule Organization in Differentiated Cells

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Abstract

The centrosome consists of a pair of centrioles surrounded by pericentriolar material. During the formation of the mitotic spindle, multi-protein complexes in the pericentriolar material are involved in the nucleation and anchorage of microtubules. In postmitotic cells of many tissues, proteins of the pericentriolar material lose their association with the centrosome and redistribute to various sites in the cytoplasm, to the cellular cortex, or to the nuclear surface. Consequently, the organization of the microtubule network is changed. Localization of centrosomal proteins and organization of microtubules follow cell type-specific patterns, to fulfill specialized functions. For example, in polarized epithelia, microtubules are involved in transcytosis and establishment of epithelial polarity, in neurons microtubules are necessary for axonal transport, or in muscle microtubules participate in the assembly of sarcomeres and in the positioning of nuclei. In this review, the principles of microtubule organization in different cell types will be described. The role of microtubules in muscle cells and the potential involvement of microtubule-dependent processes in muscular diseases will be documented in detail.

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2.1 Introduction

During division of somatic animal cells, centrosomes serve as centers for the nucleation and organization of microtubules. At each pole of the mitotic spindle, a pair of centrioles can be found, surrounded by pericentriolar material from which microtubules emanate. After completion of mitosis, the involvement of the centrosome in microtubule organization varies among different cell types. Although textbook illustrations often depict the centrosome as an organizing center of a radial microtubule network in interphase, this type of microtubule organization may only reflect a special situation seen in two-dimensional cell culture. In animals, radial organization of microtubules from the centrosome may still be detected in fibroblasts or in other migratory cell types, but in differentiated cells of many tissues, the centrosome loses its role as an organizing center. The following chapter will highlight several typical examples of non-centrosomal microtubule organization, with a particular focus on the transformation of the microtubule network in differentiating muscle cells. The potential role of microtubules in muscular tissue and potential defects in myopathies will be discussed.

2.2 Microtubule Organization in Polarized Epithelia

Among the numerous cell types that exhibit altered microtubule organization after differentiation, perhaps the best-studied objects are polarized epithelial cells of various origins. Monolayers of polarized epithelial cells are connected by intercellular connections, such as tight junctions, that form an impermeable barrier. These tight junctions separate the plasma membrane into an apical domain and a basolateral domain. Any transport processes across the epithelial layer have to occur via transcytosis, involving directed intracellular transport. Consequently, microtubules in these cells are organized in a polarized manner and serve as tracks for polarized transport. Pioneering studies on *Drosophila* wing epidermal cells revealed a uniform polarity of the microtubule cytoskeleton, with microtubule minus ends anchored in a region underlying the apical plasma membrane and plus ends terminating in the basal region of the cell (Mogensen et al. 1989). Similar organizational principles have been described for the microtubule network in other polarized epithelia, including cells from canine kidney, human intestine, rodent cochlea, *Drosophila* ommatidia, or *Drosophila* tracheal placodes (Bacallao et al. 1989; Meads and Schroer 1995; Tucker et al. 1992; Mogensen et al. 1993; Brodu et al. 2010). In all these cell types, centrioles are still visible in the apical area of the cytoplasm, but they no longer serve as major anchorage points for microtubules. Likewise, marker proteins of the centrosome are partly delocalized from the pericentriolar material. Whereas large amounts of gamma-tubulin and pericentrin are still focused around the centrioles of several epithelial cell types (Meads and Schroer 1995; Tucker et al. 1998), an increasing percentage of ninein in cochlear epithelial cells is lost from the centrosome during differentiation and accumulates at the non-centrosomal sites in the apical region of the cell. This is

particularly well visible in inner pillar cells of the organ of Corti in the cochlea, where ninein concentrates at the apical cell periphery in a ring-shaped area underlying the plasma membrane, where thousands of microtubule minus ends terminate (Mogensen et al. 2000). Since ninein is considered to play a role in anchoring microtubule minus ends (Dammermann and Merdes 2002), the data in cochlear epithelial cells have evoked the hypothesis of initial microtubule nucleation at the pericentriolar material, followed by release, translocation, and subsequent capture of microtubule minus ends at non-centrosomal apical sites containing ninein (Mogensen 1999). Release of microtubules from the centrosome in epithelial cells may involve microtubule-severing enzymes, such as spastin (Brodu et al. 2010). The maintenance of the non-centrosomal microtubule network may be further supported by a recently identified class of minus end-binding proteins termed CAMSAP, nezha, or patronin (Tanaka et al. 2012). Interestingly, a recent report on a ninein-related protein in the nematode *Caenorhabditis elegans*, NOCA-1, has shown functional redundancy with patronin, in the organization of non-centrosomal microtubule arrays in larval epidermal cells (Wang et al. 2015).

Along the basal cortex of polarized epithelial cells of the MDCK line, a separate set of microtubules has been described in addition to the apicobasal fibers, consisting of acentrosomal microtubules of mixed polarity that intersect and that interact with the cortex (Reilein et al. 2005). Small amounts of the microtubule-nucleating protein gamma-tubulin are found at branch points within this basal microtubule network.

In WIF-B cells that possess characteristics of polarized hepatocytes, neighboring cells are in close contact, except for small intercellular spaces that represent bile canaliculi (Ihrke et al. 1993). The plasma membrane surfaces outlining the bile canaliculi are equivalent to the apical membrane domains seen in columnar epithelial cells, such as intestinal or renal epithelia. In an analogous manner, gamma-tubulin is enriched underneath these membrane areas, from which microtubules radiate out toward the basolateral regions of the cells (Ihrke et al. 1993) (Fig. 2.1).

2.3 Microtubule Organization in Skin Keratinocytes

The epidermis of vertebrate skin is a stratified epithelium, i.e., an epithelium containing multiple layers of cells. The innermost layer is the “basal layer,” in contact with the basement membrane, and consists of cells that maintain proliferative activity. Differentiating keratinocytes are oriented outward and establish a dense pattern of intercellular junctions, containing desmosomes, tight junctions, and adherens junctions. During differentiation, centrosomal proteins such as ninein lose their association with the pericentriolar material and redistribute to the cell cortex (Lechler and Fuchs 2007). Ninein interacts with the desmosomal protein desmoplakin. Moreover, adherens junctions have been found to be involved in microtubule reorganization: the protein p120/catenin has been shown to interact with microtubule plus ends in basal cells, via the plus end-binding protein CLASP2 (Shahbazi et al. 2013). Other microtubule-binding proteins, such as Lis1, Ndel1,

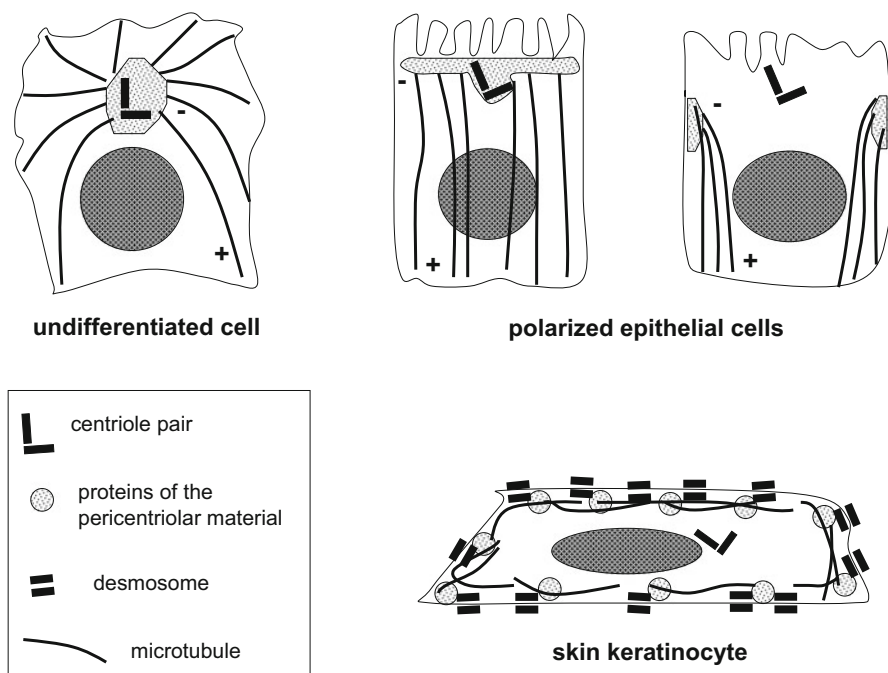


Fig. 2.1 Microtubule organization in epithelial cells. *Top left*: undifferentiated cell, with microtubules organized in a radial network. Microtubule minus ends are anchored at the pericentriolar material; plus ends are growing outward to the cell periphery. *Top middle and right*: two different examples for microtubule organization in polarized epithelial cells. *Top middle*: proteins of the pericentriolar material are redistributed to a wider apical region where microtubule minus ends are anchored. This resembles microtubule organization as seen in MDCK cells. *Top right*: Microtubule organization from specific sites underlying the apical plasma membrane, such as seen in cochlear epithelial cells. *Bottom*: microtubule organization in differentiated skin keratinocytes. Centrosome proteins such as ninein are relocalized to the cortex of the cell, binding to proteins of the desmosome. Microtubules concentrate in the cortical region of the cell

and CLIP170, equally associate with the cortex (Sumigray et al. 2011). The microtubule network is transformed during this process, from a centrosomally anchored network into a cortical array of fibers (Lechler and Fuchs 2007; Sumigray et al. 2011, 2012). Consistently, in differentiated keratinocytes of the suprabasal layer, the non-centrosomal minus end-binding protein Nezha equally localizes to the cortex (Shahbazi et al. 2013). Apparently, the density of desmosomal cell junctions and the cortical recruitment of microtubule-binding proteins are mutually dependent, as knockout of desmoplakin prevents cortical accumulation of ninein, Lis1, and Ndel1, and likewise the knockout of Lis1 provokes desmosomal defects with reduced desmosomal stability (Sumigray et al. 2011). A similar interdependence as seen for microtubules and desmosomes has been described for adherens junctions and microtubule plus ends (Shahbazi et al. 2013). Moreover, it has been

shown that the rapid incorporation of the desmosomal components Dsc2 and Dg12 into desmosomes involves microtubule-dependent transport by the motor proteins KIF3A and kinesin 1, respectively (Nekrasova et al. 2011).

In primary cultures of keratinocytes, the formation of cell junctions and the transformation of the microtubule network can be followed upon induction of “differentiation” *in vitro*, by adding calcium to the culture medium. In such cultures, centrosomes maintain their ability to nucleate microtubules, but after initial nucleation, microtubules don’t remain anchored at the pericentriolar material and redistribute in the cell (Lechler and Fuchs 2007). This observation is consistent with the “release and capture” model of microtubules that has been described for polarized epithelial cells (Mogensen 1999). Ninein could play the role of a crucial factor for anchoring microtubule minus ends to the centrosome in non-differentiated cells and to specific non-centrosomal sites after differentiation, although it still remains to be determined experimentally whether ninein takes indeed an active role in microtubule anchoring or whether it simply follows the reorganized microtubule network.

2.4 Microtubule Organization in Neurons

Neurons have a very specific morphology, with a main cell body (soma) from which one axon and multiple dendrites emanate. Within the soma, a pair of centrioles is located next to the nucleus (Sharp et al. 1982), surrounded by centrosomal proteins such as gamma-tubulin, pericentrin, or ninein (Baas and Joshi 1992; Leask et al. 1997; Baird et al. 2004). Despite the presence of a centrosome, microtubule ends are not anchored at the pericentriolar material but are found free in the cytoplasm (Baas and Joshi 1992). Arrays of overlapping microtubules are found along the length of axons and dendrites. These microtubules don’t seem to have any specific points of anchorage, nor do their ends seem to be capped by gamma-tubulin complexes (Baas and Joshi 1992). Axons possess microtubules of uniform polarity, with the minus ends oriented toward the soma and the plus ends oriented toward the growth cone of the axon (Heidemann et al. 1981). Microtubules in dendrites, on the other hand, are of mixed polarity (Baas et al. 1988; Burton 1988). It is hypothesized that during neurogenesis, microtubules are initially nucleated at the centrosome, followed by release and translocation into the extending axon and dendrites. The release of centrosomal microtubules may involve microtubule-severing proteins, such as katanin and spastin (Ahmad et al. 1999; Wood et al. 2006). The position of the centrosome within the cell body is important for neuronal polarization, by defining the site of initial axon formation (de Anda et al. 2005). Experimentally generated neurons with multiple centrosomes grew additional axons in the vicinity of each centrosome, as verified by immunolabeling of the axon-specific microtubule-associated protein tau (de Anda et al. 2005). Interestingly, at later stages of neuronal differentiation, during the formation of synaptic connections, the centrosome may no longer function as a nucleation site, since gamma-tubulin has been found to be absent in synaptically coupled neurons in the hypothalamus and cortex

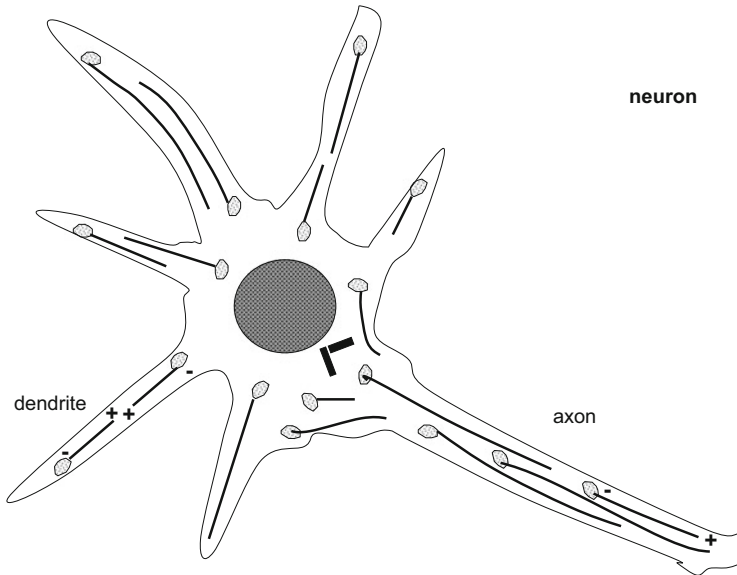


Fig. 2.2 Microtubule organization in neurons. Most microtubules are detached from the centrosome. Microtubules in the axon have a uniform polarity, with minus ends oriented toward the cell body and distal plus ends oriented toward the axonal growth cone. In dendrites, microtubules are of mixed polarity, with a subset of microtubules growing from distal sites toward the cell body. Proteins such as ninein are found widespread in the cytoplasm

(Leask et al. 1997). Moreover, in *Drosophila* neurons, the nucleation and organization of microtubules may not involve the centrosome at all (Nguyen et al. 2011). It is likely that a large number of microtubules are nucleated from cytoplasmic sites, or from the surface of existing microtubules, in particular at later stages. Specifically those microtubules in dendrites that grow from distal sites toward the cell body must originate from non-centrosomal sites, since their polarity is opposite to the polarity of microtubules growing outward from sites at or near the centrosome. Cytoplasmic microtubules may be anchored or stabilized by non-centrosomal ninein that has been detected in small particles, widespread in neurons (Baird et al. 2004). Additional information on microtubule organization in neurons can be found in the Chap. 4 by Sánchez-Huertas, Freixo, and Lüders (Fig. 2.2).

2.5 Microtubule Organization in Skeletal Muscle Cells

The microtubule network in skeletal muscle cells has been studied largely in cultures of myoblasts that undergo differentiation into myotubes upon serum starvation. Undifferentiated myoblasts possess a regular centrosome that acts as a nucleation center and that constitutes an anchorage point of a radial microtubule network. During differentiation, myoblasts elongate and subsequently fuse into

multinucleated, syncytial myotubes. In an early phase of the differentiation process, prior to fusion, proteins of the pericentriolar material accumulate at the cytoplasmic surface of the nuclear envelope (Tassin et al. 1985; Musa et al. 2003; Bugnard et al. 2005; Srsen et al. 2009). How pericentriolar proteins anchor to the nucleus is currently unknown. During myogenesis in *Drosophila*, RacGap50C, a protein that has been identified previously at the cleavage furrow of dividing cells, appears to be necessary for binding gamma-tubulin to various foci associated with the nuclear periphery (Guerin and Kramer 2009). Consistently, experiments involving regrowth of microtubules after previous depolymerization have shown that the nuclear surface can act as a nucleation center (Tassin et al. 1985; Bugnard et al. 2005; Fant et al. 2009). The centrioles are still detectable in fused myotubes during the first days of culture (Tassin et al. 1985), but individual marker proteins may be lost from the pericentriolar material in prolonged cultures (Connolly et al. 1986). Although centrioles may be partially degraded in maturing muscle, few centriolar cylinders seem to persist in adult muscle tissue, as electron microscopy of diaphragm muscle from rodents has revealed the presence of occasional centriole pairs (Kano et al. 1991). Differentiated myotubes in culture contain long, parallel arrays of microtubules, oriented along the long axis of the syncytial cell (Tassin et al. 1985). The formation of elongated microtubules depends on proteins of the EB family that are necessary for the shape of the cells and for fusion of myoblasts into myotubes (Straube and Merdes 2007; Zhang et al. 2009a). Although centrosomal proteins have largely accumulated at the nuclear surface in myotubes, most longitudinally oriented microtubules do not seem to be anchored to the nuclei in these cells (Musa et al. 2003). It is possible that the nuclear surface is involved in microtubule nucleation at an early stage of differentiation (Fant et al. 2009) and that microtubules are subsequently released and re-oriented in the cytoplasm, in a similar manner as seen in other differentiated cell types (see previous paragraphs). In muscle fibers from adult mouse tissue, a grid-like network of microtubules has been described (Kano et al. 1991; Ralston et al. 1999, 2001; Oddoux et al. 2013).

In these mature muscle fibers, the cytoplasm is filled with actin and myosin filaments that are organized into sarcomeres. Nuclei and microtubules are distributed in a thin cytoplasmic layer at the periphery of the fiber, and grids of orthogonally oriented microtubules are nucleated from elements of the Golgi complex. Clusters of the centrosome proteins gamma-tubulin and pericentrin co-localize with these Golgi elements (Oddoux et al. 2013). Growing microtubules are often guided by existing microtubules, with which they form bundles. At least in part, microtubules are also guided by dystrophin (Percival et al. 2007; Prins et al. 2009; Oddoux et al. 2013).

Interestingly, slight differences in microtubule organization exist between slow-twitch and fast-twitch fibers: in the former, bundles of microtubules are seen between nuclei, with few clear nucleation points. In the latter, more individual microtubules than bundles are visible, and these microtubules possess astral nucleation points adjacent to the nuclei (Ralston et al. 1999). The microtubule patterns in both slow- and fast-twitch muscle fibers can be slightly altered by experimental

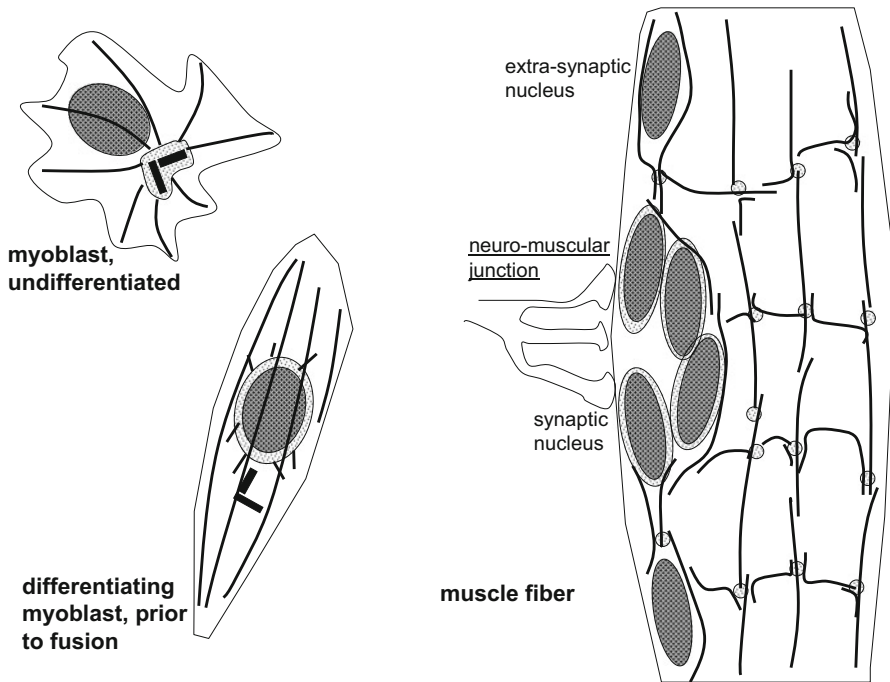


Fig. 2.3 Microtubule organization in muscle cells. In undifferentiated myoblasts, a fully functional centrosome is visible that acts as a microtubule-organizing center. At the beginning of the differentiation process, myoblasts elongate, and proteins of the pericentriolar material accumulate on the surface of the nucleus. At this stage, most microtubules are visible in long parallel arrays in the cytoplasm. Upon fusion of myoblasts into myotubes, actin and myosin organize into sarcomeres (not shown). In the fully mature muscle fibers, the microtubules are organized into a grid-like pattern, excluded from the sarcomeres. Elements of the Golgi complex associate with centrosome proteins and act as microtubule-organizing centers. The nuclei are pushed toward the periphery of the muscle fiber. Multiple synaptic nuclei are clustered near the neuromuscular junction, whereas the remaining extra-synaptic nuclei are distributed along the length of the fiber

stimulation with electrical pulses, mimicking different firing patterns of motor neurons (Ralston et al. 2001) (Fig. 2.3).

2.6 Potential Role of the Microtubule Network in Skeletal Muscle Cells

Skeletal muscle fibers are highly specialized cells that fulfill the single role of contraction and relaxation. They are postmitotic and thus unable to renew after injury. Damaged fibers are replaced by satellite cells that differentiate into new muscle cells. Since muscle fibers don't undergo any cell division, the question arises as to what specific role microtubules might play in differentiation and in the mature cell.

In early pharmacological experiments on cultures of myoblasts and myotubes, it has been shown that microtubules influence the distribution of intermediate filaments and myosin (Holtzer et al. 1975; Antin et al. 1981; Saitoh et al. 1988). More recently, direct evidence was obtained from microscopic observations of living myotubes that microtubules provide a scaffold for the transport of myosin and for the proper assembly of sarcomeres (Pizon et al. 2005).

Microtubules seem to play a second major role in myotubes, in the positioning of nuclei (Folker and Baylies 2013). After fusion of mononucleated myoblasts into multinucleated myotubes, most nuclei are distributed at equidistance along the periphery of the myotube. However, a subgroup of nuclei is found clustered beneath the neuromuscular junction. Although the specific role of these “synaptic” nuclei is still unclear, it has been suggested that they are involved in the maintenance of the synapse, for example, by an increased transcriptional activity to express acetylcholine receptors and other constituents of the postsynaptic membrane (Klarsfeld et al. 1991; Sanes et al. 1991). For the correct positioning of synaptic and extra-synaptic nuclei, microtubules likely serve as tracks, for motor-dependent transport of the nuclei. The dependence of nuclear positioning on microtubules has been shown first in cultured myotubes, in which clusters of acetylcholine receptor were experimentally induced on the plasma membrane, by treating the culture with extracts of electrical tissue from *Torpedo* fish (Englander and Rubin 1987). Once clusters of acetylcholine receptor had formed, the nearest nuclei moved to the cluster and got immobilized there. The movement of these nuclei occurred in a microtubule-dependent manner, since the microtubule poison colchicine inhibited any nuclear movement, unlike the actin poison cytochalasin D that had no such effect. More recent experiments in vivo, in mice and in *Drosophila*, confirm a role of microtubules in the positioning of myonuclei (Bruusgaard et al. 2006; Elhanany-Tamir et al. 2012).

The nuclear movement is driven both by plus end- and minus end-directed microtubule motors and might involve additional microtubule-associated proteins. In *Drosophila*, nuclear movement has been shown to involve kinesin 1 “KIF5B,” dynein, and MAP7 (Metzger et al. 2012; Folker et al. 2012, 2014). On the surface of moving nuclei, kinesin acts at the leading edge, whereas dynein acts at the lagging edge, in addition to cortically anchored dynein that generates pulling forces on microtubules (Folker et al. 2014). In mouse C2C12 cells, dynein, dynactin, as well as KIF5B have been detected on the nuclear envelope of myotube nuclei (Cadot et al. 2012; Wilson and Holzbaur 2012, 2015). Dynein and kinesin motor complexes are involved in linear translocation of nuclei along microtubules and also in the rotation of these nuclei (Wilson and Holzbaur 2012).

Altogether, these experiments suggest that microtubules play an important role in the differentiation of myotubes, during the formation of sarcomeres and during the positioning of nuclei. This raises the question whether cellular defects in any known myopathies correlate with defects in the microtubule network.

2.7 Possible Involvement of Microtubules in Muscular Defects

Duchenne muscular dystrophy is one of the most abundant muscular diseases in children. Since the locus for this recessive muscular dystrophy is on the X chromosome, encoding the protein dystrophin, mainly boys are affected. Dystrophin is a large cytoplasmic protein that links the cytoskeleton to a complex of plasma membrane proteins, including alpha- and beta-dystroglycan that are connected to the extracellular matrix. Besides binding to the actin cytoskeleton, dystrophin has also been shown to interact with microtubules (Prins et al. 2009). Interestingly, the dystrophin-deficient *mdx* mouse that is considered an animal model for Duchenne muscular dystrophy shows disorganization of microtubules in skeletal muscle (Percival et al. 2007). The grid pattern of orthogonally oriented microtubules is lost in these mutant mice. At the same time, elements of the Golgi complex are distributed abnormally. The organization of microtubules as well as the distribution of Golgi elements can be largely restored by the expression of microdystrophin, a designed form of dystrophin lacking most of the central rod domain and the carboxy-terminus (Percival et al. 2007).

Besides showing differences in microtubule organization, muscle fibers of Duchenne muscular dystrophy appear to exhibit another defect involving microtubules: biopsies from patients display an increased percentage of myofibers with incorrectly positioned nuclei. Instead of localizing along the periphery, nuclei concentrate centrally within the fiber (Bell and Conen 1968). This unusual pattern has been described in a variety of muscular diseases, also including Becker muscular dystrophy and Emery-Dreifuss muscular dystrophy (Folker and Baylies 2013). However, the significance of nuclear positioning for pathogenesis remains unclear.

In recent years, the molecular mechanisms leading to defective distribution of nuclei have been tested in mutant mice with phenotypes resembling Emery-Dreifuss muscular dystrophy (Puckelwartz et al. 2009; Zhang et al. 2007a, 2010). In these mice, exons of the *syne-1* gene were removed, encoding various parts of the carboxy-terminal region of the protein nesprin 1. As a consequence, these mice showed defects in the positioning of synaptic and extra-synaptic nuclei in skeletal muscle. Nesprins are a family of nuclear envelope proteins that provide a link between the cytoplasm and the inner nuclear membrane. Four nesprin genes exist in mammals, encoding proteins with a conserved carboxy-terminal “KASH” domain that binds to “SUN” proteins in the perinuclear space, i.e., in the lumen between the outer and inner nuclear membrane. The SUN proteins are transmembrane proteins of the inner nuclear membrane and interact with the nuclear lamina. The link to the cytoplasm is established by a nesprin transmembrane domain in the outer nuclear membrane and an amino-terminal region projecting into the cytoplasm. The amino-terminal regions of the four nesprins differ from each other, and numerous splice variants exist for each nesprin gene. They encode a varying number of spectrin repeats, besides calponin homology domains and other sequence features. The calponin homology domains are involved in binding nesprins to the actin network (Starr and Han 2002; Zhen et al. 2002; Padmakumar et al. 2004). Moreover, the protein nesprin 3 forms a connection to the intermediate filament network, by

binding to the linker protein plectin (Wilhelmsen et al. 2005). Finally, interactions between microtubule-dependent motors and nesprins or nesprin-related KASH proteins have been documented in a variety of experimental systems, including muscular and nonmuscular cell types from vertebrates as well as *Caenorhabditis elegans* (Malone et al. 2003; Meyerzon et al. 2009; Roux et al. 2009; Zhang et al. 2009b; Zhou et al. 2009; Fridolfsson et al. 2010; Yu et al. 2011; Wilson and Holzbaaur 2012). A possible mechanism for nuclear positioning may involve nesprins, binding directly or indirectly to microtubule motor proteins that drive the translocation of nuclei along longitudinal arrays of microtubules along the muscle fiber. Interestingly, synaptic nuclei accumulate significantly more nesprin 1 on the nuclear envelope than extra-synaptic nuclei (Apel et al. 2000), raising the possibility that their clustering and retention at the neuromuscular junction requires increased interactions between the nuclear envelope and microtubule motor proteins. While it is unknown whether defects in the microtubule network or defects in microtubule-dependent transport may be causally involved in the pathogenesis of muscular diseases, it is clear that mutations in nesprin-encoding genes correlate with different myopathies, such as Emery-Dreifuss muscular dystrophy or autosomal recessive arthrogryposis (Zhang et al. 2007b; Wheeler et al. 2007; Attali et al. 2009).

2.8 Conclusion

Microtubule organization in differentiating cell types is generally characterized by a loss of microtubule anchoring to the centrosomal surface and by cell type-specific remodeling of the microtubule network from various cytoplasmic and cortical sites. It is unclear how this loss of centrosomal anchoring is regulated. It is possible that upon differentiation, centrosomes maintain microtubule nucleation activity, but that new microtubules are no longer firmly anchored to the pericentriolar material or that they are actively disconnected from the centrosome by severing enzymes. The resulting free minus ends may then permit translocation of these microtubules to novel sites or may lead to increased polymer turnover and disappearance or remodeling. Likely, proteins of the pericentriolar material may be lost from centrosomes in various differentiating cell types, such as seen in muscle cells, and as a consequence, centrosomal microtubule nucleation may be lost with time. Moreover, it remains so far largely unknown how novel non-centrosomal microtubule organizing centers form. Strikingly, proteins that are part of the pericentriolar material in undifferentiated cells, such as ninein, are now found enriched at new microtubule-organizing centers, without centrioles being present. Identifying mechanisms that lead to this relocalization and identifying “receptor” proteins for the pericentriolar material at the sites of non-centrosomal microtubule organization will be a challenge for future research activities. Finally, the notion of a “centrosomal protein” will need to change: since proteins of the pericentriolar material can equally be found at non-centrosomal locations upon differentiation, they should be considered “microtubule-organizing proteins” in a broader sense.

References

- Ahmad FJ, Yu W, McNally FJ, Baas PW (1999) An essential role for katanin in severing microtubules in the neuron. *J Cell Biol* 145:305–315
- Antin PB, Forry-Schaudies S, Friedmann TM, Tapscott SJ, Holtzer H (1981) Taxol induces postmitotic myoblasts to assemble interdigitating microtubule-myosin arrays that exclude actin filaments. *J Cell Biol* 90:300–308
- Apel ED, Lewis RM, Grady RM, Sanes JR (2000) Syne-1, a dystrophin- and Klarsicht-related protein associated with synaptic nuclei at the neuromuscular junction. *J Biol Chem* 275:31986–31995
- Attali R, Warwar N, Israel A, Gurt I, McNally E, Puckelwartz M, Glick B, Nevo Y, Ben-Neriah Z, Melki J (2009) Mutation of SYNE-1, encoding an essential component of the nuclear lamina, is responsible for autosomal recessive arthrogryposis. *Hum Mol Genet* 18:3462–3469
- Bacallao R, Antony C, Dotti C, Karsenti E, Stelzer EHK, Simons K (1989) The subcellular organization of Madin-Darby canine kidney cells during the formation of a polarized epithelium. *J Cell Biol* 109:2817–2832
- Baas PW, Deitch JS, Black MM, Banker GA (1988) Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. *Proc Natl Acad Sci U S A* 85:8335–8339
- Baas PW, Joshi HC (1992) Gamma-tubulin distribution in the neuron: implications for the origins of neuritic microtubules. *J Cell Biol* 119:171–178
- Baird DH, Myers KA, Mogensen M, Moss D, Baas PW (2004) Distribution of the microtubule-related protein ninein in developing neurons. *Neuropharmacology* 47:677–683
- Bell CD, Conen PE (1968) Histopathological changes in Duchenne muscular dystrophy. *J Neurol Sci* 7:529–544
- Brodu V, Baffet AD, Le Droguen PM, Casanova J, Guichet A (2010) A developmentally regulated two-step process generates a noncentrosomal microtubule network in *Drosophila* tracheal cells. *Dev Cell* 18:790–801
- Bruusgaard JC, Liestøl K, Gundersen K (2006) Distribution of myonuclei and microtubules in live muscle fibers of young, middle-aged, and old mice. *J Appl Physiol* 100:2024–2030
- Bugnard E, Zaai KJ, Ralston E (2005) Reorganization of microtubule nucleation during muscle differentiation. *Cell Motil Cytoskeleton* 60:1–13
- Burton PR (1988) Dendrites of mitral cell neurons contain microtubules of opposite polarity. *Brain Res* 473:107–115
- Cadot B, Gache V, Vasyutina E, Falcone S, Birchmeier C, Gomes ER (2012) Nuclear movement during myotube formation is microtubule and dynein dependent and is regulated by Cdc42, Par6 and Par3. *EMBO Rep* 13:741–749
- Connolly JA, Kiousses BW, Kalnins VI (1986) Centrioles are lost as embryonic myoblasts fuse into myotubes in vitro. *Eur J Cell Biol* 39:341–345
- Dammermann A, Merdes A (2002) Assembly of centrosomal proteins and microtubule organization depends on PCM-1. *J Cell Biol* 159:255–266
- de Anda FC, Pollarolo G, Da Silva JS, Camoletto PG, Feiguin F, Dotti CG (2005) Centrosome localization determines neuronal polarity. *Nature* 436:704–708
- Elhanany-Tamir H, Yu YV, Shnayder M, Jain A, Welte M, Volk T (2012) Organelle positioning in muscles requires cooperation between two KASH proteins and microtubules. *J Cell Biol* 198:833–846
- Englander LL, Rubin LL (1987) Acetylcholine receptor clustering and nuclear movement in muscle fibers in culture. *J Cell Biol* 104:87–95
- Fant X, Srsen V, Espigat-Georger A, Merdes A (2009) Nuclei of non-muscle cells bind centrosome proteins upon fusion with differentiating myoblasts. *PLoS One* 4:e8303
- Folker ES, Schulman VK, Baylies MK (2012) Muscle length and myonuclear position are independently regulated by distinct Dynein pathways. *Development* 139:3827–3837

- Folker ES, Baylies MK (2013) Nuclear positioning in muscle development and disease. *Front Physiol* 4:363
- Folker ES, Schulman VK, Baylies MK (2014) Translocating myonuclei have distinct leading and lagging edges that require kinesin and dynein. *Development* 141:355–366
- Fridolfsson HN, Ly N, Meyerzon M, Starr DA (2010) UNC-83 coordinates kinesin-1 and dynein activities at the nuclear envelope during nuclear migration. *Dev Biol* 338:237–250
- Guerin CM, Kramer SG (2009) RacGAP50C directs perinuclear gamma-tubulin localization to organize the uniform microtubule array required for *Drosophila* myotube extension. *Development* 136:1411–1421
- Heidemann SR, Landers JM, Hamborg MA (1981) Polarity orientation of axonal microtubules. *J Cell Biol* 91:661–665
- Holtzer H, Croop J, Dienstman S, Ishikawa H, Somlyo A (1975) Effects of cytochalasin-B and colcemid on myogenic cultures. *Proc Natl Acad Sci U S A* 72:513–517
- Ihrke G, Neufeld EB, Meads T, Shanks MR, Cassio D, Laurent M, Schroer TA, Pagano RE, Hubbard AL (1993) WIF-B cells: an in vitro model for studies of hepatocyte polarity. *J Cell Biol* 123:1761–1775
- Kano Y, Fujimaki N, Ishikawa H (1991) The distribution and arrangement of microtubules in mammalian skeletal muscle fibers. *Cell Struct Funct* 16:251–261
- Klarsfeld A, Bessereau JL, Salmon AM, Triller A, Babinet C, Changeux JP (1991) An acetylcholine receptor alpha-subunit promoter conferring preferential synaptic expression in muscle of transgenic mice. *EMBO J* 10:625–632
- Leask A, Obrietan K, Stearns T (1997) Synaptically coupled central nervous system neurons lack centrosomal gamma-tubulin. *Neurosci Lett* 229:17–20
- Lechler T, Fuchs E (2007) Desmoplakin: an unexpected regulator of microtubule organization in the epidermis. *J Cell Biol* 176:147–154
- Malone CJ, Misner L, Le Bot N, Tsai MC, Campbell JM, Ahringer J, White JG (2003) The *C. elegans* hook protein, ZYG-12, mediates the essential attachment between the centrosome and nucleus. *Cell* 115:825–836
- Meads T, Schroer TA (1995) Polarity and nucleation of microtubules in polarized epithelial cells. *Cell Motil Cytoskeleton* 32:273–288
- Metzger T, Gache V, Xu M, Cadot B, Folker ES, Richardson BE, Gomes ER, Baylies MK (2012) MAP and kinesin-dependent nuclear positioning is required for skeletal muscle function. *Nature* 484:120–124
- Meyerzon M, Fridolfsson HN, Ly N, McNally FJ, Starr DA (2009) UNC-83 is a nuclear-specific cargo adaptor for kinesin-1-mediated nuclear migration. *Development* 136:2725–2733
- Mogensen MM (1999) Microtubule release and capture in epithelial cells. *Biol Cell* 91:331–341
- Mogensen MM, Malik A, Piel M, Bouckson-Castaing V, Bornens M (2000) Microtubule minus-end anchorage at centrosomal and non-centrosomal sites: the role of ninein. *J Cell Sci* 113:3013–3023
- Mogensen MM, Tucker JB, Baggaley TB (1993) Multiple plasma membrane-associated MTOC systems in the acentrosomal cone cells of *Drosophila* ommatidia. *Eur J Cell Biol* 60:67–75
- Mogensen MM, Tucker JB, Stebbings H (1989) Microtubule polarities indicate that nucleation and capture of microtubules occurs at cell surfaces in *Drosophila*. *J Cell Biol* 108:1445–1452
- Musa H, Orton C, Morrison EE, Peckham M (2003) Microtubule assembly in cultured myoblasts and myotubes following nocodazole induced microtubule depolymerisation. *J Muscle Res Cell Motil* 24:301–308
- Nekrasova OE, Amargo EV, Smith WO, Chen J, Kreitzer GE, Green KJ (2011) Desmosomal cadherins utilize distinct kinesins for assembly into desmosomes. *J Cell Biol* 195:1185–1203
- Nguyen MM, Stone MC, Rolls MM (2011) Microtubules are organized independently of the centrosome in *Drosophila* neurons. *Neural Dev* 6:38
- Oddoux S, Zaai KJ, Tate V, Kenea A, Nandkeolyar SA, Reid E, Liu W, Ralston E (2013) Microtubules that form the stationary lattice of muscle fibers are dynamic and nucleated at Golgi elements. *J Cell Biol* 203:205–213

- Padmakumar VC, Abraham S, Braune S, Noegel AA, Tunggal B, Karakesisoglou I, Korenbaum E (2004) Enaptin, a giant actin-binding protein, is an element of the nuclear membrane and the actin cytoskeleton. *Exp Cell Res* 295:330–339
- Percival JM, Gregorevic P, Odum GL, Banks GB, Chamberlain JS, Froehner SC (2007) rAAV6-microdystrophin rescues aberrant Golgi complex organization in mdx skeletal muscles. *Traffic* 8:1424–1439
- Pizon V, Gerbal F, Diaz CC, Karsenti E (2005) Microtubule-dependent transport and organization of sarcomeric myosin during skeletal muscle differentiation. *EMBO J* 24:3781–3792
- Prins KW, Humston JL, Mehta A, Tate V, Ralston E, Ervasti JM (2009) Dystrophin is a microtubule-associated protein. *J Cell Biol* 186:363–369
- Puckelwartz MJ, Kessler E, Zhang Y, Hodzic D, Randles KN, Morris G, Earley JU, Hadhazy M, Holaska JM, Mewborn SK, Pytel P, McNally EM (2009) Disruption of nesprin-1 produces an Emery Dreifuss muscular dystrophy-like phenotype in mice. *Hum Mol Genet* 18:607–620
- Ralston E, Lu Z, Ploug T (1999) The organization of the Golgi complex and microtubules in skeletal muscle is fiber type-dependent. *J Neurosci* 19:10694–10705
- Ralston E, Ploug T, Kalhovde J, Lomo T (2001) Golgi complex, endoplasmic reticulum exit sites, and microtubules in skeletal muscle fibers are organized by patterned activity. *J Neurosci* 21:875–883
- Reilein A, Yamada S, Nelson WJ (2005) Self-organization of an acentrosomal microtubule network at the basal cortex of polarized epithelial cells. *J Cell Biol* 171:845–855
- Roux KJ, Crisp ML, Liu Q, Kim D, Kozlov S, Stewart CL, Burke B (2009) Nesprin 4 is an outer nuclear membrane protein that can induce kinesin-mediated cell polarization. *Proc Natl Acad Sci U S A* 106:2194–2199
- Saitoh O, Arai T, Obinata T (1988) Distribution of microtubules and other cytoskeletal filaments during myotube elongation as revealed by fluorescence microscopy. *Cell Tissue Res* 252:263–273
- Sanes JR, Johnson YR, Kotzbauer PT, Mudd J, Hanley T, Martinou JC, Merlie JP (1991) Selective expression of an acetylcholine receptor-lacZ transgene in synaptic nuclei of adult muscle fibers. *Development* 113:1181–1191
- Shahbazi MN, Megias D, Epifano C, Akhmanova A, Gundersen GG, Fuchs E, Perez-Moreno M (2013) CLASP2 interacts with p120-catenin and governs microtubule dynamics at adherens junctions. *J Cell Biol* 203:1043–1061
- Sharp GA, Weber K, Osborn M (1982) Centriole number and process formation in established neuroblastoma cells and primary dorsal root ganglion neurones. *Eur J Cell Biol* 29:97–103
- Srsen V, Fant X, Heald R, Rabouille C, Merdes A (2009) Centrosome proteins form an insoluble perinuclear matrix during muscle cell differentiation. *BMC Cell Biol* 10:28
- Starr DA, Han M (2002) Role of ANC-1 in tethering nuclei to the actin cytoskeleton. *Science* 298:406–409
- Straube A, Merdes A (2007) EB3 regulates microtubule dynamics at the cell cortex and is required for myoblast elongation and fusion. *Curr Biol* 17:1318–1325
- Sumigay KD, Chen H, Lechler T (2011) Lis1 is essential for cortical microtubule organization and desmosome stability in the epidermis. *J Cell Biol* 194:631–642
- Sumigay KD, Foote HP, Lechler T (2012) Noncentrosomal microtubules and type II myosins potentiate epidermal cell adhesion and barrier formation. *J Cell Biol* 199:513–525
- Tanaka N, Meng W, Nagae S, Takeichi M (2012) Nezha/CAMSAP3 and CAMSAP2 cooperate in epithelial-specific organization of noncentrosomal microtubules. *Proc Natl Acad Sci U S A* 109:20029–20034
- Tassin AM, Maro B, Bornens M (1985) Fate of microtubule-organizing centers during myogenesis in vitro. *J Cell Biol* 100:35–46
- Tucker JB, Mogensen MM, Henderson CG, Doxsey SJ, Wright M, Stearns T (1998) Nucleation and capture of large cell surface-associated microtubule arrays that are not located near centrosomes in certain cochlear epithelial cells. *J Anat* 192:119–130

- Tucker JB, Paton CC, Richardson GP, Mogensen MM, Russell IJ (1992) A cell surface-associated centrosomal layer of microtubule-organizing material in the inner pillar cell of the mouse cochlea. *J Cell Sci* 102:215–226
- Wang S, Wu D, Quintin S, Green RA, Cheerambathur DK, Ochoa SD, Desai A, Oegema K (2015) NOCA-1 functions with gamma-tubulin and in parallel to patronin to assemble non-centrosomal microtubule arrays in *C. elegans*. *Elife* 4. doi:[10.7554/eLife.08649](https://doi.org/10.7554/eLife.08649)
- Wheeler MA, Davies JD, Zhang Q, Emerson LJ, Hunt J, Shanahan CM, Ellis JA (2007) Distinct functional domains in nesprin-1alpha and nesprin-2beta bind directly to emerin and both interactions are disrupted in X-linked Emery-Dreifuss muscular dystrophy. *Exp Cell Res* 313:2845–2857
- Wilhelmsen K, Litjens SH, Kuikman I, Tshimbalanga N, Janssen H, van den Bout I, Raymond K, Sonnenberg A (2005) Nesprin-3, a novel outer nuclear membrane protein, associates with the cytoskeletal linker protein plectin. *J Cell Biol* 171:799–810
- Wilson MH, Holzbaur EL (2012) Opposing microtubule motors drive robust nuclear dynamics in developing muscle cells. *J Cell Sci* 125:4158–4169
- Wilson MH, Holzbaur EL (2015) Nesprins anchor kinesin-1 motors to the nucleus to drive nuclear distribution in muscle cells. *Development* 142:218–228
- Wood JD, Landers JA, Bingley M, McDermott CJ, Thomas-McArthur V, Gleadall LJ, Shaw PJ, Cunliffe VT (2006) The microtubule-severing protein Spastin is essential for axon outgrowth in the zebrafish embryo. *Hum Mol Genet* 15:2763–2771
- Yu J, Lei K, Zhou M, Craft CM, Xu G, Xu T, Zhuang Y, Xu R, Han M (2011) KASH protein Syne-2/Nesprin-2 and SUN proteins SUN1/2 mediate nuclear migration during mammalian retinal development. *Hum Mol Genet* 20:1061–1073
- Zhang J, Felder A, Liu Y, Guo LT, Lange S, Dalton ND, Gu Y, Peterson KL, Mizisin AP, Shelton GD, Lieber RL, Chen J (2010) Nesprin 1 is critical for nuclear positioning and anchorage. *Hum Mol Genet* 19:329–341
- Zhang Q, Bethmann C, Worth NF, Davies JD, Wasner C, Feuer A, Ragnauth CD, Yi Q, Mellad JA, Warren DT, Wheeler MA, Ellis JA, Skepper JN, Vorgerd M, Schlotter-Weigel B, Weissberg PL, Roberts RG, Wehnert M, Shanahan CM (2007a) Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity. *Hum Mol Genet* 16:2816–2833
- Zhang T, Zaal KJ, Sheridan J, Mehta A, Gundersen GG, Ralston E (2009a) Microtubule plus-end binding protein EB1 is necessary for muscle cell differentiation, elongation and fusion. *J Cell Sci* 122:1401–1409
- Zhang X, Lei K, Yuan X, Wu X, Zhuang Y, Xu T, Xu R, Han M (2009b) SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice. *Neuron* 64:173–187
- Zhang X, Xu R, Zhu B, Yang X, Ding X, Duan S, Xu T, Zhuang Y, Han M (2007b) Syne-1 and Syne-2 play crucial roles in myonuclear anchorage and motor neuron innervation. *Development* 134:901–908
- Zhen YY, Libotte T, Munck M, Noegel AA, Korenbaum E (2002) NUANCE, a giant protein connecting the nucleus and actin cytoskeleton. *J Cell Sci* 115:3207–3222
- Zhou K, Rolls MM, Hall DH, Malone CJ, Hanna-Rose W (2009) A ZYG-12-dynein interaction at the nuclear envelope defines cytoskeletal architecture in the *C. elegans* gonad. *J Cell Biol* 186:229–241

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