Cytologia 52: 189–198, 1987

Cytoskeleton, Microtubules, Tubulin and Colchicine: a Review

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Accepted November 22, 1985

Cytoskeleton: *Its chemical and physical organization*

Cytoskeleton was first visualized as fluorescence shape reflecting the distributions within non-muscle cells of subunit proteins forming filamentous subcellular structures, which is a network with associated nucleus (Lazarides and Weber 1974, Weber and Groeschel-Stewart 1974, Weber *et al.* 1975). Therefore this structure has been known as a fibrous and contractile skeleton of cells. Proceeding studies on different kinds of cells increased the number of protein subunits associated with cytoskeletons more of which belong to the filamentous structures and its connections (Satir 1984, Weber and Osborn 1982).

However, cytoskeleton is described in a broad sense the three dimensional network formed by the nucleus, organelles, fibrous systems and membranes (Weber and Osborn 1982). Since the data concerning the proteinous organization of fibrous part of cytoskeleton had already been cited, in this article evidences concerning other mentioned aspects of cytoskeletal organization will be presented.

Cellular proteins that constitute cytoskeletal filaments are not specific for these substructures. Even they are contained in cellular membranes and nucleus (Douvas *et al.* 1975, Gruenstein *et al.* 1975, Burridge and Phillips 1975, Blitz and Fine 1974, Bhattacharyya and Wolff 1975). Recent reports expand these findings and lead to the concept that cytoskeleton and remaining part of cells share similar protein components (Carraway *et al.* 1982, Nakayasu and Ueda 1983, Hubbard and Ma 1983, Rotman *et al.* 1982, Phillips *et al.* 1980, Lehto 1983, Wilkins and Lin 1981, Armbruster *et al.* 1983). Morphological studies showed that cytoskeletal systems are associated with ribosomes (Wolosewick and Porter 1976, Lenk *et al.* 1977, Fulton *et al.* 1980, Cervera *et al.* 1981, Jeffery 1982). Myofibrils that are analogue to cytoskeleton in muscle cell are associated with ribonucleic acids and contain phospholipids (Zak *et al.* 1967, Özgünes and Artvinli 1982). Besides phospholipids, other components that assemble to membranes also retained in Triton X-100 extracted cells which comprise cytoskeletons (Schick *et al.* 1983, Nagai and Sakakibara 1982, Ben-Ze'ev and Abulafia 1983, Wheeler *et al.* 1984, Carraway *et al.* 1983, Moss 1983). In detergent-treated cytoskeletal residues, some receptor and enzyme activities tested could also be detected (Prives *et al.* 1982, Streuli *et al.* 1981, Sahyoun *et al.* 1981, Tuszynski *et al.* 1984, Tashiro and Ishizaki 1982). Sphingolipids and creatine phosphokinase activity in cytoskeletons of intact cells were showed by immunoflourescence microscopy (Sakakibara *et al.* 1981, Eckert *et al.* 1980).

In addition to the data that reveal contribution of membranous components to cytoskeletal organization, those that and surface membrane were reported (Nicolson 1976). Subsequently, the presence of such associations were observed (Koch and Smith 1978, Lehto *et al.* 1983, Williams *et al.* 1979, Ash and Singer 1976, Ash *et al.* 1977, Flanagan and Koch 1978).

Microtubules: *Other aspects of their organization*

Microtubules have been accepted as separate fibrous substructures of cells until cytoskeleton could be visualized by using antibody to its principal component, tubulin. Now, it is believed that these fibrous structures belong to the fibrous part of cytoskeleton, besides microfilaments

and intermediate filaments. The first isolated constituent of microtubules was named as tubulin (Mohri 1968). The other integral components of microtubules are microtubule accessory proteins (Sandoval and Cuatrecasas 1976, Brinkley *et al.* 1980). Some authors' ideas that non-protein and enzymic components which could be found in microtubule extracts are impurities have been the current opinion in this subject (Murphy *et al.* 1983, Snyder and McIntosh 1976, Eipper 1972). However, clear structural associations between these fibrous protein assemblies and membranes are present (Marchant 1978, Esau and Hoefert 1980, Allen 1975, Franke 1971, Bird 1976, Aufderheide 1980, Heggeness *et al.* 1978, Raine *et al.* 1971, Ball and Singer 1982, Bell 1978, Smith *et al.* 1977, Jarlfors and Smith 1969). Therefore, evidences that emphasize membranous nature of these fibrous assemblies were added to this review.

Usual conformation of microtubules which is very important for their function is affected *in vivo*, by membrane-active detergent digitonin and by halothane, the drug acting on fluidity of membranes (Hinkley and Samson 1972, Hanzely and Olah 1970, Livingstone and Vergara 1979). *In vitro* microtubule polymerization inhibited by phospholipase A (Bryan 1975), and hydrophobic trialkyltin compounds (Tan *et al.* 1978) and changed by halothane (Hinkley 1978). Subunit protein tubulin polymerized to membranous forms (Feit and Shay 1980). Some enzymic activities which were investigated are found preparations containing microtubules. These tested enzymes are glyceraldehyde 3-phosphate dehydrogenase (Kumagai and Sakai 1983), nucleotide-dependent enzymes (Terry and Purich 1982), DNA polymerase (Avila 1980), and acid-alkaline phosphatases (Prus and Wallin 1983, Larsson *et al.* 1979). Daleo *et al.* (1974) showed phospholipids, diglycerides and phospholipid synthesizing enzyme, diglyceride kinase, in microtubule extracts. ATP-ase and acetylcholine esterase which are known to be glycolipoprotein enzymes were found biochemically or histochemically in microtubules (Bradford 1979, Tominaga and Kaziro 1983, Darin de Lorenzo *et al.* 1969)

Tubulin: *Its chemistry, transport and secretion*

Tubulin (microtubular protein) is the name of the principal subunit protein of flagella (Mohri 1968). It was first isolated as the subunit protein of cilia, flagella, and of mitotic apparatus with colchicine-binding ability (Shelanski and Taylor 1967, Borisy and Taylor 1967b). Therefore, it is also known as colchicine-binding protein (Weisenberg *et al.* 1968). Other localizations of tubulin in which labile microtubules and subcellular membranes are included have been detected by studying colchicine-binding abilities of cell fractions (Borisy and Taylor 1967b, Schimmel 1975).

After physicochemical properties of tubulin was established, membrane-bound forms of tubulin could be isolated from tissue cells studied without using colchicine (Bhattacharyya and Wolff 1975, Bhattacharyya and Wolff 1976, Blitz and Fine 1974, Kelly and Cotman 1978, Strocchi *et al.* 1981, Babitch 1981, Bernier-Valentin *et al.* 1983, Steiner 1983, Wiedenmann and Mimms 1983, Soifer and Czosnek 1980). As expected from a membranous protein, purified tubulin was found in association with other membranous components, namely glyco- and lipo-conjugates. Margolis *et al.* (1972) found 1.3 per cent carbohydrate containing glucosamine, galactosamine, galactose, mannose, fucose and sialic acid in microtubule protein obtained from 100,000xg supernatants of brain homogenates. Incorporation of ^{14}C glucosamine into the major protein species present in tubulin preparations purified from high speed supernatants of brain homogenates (Feit and Shelanski 1975), and of ^{14}C fucose into the particulate tubulin of neurite explants (Estridge 1977), were shown *in vivo*. Tubulin purified from high speed supernatants of brain homogenates contained phospholipids and polymerized to membranous forms (Feit and Shay 1980). In addition, examination of some reports emphasized the presence of lipo-conjugates of tubulin. Treatment of reduced and carboxamidomethylated tubulin with organic solvents dramatically increased the number of peptides resulting from tryptic digestion

(Nelles and Bamburg 1979). By using phospholipid vesicles, Kumar *et al.* (1981) and Klausner *et al.* (1981) showed that tubulin can be found in lipid-soluble or water-soluble forms.

Assuming that tubulin with glyco- and lipo-components performs fibrous cytoskeletal function, its metabolic fate would be somewhat different from secretory glyco- or lipoproteins. However, the literature revealed the transport (Tamura 1971, Feit *et al.* 1971, Komiya and Kurokawa 1980, Goodrum and Morell 1982), and secretion of tubulin (Bachvaroff *et al.* 1980, Bachvaroff and Rapaport 1980), and could not distinguish it from secretory cellular proteins.

Colchicine: Its effect on mobility of the component of microtubules and membranes

Until 1967, colchicine has been observed as a drug which produces effect on diverse cell activities, especially on mitosis. Mechanism of its action was sometimes thought to alter viscosity of cytoplasm through gel-sol dynamic equilibrium (Malawista 1965, Erbe *et al.* 1966, Chakraborty and Biswas 1965, Affonso *et al.* 1967).

Thereafter colchicine-binding site or its receptor has been determined to be a protein in stable microtubules (Shelanski and Taylor 1967, Borisy and Taylor 1967a), labile microtubules (Borisy and Taylor 1967b), and in subcellular membranes (Feit and Barondes 1970, Lagnado *et al.* 1971, Stadler and Franke 1972), which corresponds to tubulin. Last fifteen years, mechanism of action of the drug was searched by means of morphological and functional analyses on colchicine treated cells, which showed that in almost all cells studied, functional disturbances occur simultaneously with depolymerization of cytoplasmic, labile microtubules. Therefore, general idea on this subject is that colchicine binds to its receptor tubulin on labile microtubules and shifts assembly-disassembly equilibrium to the right. Thus, cell functions dependent on assembled microtubules and cytoskeleton are affected. However, such a mechanism neglects the effect of its binding to receptor tubulin on membranes. Examination of the reports of some authors will lead us to a mechanism which concerns both binding to membranes and microtubules. Wunderlich *et al.* (1973), Furcht and Scott (1975) and Furcht *et al.* (1976) showed that colchicine alters mobility or topography of membrane components on normal or transformed cells. On the other hand, Tamura (1971) showed blockade of transference of newly synthesized microtubule protein to particulate fraction within colchicine treated cells. Komiya and Kurokawa (1980) also observed the same effect of colchicine. Considering both membranous and cytosolic effects of colchicine, it will be clear that common mechanism of effect of its binding to all the receptors is to prevent mobility of constituent tubulin. Such a mechanism is able to explain the effect of colchicine on isolated nuclei (Agutter and Suckling 1982, Schumm and Webb 1982, Agutter *et al.* 1979). In addition, it also agrees with the author's idea that they do not ascribe the effect of colchicine to microtubule depolymerization (Katz 1972, Turkanis 1973, Redman *et al.* 1975, Whittaker *et al.* 1981a, Azhar *et al.* 1983, Sokka and Patton 1983, O'Leary and Suszkiw 1983).

Conclusion

Assuming that colchicine depolymerizes microtubules and thus disturbs fibrous skeletal function of cytoskeleton, it will have an effect, directly or indirectly, on molecular and supramolecular events in cells. Cell functions affected at molecular level are enzyme activities (Volpe 1979, Wilfred 1977, Ewart 1982, Chen *et al.* 1976, Nicklas *et al.* 1973), redistribution of enzymes and proteins (Borensztajn *et al.* 1975, Brimijoin 1974, Parish and Pelli 1974, Raymackers and Hugon 1973, Thyberg *et al.* 1980, Kreutzberg 1969, Sjöstrand *et al.* 1970, James *et al.* 1970), permeability of cells (Berlin 1973, Mizel and Wilson 1972, Cheng and Katsoyannis 1975, Tauber and Reutter 1980), distribution of receptors on membrane (Whittaker *et al.* 1981b),

release of secretory products (Busson-Mabillot *et al.* 1982, Jansen and Bornstein 1974, Gordon and Werb 1976, Ehrlich *et al.* 1974, Reaven and Reaven 1980), and DNA and protein synthesis (Piatigorsky *et al.* 1972, Daniels 1972, Redman *et al.* 1978, Friedkin and Rozengurt 1980). Movement or shape changes of cells, division, phagocytosis, endocytosis, and translocations of organelles are examples of cell events affected at supramolecular level (Mori *et al.* 1982, Bhisey and Freed 1971, Malawista 1975).

Since cytoskeleton, microtubules, tubulin and colchicine contribute, in any way, to the regulation of cell events, to make a synthesis from the collected data concerning them leads to understanding of the regulation of cell events.

Tubulin is believed to be a dimeric globular protein. However, biochemical methods could not obtain it free from carbohydrates and lipids but could find it in membranous structures. On the other hand, additional evidences showed that as it is synthesized in the cell, it is transported and secreted like a secretory glyco- or lipoprotein. Consequently, with its biochemical properties and metabolic fate tubulin resembles a membranous component rather than a fibrous skeletal component.

Microtubules which are constituted from tubulin and contribute to the organization of cytoskeleton do not resemble unconjugated protein assemblies. Their polymerization, *in vitro* and *in vivo*, are susceptible to agents that react with non-protein membranous components. They contain several enzymic activities in addition to ATP-ase activity which is believed to be a characteristic of motile structures. Moreover, these fibrous structures are linked to membranes.

Cytoskeleton with its elements is clearly related with membranous structures considering biochemical and morphological findings. Therefore, it would be a membranous skeleton rather than a fibrous skeleton. Considering that the cell is a membranous network (Artvinli 1980), the membranous skeleton should be its fragile inner part, and tubulin should be one of its components. Thereby tubulin will be synthesized, transferred and secreted within this network.

Lastly, the colchicine effect should be placed in this network, which will complete the synthesis from the collected data. As colchicine binds to the tubulins on the network, it prevents their transfer, thus indirectly disturbs transfer of other membranous components. Finally, depending on dose and duration of colchicine treatment, biomolecular traffic is disturbed allover the cell, which appears as abnormalities in metabolism and shape changes of cells.

Perspective and summary

Microtubules are substructures detected in eucaryotic, even procaryotic cells, since glutaraldehyde fixatives have been introduced into microscopy in 1962. Subject heading of microtubules was first introduced into index medicus in 1972. Structural units of microtubules, namely tubulin (microtubuler protein) have been first identified as colchicine-binding protein in 1967, and the reports including microtubules and tubulin appeared under the heading of colchicine. The reports of tubulin began to appear in the subjects of microtubules, glycoproteins and tubulin in 1972, 1974, 1980 respectively. While microtubules are seen as straight, cylindrical structures by using direct electron microscopy, indirect immuno-fluorescence microscopy showed them as a network by using antibody to tubulin since 1975. This tubulin containing network is cytoskeleton. The reports relating this subject are placed in subject headings of cytoplasm, cytoplasmic filaments, and of microtubules.

Over fifty review articles have refined microtubules by their several aspects. However, the question of how these fibrous labile structures are able to affect many different cell activities

remained unsolved. In this review, evidences concerning the subjects of cytoskeleton, microtubules, tubulin and colchicine which are intimately related are collected to make a synthesis.

The review will clarify the matter by suggesting that tubulin and microtubules do not contribute to a fibrous cytoskeleton directing diverse cell functions through a dynamic assembly-disassembly cycle. The presence of membranous constituents is established for tubulin, microtubules and cytoskeleton. They constitute a membranous skeleton associated with the known membranous parts, forming a network. While cell is alive, tubulin and other membranous components are transferred through this membranous network, by which the cellular components rearrange. As tubulin transport is blocked, the cellular components disarrange, which results in disturbances in metabolic activities and changes of shape of cells with simultaneous disassembly of labile microtubules.

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