Anais da Academia Brasileira de Ciências
ISSN: 0001-3765
aabc@abc.org.br
Academia Brasileira de Ciências
Brasil

Mermelstein dos Santos, Claudia; Costa, Manoel Luis; Neto Moura, Vivaldo
The cytoskeleton of the electric tissue of Electrophorus electricus, L.
Anais da Academia Brasileira de Ciências, vol. 72, núm. 3, set., 2000, pp. 341-351
Academia Brasileira de Ciências
Rio de Janeiro, Brasil

The cytoskeleton of the electric tissue of *Electrophorus electricus*, L.*

CLAUDIA DOS SANTOS MERMELSTEIN[1], **MANOEL LUIS COSTA**[1] **and VIVALDO MOURA NETO**[2]

[1]Departamento de Histologia e Embriologia
[2]Departamento de Anatomia, Instituto de Ciências Biomédicas
Universidade Federal do Rio de Janeiro – 21949-590 Rio de Janeiro, RJ, Brazil

Manuscript received on May 3, 2000; accepted for publication on May 9, 2000; contributed by VIVALDO MOURA NETO**

ABSTRACT

The electric eel *Electrophorus electricus* is a fresh water teleost showing an electrogenic tissue that produces electric discharges. This electrogenic tissue is distributed in three well-defined electric organs which may be found symmetrically along both sides of the eel. These electric organs develop from muscle and exhibit several biochemical properties and morphological features of the muscle sarcolema. This review examines the contribution of the cytoskeletal meshwork to the maintenance of the polarized organization of the electrocyte, the cell that contains all electric properties of each electric organ. The cytoskeletal filaments display an important role in the establishment and maintenance of the highly specialized membrane model system of the electrocyte. As a muscular tissue, these electric organs expresses actin and desmin. The studies that characterized these cytoskeletal proteins and their implications on the electrophysiology of the electric tissues are revisited.

Key words: *Electrophorus electricus*, cytoskeleton, desmin, actin, alpha-actinin, vinculin.

INTRODUCTION

The electric eel *Electrophorus electricus* L. is a fresh water teleost that lives in the basins of the Amazonas and the Orenoco River. It belongs to the family of the gymnotidae, being the only representative of the genus and the species.

Electrophorus electricus L. has an electrogenic tissue that produces electric discharges and which is used in predation and defense. The electrogenic tissue is distributed in three well-defined electric organs that may be found symmetrically along both sides of the animal. The main electric organ extends from behind the peritoneal cavity of the viscera down the tail of the animal where it gives rise to Sach's organ, which occupies the remainder of the caudal portion. Hunter' organ is located subjacent to the other electric organs and dorsal to the long swimming fin on the ventral surface of the animal (Gotter *et al.* 1998). It is possible that each organ is involved in separate behavioral electric activities (Keynes & Martins-Ferreira 1953).

The morphological and functional cellular unit of the electric organ is a multinucleated syncytium called electroplaque or electrocyte. Each electrocyte

Professor Carlos Chagas Filho was our advisor during his last years. He gave us more than scientific and cultural guidance, he was an example and a friend.
*Invited paper
**Member of the Academia Brasileira de Ciências
Correspondence to: Vivaldo Moura Neto
E-mail: vivaldo@anato.ufrj.br

is placed in the interior of a insulating septa composed of connective tissue, and disposed by stacks in files superposed one after another (Luft 1957). In an adult animal, there are thousands of electrocytes. The whole discharge is assured by a complex system of coordination that allows the synchronization and distribution of the discharges of the electroplaques (120mV). The electric discharge is produced by nerve excitation and is composed of a certain number of spikes, attaining quite high voltages (400 to 600 volts) in open circuits (Albe-Fessard & Chagas 1955), depending on the eel's size. The electrocytes are flatten cellular structures with six surfaces, two of which are well-defined: the posterior membrane, innervated and flat, and the anterior membrane, non-innervated and with undulations. The papillae of the anterior face are quite prominent while those on the posterior surface are much less pronounced. The anterior surface is rich in Na^+, K^+ - ATPase (Somló *et al.* 1977, Hassón-Voloch *et al.* 1993, Araujo *et al.* 1993) and is rich in acetylcholine receptors (AChR) (Changeux *et al.* 1969, Meunier *et al.* 1974) and responsible for the discharge (Keynes & Martins-Ferreira 1953). The electric organ expresses high levels of membrane receptors, ion channels and ATPases, and has been used as a tissue source for the purification and studies of these proteins.

The electrocyte morphology and the overall cellular distribution in the tissue reflects the specialized physiological function of these cells, which is to produce pronounced hyperpolarizations in membrane potential. This cellular morphology is maintained by a cytoskeletal meshwork (Cartaud *et al.* 2000).

Cytoskeleton

All eucaryotic cells possess a three-dimension cytoskeleton composed of a complex network of filaments that provides the cells with shape, rigidity, elasticity, internal spatial organization and motility (Machesky & Schliwa 2000). These filaments are classified, based in their diameter, in three types: microtubules, microfilaments and intermediate filaments (IFs). Each type of filament is formed from a different protein subunit: actin for microfilaments, tubulin for microtubules, and a family of related fibrous proteins for IFs. Actin and tubulin have been highly conserved throughout the evolution of eucaryotes.

Microtubules consist of a dynamic, highly polarized network of microtubule filaments, microtubule-associated proteins (MAPs), microtubule motors and microtubule-organizing proteins (Cassimeris 1999). Microtubules are long and hollow cylinders with an outer diameter of 25 nm. They are made of the protein tubulin, which exists in two closely related globular polypeptides called alpha-tubulin and beta-tubulin. The alpha- and beta-tubulins are present in all eukaryotic cells, but are highly expressed in nervous system with different subtypes distributed in the neural and glial cells (Moura Neto *et al.* 1983). In fact, more than seven isoelectric variants were described for alpha-tubulin and fourteen beta-tubulin variants were found in the nervous system (Regnard *et al.* 1996). Since there are few tubulin genes, these variants are provided by post-translational modifications as tyrosylation, acetylation and glycosilation. The tubulin superfamily also comprises gamma-tubulin, which is involved in the nucleation of new microtubules (Oakley *et al.* 1990) and more recently delta and epsilon-tubulin have been identified as components of centrosomes (Chang & Stearns 2000). Microtubules are implicated in various cellular functions such as: cellular division, secretion and transport of vesicles and organelles (Vale & Milligan 2000). Tubulin molecules have sites that can interact with microtubule-based motor proteins, like dynein and kinesin, and they are implicated in the transport of vesicles in the cell (Allan 1996).

Microfilaments are two-stranded helical polymers with a diameter of 5-9 nm. Its major protein component is actin (43 kd), which is present in all eucaryotic cells. Higher eucaryotes have six different types of actin that are expressed in a tissue-specific way. These isoforms fall into three classes, depending on their isoelectric point. Alpha-actins

are found in muscle cells, whereas in non-muscle cells there are two isoactins named beta and gamma-cytoplasmic actins (Vandekerckhove *et al.* 1986, Otey *et al.* 1988). Although there are differences in the properties of different forms of actin, the amino-acid sequences have been highly conserved in evolution. Polymerization of monomeric globular actin (G-actin) requires ATP, K^+ and Mg^{2+}. Like microtubules, microfilaments are polar structures, with two different ends: a slow-growing minus end and a fast-growing plus end. The ability of G-actin to polymerize into filamentous actin (F-actin) gives a dynamic role to this protein in cellular functions such as cytokinesis, secretion and cell locomotion (Singer *et al.* 1986).

Actin filaments in animal cells are organized into three general types of arrays: in parallel bundles, in contractile bundles and in gel-like networks. An understanding of the functional role of actin in the cell requires detailed knowledge of the expression of several proteins related with the actin network. These proteins are referred to as actin-binding proteins (Hartwig & Kwiatkowski 1991). They control and modulate the length of actin filaments, their stability and the attachment of these filaments with one another and to other components of the cell (like the plasma membrane). Alpha-actinin (100 kd) is an actin-bundling protein that participates in the cross-linking of actin filaments and helps to form the anchorage for the ends of actin filaments where they terminate on the plasma membrane (Viel 1999). Filamin (250 kd) is a gel-forming protein enriched in the cortex of cells, which promotes the formation of a loose network of actin filaments (Matsudaria 1994). Vinculin (130 kd) is an attachment protein present in actin-containing cell junctions, which associates with alpha-actinin helping to anchor actin filament on the plasma membrane (Rudiger 1998).

Intermediate filaments are highly stable protein fibers found in the cytoplasm of most animal cells. They have a diameter of 8-10 nm, between that of microfilaments and microtubules. In most animal cells an extensive network of IFs surrounds the nucleus and extends out to the cell periphery. They are made of intermediate filament proteins, which constitute a large and heterogeneous multigene family (Osborn & Weber 1986). In spite of the ubiquitous characteristics of tubulin and actin, intermediate filament proteins have a cellular specific distribution. Eight major types of IFs have been distinguished by their polypeptide composition: 1) vimentin (54 kd) is present in cells of mesenchymal origin, often expressed transiently during development and normally expressed in tumoral cells; 2) keratins (40-70 kd) are found characteristically in epithelial cells and their derivatives, and are subdivided in type I (acidic) and type II (basic/neutral); 3) neurofilament proteins (60-200 kd) are a triplet of polypeptides (NF-L, NF-M and NF-H) which are present in neurons; 4) GFAP - glial fibrillary acidic protein (50 kd) is expressed in glial cells; 5) desmin (53 kd) is found in muscle cells; 6) nestin is expressed in the central nervous system and muscle precursor cells; 7) peripherin is found in neurons; and 8) nuclear lamins (65-75 kd) are composed of the three polypeptides lamins A, B and C, which are present in the nuclear lamina of all eucaryotic cells (Galou *et al.* 1997, Gomes *et al.* 1999). IF proteins show similarities in peptidic composition, as analyzed by amino acid sequence analysis (Geisler *et al.* 1982) and immunological studies using monoclonal antibodies (Pruss *et al.* 1981). However, very little is known about their function. The major function attributed to IFs is to provide mechanical stability to animal cells. The structure of IFs is ideally suited for such function, because the fibrous subunit associate side by side in overlapping arrays (Herrmann & Aebi 2000).

Desmin, the IF protein specifically found in muscle cells, is distributed throughout the cytoplasm of smooth muscle cells, and it links together adjacent myofibrils in skeletal and cardiac muscle cells. Desmin is the first muscle structural protein to be expressed during development, and is always expressed in muscle cells, even in muscle de-differentiation or hypertrophy. For instance, TPA-treated chick skeletal muscle cells lose their myofibrils, but still express desmin (Mermelstein *et al.* 1996). Furthermore, the function of desmin is un-

clear: some reports suggest that it is involved in regulating the proper size of the sarcomeres and muscle striation, but cells expressing truncated-desmin in culture, which lack the intermediate filament network, have normally spaced striations, and contract normally (Schultheiss *et al.* 1991). Desmin null transgenic mice show normal overall muscle structure but exhibit minor physiological differences such as less resistance to fatigue (Li *et al.* 1997). A regulatory role for desmin, as a nuclear binding protein, has also been proposed (Li *et al.* 1994): it has potential association sites for the muscle regulatory master switch gene MyoD, and unclear nuclear localization.

Cytoskeleton of the electrocyte of *Electrophorus electricus*

The first observation of the cytoskeletal organization of the electrocyte of *Electrophorus electricus* was obtained by optical and electron microscopy. The presence of myofibrils was observed using young animals (Esquibel *et al.* 1971). In older eels, the electron microscopy does not show a myofibril organization (Machado *et al.* 1976). Noteworthy is the fact that microtubules were observed as well as a pattern of filaments (with 7 nm in diameter) which might be characterized as parts of microfilaments (Machado *et al.* 1976, Benchimol *et al.* 1979).

Since myofibrils were observed in the electrocytes, we decided to analyze the expression of typical muscle proteins, like desmin and actin, in order to understand the cellular origin and differentiation of these cells. We started our study of the eel's cytoskeleton by the biochemical identification of desmin, which has a muscle-specific cell expression. We purified desmin from the main electric organ of *E. electricus*, and characterized its molecular weight, peptidic map and immunological identity (Costa et al. 1986). The presence of desmin in the electric organ of *Electrophorus electricus* described by us (Costa *et al.* 1986, 1988) supports the concept of a muscular origin of the electrogenic tissue (Mathewson *et al.* 1961). This result leads to view this electric organ as a study model for muscle differentiation; particularly suitable for biochemical analysis, since one large eel can have a huge mass of electric (muscle-derived) tissue.

When desmin from the main electric organ of *E. electricus* was analyzed in an isoelectric focusing gel electrophoresis system (IEF), five isoelectric variants were detected. The same observations were obtained from tissue portions taken from Hunter's or Sach's organs (Costa *et al.* 1988, Fig. 1). Opposed to the five variants found in preparations obtained from the electric organ, only four desmin isoforms were found in extracts from the dorsal muscle of the eel (Cordeiro *et al.* 1995).

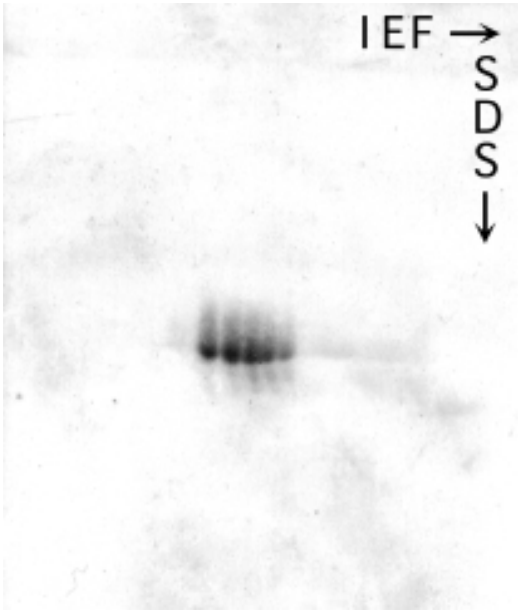

Fig. 1 – Bidimensional gel electrophoresis of purified desmin from Sachs' electric organ of *E. electricus*, showing five isoforms.

Desmin is coded by a single gene (Quax *et al.* 1985) and shows only two or three isoelectric variants in muscle tissues of mammals and birds (Lazarides & Balzer 1978). This contrasts with the five isoelectric desmin variants found in the electric tissue of the *E. electricus*. It is also interesting that five desmin variants were described in conductive Purkinje fibers of some mammalian hearts. These fibers have a myogenic origin but have nerve-like

properties (Thornell *et al.* 1985). Furthermore, in some human cardiac hypertrophies, the number of isodesmins is increased up to six isovariants (Rappaport *et al.* 1988). All the above experimental models have in common the loss of myofibrils and changes in the overall shape and electrical properties. Desmin could have only a structural role in the cell, and could be maintained as a mechanical substitute for the myofibrils. The presence of more isoforms suggests that there are some special physiological conditions in these models that promote the generation of the unusual desmin variants. The regulation and physiological differences between desmin isoforms is not known, but we would like to correlate the extra isoforms described in electrocytes with the muscle dedifferentiation of these cells.

At least some of the isoelectric variants of desmin may be produced by post-translational modifications, including phosphorylation (O'Connor *et al.* 1981, Leão Ferreira *et al.* 1994). Differences in isodesmins indicate different degrees of desmin phosphorylation in the electric tissue. Some of the five isoelectric variants of desmin found in the electric tissue are phosphorylated (Cordeiro *et al.* 1995). The mechanism of post-translational modifications could play a role in the organization of desmin filaments network and might be responsible for the interactions of desmin with other components of the cytoskeleton or with other cell constituents like membranes. The same has been suggested for the IF phophorylation in general (Traub 1985).

Interestingly, the five isodesmins found in the electric tissue are expressed in a characteristic and different pattern in the three electric organs (main, Hunter and Sachs), as showed by statistical analysis and quantitative densitometry (Figs. 2 and 3) using Coomassie blue-stained bands in IEF (Costa *et al.* 1998). The organ-specific isodesmin pattern could correlate with differences in each organ's physiology, that would reflect differences in their behavioral electric activities (Keynes & Martins-Ferreira 1953).

Using electron microscopy (Cordeiro *et al.* 1996) we were able to show a dense network of

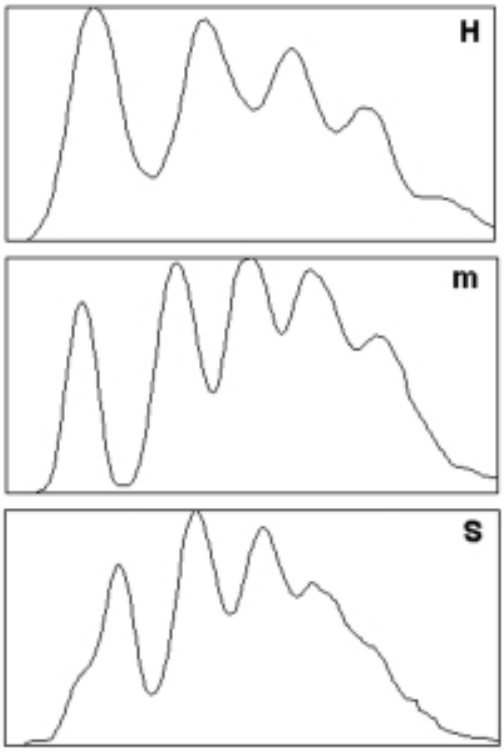

Fig. 2 – Typical densitometry profile for each organ (H - Hunter, m - main, S- Sachs) of *E. electricus*, obtained from the average images of isoelectricfocusing gels (left = basic, right = acidic).

filaments in the electric tissue of the eel (Fig. 4).

The presence of desmin in fractions of membrane of the main electric organ (Mermelstein *et al.* 1988, 1997) suggests a possible association between desmin and membranes of electrocytes. In striated muscle, desmin is concentrated in the Z line (and in intercalated disks in cardiac muscle) and on the non-myofibrillar region. In smooth muscle and in young myoblasts, desmin is more abundant around the nucleus, similar to what happen to all intermediate filaments.

Actin also has a specific pattern of isoform expression in different tissues. We also purified actin from the main electric organ of *E. electricus* (Ayres Sá *et al.* 1991). Analysis by isoelectric focusing shows two different isoelectric points for actin, as opposed to the unique isoelectric form of actin

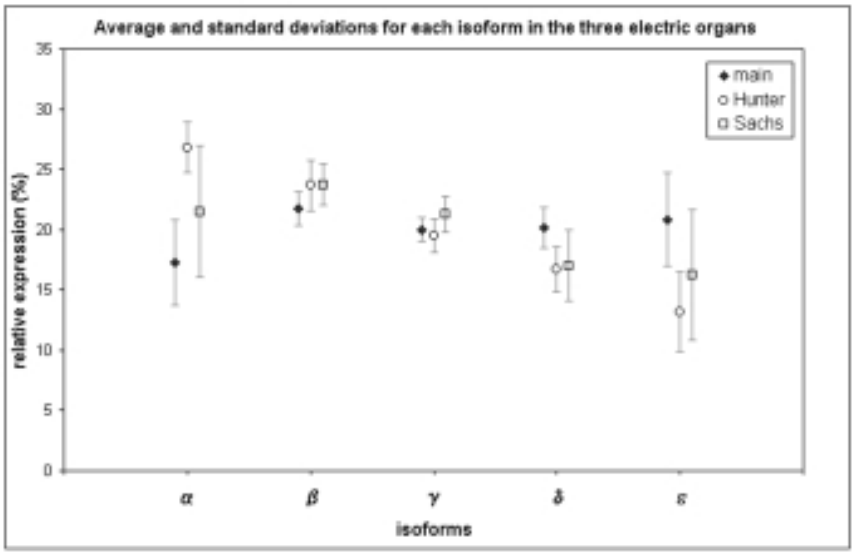

Fig. 3 – Relative expression (% of total of densitometry ± S.D.) of each isodesmin in each electric organ of *E. electricus* (number of observations: six for main, six for Hunter and four for Sachs).

extracted from the dorsal muscle of *Electrophorus electricus*. Only one actin variant found in the electric organ has, on the gel system used, the same isoelectric point of the alpha-actin from eel's dorsal muscle and of the alpha-actin from rabbit striated muscle. The other actin variant from the electric organ has the same isoelectric point of gamma-actin from smooth muscle (Ayres Sá *et al.* 1991). The expression of these two actin isoforms could be related to transient steps of the electrocyte differentiation program.

Actin was found distributed in the cytoplasm of the electrocyte by immunohistochemical methods using an antibody anti-actin and NBD-phallacidin, a probe that binds specifically to F-actin (Taffarel *et al.* 1985). However, only few and short actin filaments were seen in the electrocyte's cytoplasm. It seems that the major portion of actin is occurring in a globular form in these cells.

The presence of alpha-actinin, filamin and vinculin was confirmed in cytoskeletal-enriched membrane fractions from the main electric organ of *E. electricus* (Mermelstein *et al.* 1988), by SDS-PAGE and immunoblotting (Fig. 5). These fractions were obtained according to Kordeli *et al.* (1986). It is interesting to keep in mind that microfilaments need the physiological regulation of associated proteins. Proteins like alpha-actinin, filamin and vinculin regulate the overall distribution and architecture of the microfilaments, particularly during myofibrillogenesis, where they nucleate the nascent myofibrils, by aligning microfilaments (stress-fiber like structures). As they are important in myofibrillogenesis, they should be also involved in the breakdown of myofibrils during muscle dedifferentiation. When these cytoskeletal-enriched membrane fractions from the main electric organ were examined by transmission electron microscopy, a network of cytoskeletal filaments associated with membranes could be easily observed (Mermelstein *et al.* 1997). The analysis of these cytoskeletal-enriched membrane fractions by polyacrylamide gel electrophoresis, showed that actin and desmin could be the cytoskeletal components of these preparations. In fact, a peptidic map

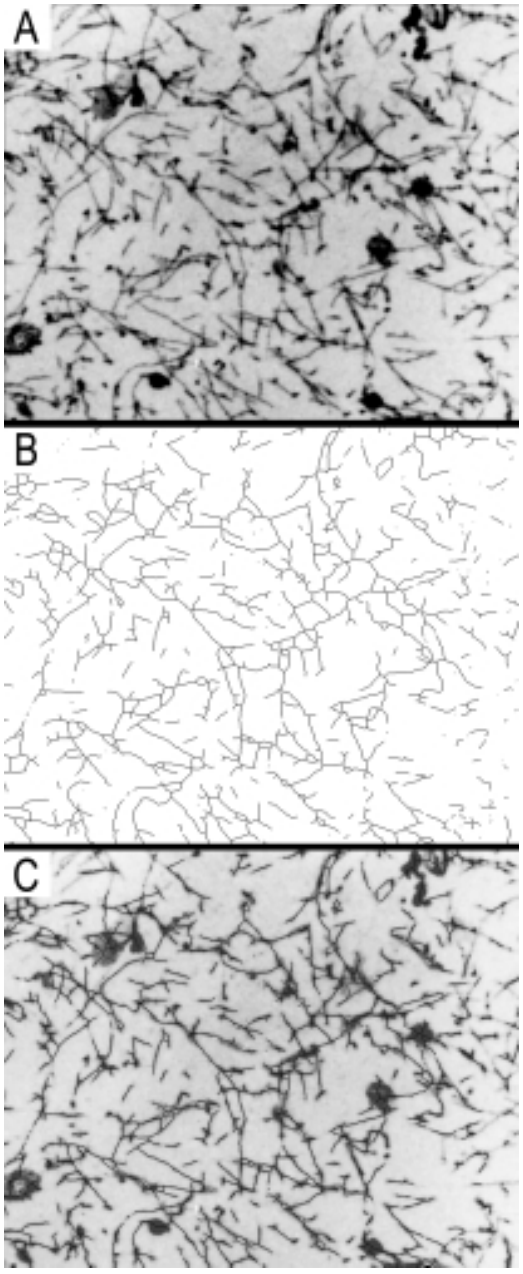

Fig. 4 – A= Transmission electron microscopy of cytoskeletal filaments from the main electric organ of *E. electricus*; B = Digital processing of the image A, using an erosion morphing procedure (skeletonize) to outline the filaments; C = Superimposition of images A and B. We used the programs NIH Image and Adobe PhotoShop. Picture A was given by Dr. Marlene Benchimol.

produced with these proteins extracted from the gel, confirmed their identity as been actin and desmin (Mermelstein *et al.* 1988, 1997).

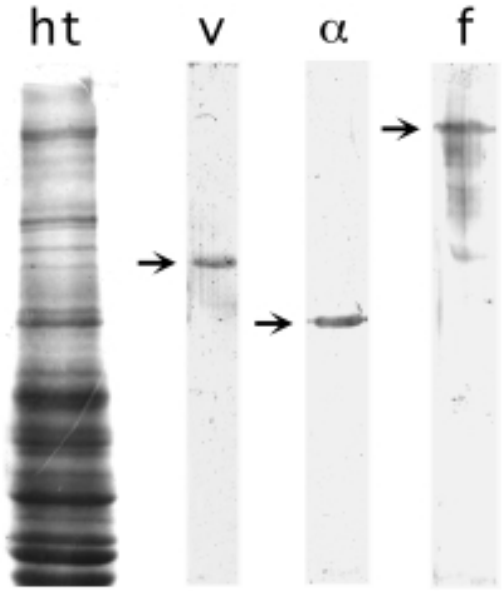

Fig. 5 – Polyacrylamide gel electrophoresis with SDS of a total homogenate (ht) from the main electric organ of *E. electricus*, stained with Coomassie blue; Immunoblottings using antibodies anti-vinculin (v), anti-alpha-actinin (α) and anti-filamin (f) against a cytoskeletal-enriched membrane fraction. Arrows indicate the main protein bands recognized by the antibodies.

We have not found long actin filaments in the cytoskeletal network of the electrocytes. It is difficult to think that actin filaments cross the whole cytoplasm of the cell. However, actin-binding proteins such as alpha-actinin, filamin and vinculin, are present in the electrocyte tissue. It is possible that actin, organized in short filaments, is linked to the membrane by interactions with alpha-actinin and vinculin. Another possible role for actin is to interact with desmin filaments, allowing this IF protein to interact with membranes. Thus, desmin could indirectly anchor protein receptors in the membrane, participating in the electric stimulation of the electrocytes. In this hypothetical picture, desmin could organize a meshwork with actin and actin-binding

TABLE I
Comparison between the biochemical purification, molecular weight, isoform expression and cellular localization of five cytoskeletal proteins from the electric organs of E. electricus. ND = not determined.

protein	biochemical purification	molecular weight	isoform expression	cellular localization
actin	yes	43 kd	2 (alpha sarcomeric and beta non-muscle), compared to 3 in other vertebrates	sparse and diffuse network
desmin	yes	53 kd	5, compared to 2-3 in other vertebrates	dense and diffuse network
alpha-actinin	yes	100 kd	1	close to plasma membrane
filamin	yes	250 kd	ND	ND
vinculin	yes	130 kd	ND	ND

proteins with attachment points in the electrocyte membrane, establishing and maintaining the functional polarity of the cell.

CONCLUDING REMARKS

Electrocytes are derived from skeletal muscle, which has a very specialized cytoskeletal organization. The observed changes in the cytoskeleton of electrocytes could be an indirect consequence of the induced modification of the tissue during the processes of acquisition of electric properties and loss of contractile function. On the contrary, the specific morphology suggests an active and important role for the cytoskeleton rearrangement. We studied structural and biochemical changes in two of the most abundant cytoskeletal proteins in muscle cells: actin and desmin (Table I). Microfilaments change from a myofibrillar structure in skeletal muscle cells into a loose network found in electrocytes (Ayres Sá *et al.* 1991) and desmin IFs change from a Z-band localization into a diffuse network in electrocytes (Cordeiro *et al.* 1996, Costa *et al.* 1988). One interesting question about desmin and its isovariants in the electric tissue is to understand if its presence is the cause or consequence of the electrocyte's specialization. Why there are five isoforms of desmin in the electric organ of *Electrophorus electricus*? Are the different degrees of phosphorylation of these isoforms related to different metabolical signalization in electrocytes?

The study of the cytoskeleton of the electric tissue of *Electrophorus electricus* may help to understand its role in keeping the polarized organization of the electrocyte. This study may also provide information about a possible role of the cytoskeleton on the bioelectrogenic property of the electric tissue.

ACKNOWLEDGEMENTS

We wish to thank Angela Maria Alves Langer for technical assistance. This work was supported by Conselho de Ensino e Pesquisa para Graduados/Universidade Federal do Rio de Janeiro (CEPG/UFRJ); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ); Financiadora de Estudos e Projetos (FINEP); Fundação Universitária José Bonifácio/Universidade Federal do Rio de Janeiro (FUJB/UFRJ) and PRONEX 052/97, Brasil.

REFERENCES

ALBE-FESSARD D & CHAGAS C. 1955. Études par dérivation intracellulaire des effets sommatifs de deux stimulations successives au niveau d'une électroplaque du gymnote. *C R Acad Sci* (Paris) **239:** 1951-1954.

ALLAN V. 1996. Motor protein: a dynamic duo. *Curr Biol* **6:** 630-633.

ARAUJO GMN, PEDRENHO AR & HASSÓN-VOLOCH A. 1993. The effect of mercury and aluminum on sodium-potassium-Mg^{2+} dependent-adenosine triphosphatase activity of *Electrophorus electricus* (L.) electrocyte. *Int J Biochem* **25:** 1729-1735.

AYRES SÁ L, MOURA NETO V, OLIVEIRA M M & CHAGAS C. 1991. Heterogeneity of purified actin in the electric organ of the electric eel *Electrophorus electricus. J Exp Zoology* **257:** 43-50.

BENCHIMOL M, MACHADO RD & SOUZA W. 1979. Staining of microtubules of the electrocyte of *Electrophorus electricus* L. by alcian blue and lanthanum. *Experientia* **35:** 670-671.

CARTAUD J, CARTAUD A, KORDELI E, LUDOSKY MA, MARCHAND S & STETZKOWSKI-MARDEN F. 2000. The Torpedo electrocyte: a model to study membrane-cytoskeleton interactions at the postsynaptic membrane. *Microsc Res Tech* **49:** 73-83.

CASSIMERIS L. 1999. Accessory protein regulation of microtubule dynamics throughout the cell cycle. *Curr Opin Cell Biol* **11:** 134-141.

CHANG P & STEARNS T. 2000. Gamma-tubulin and epsilon-tubulin: two new human centrosomal tubulins reveal new aspects of centrosome structure and function. *Nature Cell Biol* **2:** 30-35.

CHANGEUX JP, GAUTRON J, ISRAËL M & PODLESKI T. 1969. Séparation de membranes excitables à partir de l'organe électrique de l'*Electrophorus electricus. C R Acad Sci* (Paris) **269:** 1788-1791.

CORDEIRO MCR, MOURA NETO V, BENCHIMOL M, FARIA MVC & CHAGAS C. 1995. Microheterogeneity of desmin in the electric organ and dorsal muscle of the *Electrophorus electricus. Comp Biochem Physiol* **111A:** 345-350.

CORDEIRO MCR, BENCHIMOL M, MERMELSTEIN CS, SÁ LA, FARIA MVC, CHAGAS C & MOURA NETO V. 1996. Desmin filaments in the electrocytes of the electric organ of the *Electrophorus electricus. Cell Tissue Res* **285:** 387-393.

COSTA ML, OLIVEIRA MM, ALBERTI JR O, NETO VM & CHAGAS C. 1986. Caractérisation de la desmine dans l'organe électrique de l'*Electrophorus electricus* L. *C R Acad Sci* (Paris) **303:** 547-550.

COSTA ML, NETO VM & CHAGAS C. 1988. Desmin heterogeneity in the main electric organ of *Electrophorus electricus* (Linnaeus). *Biochimie* **70:** 783-789.

COSTA ML, MERMELSTEIN CS, FRÓES MM, CHAGAS C & NETO VM. 1998. Differences in the isodesmin pattern between the electric organs of *Electrophorus electricus* L. *Comp Biochem Physiol* **119:** 715-719.

ESQUIBEL MA, ALONSO I, MEYER H, CASTRO GO & CHAGAS C. 1971. Quelques aspects de l'histogenese et de l'ontogenese des organes électriques chez l'*Electrophorus electricus* L. *C R Acad Sci* (Paris) **273:** 196-199.

GALOU M, GAO J, HUMBERT J, MERICSKAY M, LI Z, PAULIN D & VICART P. 1997. The importance of intermediate filaments in the adaptation of tissues to mechanical stress: evidence from gene knockout studies. *Biol cell* **89:** 85-97.

GEISLER N, PLESSMAN U & WEBER K. 1982. Related amino acid sequences in neurofilaments and non-neuronal intermediate filaments. *Nature* **296:** 448-450.

GOMES F, PAULIN D & MOURA NETO V. 1999. Glial fibrillary acidic protein (GFAP): modulation by growth factors and its implication in astrocyte differentiation. *Braz J Med Biol Res* **32:** 619-631.

GOTTER AL, KAETZEL MA & DEDMAN JR. 1998. *Electrophorus electricus* as a model system for the study of membrane excitability. *Comp Biochem Physiol* **119:** 225-241.

HARTWIG JH & KWIATKOWSKI DJ. 1991. Actin-binding proteins. *Curr Opin Cell Biol* **3:** 87-97.

HASSÓN-VOLOCH A, SOMLÓ C, NUNES-DE-ARAUJO GM & GOMES-QUINTANA E. 1993. Mg^{2+}-dependent ATPases in the electrocyte of *Electrophorus electricus* (L.). *Brazilian J Med Biol Res* **26:** 351-354.

HERRMANN H & AEBI U. 2000. Intermediate filaments and their associates: multi-talented structural elements specifying cytoarchitecture and cytodynamics. *Curr Opin Cell Biol* **12:** 79-90.

KEYNES RD & MARTINS-FERREIRA H. 1953. Membrane potentials in the electroplates of the electric eel. *J Physiol* **119:** 315-351.

KORDELI E, CARTAUD J, NGHIÊM HD, PRADEL LA, DUBREUIL C, PAULIN D & CHANGEUX JP. 1986. Evidence for a polarity in the distribution of proteins from the cytoskeleton in *Torpedo marmorata* electrocytes. *J Biol Chem* **102:** 748-761.

LAZARIDES E & BALZER DR. 1978. Specificity of desmin to avian and mammalian muscle cells. *Cell* **14:** 429-438.

LEÃO FERREIRA LR, MOUSSATCHÉ N & MOURA NETO V. 1994. Rearrangement of intermediate filament network of BHK-21 cells infected with vaccinia virus. *Arch Virol* **138:** 273-285.

LI H, CHOUDHARY SK, MILNER DJ, MUNIR MI, KUISK, IR & CAPETANAKI Y. 1994. Inhibition of desmin expression blocks myoblast fusion and interferes with the myogenic regulators MyoD and Myogenin. *J Cell Biol* **124:** 827-841.

LI Z, MERICSKAY M, AGBULUT O, BUTLER-BROWNE G, CARLSSON L, THORNELL L-E, BABINET C & PAULIN, D. 1997. Desmin is essential for the tensile strength and integrity of myofibrils but not for myogenic commitment, differentiation, and fusion of skeletal muscle. *J Cell Biol* **139:** 129-144.

LUFT JH. 1957. The histology and cytology of the electric organs of electric eel (*Electrophorus electricus* L.). *J Morph* **100:** 113-139.

MACHADO RD, DE SOUZA W, COTTA PEREIRA G & DE OLIVEIRA G. 1976. On the fine structure of the electrocyte of *Electrophorus electricus* L. *Cell Tissue Res* **174:** 355-366.

MACHESKY LM & SCHLIWA M. 2000. Cell dynamics: a new look at the cytoskeleton. *Nature Cell Biol* **2:** E17-E18.

MATHEWSON R, WACHTEL A & GRUNDFEST H. 1961. Fine structure of electroplaques. In: CHAGAS C & PAES CARVALHO A (Ed.); *Bioelectrogenesis*. Amsterdam: Elsevier Publishing Co., p. 25-53.

MATSUDARIA P. 1994. Actin crosslinking proteins at the leading edge. *Semin Cell Biol* **5:** 165-174.

MERMELSTEIN CS, MOURA NETO V & CHAGAS C. 1988. *C*. Alpha-actinine dans l'organe electrique de l'*Electrophorus electricus,* L. *C R Acad Sci* (Paris) **307:** 717-721.

MERMELSTEIN CS, COSTA ML, CHAGAS C & MOURA NETO V. 1996. Intermediate filament proteins in TPA-treated skeletal muscle cells in culture. *J Muscle Res Cell Mot* **17:** 199-296.

MERMELSTEIN CS, BENCHIMOL M, TAFFAREL M, CORDEIRO MCR, CHAGAS C & MOURA NETO V. 1997. Desmin and actin filaments in membrane-cytoskeletal preparations of the electric tissue of *Electrophorus electricus,* L. *Arch Histol Cytol* **60:** 445-452.

MEUNIER JC, SEALOCK R, OLSEN R & CHANGEUX JP. 1974. Purification and properties of the choline receptor protein from *Electrophorus electricus* electric tissue. *Eur J Biochem* **45:** 371-394.

MOURA NETO V, MALLAT M, JEANTET C & PROCHIANTZ A. 1983. Microheterogeneity of tubulin proteins in neuronal and glial cells from the mouse brain in culture. *EMBO J* **2:** 1243-1248.

OAKLEY BR, OAKLEY CE, YOON Y & JUNG MK. 1990. Gamma-tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergilla nidulans. Cell* **61:** 1289-1301.

O'CONNOR M, GARD DL & LAZARIDES E. 1981. Phosphorylation of intermediate filament protein by cAMP-dependent protein quinases. *Cell* **23:** 135-143.

OSBORN M & WEBER K. 1986. Intermediate filament proteins: a multigene family distinguishing major cell lineages. *TIBS* **11:** 469-472.

OTEY CA, KALNOSKI MH & BULINSKI JC. 1988. Immunolocalization of muscle and nonmuscle isoforms of actin in myogenic cells and skeletal muscle. *Cell Mot Cytoskeleton* **9:** 337-348.

PRUSS RM, MIRSKY R & RAFF MC. 1981. All classes of intermediate filaments share a common antigenic determinant defined by a monoclonal antibody. *Cell* **27:** 419-428.

QUAX W, VAN DEN BROCK L, VREE EGHERTS W, RA-

MAEKERS F & BLOEMENDAL H. 1985. Characterization of the hamster desmin gene: expression and formation of desmin filaments in non-muscle cells after gene transfer. *Cell* **43:** 327-328.

RAPPAPORT L, CONTARD F, SAMUEL JL, DELCAYRE C, MAROTTE F, TOME F & FARDEAU M. 1988. Storage of phosphorylated desmin in a familial myopathy. *FEBS Lett* **231:** 421-425.

REGNARD C, AUDEBERT S, BOUCHER D, LARCHER JC, EDDE B & DENOULET P. 1996. Les microtubules: polymorphismes fonctionnels de la tubuline et des protéines associées (MAPs structurales et motrices). *Compt Rend Soc Biol* **190:** 255-268.

RUDIGER M. 1998. Vinculin and alpha-catenin: shared and unique functions in adherens junctions. *Bioessays* **20:** 733-740.

SCHULTHEISS T, LIN Z, ISHIKAWA H, ZAMIR I, STOECKERT CJ & HOLTZER H. 1991. Desmin/vimentin intermediate filaments are dispensable for many aspects of myogenesis. *J Cell Biol* **114:** 953-966.

SINGER SJ, MALHER PA, ROGALSKI AA, KUPPER A & COX GF. 1986. Progress in the study of membrane cytoskeletal associations. In: BENETT V *et al.* (Ed.); *Membrane skeletons and cytoskeletal-membrane associations*. Alan R Lins Inc, p. 261-268.

SOMLÓ C, DE SOUZA W, MACHADO RD, HASSÓN-VOLOCH A. 1977. Biochemical and cytochemical localization of ATPases on the membranes of the electrocyte of *Electrophorus electricus. Cell Tissue Res* **185:** 115-128.

TAFFAREL M, DE SOUZA MF, MACHADO RD & DE SOUZA W. 1985. Localization of actin in the electrocyte of *Electrophorus electricus* L. *Cell Tissue Res* **242:** 453-455.

THORNELL L-E, ERIKSSON A, JOHANSSON B, KJÖRELL U, FRANKE WW, VIRTANEN I & LEHTO V-P. 1985. Intermediate filament and associated proteins in heart Purkinje fibers: a membrane-myofibril anchored cytoskeletal system. *Ann N Y Acad Sci* **455:** 213-239.

TRAUB P. 1985. *Intermediate filaments: a review.* North Holland: Elsevier.

VALE RD & MILLIGAN RA. 2000. The way things move: looking under the hood of molecular motor proteins. *Science* **288:** 88-95.

VANDEKERCKHOVE J, BUGAISKY G & BUCKINGHAM M. 1986. Simultaneous expression of skeletal muscle and heart actin proteins in various striated muscle tissues and cells. *J Biol Chem* **261:** 1838-1843.

VIEL A. 1999. Alpha-actinin and spectrin structures: an unfolding family story. *FEBS Lett* **5:** 391-394.