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Cytoskeleton and calcium. A review

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SUMMARY

The aim of the present paper was to summarize the main features about cytoskeleton in order to understand the possible interactions between this system of filamentous, microfilaments structures (including microtubules, intermediate filaments, microfilaments) and calcium in mesenchymal cells of the oral cavity.

KEY WORDS:

Microtubules - Intermediate filaments - Microfilaments - Calcium.

RÉSUMÉ

Cet article fait le point des connaissances actuelles sur le cytosquelette et vise à mettre en évidence les interactions possibles entre ce système de structures fibrillaire (microtubules, filaments intermédiaires, microfilaments) et le calcium, pour ce qui concerne les cellules mesenchymateuses de la cavité buccale.

MOTS-CLÉS:

Microtubules - Filaments intermédiaires - Microfilaments - Calcium.

INTRODUCTION

Structural, ultrastructural and molecular organization of cytoskeleton in eukaryotic cells is well documented now, but little is known about the physiological properties of the cytoskeleton especially in mesenchymal cells of the oral cavity, which are often located near or within calcium sources.

The aim of the present paper was to summarize the main features about cytoskeleton in order to understand the possible interactions between cytoskeleton and calcium in mesenchymal cells of the oral cavity.

The term «cytoskeleton» refers to the system of filamentous structures (microtubules, intermediate filaments, microfilaments) found in all types of eukaryotic cells. The cytoskeleton can be thought as the integrated system of molecules that gives cells their shape, internal spatial organization, motility and communication routes with other cells and environment (Schliwa, 1986).

Activities related to cytoskeleton are controlled by intracellular signalling systems. One of the most important signal seems to be a change in the concentration of free intracytoplasmic calcium ions. Calcium may exert its effect alone or combined with others signals (Bennett and Weeds, 1986).

MICROTUBULES

Microtubules, the universal components of all eukaryotic cells (Roberts and Hyams, 1979), have the largest diameter (about 25 nm) of all cytoskeletal fibrils. The microtubules wall, about 5 nm wide, is made of a single protein, the tubulin. Each tubulin molecule is a hetero-dimer consisting of one α and one β chains. Protofilaments are constituted of these non identical tubulin polypeptide chains, α and β (Schultheiss and Mandelkow, 1983), most often isolated from brain tissue (Field et al., 1984). Tubulin polypeptide chains have a molecular weight of about 50 kD. Brain α tubulin contains 451 amino acid residues, β tubulin 445 residues (Krauhs et al., 1981). These two subunits have about 40% of homologous residues.

Structural studies have shown that protofilaments are slightly staggered (Hirokawa, 1982; Murray, 1984). Subunits of neighboring protofilaments are shifted about 1 nm along the microtubule axis, so that these subunits can be connected by a helical line. Microtubules in most animal tissues have 13 protofilaments (Tucker, 1984).

Some of the proteins attached to the outer surface of the microtubule wall are called microtubule-associated proteins (MAPs) (Vallée et al., 1984). Those isolated from the brain are most well known. These proteins form three main groups: the MAP-1 with a molecular weight of 300-350 kD, the MAP-2 (Sloboda et al., 1975) with a molecular weight of 270-285 kD and tau group (Cleveland et al., 1977) with a molecular weight of about 60 kD.

When these molecules are attached to the microtubule wall, they look like filamentous projections, 80-100 nm long (Voter and Erikson, 1982). MAPs are suspected to participate in microtubular interactions (Olmsted et al., 1984). All types of MAPs added to pure tubulin solutions promote nucleation of the microtubules in vitro (Murphy et al., 1977).

A hypothetical nucleation and elongation scheme concerning microtubules has been developped (Bershadsky and Vasiliev, 1988): fragments of protofilaments would be formed first, followed by the formation of the bidimensional fragment of the microtubule wall, and finally a cylindrically closed short microtubule would grow from its ends.

Attachment of subunits to the end of the fibril is followed by hydrolysis of the bound nucleotide. Elongation of microtubules is GTP-GDP (guanine-tri, di-phosphate) bounded (Carlier and Pantaloni, 1981).

Bound exchangeable GTP in the wall of microtubule is hydrolysed into GDP. Elongation is efficient in the presence of high concentrations of GTP-tubulin but proceeds very slowly with similar concentrations of GDP-tubulin (Hill and Carlier, 1983). Since a microtubule grows from its ends, «GTP-caps» are necessary to induce elongation and to maintain the polymer stability (Carlier et al., 1984a). Some authors consider that this stability is in fact a dynamic instability of microtubules (Mitchison and Kirschner, 1984; Sammak and Borisy, 1988).

This «capping model» of elongation (Bershadsky and Vasiliev, 1988) suggests that a population of microtubules at low GTP-tubulin concentrations consists of two fractions: growing capped microtubules and shrinking uncapped microtubules. The amount of polymerised tubulin in these conditions is not changed because the growth of some microtubules is compensated by the shrinkage of others.

It is likely that cells usually contain not only polymerized microtubules, but also a large pool of unpolymerized tubulin.

Cells seem to regulate not only the extent of microtubules formation but also the geometric organisation of polymers within the cytoplasm. It has been shown that microtubules possess an intrinsic polarity (Heidemann and Mc Intosh, 1980; Euteneuer et al., 1983) and that subunits add preferentially to one end (calles «plus end») of the microtubule and are lost from the other (called «minus end») (Margolis et al., 1978; Bergen and Borisy, 1980).

Thus, once a microtubule is initiated, elongation in a given direction is dictated. The sites from which microtubules initiate or the areas with which microtubules interact are called microtubule organizing centers (MTOC) (Pickett-Heaps, 1969; Brinkley et al., 1981; Tucker, 1984). The two most prominent organizing centers in cultured mammalian cells are the centrosome and the kinetochore (Bergen et al., 1980). However little is known about the mechanism by which tubulin becomes associated with these areas and how the ability to organise microtubules is mediated.

Motility is a characteristic feature of many types of microtubular systems, such as cilia. Mutual slidding of microtubules is caused by cyclic interactions of a major microtubule-associated ATPase, dynein with microtubule walls (Johnson, 1983; Goodenough and Heuser, 1984).

A dynein-like ATPase called kinesin, is suspected to be responsible of organelle movements along microtubules (Vale et al., 1985).

Cell microtubules are very sensitive to calcium, especially in the presence of calmodulin, a calcium-binding protein (Alberts et al., 1983; Gratzer and Baines, 1988). It is likely that the calcium-calmodulin complex acts on the microtubules via MAPs (Weisenberg, 1972), activating one of the MAP-phosphorylating kinases or via dynein, by modulating its ATPase activity (Blum et al.).

Phosphorylated MAPs are less bound to the microtubules and cannot promote tubuline polymerization or stabilise microtubules (Greene et al., 1983; Wolff, 1988).

INTERMEDIATE FILAMENTS

Intermediate filaments (IF) form a class of insoluble cytoplasmic fibrils. They are thinner than microtubules in electron microscopic sections, 8-12 nm in diameter (Granger and Lazarides, 1982). Although the intermediate dilaments may not be universal components of the cytoskeleton of all eukaryotic cells, they are however abundant in most cell types of vertebrates.

The most typical structures formed by intermediate filaments are three-dimensional loose networks distributed throughout the cytoplasm and intermixed with other cell components (Steinert et al., 1984).

The central part of these networks is concentrated around the nucleus, while the peripheral parts radiate toward the plasma membranes (Bershadsky and Vasiliev, 1988).

The morphology of intermediate filaments networks in various tissue cells presents some specificities. The filaments present in the cells of various tissues are composed of different proteins. There are five tissue-specific classes of intermediate filaments proteins (Lazarides, 1980; Lararides, 1982, Steinert et al., 1983): vimentin, keratins, glial fibrillary acidic protein (GFAP), desmin, neurofilament proteins.

Vimentin is present in all mesenchymal tissues including connective tissue cells, blood cells, bone, and cartilage cells.

Since the present paper is intended to emphasize the relationship between calcium and cytoskeleton in mesenchymal cells of the oral cavity, it will be restricted to vimentin only.

Vimentin whose molecular weight is about 54 kD (Starger et al., 1978) is a rod-shaped protein with a large central core domain of about 40 kD, consisting of several-helical subdomains interspersed by short non helical inclusions. The central core domain is flanked by two non helical terminal domains. Although the complete sequence of the gene, coding for vimentin is known (Quax et al., 1983, Quax-Jeuken et al., 1983) well-controlled experiments are still needed to relate the molecular structure of vimentin to specific cytoplasmic events (Geiger, 1987). Georgatos and Blobel (1987a, 1987b) have shown that purified vimentin binds to different fractions of avian erythrocyte membranes through two distinct domains. First sites (lamin B or lamin A-lamin B hetero-oligomers), located at the carboxy-terminal tail of the vimentin molecule, bind specifically to the endofacial and presumably exofacial surfaces of nuclear envelopes.

The second-type of binding sites on the plasma membrane at the amino-terminal head of the vimentin molecule, is provided by ankyrin (Georgatos et al., 1987).

On the basis of these results Geiger (1987) hypothesizes that vimentin filaments are associated with the cell nucleus, interacting with the nuclear lamina through the nuclear pores. At the cell periphery the same intermediate filaments are apparently associated with the membrane.

It is likely that cell is provided with an elaborate system of nucleolemmalplasmalemmal interactions (Geiger, 1987).

Most intermediate filaments protein subunits are usually assembled in filaments within the cell. Non polymerized, newly synthesized subunits are rapidly incorporated into the filaments. Once formed, the intermediate filaments are almost insoluble under physiological conditions.

Enzymatic phosphorylation of IF-proteins may be one of the mechanisms regulating the state of filament assembly (Wong et al., 1984). Proteolysis is another mechanism that probably plays an important role in IF regulation. Some calcium-activated proteases have been found to be specific for particular types of IF-proteins (Traub and Nelson, 1981). A calcium-dependent protease which degrades vimentin has been isolated, but its calcium requirement is about 10M which is considerably high (Nelson and Traub, 1981).

Vimentin synthesis seems to be regulated mainly by cell-substrate contacts (Ben-Ze'ev, 1984).

MICROFILAMENTS

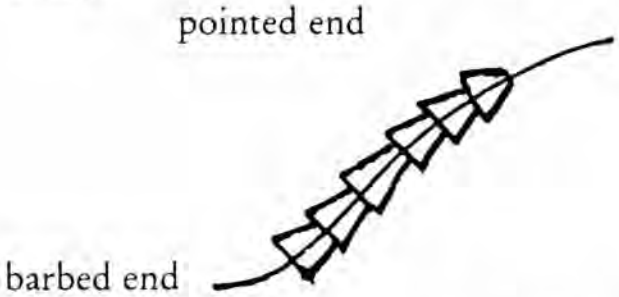
Actin filaments are the main components of cytoskeletal structures (Stossel, 1984). These filaments, whose diameter is approximately 7 nm, are also called microfilaments or Filamentous actin (F-actin). They are polymerized from globular actin monomers (G-actin). G-actin is a single polypeptide chain with a molecular weight of about 42 kD, actin has 375 amino acid residues (Vandekerckhove and Weber, 1978a). Actin in vertebrates falls into three electrophoretic classes: α, β and γ (Vandekerckhove and Weber, 1978b) whose sequences are closely related. Each variant of actin is encoded by a separate gene (Kaine and Spear, 1982; Chang et al., 1984). Elongated G-actin consists of a larger and a smaller domain separated by a cleft (Suck et al., 1981).

Each actin molecule contains one molecule of bound nucleotide (ATP or ADP) and one bound ion (Ca^{2+} or Mg^{2+}). Most molecule of G-actin in vivo are likely to contain bound ATP and Mg (Pollard, 1984).

Molecules of G-actin form subunits of actin filaments. The filament may be regarded as a helical polar structure made of elongated subunits. Data on angular disorder indicate that the position of each subunit within the filament has some degree of freedom. Owing to this structure, the filament has flexibility and ability for torsion (Smith et al., 1983). Addition of about 1 mM Ca^{2+} or 100 mM K^{+} to a solution of monomeric actin induces polymerization which occurs in two different stages: nucleation and elongation (Pollard and Graig, 1982; Tobacman and Korn, 1983; Pantaloni et al., 1985).

The nucleus formed at the first stage is a trimer of G-actin (Barden et al., 1982). Carlier et al. (1984b) have developped a theoretical model of polymerization, according to which, actin filaments would be dynamic structures that might rapidly decrease in length when their ATP caps disappear.

The elongation rates at the two ends of a filament are not the same (Wegner, 1976). Measurements made at high concentrations of monomers have shown that the elongation rates at the barbed ends are faster than dose recorded at the pointed ends (Bonder et al., 1983).



d'après Bershadsky et Vasilief (1988)

The organisation of actin filaments in cells, as well as their changes in response to intracellular Ca^{2+} concentrations and other intracellular signals, depend on the interactions of various actin-binding proteins (Schliwa, 1981, Weeds, 1982).

In muscle, interactions of actin-binding proteins with actin are affected by calcium. All of them have similar subunit molecular weights ($\approx$ 100 kD). At least two classes of non-muscle actin-binding proteins may be found in this molecular weight range: one corresponds to non muscle α-actinins, the other to proteins such as gelsolin and villin (Burridge and Feramisco, 1981). Non-muscle α-actinin and villin are calcium sensitive and bind to actin filaments with higher affinity at low calcium concentration (Bretscher and Weber, 1980, Glenney et al., 1981). The activity of gelsolin, a calcium dependent barbed end capping protein, is fully expressed at micromolar intracellular calcium concentrations (Yin and Stossel, 1979; Yin et al., 1981). Gelsolin also may stop actin filaments polymerization, blocking the newly generated barbed ends thus reducing filament length and disrupting isotropic actin gels.

If this process is reversible, the gelsolin would be expected to dissociate from the filament ends as intracellular Ca^{2+} concentrations decrease (Kurth and Bryan, 1984).

Actin filaments are grouped together within the cells, forming three different types of structures: bundles of parallel filaments with uniform polarity, three-dimensional networks of filaments, and bidimensional submembranous actin-spectrin networks (Mangeat and Burridge, 1984; Schliwa, 1985; Mangeat, 1988). Bundles with uniform polarity present densely and regularly packed filaments which observed in transverse sections, present a hexagonal order. These bundles form the cores of some stable, specialized surface extensions.

Bundles with alternate polarities are found in myofibrils of striated muscles which usually contain myosin on the sarcomere central zone.

Three-dimensional networks consist of filaments crossing one another at various angles and separated from one another by varying distances. The density of the network can vary in different areas of the same cell.

Since many of the proteins that form part of the cytoskeleton or interact with it are subject to phosphorylation, it is not unlikely that calmodulin may be implicated in some aspects of cytoskeletal function (Gratzer and Baines, 1988).

Although the regulation of smooth muscle and non-muscle myosin ATPase through the calmodulin-dependent myosin light chain kinase is now well established (Adelstein and Eisenberg, 1980), there is no conclusive evidence that calmodulin-dependent phosphorylation of any other cytoskeletal elements is important in the regulation of cytoskeletal structures.

It is however certain that many cytoskeletal proteins including MAP2 (Bennet et al., 1983; Goldenring et al., 1983), tau (Lindwall and Cole, 1984; Schulman, 1985), tubulin (Burke and De Lorenzo, 1982; Yamamoto et al., 1983), intermediate filament proteins (Vallano et al., 1985, Schulman et al., 1985) are subject to calmodulin-dependent phosphorylation.

CONCLUSION

The variations of intracellular calcium concentrations may occur in the vicinity of the plasma membrane or close to intracellular sites of calcium release.

There are major difficulties in trying to explain in more than general terms how the cytoskeletal changes may be mediated by changes in calcium concentrations. We are a long way from knowing all the components, let alone control processes regulating the cytoskeleton.

Our knowledge of intracellular signals is rudimentary. Calcium may exert its effect alone or in combination with other signals that are less defined at the present time. There is little information about the spatial range over which calcium is elevated, or the maximum concentration attained in local regions (Bennett and Weeds, 1986).

In oral cavity where mesenchymal cells are near or within calcium sources, membrane transport performs a role of paramount importance in the regulation of the signaling function of Ca^{2+}. Three of the seven known Ca^{2+} transporting systems can be recognized within the plasma membrane of normal eukaryotic cells: Ca^{2+} - ATPase, Na^{+}/CA^{+} exchanger, Ca^{2+} - channel (Carafoli, 1988).

What relations exist between membrane and cytoskeleton? What relation exist between Ca^{2+} transporting systems and cytoskeleton? Response to these questions may clarify the difficult question of interactions between cytoskeleton and calcium in some mesenchymal cells of the oral cavity.

To our knowledge dental pulp fibroblasts and osteoblasts seem to be the most interesting oral cells to perform studies about cytoskeleton and cytoskeleton-extracellular matrix-Ca^{2+} interactions.

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