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EC NEUROLOGY
Review Article

Microtubule and the Discovery of Tubulin

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Received: February 12, 2019; **Publiahed:** March 26, 2019

Abstract

A cell is the building unit of any living organism. It is composed of cell membrane, organelles, and cytoplasm. Those components are held in their place by the cytoskeleton that is present in the cytoplasm. In fact, cells have special and different shapes because of those cytoskeletal fibers. The eukaryotic cytoskeleton includes: microfilaments (actin filaments) intermediates filaments and microtubules. Each cytoskeleton type was discovered at different time. The latest one to be discovered was the microtubule, which plays important roles in the eukaryotic cells. Microtubules, polymers that are built up by the polymerization of tubulin, are essential inside the eukaryotic cells due to their functions in division, motility, and transport. In fact, numerous research studies have shown how to polymerize and study microtubules *in vitro* using purified tubulin.

Keywords: *Cytoskeleton; Microtubule; Tubulin; Cell*

Introduction

In 1950, the process of cell division was being studied by Gary Borisy and Edwin Taylor. In order to understand the formation of the mitotic spindle and identify the molecule that is responsible for it, Taylor and Borisy stopped cell division using colchicine, where the mitosis inhabitation is occurred due to it. They found that in most of cells, there was a protein that well binds to colchicine which is very abundant in the brain. Therefore, they realized that spindles are made by a protein that is not responsible only for cell division as in brain cells, there is almost no cell division [1].

The microtubules that make the mitotic spindle disassembled in the presence of colchicine. Therefore, it was realized that the protein discovered by Taylor and Borisy was the subunit that self-assembles into microtubules. In 1968, this protein was given the name tubulin by Mohri [2,3].

Building unit: Tubulin

Tubulin is a structural protein that is important for cell division and exists as a dimer of α and β subunits. The polymerization and depolymerization of aβ-tubulin is regulated based on the biological function that is needed. There are other tubulin genes, in addition to aβ-tubulin, have been discovered, and aβ-tubulin can be post-translationally modified to regulate its [4,5].

In the microtubule, tubulin self-assembles longitudinally forming protofilaments, and 13 protofilaments associate laterally making the wall of the microtubules. When microtubules polymerize, they break up into protofilaments and eventually can end up as protofilament and individual tubulin subunits [6].

Microtubules undergo dynamic instability, which is caused by the hydrolysis of the Guanosine Triphosphate (GTP) bound within β-tubulin. Hydrolysis controls whether the microtubule grows or shortens and can be affected by other proteins to adapt to different biological functions. With high concentration of tubulin bound to GTP, the microtubule grows, as tubulin molecules which bind with GTP are added faster than the hydrolysis of GTP. On the other hand, if the addition of tubulin molecules that bind with GTP is slower than the rate of GTP hydrolysis, tubulin molecules bind with GDP are exposed at the end of the polymer, which results to shrinkage [6,7].

Microtubule

Microtubule is a polymer made of α- and β-tubulin, with cylindrical shape, that is found in the cytoplasm as part of cell's cytoskeleton. Microtubules give the cell its specific shape, and keep its organelles in place. Microtubules are the thickest structure in the cytoskeleton, with 12 nm inner diameter and 24 nm outer diameter. They have a polar structure, where one end is capped with α-tubulin (the minus end) and the other end with β-tubulin (the plus end) (Figure 1) [8]. Due to the dynamic nature of microtubules, they can switch between shrinking and growing phases that are very important for most microtubule functions. This non-equilibrium behavior is more dramatic for the plus end than for the minus end [9].

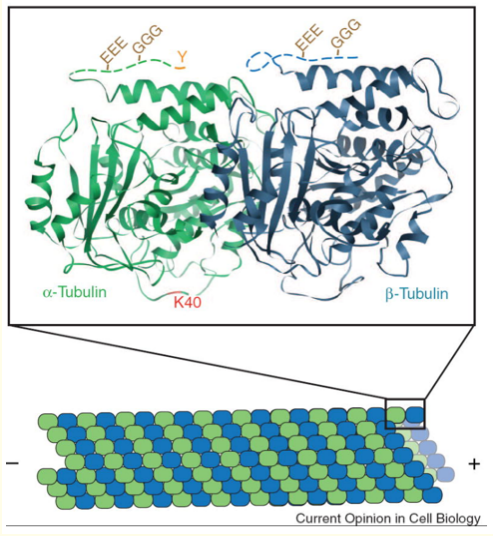

Figure 1: *Microtubule structure.*

Functions

Microtubules are essential polymers for many important eukaryotic cell functions, such as intracellular transport, axonal transport, cell division, and mobility. Microtubule functions require the interaction of microtubules with several microtubule-associated proteins (MAPs). MAPs are necessary for the regulation and distribution of microtubules inside the cell.

Throughout the lifetime of the cell, many molecules are transported inside the cell by motor proteins which move on cytoskeletal filaments powered by hydrolysis of adenosine triphosphate (ATP). There are motors of three types: kinesin, dynein, and myosin. Kinesin and dynein use microtubule as a network of tracks to travel along its surface in opposite directions [10]. Kinesin motors travel toward the 'plus' end of microtubules and have the tendency to move cargoes away from the nucleus. Dynein travels toward the 'minus' end, and therefore transports cargoes towards the nucleus. Improper sorting of cargoes into transport carriers can lead to proteins aggregation.

It has been confirmed that microtubules are more abundant in nerve cells than other cells. The structure of neurons can be divided into three parts: soma, axon, and synaptic terminal. Microtubules form a network from the soma to the synaptic terminal where kinesin and dynein proteins move substances between the soma, where they are made, to the synaptic terminal, and vice versa. Microtubules are used for the transport of synaptic vesicles that contain the neurotransmitters and that are then released into the synapse to transmit nerve signals. Proteins, lipids ,and organelles such as mitochondria, all can travel via microtubules. This mode of transport is called axonal transport or axoplasmic transport [11]. The disruption of the microtubule polarity would send carried molecules or ions in the wrong direction and that potentially can cause traffic jams [12]. Conserving the microtubules polarity in nerve fibers requires dynein to move microtubules that are mis-oriented back to the cell body [13]. Factors that promote the formation of mis-oriented microtubules or the flipping of short microtubules might weak this clearing mechanism. This problem can contribute to disease [12]. The accumulation of proteins, which is caused by improper transport, has been proposed to be a cause for the development of Alzheimer's and Dementia diseases [14].

Even though intracellular transportation is an important phenomenon, cell division is an equally important phenomenon for the survival of living organisms. The function of microtubules is essential for mitosis. Most cells have centrosomes, Microtubules Organizing Centers (MTOC), made up of proteins and two centrioles. Each centriole has 27 microtubules (9 triplets microtubules). With the beginning of mitosis, the centrioles duplicate and move to the opposite ends of the cell, so that each pole has two centrioles [1,2]. The microtubules then grow and radiate from MTOC giving rise to three subtypes of microtubules: inter-polar, astral and kinetochore that define the mitotic spindle. Inter-polar microtubules are located between the cell poles clinging to centrioles. Astral microtubules radiate from the MTOC to the cell membrane to keep the mitotic spindles in place. On the other hand, kinetochore fibers serve as an anchoring site for mitotic spindles and chromosomes [15]. In metaphase the mitotic spindle will attaches to chromosomes with the help of kinetochore fibers. Mitotic spindles during anaphase due to depolymerization of microtubules will separate the chromosomes in equal numbers towards the poles to form two daughter cells [11]. Microtubules are the target for anticancer drugs such as Taxol because they are very important for cell division [16,17].

Certain cell types use cilia or flagella for cell motility [18]. Cilia and flagella grown from basal bodies and have a (9+2) microtubule arrangement of nine doublets and two additional microtubules in the center [19]. The nine doublets are connected by dynein that breaks down ATP and uses its energy to help microtubule doublets to slide past each other and generate movement of cilia and flagella [11]. Cells with cilia are found in the fallopian tube, where they move the ovum from the ovaries to the uterus with the help of an epithelium sodium channel (ENAC) that controls optimal fluid levels inside the cilia. Microtubule absence leads to the absence of cilia in the fallopian tube, which in turn can cause ectopic pregnancy. Dynein arm defects are involved in Kartagener's syndrome, which causes abnormalities in the respiratory tract due to ciliary failure to move.

Thus, it has been shown that microtubule structures are critical for cell functions in transportation, division, and movement, and any defect or problem that stops microtubules function may lead to serious diseases. It should be noted that all cells mentioned before are eukaryotic cells.

Building the model

Microtubules are difficult to characterize structurally due to dynamic instability. In addition to αβ-tubulin, which makes microtubules, there are a growing number of members of the tubulin superfamily [20-22]. Microtubule are made of tubulin heterodimers, containing one α-tubulin and one β-tubulin, that self-assemble into a helical tubular structure. In cells, γ-tubulin is found within the microtubule organizing center that plays an important role in allowing the cell to control microtubule polymerization. Microtubule can be many mm in length, and their diameter is about 24 nm. To build a realistic model that can well describe the microtubule structure you can use a cylindrical can, glue, red balls, black balls and a ruler.

Method

A 3D model can be built in which the basement (cylindrical can) has the right ratio of diameter and length by zooming the actual measurements 10 million times (10^7). Therefore, the diameter is 24 cm and the length will be 250 cm. Red balls are fixed into a helical tubular structure using glue. Above the red balls, black balls are fixed into a helical tubular structure too. Each turn contains 13 heterodimers. Having two different colors of balls is to distinguish between α-tubulin and β-tubulin. Balls are then fixed alternatively in helical structure.

The model ended with α-tubulin and started with β-tubulin. α-tubulin identifies the minus end, and β-tubulin is for the plus end.

Discussion

The dynamic nature of microtubules, which is instable, makes it difficult to study the polymer *in vitro*. However, studying the structure and chemical nature are needed to understand this polymer. Different structures can be observed, that may give rise to different functions.

On one hand, protofilament structure can be gained using electron crystallography (EC) and microtubule structure using cryo-electron microscopy. On the other hand, the structure of stabilized, unpolymerized tubulin can be gained using X-ray crystallography [23,24].

As a polar polymer, when the growth phase occurs at one end and dominant there ,and the shrinkage phase at the other end, treadmilling phenomenon will occur (Figure 2) [25].

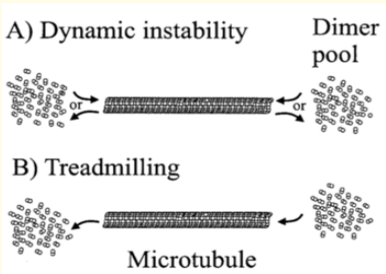

Figure 2: *Comparison of two views of microtubules assembly.*

In 1978, Margolis and Wilson proved that treadmilling can occur *in vitro* at steady-state assembly. In 1980, the idea of treadmilling could occur in the plus end at slow velocities was predicted by Bergen and Borisy. Hotani and Horio observed the link between dynamic instability and treadmilling of microtubules.

The method was based on Panda., *et al.* discoveries in 1999. At the beginning, porcine brain tubulin was purified and then it was stored at -80°C. Near steady state, microtubules started to assemble. To make lattice marks available to be noticed, fluorescent tubulin was added. Microtubules seeds were prepared at 30°C for 30 minutes to achieve steady state assembly where 30 μM tubulin was polymerized in PME (Particle mesh Ewald, an algorithm used in calculating) buffer that contained 10% glycerol, 5 mM NaCl and 1 mM GTP. The seeds then were sheared. A mixture of tubulin was diluted in PME that had 5 mM NaCl, 1 mM GTP and 10 μM ATP. NaCl and ATP with low concentration were added to slow kinesin motility.

The seeds, finally, were added to the mixture with 1:5 volume ratio.

As a result, microtubules essentially assembled in a test tube *in vitro* at 30°C [25].

Conclusion

Microtubules, polymers that are built up by the polymerization of tubulin, are essential inside the eukaryotic cells due to their functions in division, motility and transport. In fact, numerous research studies have shown how to polymerize and study microtubules *in vitro* using purified tubulin.

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Volume 11 Issue 4 April 2019