How Cellular Membranes Can Regulate Microtubule Network

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Abstract—Microtubule array in eukaryotic cells supports directed transport of various cargoes driven by motor proteins. The arrangement of microtubules in cytoplasm is not stochastic; they are organized in a certain way setting a system of coordinates for intracellular transport. Most cultured fibroblast-like cells possess a radial microtubule array with the minus ends of microtubules gathered on the centrosome and plus ends directed towards the periphery of the cell. Mechanisms that regulate the formation of radial microtubule system remain unclear. Usually centrosome works as a microtubule-organizing center; however, the radial system of microtubules can be formed without centrosome participation. At least in some cases microtubule network can be organized by dynein–dynactin complexes associated with membrane vesicles. Membrane vesicles can nucleate microtubules, anchor them and move along them. However, the role of membrane organelles in microtubule organization began to attract attention of researchers only recently. In this review we summarize the data indicating that membrane organelles can organize microtubules, providing “tracks” for their subsequent transport.

Key words: microtubules, vesicular transport, Golgi apparatus, endoplasmic reticulum.

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NUCLEATION AND ANCHORING OF MICROTUBULES ON THE CENTROSOME

Radial array of microtubules is ensured by a single powerful microtubule-organizing center, the centrosome. Microtubules are highly dynamic cytoskeletal structures composed of tubulin protein that continuously undergoes cycles of polymerization and depolymerization. Cellular microtubules appear, grow, shorten, and disassemble at the end of their life cycle. The assembly of microtubules from soluble tubulin begins with nucleation, and it is a limiting stage of microtubule lifecycle. Microtubules are spontaneously nucleated in the cytoplasm; their polymerization is initiated on intracellular particles or vesicles. Nucleation is promoted by special proteins, mostly by \( \gamma \)-tubulin [5, 6], which interacts with other proteins. Two complexes have been described: small \( \gamma \)-tubulin complex, \( \gamma \)TuSC, where \( \gamma \)-tubulin is associated with GCP2 and GCP3 proteins, and ring complex, \( \gamma \)TuRC, composed of 6–7 \( \gamma \)TuSCs bound to GCP4, GCP5, GCP6 and GCP-WD proteins [6]. Ring complexes \( \gamma \)TuRCs are considered to work as templates for microtubule growth. They are concentrated on the centrosome but can be recruited to other intracellular structures as well [7]. In addition to \( \gamma \)-tubulin, nucleation of microtubules is also promoted by some other structural fibrillar proteins, such as CLASP2, chTOG/XMAP215 and TACC [9, 10]. A particular
mechanism of microtubule nucleation by these proteins still remains unknown. TOG/XMAP215 and TACC, accumulated on the centrosome, regulate microtubule nucleation in cooperation with γTuRCs by stabilizing microtubules newly formed on γTuRCs [9]. Motor protein dynein and its cofactor dynactin are also considered to be directly involved in nucleation [11, 12]. The nucleation of microtubules in mitotic spindle requires several nucleating proteins, and the lack of any of them causes spindle defects [3].

After the nucleation microtubules grow, mostly at their plus-end, located distally to the nucleating structure. If the minus-end is not capped it disassembles in the cytoplasm, and the microtubule detaches from the nucleating complex. The capping (anchoring) of minus-end by some proteins or protein complexes prevents microtubule disassembly. Minus-end can be capped by γTuRC [12], usually associated with other proteins, such as dynactin [14], ninein [15], PCM1 [16], Nlp [17], etc. The details of capping mechanism remain obscure. The inhibition of anchoring proteins causes chaotization of microtubule array radiating from the centrosome, though the centrosome can still nucleate microtubules under these conditions [15–18]. Specific non-centrosomal intracellular structures involved in anchoring of microtubule minus-ends have been described, e.g. for spiral organ. Such structures are located in the apical part of the oblong cell, and organize the bundle of microtubules directed along the longitudinal axis of the cell [15]. In hepatocytes microtubules form a loose bundle, piercing the cell, with minus-ends directed towards bile capillary [19, 20]. The radial array of microtubules in such cells remains even in the absence of the centrosome.

ORGANIZATION OF MICROTUBULES IN THE ABSENCE OF THE CENTROSOME

Cytoplasts, i.e., nucleus-free cells, represent a good system for studying microtubule organization in the absence of the centrosome. Nucleus can be removed under certain experimental conditions, such as centrifugation in the presence of actin-depolymerizing drug cytochalasin B or as a result of microsurgical manipulations. Centrosome-free cytoplasts are obtained after depolymerization of microtubules with nocodazole prior to cells’ centrifugation [21–23]. Centrosome-free cytoplasts obtained from CHO cells have randomly organized microtubules, while centrosome-free cytoplasts from BSC-1 cells possess a radial array of microtubules, similar to that in intact cells [23]. The formation of microtubule array may be determined by membrane structures that accumulate microtubule-interacting proteins and protein complexes on their surface. Such interaction of membranes and microtubules might contribute to microtubule organization even in the presence of the centrosome.

Centrosome-free cytoplasts obtained from fish pigment cells (melanophores) represent a remarkable system. Such cytoplasts are filled with evenly distributed pigment granules (melanosomes) and contain a chaotic network of microtubules. Under adrenalin treatment pigment granules aggregate around the geometrical center of the cytoplast, microtubule network becomes more dense and radial, with the center in the aggregate of pigment granules [11, 12, 24–27]. Dynein on the surface of melanosomes is considered to regulate the formation of radial microtubule array in this system [11, 12, 24, 26, 27]. It is involved in nucleation and, probably, in anchoring of microtubules on pigment granules. The ability of melanosomes to nucleate microtubules and move along them contributes to granule and microtubule self-organization into a radial array [11, 12, 26, 27]. Such self-organization is well-reproduced in vitro and can be described with a simple computational model [26, 27]. Nucleation of microtubules on pigment granules in intact melanophores accelerates aggregation and may play an adaptive role. The inhibition of dynein prevents self-organization of microtubules and aggregation of pigment granules [24].

Most animal cells lack pigment granules; however, other membrane organelles can be involved in microtubule organization. Such organelles should be covered with proteins that enable microtubule nucleation or anchoring. The interaction of microtubules irradiating from such organelles with those originating from the centrosome, might result either in the disturbance of microtubule network or in the promotion of radial organization, and regulate intracellular transport. Organelles that can organize microtubules include Golgi apparatus, endoplasmic reticulum (ER), endoplasmic reticulum—Golgi intermediate compartment (ERGIC), endosomes, etc. Most probably, mitochondria transported along microtubules are not involved in microtubule organization; we have not found any data concerning their role in this process. Most if not all membrane organelles of the cell can be transported along microtubules by motor proteins. A great number of works address the problem of intracellular transport. Here we focus on the organization of microtubules by membrane organelles.

ENDOPLASMIC RETICULUM AND ORGANIZATION OF MICROTUBULES

ER tubules are extended along microtubules. The process can be reconstructed in the extract from Xenopus oocytes by the addition of microtubules and ER membranes isolated from liver cells [28]. In such system the addition of ATP induces an extension of ER membranes along microtubules, their fusion at intersections, and the formation of a distinct network with T-like intersections [28] similar to the ER network in cells. The extension of ER membranes depends on microtubules and can be observed in cells in vivo [29].