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THE CYTOSKELETON OF TRYPANOSOMATIDS

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The cytoskeleton of trypanosomatids differs from that found in vertebrate cells where microtubules, intermediate filaments, microfilaments and microtrabeculae elements are observed (Porter, 1984). Only microtubules forming a layer below the plasma membrane, and known as sub-pellicular microtubules, are seen.

A. Microtubules

It has been observed that the sub-pellicular microtubules are connected with each other and with the plasma membrane by short filaments of still unknown nature (Hunt and Ellar, 1974; De Souza 1976; Linder and Staehelin, 1977; Dwyer, 1980). This association is probably responsible for the rigidity of the cell and the difficulties found in the disruption of the cell by mechanical means. Bridges between adjacent microtubules have been reported in a variety of systems such as in the flagellar axoneme, in the axostyle, etc. It has been shown that dynein, a microtubule-bound ATP-ase, can bind to and induce cross-bridging between adjacent in vitro polymerized microtubules (Haimo et al., 1979). Dwyer

(1980) suggested that the arms connecting the subpellicular microtubules to the plasma membrane of Leishmania donovani might represent dynein, although no data were presented so far to support this suggestion. His results indicate that the microtubule-plasma membrane linkage is not mediated by either divalent cations or disulfide-type bonds since the complex was not altered when it was treated with EDTA, 2-mercaptoethanol, or dithiothreitol.

By using surface tension disruption of various trypanosomatids, followed by critical point drying, Angelopoulos (1970) showed that the microtubules follow a longitudinal helical pathway in the protozoan cortex. Such array can also be seen in whole cells adhering to a polylysine- Formvar-coated grid, treated slightly with Triton X-100, fixed in glutaraldehyde, dehydrated, critical point dried, and observed in the high voltage electron microscope (De Souza and Benchimol, 1983). The nucleating center of the subpellicular mitrotubules has not yet been identified.

Transversal sections through different regions of the trypomastigote form of T. cruzi show that the microtubules are regularly spaced, with a distance equalling 44 nm (center to center). Occasionally one or more microtubules may be lacking, specially in the region of attachment of the flagellum to the protozoan body (Meyer and De Souza, 1976). It was observed that the number of microtubules is related to the diameter of the cell. At the posterior and anterior regions of the protozoan the number of microtubules is smaller while the largest number is found in the region between the nucleus and the kinetoplast, where the Golgi complex is located. Observation of tangential sections of trypomastigotes never showed branching of microtubules,

which could lead to an increase in number, or confluence which would result in reduction of this number. Observation of transversal sections through spheromastigotes localized inside the host cells shows that the number of subpellicular microtubules varies according to the stage of the cell cycle. The maximum number of microtubules in trypomastigotes (120) is virtually the same as the minimum number of these structures in spheromastigotes. In transverse sections of dividing forms, however, it was possible to count up to 220 subpellicular microtubules (Meyer and De Souza, 1976.

Cross-sections of the subpellicular microtubules of T. cruzi show that they are hollow structures. Their wall has a thickness of about 5 nm and the central portion shows a diameter of about 20 nm. Based on the use of the image reinforcement technique, it was reported that the wall of the microtubules of T. brucei was composed of 12 subunits, called protofilaments (Fuge, 1968). With the use of an association of glutaraldehyde with tannic acid (Mizuhira and Futeasaku, 1972) for fixation of T. cruzi and H. samuelpessoai it was shown that each subpellicular microtubule is composed by 13 protofilaments (Baeta Soares and De Souza, 1977), as in most of the microtubules found in other cells.

Biochemical analysis have shown that tubulin represents a major protein of trypanosomatids, accounting for about 20% of the total protein found in a fraction containing the elements of the cell surface of L. tropica (Bordier et al. 1982). α-tubulin migrates slower than the ß-sub-unit. Peptide mapping, after V8 protease digestion, showed homology between ß-tubulin from L. tropica and pig tubulin. α -tubulin of the two

different cells contains few if any common peptides (Bordier et al., 1982) a observation which confirm results reported by Little et al. (1985) showing that ß sub-units of different species are conserved while α -subunits have evolved divergently. Russel et al. (1984) analyzed three different tubulin populations originated from the sub-pellicular microtubules, the flagellum and the cytoplasmic pool of C. fasciculata. α_1 and α_2 tubulin isotypes were found in the cytoplasmic tubulin and formed most of the tubulin found in the pellicular fraction. α_3 tubulin was the sole isotype found in the flagellum, appearing in small amounts in the pellicular fraction. It was also shown by Russel and Gull (1984) that α_3 was a post-translational modification of cytoplasmic tubulin. More recently results carried out in T. brucei (Schneider et al., 1987) showed that α_1-tubulin represents the primary translation product while α_3-tubulin is a post translational acetylated derivative of α_1 tubulin. This fact was shown in experiments in which the parasites were radiolabeled with ^{3}H-acetate and the proteins subsequently analyzed by two dimensional gel electrophoresis and fluorography. They observed that under these conditions only the α_3 tubulin becomes radiolabeled. Analysis of different microtubules of T. brucei showed that flagellar microtubules contain almost exclusively the α_3 tubulin isoform. The sub-pellicular microtubules contain both α_1 and α_3 isoforms. Immunocytochemical observations using a monoclonal antibody which specifically recognizes acetylated α -tubulin (Piperno and Fuller, 1985) showed a strong labeling of the sub-pellicular microtubules of T. cruzi (Souto-Padrón et al., submitted). Post-translational tyrosinolation of tubulin

has been shown to occur in all tubulin isoform of T. brucei (Sherwin et al., 1987). A strong fluorescence found in the posterior portion of the cell during the early stages of the cell cycle of T. brucei would suggest that tyrosinolation of ∝-tubulin may represent a marker for newly formed microtubules.

Besides tubulin, the microtubules also have other proteins which are necessary for the formation and function of the structure. Usually these proteins are involved in the generation of motility, in the creation of a connection between microtubules and other structures, or in controlling the assembly and disassembly of the microtubules. Some of the microtubule-associated proteins are known, such as dynein and nexin which are associated with the flagellar microtubules. Associated with cytoplasmic microtubules of mammalian cells, two groups of proteins have been detected: (1) some high-molecular-weight proteins, which have been designated as HMW and have a molecular weight of about 300,000, and (2) some proteins with a molecular weight around 70,000 which have been designated as tau. These microtubule-associated proteins, which are known as MAPs, are structural components of microtubules which form thin projections attached to the tubules at regular intervals along their length and which are visualized by electron microscopy . Although no biochemical studies exist on the presence of MAPs in the subpellicular microtubules structural observations indicated that these microtubules seem to be similar to the other well characterized microtubules mentioned above. More recently a microtubule-associated protein was found in T. brucei and designated as tripontin in view of its possible role in the linkage between the sub-pellicular microtubules (Seebeck et al., 1987). It behaves as a MAP

since it can copolymerise with tubulin and binds to performed microtubules in vitro.

In vivo, the cells would need a control system to determine the places of assembly of tubulin into microtubules, their elongation, and their spatial orientation. The centers which would be able to organize the assembly of microtubules have been designated as microtubule-organizing centers (MTOC), some of which have been identified as the basal body, centriole, kinetochore, etc. Up to the present time we do not have a precise knowledge about the localization of the organizing center for the subpellicular microtubules of trypanosomatids. Angelopoulos (1970) showed that after the disruption of the body of the protozoan the remaining microtubules were always connected to those of the flagellum in the region of the basal body. Further studies are necessary to clarify this point.

The analysis of the various microtubules found in different cell types and their response to treatment of the cells with low temperature and with some alcaloids indicate that there are two main groups of microtubules: those which are in constant equilibrium with the tubulin pool through a dynamic process of polymerization and depolymerization and those which are permanent structures and usually do not depolymerize. The microtubules of the first group are labile while those of the second group are called stabile. Usually the cytoplasmic microtubules are labile while those which form the flagellum are stabile. Based on experiments with various physical and chemical agents we may consider the subpellicular microtubules of trypanosomatids as of the stabile group (Messier, 1971; De Souza, unpublished observations). This idea is supported by recent biochemical (Schneider et al., 1987)

and immunocytochemical (Souto-Padrón et al., submitted for publicaton) observations showing the presence of α_3 or acetylated α-tubulin in the sub-pellicular microtubules of trypanosomatids. However, it has been shown that the sub-pellicular microtubules of trypanosomatids can be desintegrated by phenotiazine derivatives (Seebeck and Gehr, 1983). The same occurs when the parasites are incubated in the presence of Ca^{++} (Dolan et al., 1986). Results reported by Baum et al (1981) showed that incubation of T. cruzi in the presence of taxol inhibits parasite division, suggesting a process of assembly-disassembly of the sub-pellicular microtubules during the parasite's cell cycle. Taken together these observations indicate that the sub-pellicular microtubules of trypanosomatids have a behaviour different from that observed in other microtubules.

The observation that tubulin has a binding site for the alcaloid drug colchicine opened the possibility of performing various biochemical studies. Using ^{3}H-colchicine the amount of tubulin can be easily assayed. Experiments made on C. fasciculata show that colchicine did not penetrate the cells but bound in a nonsaturable fashion to the membrane (Rembold and Langenbach, 1978) perhaps by a hydrophobic interaction with membrane lipids.

In mammalian cells microtubules are believed to have two basic functions: (1) providing the structural support acting as a sort of cellular skeleton and (2) as part of the machinery recquired for certain types of movement of the whole cell, of intramembranous macromolecules and of some intracellular structures.

As to the role as a cytoskeleton which gives a certain form to the protozoan, it has been shown that

when a trypanosomatid changes its form in consequence of environmental factors or during the process of differentiation or dedifferentiation, the microtubules also change their spatial orientation (Meyer and De Souza, 1976). It is possible, therefore, that such changes are related to the grade of spiralization of the microtubules. A detailed study was performed to analyze the changes in shape which can be induced in H. samuelpessoai. A model was then presented to demonstrate the relationship between microtubule arrangement, changes in the cell shape, and changes in the cell volume (De Andrade and De Almeida, 1980). Its central feature consists of an asymmetric departure from the regularly helicoidal distribution of the microtubules upon induction of shape changes. While some microtubules become more linear, other assume a compensatory overspiralized course. Observations of the profile of the subpellicular microtubules made on both longitudinal and transversal sections of different forms of the protozoan support the model. Such studies should be extended to analyze systems in which the change in shape is more evident such as occurs during spheromastigote-promastigote and spheromastigote--trypomastigote transformation in Leishmania and in T. cruzi, respectively. It is possible that the short filaments which connect the microtubules to each other and to the plasma membrane are,through a mechanism of sliding, involved in the changes of shape which occur in the life cycle of trypanosomatids, causing dramatic changes in the relative position of certain intracellular structures as the kinetoplast, the Golgi complex and the nucleus. Indeed, preliminary observations indicate that when bloodstream trypomastigotes of T. cruzi are incubated in the

presence of cytochalasin B, a change occurs in the form of the protozoan. Ultrastructural analysis of such cells shows that some subpellicular microtubules are displaced, appearing below the normal layer of microtubules (unpublished observations).

The available data do not indicate a participation of the subpellicular microtubules in the control of the mobility of intramembranous components of the plasma membrane of trypanosomatids. As mentioned in another section, treatment of T. cruzi and L. donovani with microtubule- disrupting drugs did not influence the capping of antigenic sites and concanavalin A receptors (Schmunis et al., 1980; Dwyer, 1976). If we remember that the microtubules of trypanosomatids are not altered by such drugs, these results could be explained.

The subpellicular microtubules of T. cruzi and other trypanosomatids are seen running throughout the whole protozoan body, with the exception of the region of attachment of the flagellum to the cell body and of the region of the flagellar pocket. This fact is of importance when one considers that endocytosis and/or exocytosis does not occur in any region of the protozoan which contains microtubules (De Souza et al., 1978). It occurs only at the region of the flagellar pocket.

B. Filaments

In most of the eukaryotic cells we find elongated structures with a diameter varying between 4 and 12 nm which have been designated as filaments. The filaments, with a diameter of 4 to 7 nm, are formed by actin. They can be observed free in the cytoplasm, mainly at the cell cortex or in bundles forming the so-called stress fibers. The filaments, with a diameter of 8-9 nm, are

known as intermediate filaments. Their composition varies from one cell to the other. The filaments of 10 to 12 nm are composed by myosin. All these filaments are found not only in muscle cells but in almost all eukaryotic cells studied up to now (Schliwa and Van Blerkan, 1981). The study of the distribution of these filaments in different cell types has received great attention in the last years, since it is possible to visualize them by immunofluorescence using specific antibodies labeled with fluorescein or rhodamine . These experiments indicated that the microfilaments play important role in various cell processes such as the maintenance of the cell shape, control of the movement of intramembranous macromolecules, movement of intracellular structures, and movement of the whole cell.

Few data are available about the presence of microfilaments in trypanosomatids. They are observed in the flagellum of almost all species of the Trypanosomatidae family forming the paraxial structure (see Fuge, 1969; Vickerman and Preston, 1976; De Souza and Souto-Padrón, 1980). As mentioned previously, short filaments 6 nm thick connect the subpellicular microtubules with each other and with the plasma membrane of all trypanosomatids (Souto-Padron et al., 1984). In some trypanosomatids a microfibrillar structure has been observed in the region of attachment of the flagellum to the cell body or to the wall of the intestinal tube of the invertebrate host (Vickerman and Preston, 1976).

Filamentous tracts were also observed in the cytoplasm of C. fasciculata (Brooker, 1970) and T. brucei (Vickerman and Preston, 1976). However, in any of the above mentioned sites the nature of the

microfilaments was established. In the case of T. cruzi the presence of microfilaments was never observed in the cytoplasm. However, cytochalasin B, a drug which interferes with actin microfilaments, induces marked changes in the general morphology of bloodstream trypomastigotes and inhibits partially the parasites's movement. The cytochalasin B effect is readily reversed by washing the cells (unpublished observations). However, treatment of the parasites with this drug does not interfere with the process of capping of antigenic sites (Schmunis et al., 1980). Further studies using fluorescein-labeled antibodies against actin, myosin, vimentin, etc. could help in to clarify the possible participation of microtubules in the structural organization of trypanosomatids. Using anti-actin antibodies, actin was recently observed by immunofluorescence in the cell body and mainly in the flagellum of T. cruzi. However, with the resolution provided by the light microscope it was not possible to identify the structures which reacted with the antibodies (De Souza et al., 1983). Further studies at the ultrastructural level need to be carried out to clarify this point. More recently (Mortara, submitted for publication) actin was biochemically detected in trypanosomatids and showed to display an unusual DNAse-I binding behavior when compared to actin from higher eukaryotes.

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