Echinococcus granulosus: Cellular territories and morphological regions in mature protoscoleces

Mario Galindo [a], Gloria Schadebrodt [b], Norbel Galanti [a,*]

[a] *Program of Cellular and Molecular Biology, Institute of Biomedical Sciences (I.C.B.M.), Faculty of Medicine, University of Chile, Av. Independencia 1027, P.O. Box 70061, Santiago, Chile*
[b] *Laboratorio de Diagnostico Veterinario, Servicio Agrícola y Ganadero (S.A.G.), Avda Ogana 1060, Coyhaique, Chile*

A B S T R A C T

Basic aspects of the generation, structure and function of *Echinococcus granulosus* protoscoleces are unknown. We review the work done on the structure and ultrastructure of the *E. granulosus* protoscolex and provide new data together with a comprehensive view of this form of the parasite. The surface, as observed by scanning electron microscopy, tightly correlates with five cellular territories characterized in the interior using light and transmission electron microscopy as well as a histochemical technique. Three of these territories are surrounded by a basal lamina that is also present in the internal side of the tegument, suggesting a complex internal organization. These cellular territories correlate with the expression of specific genes and the regionalization of DNA synthesis in protoscoleces. Additionally, a proposal to explain movements of the body of this form of the parasite in relation to the neck or to the germinal layer of the hydatid cyst is provided.

Index Descriptors and Abbreviations:
E. granulosus
Protoscolex
Cellular territories
SEM, scanning electron microscopy
TEM, transmission electron microscopy
LM, light microscopy
RR, rostellar region
SR, sucker region
NR, neck region
BR, body region
RC, rostellar cone
H, hooks
S, suckers
RCCT, rostellar cone cellular territory
RPCT, rostellar pad cellular territory
SCT, sucker cellular territory
RICT, rostellar intermediate cellular territory
BCT, body cellular territory
DNA, deoxyribonucleic acid

1. Introduction

The protoscolex of *Echinococcus granulosus* plays a key role in the life cycle of this parasite, being the only infective form to canine and other carnivores, when ingested in a meal of organs infected with hydatid cysts. In spite of its importance, many basic aspects of the generation, structure and function of *E. granulosus* protoscoleces remain obscure. It is formed in the germinal layer of fertile hydatid cysts by means of cellular and biochemical processes which still are not fully known, defining the fertility of the cysts and its infectivity to carnivores (Bortoletti and Ferretti, 1973; Thompson, 1976; Galindo et al., 2002, 2003). However, it is not known whether buds that form in the germinal layer and become protoscoleces are built up from undifferentiated cells, from a specific cell line or lines, or from any indefinite cellular territory of the germinal layer (Galindo et al., 2003). Cell differentiation in the growing bud has not been carefully studied, the cellular origin of the different anatomical regions of the protoscolex being obscure (Martinez et al., 2005). Although it is well known that in the protoscolex a number of different cells are present (Morseth, 1967), their origin and arrangement in the final mature protoscolex is not fully understood. In spite of important efforts (Smyth, 1962, 1967; Smyth et al., 1966), it is also not known with any precision which are the cells involved in the differentiation of the protoscolex to the adult worm, or those implicated in the reversion to the hydatid cyst.

Thus, more information is indeed necessary in order to reach a comprehensive knowledge of the *E. granulosus* protoscolex generation, growth, differentiation and internal cellular organization.

* Corresponding author. Fax: +56 2 7353510.
E-mail address: ngalanti@med.uchile.cl (N. Galanti).

Lack of this information is a challenge for finding a rationale way to control this parasite.

In this work we present a review of the work done on the structure and ultrastructure of the protoscolex of *E. granulosus* and provide a comprehensive view of this form of the parasite. Using light, scanning and electron transmission microscopy, as well as a histochemical reaction designed to stain basal lamina, we define five internal cellular territories in the mature, evaginated protoscolex. The exterior of the protoscolex observed by scanning electron microscopy tightly correlates with cellular territories described in the interior using light and transmission electron microscopy. Three of these territories are surrounded by basal lamina, suggesting a complex internal organization of the protoscolex. A basal lamina also seems to be present on the internal side of the tegument. Additionally, ultrastructural analysis of the anatomical regions of the protoscolex provides an understanding of processes such as the up–down, circular and oscillatory movements of the body in relation to the neck in mature protoscoleces fixed at the mucosa of the intestinal villi and movements of the nascent protoscolex when still attached to the germinal layer of the hydatid cyst. Moreover, the presence of bundles of muscle fibers in the cellular pad and in the sucker territories may be related to opening/closing movements of hooks and to the fixation mechanism of the protoscolex to the intestinal mucosa of the dog, respectively. The cellular territories described here strictly correlate with previous work on the regionalization of DNA synthesis observed in the protoscoleces (Galindo et al., 2003). Finally, the presence of a basal lamina surrounding several cellular territories in the adult invasive protoscolex tackles the problem of movement between the different territories of cellular products throughout the basal lamina.

2. Materials and methods

2.1. Samples

Echinococcus granulosus fertile hydatid cysts were obtained from livers or lungs of naturally infected sheep at the Lo Valledor slaughter house in Santiago and from Coyhaique and Puerto Porvenir in the south of Chile. Cyst fertility was determined by the presence of free protoscoleces in the hydatid fluid. Protoscoleces were decanted by gravity, washed in PBS, pH 7.2 at 38.5 °C and treated with 0.1% pepsin in Hanks' salt solution, pH 2.0, at 38.5 °C for 15 min, to eliminate remnants of germinal layer. Pepsin was removed by four washings with Hanks' medium. Viability of the protoscoleces was evaluated on the basis of body movements and flame cell activity as observed under a light microscope.

2.2. Light microscopy

For light microscopy studies, protoscoleces were further washed three times in 0.1 M sodium cacodylate, pH 7.4 (buffer A) at 4 °C and fixed in 2.5% glutaraldehyde in the same buffer at 4 °C for 3 h. The samples were then washed three times in buffer A at 4 °C, postfixed in 1% OsO_4 prepared in buffer A at room temperature for 1 h, washed three times in buffer A at 4 °C and observed directly. Alternatively, samples fixed in glutaraldehyde and postfixed in OsO_4 were dehydrated with 100% ethyl alcohol and propylene oxide, embedded in Epon-812, and 0.5–1.0 μm sections were cut in an automatic Zeiss ultramicrotome. These sections were stained in toluidine blue. In some experiments, protoscoleces were fixed in alcoholic Bouin, embedded in paraffin, and 5 μm sections were stained in hematoxylin–eosin. All these specimens were observed under a Nikon light microscope.

2.3. Electron microscopy

For scanning electron microscopy work, protoscoleces were fixed in glutaraldehyde, postfixed in OsO_4 and dehydrated, as described above and were further dehydrated in acetone, dried with CO_2 in a Polaron E 3000 apparatus and then sputter-coated with gold under a Sputtering Device Polaron E 5000. Samples were observed under a Zeiss DSM-940 scanning electron microscope. For transmission electron microscopy, samples fixed in glutaraldehyde, postfixed in OsO_4, dehydrated and embedded in Epon were cut in 0.2 μm sections in a Sorvall Mt-2B ultramicrotome. These sections were placed on 200-mesh copper grids, stained in lead uranyl acetate and observed using a Zeiss EM-109 transmission electron microscope.

The panoramic view of whole protoscoleces and some cellular territories, either at light or electron microscopy, correspond to reconstructions based in contiguous microphotographs.

2.4. Arteta's histochemical reaction

Protoscoleces fixed in alcoholic Bouin were embedded in paraffin. Slices (5 μm) were sequentially stained with Harris haematoxylin, erythrosine-Orange G and after treatment with phosphotungstic acid, with aniline blue. Collagen from basal lamina is identified by a blue color (Humason, 1979).

3. Results

Fig. 1A and B shows evaginated protoscoleces as observed by SEM and LM, respectively. Following the anterior–posterior axis, four morphological regions are clearly distinguished, namely rostellar (RR), sucker (SR), neck (NR) and body (BR). Rostellar cone (RC), hooks (H) and suckers (S), are indicated.

Fig. 1C and D shows longitudinal sections of protoscoleces observed under LM and after Arteta's staining, respectively. Cellular territories such as rostellar cone (RCCT), rostellar pad (RPCT) and suckers (SCT) are easily distinguished because they are surrounded by basal membranes (Fig. 1C and blue[1] lines in Fig. 1D); by exclusion and morphological location, rostellar intermediate (RICT) and body (BCT) cellular territories are defined. These last two cellular territories are contiguous, limiting at the neck of the protoscolex. Basal lamina is clearly evidenced by the Arteta's blue staining (Fig. 1D), surrounding the RPCT and the SCT. It is also present under the tegument in the body cellular territory (1D).

Fig. 2A shows a lateral view of the scolex as observed by SEM. Hooks, rostellum (R), rostellar base (RB) (boxed area) and suckers are clearly distinguished. In Fig. 2B an upper view of the scolex shows the rostellar cone, above hooks and suckers. Fig. 2C and D corresponds to lateral views of the scolex showing the rostellar cone and hooks (2C). A dense network of long, filamentous and branched microtriches are observed, covering the rostellar cone and hooks (2C); these structures are magnified in Fig. 3D (squared area in 3C). These types of microtriches are restricted to the hooks and cone; they are not observed in the base of the rostellum (Fig. 2E). The surface of the rostellar base is covered by short, non-branched microtriches (Fig. 2F). In the same Figure, branched hook microtriches are shown.

Fig. 2G shows a lateral view of the scolex, specifically a zone between suckers. Microtriches covering this area as well as those found inside the suckers (Fig. 2H) are similar to the ones described for the rostellar base. In Fig. 2I a fracture in the neck (NE) of the protoscolex, between the scolex (SC) and the body (B), is shown,

[1] For interpretation of the references to color in this figure, the reader is referred to the web version of this article.

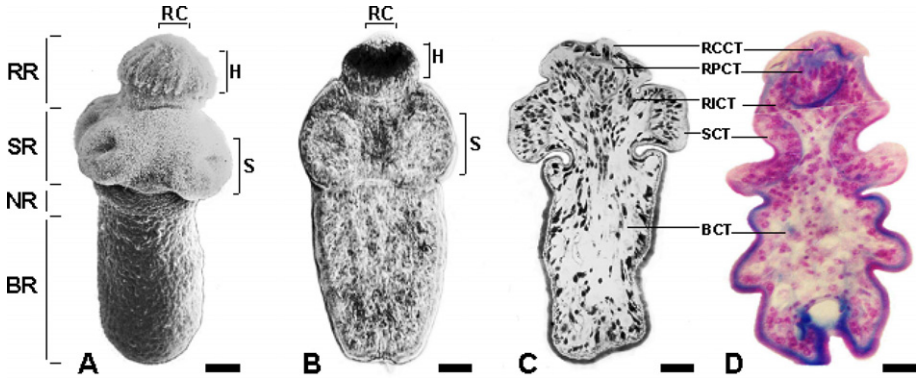

Fig. 1. Morphological regions and cellular territories of the *E. granulosus* protoscolex. (A and B) Whole protoscoleces observed by scanning electron microscopy and light microscopy, respectively. RR, rostellar region; SR, sucker region; NR, neck region; BR, body region; RC, rostellar cone; H, hooks; S, suckers. (C and D) Light microscopy reconstructed sections stained with toluidine blue and with the Arteta technique, respectively. RCCT, rostellar cone cellular territory; RPCT, rostellar pad cellular territory; RICT, rostellar intermediate cellular territory; SCT, sucker cellular territory; BCT, body cellular territory. Scale bars: 10 μm.

which is magnified in Fig. 2J. The neck is an articulation structure with similarity to a telescopic fishing rod, suggesting that it may allow both up and down as well as restricted rotating and pendular movements of the body in relation to the scolex, probably by fixation of the protoscolex to the intestine mucosa. Inside the neck (IN), a complex network of cellular structures is observed (Fig. 2J,

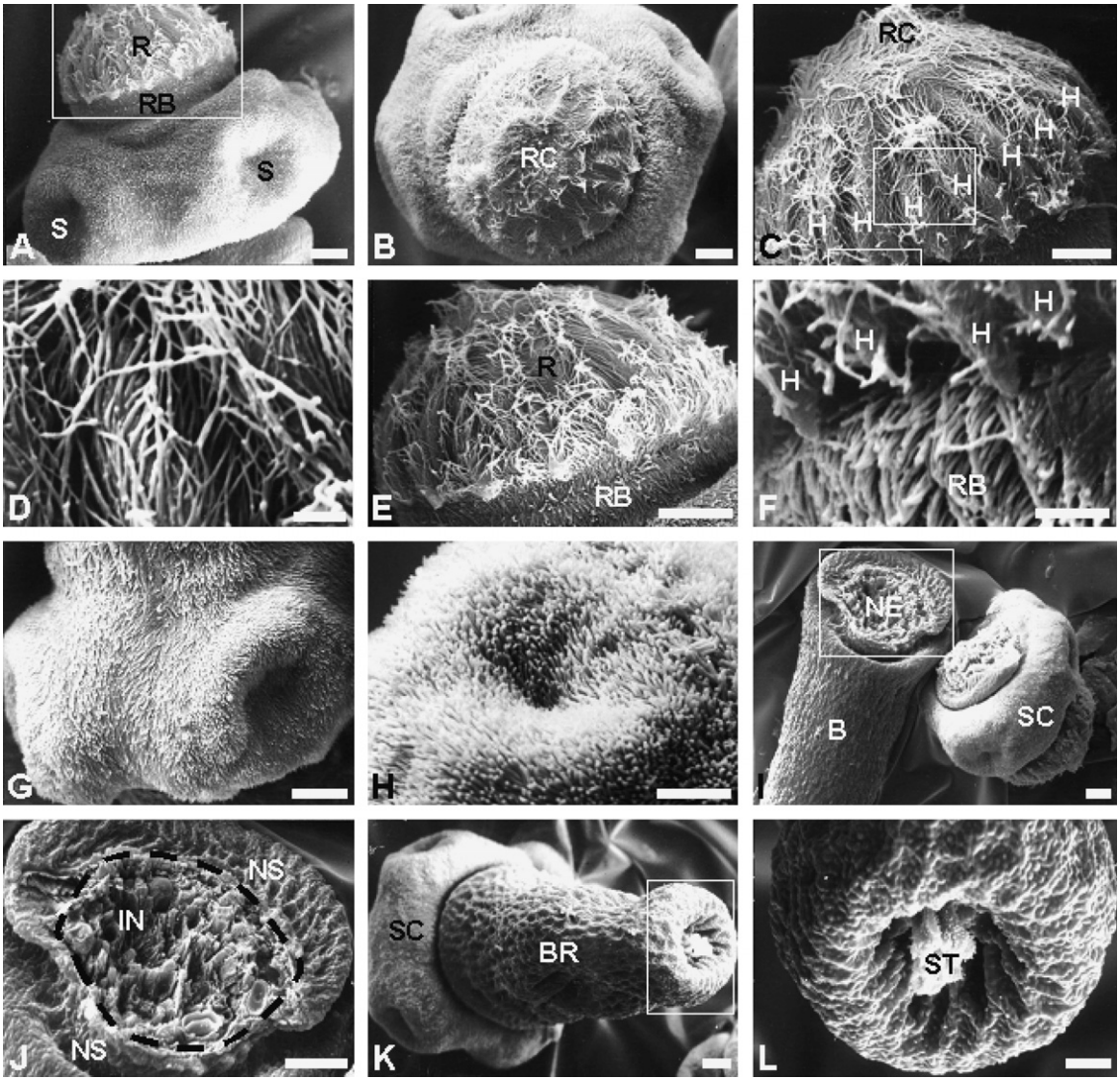

Fig. 2. Specific features of the surface in different regions of the *E. granulosus* protoscolex. (A) Scolex, lateral view. R, rostellum; RB, rostellar base; S, sucker. (B) Scolex, top view. RC, rostellar cone. (C) Rostellar cone and hooks, lateral view. H, hooks. Filamentous microtriches from rostellar cone and hooks are shown. (D) Amplified square area of (C). (E) Rostellum and rostellar base. (F) Microtriches from hooks (upper) and rostellar base (down), corresponding to the small square area of (C). (G) Sucker region. (H) Sucker microtriches. (I) Fractured protoscolex at the neck region (NE). B, body; SC, scolex. (J) Amplified square region of (I). Neck surface (NS) and interior structure of the neck (IN, dashed lines). (K) Body region (BR), lateral view. (L) Amplified square area of (K). Distal body region and stalk (ST). Scale bars: 10 μm.

boxed area in 2I, dashed lines). On the surface of the neck region (NS) microtriches seem to be absent; this surface is ruffled and uneven. The folded aspect of the tegument observed in this area is probably derived from the normal retraction of this structure which follows the up and down movement of the body.

Fig. 2K shows the body region which represents ≈50–60% of the length of the protoscolex (see also Fig. 1). The surface of this region is similar to that of the neck, being corrugated and covered with knob-like projections, which are better observed at higher magnification (Fig. 2L). These projections were previously described (Morseth, 1967). In the center of the base of the protoscolex, a stalk or pedunculus is observed (Fig. 2K) which is magnified in Fig. 2L (ST, boxed area in 2K). Again this structure reminds one of a telescopic fishing rod, permitting up and down as well as rotating and pendulous movements of the protoscolex, when it is forming or still fixed to the germinal layer of the hydatid cyst.

Fig. 3A shows a longitudinal light microscopy view of a section from the tip of the rostellum. Rostellar cone and rostellar pad cellular territories are clearly distinguished; this last one is surrounded by a basal lamina (arrows) that is also observed in Fig. 3B (arrows). Around the rostellar pad cellular territory cells from the rostellar intermediate territory are observed. At both

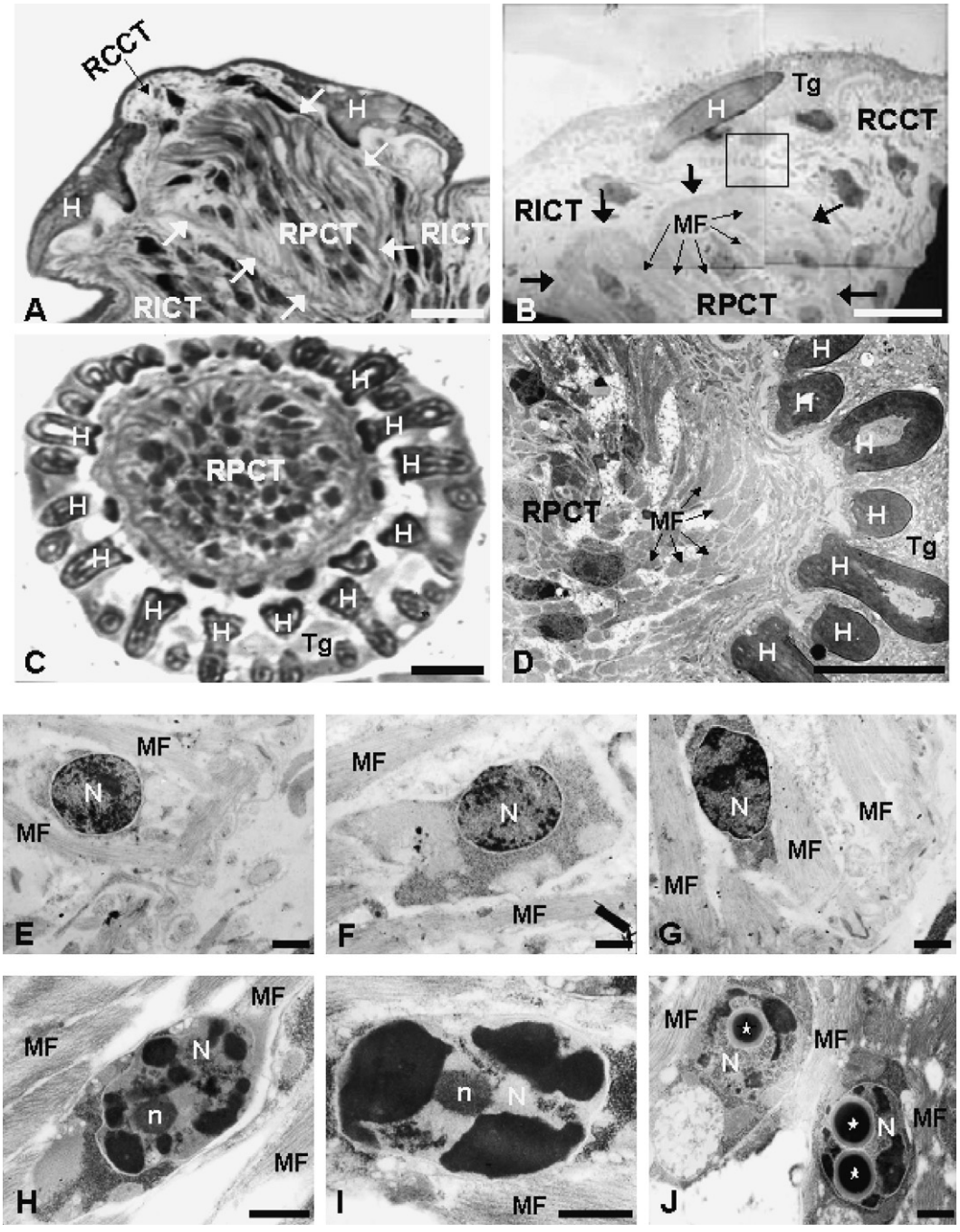

Fig. 3. Rostellar cone (RCCT), pad (RPCT) and intermediate (RICT) cellular territories observed by (A) light microscopy, longitudinal section and (B) transmission electron microscopy, longitudinal section. H, hooks; MF, muscle fibers; Tg, tegument; arrows, basal lamina of RPCT. Rostellar pad cellular territory (RPCT) and hooks H observed by (C) light microscopy, transversal section and (D) transmission electron microscopy, transversal section. (E–J) Representative cells from the rostellar pad cellular territory, transmission electron microscopy sections. N, nuclei; n, nucleoli; MF, muscle fibers; stars, nuclear inclusions. Scale bars: (A–D) 10 μm; (E–J) 1 μm.

sides of the rostellar cone hooks are present. A similar view but at TEM is observed in Fig. 3B. Cellular continuity between the RCCT and the RICT is restricted by a narrowing between the basal membrane of the RPCT and a thickening of the tegument around the hooks (squared area); this is better observed in Fig. 3C.

In the RPCT, muscle fibers are observed along the anterior–posterior axis (Fig. 3B, MF) which also can be seen in Fig. 3D in a transversal section. These fibers may contribute to the extension-retraction movements of the hooks upon fixation of the protoscolex to the intestinal mucosa; then, the RPCT would function as a muscle-like organ. Fig. 3C clearly shows that RPCT is an individual compartment; it also shows the spatial relationship of RPCT with the hooks, reinforcing the proposal of its contribution to the control of the movements of the hooks. The tegument (Tg) becomes thick at the region of the scolex, containing and surrounding the hooks (Fig. 3B–D). Fig. 3E–J shows cells surrounded by muscle fibers; most if not all of them being muscle cells, with fibers inside their cytoplasm. Cells observed in the RPCT are similar in structure; however, differences in chromatin compaction are evident. Dense spherical bodies (star) are observed inside the nucleus (N) of some cells (Fig. 3J); these structures were previously described (Herbaut et al., 1988). The function of these nuclear dense bodies is not known although we discard the possibility that they correspond to apoptotic cells considering the healthy appearance of the nuclear envelope. Functional, non-segregated nucleoli (n) are observed in Fig. 3H and I, suggesting that they are actively involved in rRNA synthesis. A granular aspect of the cytoplasm, possibly corresponding to ribosomes, is observed in all cells of the RPCT.

Though most cells are related to muscle fibers, the differences in chromatin compaction observed in some nuclei suggest the existence of different kinds of cells in this territory.

Fig. 4A shows the rostellar cone cellular territory observed under light microscopy in a longitudinal section; it is surrounded by hooks at it sides and by the rostellar pad cellular territory located bellow. A basal lamina, that is clearly evident surrounding the RPCT (arrows), is not distinguishable as part of the RCCT. The rostellar pad cellular territory is surrounded by the rostellar intermediate cellular territory. Fig. 4B shows a region similar to A in a sagittal view under TEM. The RCCT, surrounded by dashed lines, shows scarce cells located between the tegument and the basal membrane of the rostellar pad cellular territory; no muscle fibers are observed in RCCT. However, they are present in RPCT. Tegu-

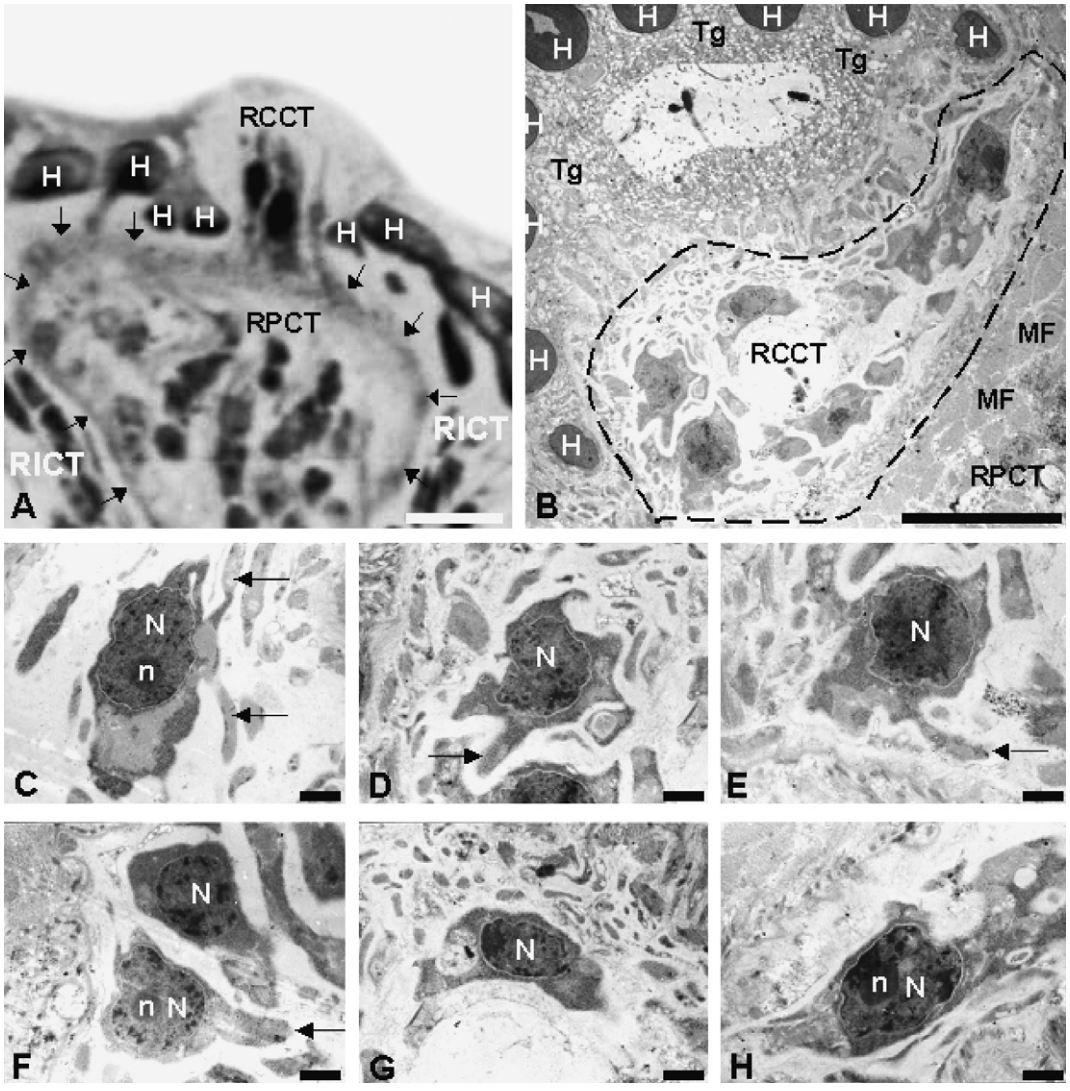

Fig. 4. (A) Light microscopy of a longitudinal section of the scolex. H, hooks; RCCT, rostellar cone cellular territory; RPCT, rostellar pad cellular territory; RICT, rostellar intermediate cellular territory. (B) Electron microscopy of a sagittal section of a similar territory as indicated in (A). Tg, tegument; MF, muscle fibers. (C–H) Representative cells from the rostellar cone cellular territory, transmission electron microscopy sections. N, nuclei; n, nucleoli; arrows, cytoplasmic processes. Scale bars: (A and B) 10 μm; (C–H) 1 μm.

ment is shown surrounding the hooks. In Fig. 4C–H, cells of the RCCT are shown. They present similar structures, with cytoplasmic processes (arrows). Nuclei show scarce perinuclear heterochromatin and a nucleolus. Similarity among these cells suggests a limited number of functions of this territory. Again, no muscle fibers are observed, either outside or inside the cells. Consequently, the RCCT cannot be considered a muscle organ. A secretory function of this cellular territory was proposed in the adult worm (Thompson et al., 1979). However, we were not able to observe secretory cells or secretory vesicles in the RCCT.

Fig. 5 shows a longitudinal section of the sucker cellular territory at light (A) and transmission electron (B) microscopy. A basal lamina containing the cells of this territory is evident in both figures (small arrows). In 5A it is observed that this basal lamina is separated from the tegument, reinforcing the condition of a segregate territory for the suckers. Cells and longitudinal as well transversal muscle fibers (Fig. 5B) are observed in this cellular territory, suggesting a network of these structures in the suckers. Indeed, muscle bundles were previously described in the suckers (Morseth, 1967). The SCT is surrounded on one side by the tegument and on the others by cells of the rostellar intermediate cellular territory; calcareous corpuscles (cc) are observed in this territory. In Fig. 5B cells with different structures are shown, that are better described below. In Fig. 5B and C microtriches, that were described in Fig. 2G and H are evident, projecting from the tegument. The structure of these microtriches at the TEM level were described elsewhere (Rogan and Richards, 1987).

In Fig. 5C–E, characteristic cells showing long cytoplasmic projections (CP) associated to bundles of muscle fibers projecting to the sucker tegument are shown. The bundles of MF are numerous, filling the space between cells and the basal lamina (BL) that surrounds the sucker region. Nuclei of these cells show an irregular

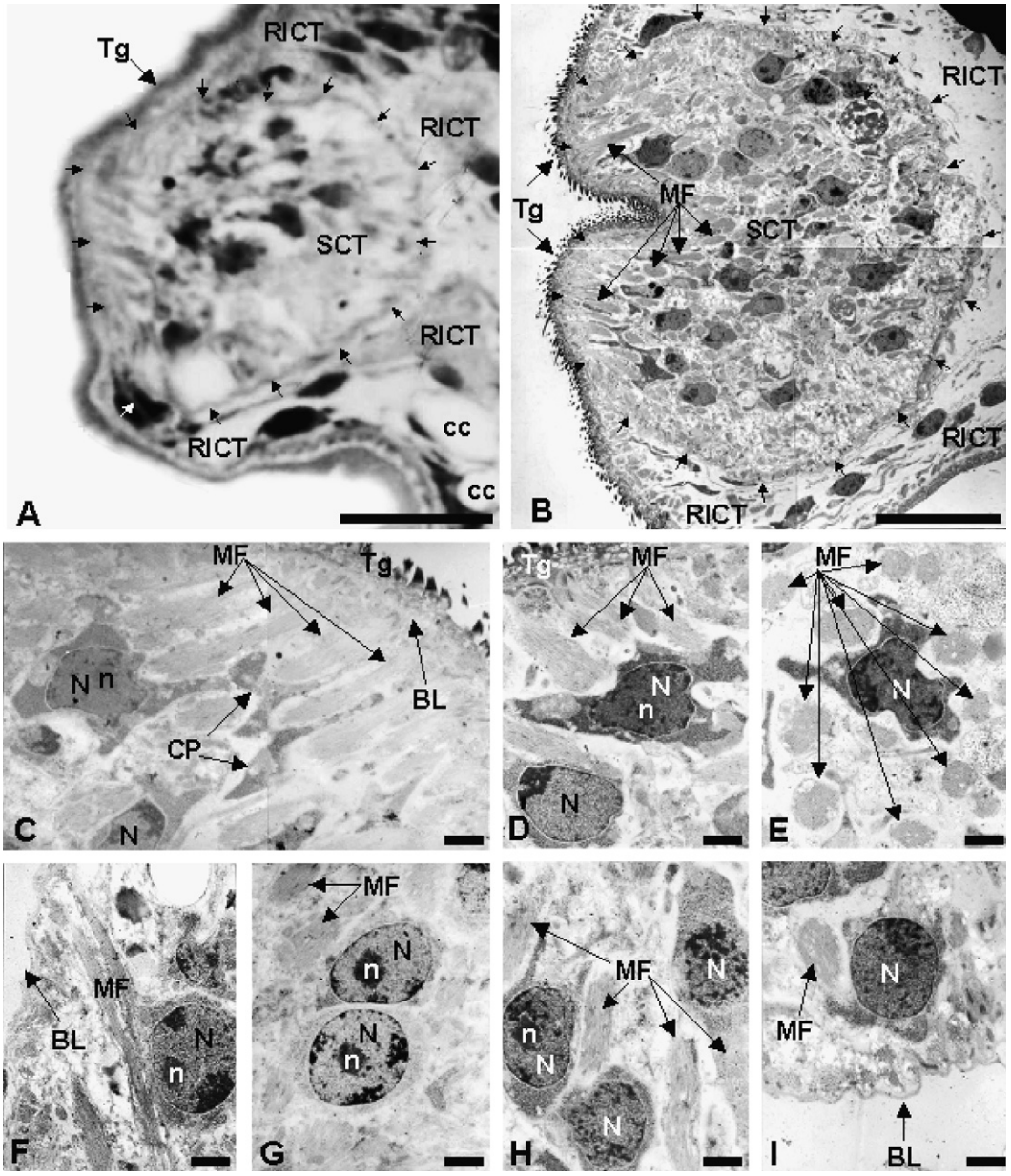

Fig. 5. (A and B) Light microscopy and electron microscopy, respectively, of longitudinal sections of the sucker territory (SCT). Arrows, basal membrane of the sucker territory; cc, calcareous corpuscles; MF, muscle fibers; Tg, tegument; RICT, rostellar intermediate cellular territory. (C–I) Representative cells from the sucker cellular territory, transmission electron microscopy sections. MF, muscle fibers; N, nuclei; n, nucleoli; CP, cytoplasmic processes; Tg, tegument; BL, basal lamina. Scale bars: (A and B) 10 μm; (C–I) 1 μm.

shape, with patches of heterochromatin mostly distributed attached to the internal nuclear envelope. A non-segregated nucleolus is evident in most of these cells, suggesting an active rRNA synthesis. Other type of cells, also showing cytoplasmic projections and association to bundles of muscle fibers, are shown in Fig. 5F–I. These cells show spherical nuclei, with patches of condensed chromatin; the cytoplasm seems to be filled up with structures resembling ribosomes. Considering their structure, these cells may be those involved in DNA synthesis, as was reported (Galindo et al., 2003). Cells showing irregular and spherical nuclei are intermixed and present in all regions of the sucker cellular territory; these cells seem to be metabolically active.

In Fig. 6A, a longitudinal section of the rostellar intermediate cellular territory is shown under light microscopy. Hooks, rostellar cone cellular territory and rostellar pad cellular territory are indicated. The RICT is reach in cells, with bundles of longitudinal and transversal muscle fibers located near the hooks and tegument (Fig. 6B, TEM). In Fig. 6C a bunch of cells is shown located towards

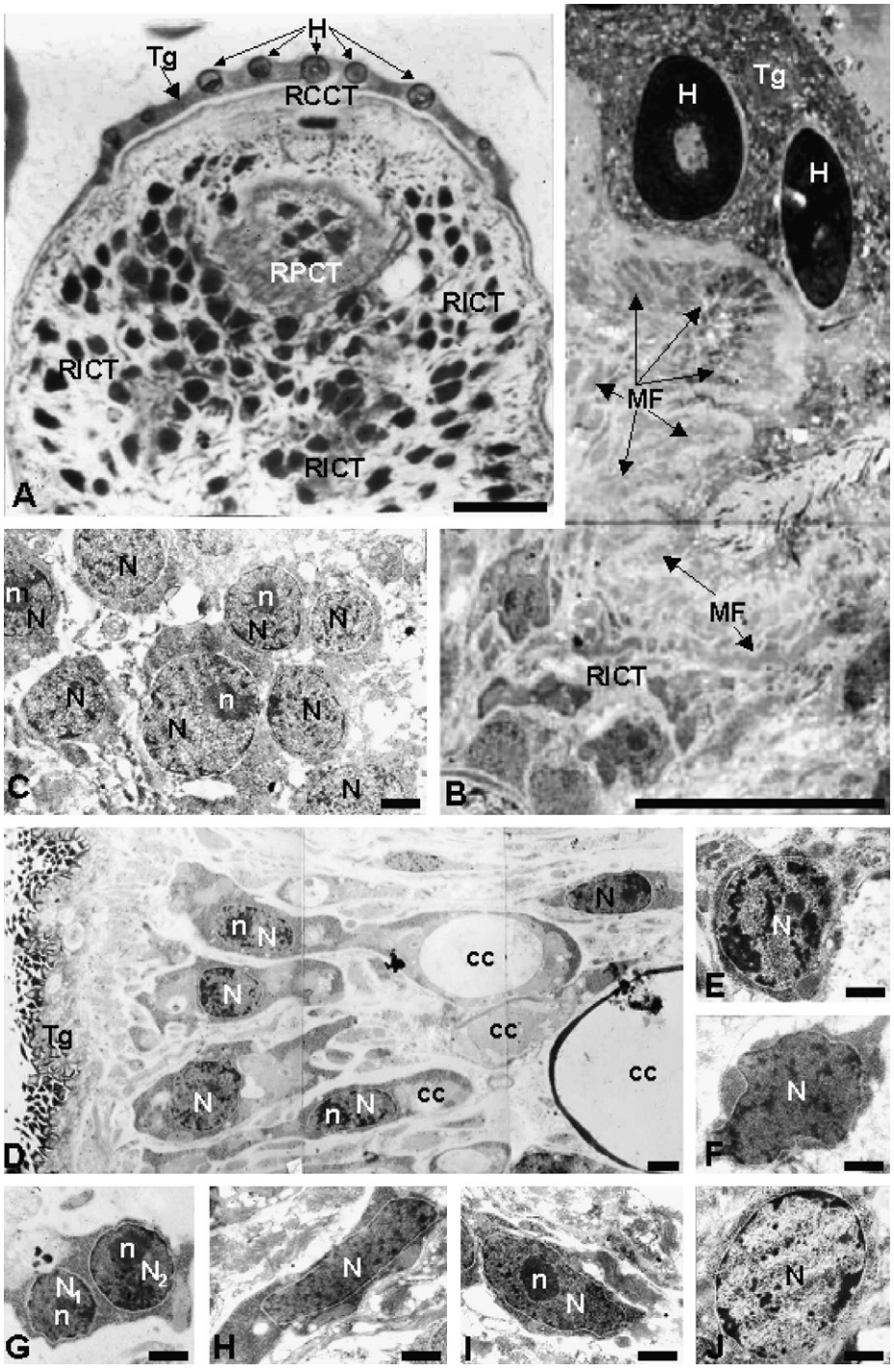

Fig. 6. (A) Electron microscopy of a rostellar intermediate cellular territory (RICT), surrounding the rostellar pad cellular territory (RPCT) and adjacent to the rostellar cone cellular territory (RCCT), longitudinal section. Tg, tegument; H, hooks. (B) Electron microscopy of a rostellar intermediate cellular territory, adjacent to the tegument (Tg) and hooks (H), longitudinal section. MF, muscle fibers. (C–J) Representative cells from the rostellar intermediate cellular territory, transmission electron microscopy sections. Tg, tegument; N, nuclei; n, nucleoli; cc, calcareous corpuscles. Scale bars: (A and B) 10 μm; (C–J) 1 μm.

the center of the RICT, with scarce cytoplasm, spherical nuclei, lax chromatin and a prominent non-segregated nucleolus. These cells seem to be multinuclear and undifferentiated, corresponding to a cellular territory with active DNA synthesis (Galindo et al., 2003). Multinuclear cells were described associated to the *in vitro* larval development of protoscoleces (Morseth, 1967; Casado and Rodriguez-Caabeiro, 1989). Fig. 6D shows a group of elongated cells, with similar morphological features among them, located near the tegument. These cells are involved in the formation of calcareous corpuscles, confirming that this cellular territory produces these mineral structures (also observed in Fig. 5A). Fig. 6E–J shows cells located in the RICT differing in the shape of nuclei as well as in the compaction and pattern of distribution of chromatin, among other features. Nucleolus is observed in cells shown in Fig. 6D, G and I; this structure may not be observed in other cells of the RICT due to the level of the section. Some cells show two nuclei (Fig. 6G)

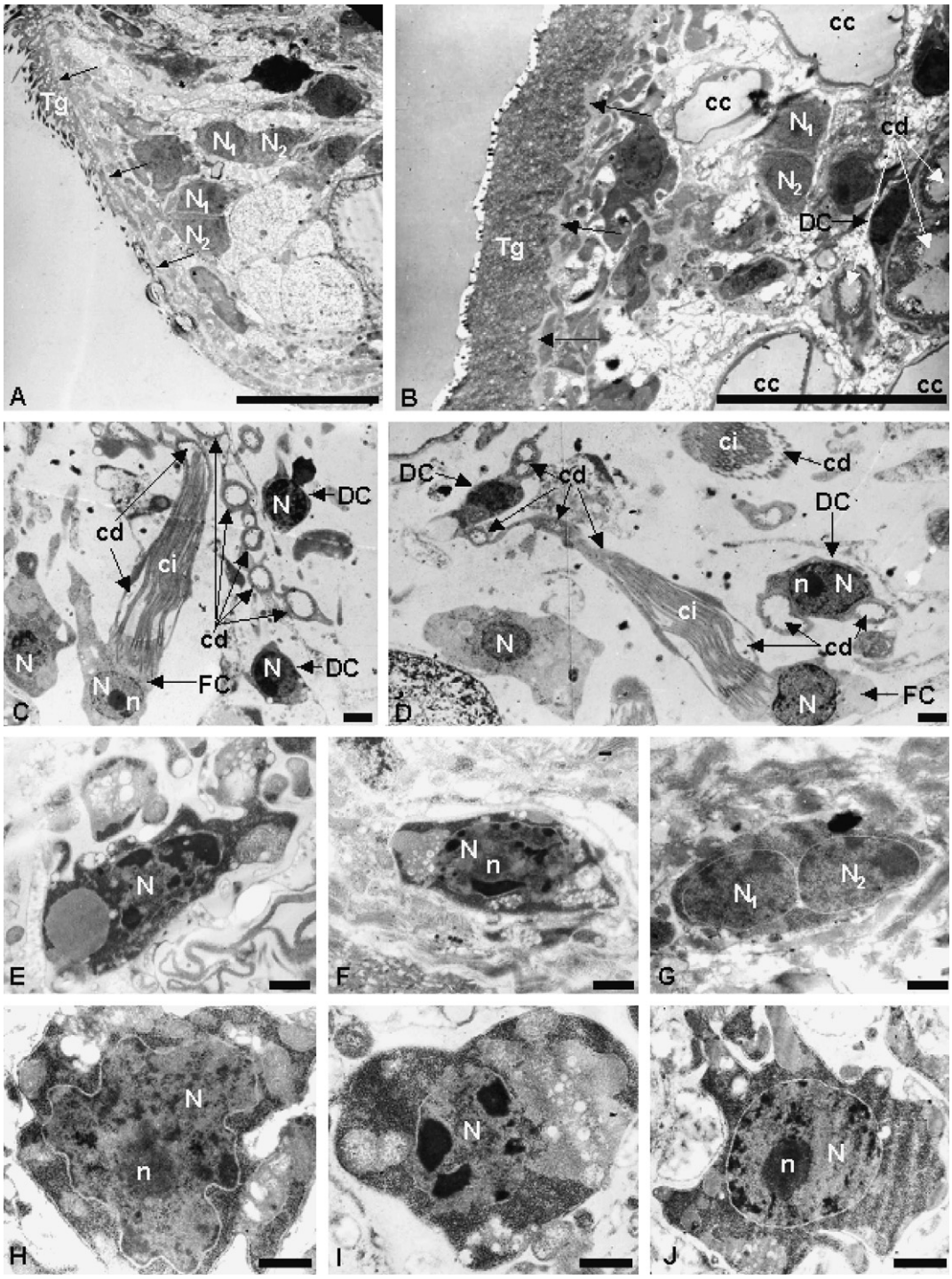

Fig. 7. (A and B) Electron microscopy of longitudinal sections of the body cellular territory, proximal and distal to the neck, respectively. Tg, tegument; N, nuclei; cc, calcareous corpuscles; DC, duct cell; cd, collecting ducts; arrows, basal lamina underlying the tegument of the body. (C–J) Representative cells from the body cellular territory, transmission electron microscopy sections. In (C) and (D), flame (FC) and duct (DC) cells are indicated. ci, bundles of cilia in flame cells; cd, collecting ducts. From (E–J), N, nuclei; n, nucleoli. Scale bars: (A and B) 10 μm; (C–J) 1 μm.

probably as a result of cell division. This is in agreement with the important proliferative activity observed in this cellular territory, as previously described (Galindo et al., 2003).

In Fig. 7 cells and structural features of the protoscolex body are shown. In Fig. 7A and B, tegument shows short microtriches. A basal lamina (arrows) is evident limiting on one side the tegument and in the other side the interior of the body. This is in agreement with results shown in Fig. 1D. Collecting ducts (cd) and calcareous corpuscles are numerous at the analyzed region (Fig. 7B–D). Transversal sections of collecting ducts are evident in Fig. 7C. Binucleate cells (N_1, N_2) are observed (7A, 7B and 7G), again in correspondence with the active DNA synthesis described in this cellular territory (Galindo et al., 2003). Cells (DC) forming collecting ducts are shown in Fig. 7B and D. Flame cells (FC), showing bundles of cilia (ci) in the cytoplasm, spherical nucleus and a prominent non-segregated nucleolus, are typical of this cellular territory (Fig. 7C and D). As previously described (Morseth, 1967), these cells are tightly associated to duct cells. In Fig. 7E–J cells showing different forms with diverse nuclei shape are observed; this diversity is probably connected to particular functions. In 7A, 7B and 7G binucleated cells, probably related to the active DNA synthesis that was described in this cellular territory (Galindo et al., 2003) are shown. The compaction pattern of nuclear chromatin differs in these cells. In some cells a nucleolus is observed.

4. Discussion

Structural and ultrastructural research in *E. granulosus* has been focused mainly on the germinal layer of hydatid cysts, protoscoleces induced to differentiate to hydatid cyst *in vitro* and during protoscolex generation from the germinal layer of hydatid cysts (Morseth, 1967; Bortoletti and Ferretti, 1973, 1978; Colli and Schantz, 1974; Lascano et al., 1975; Heath and Osborn, 1976; Thompson, 1977; Bortoletti and Diaz, 1978; Coltorti and Varela-Diaz, 1978). Careful studies of the neck structure of adult forms (Gustafsson, 1976) and of whole oncospheres (Swiderski, 1981) of *E. granulosus* were also pursued. Some of these studies have focused on specific aspects of their structure (Morseth, 1967; Rogan and Richards, 1987; Herbaut et al., 1988; Casado and Rodriguez-Caabeiro, 1989). The study of the effect of drugs on protoscoleces has provided additional information on some structural aspects of this form of the parasite (Conder et al., 1981; Hemphill and Walker, 2004; Walker et al., 2004).

However, to our knowledge, to date there are no precise descriptions of the cellular territories that underly or define the anatomical parts of the mature, infecting protoscolex. This level of information is of importance in order to localize specific functions in this form of the parasite. The presence of a basal lamina, detected by a specific histochemical reaction and observed under LM and TEM, supports the existence of defined and particular cellular territories. The basal lamina surrounds some cellular territories in the protoscolex, such as RCCT, RPCT and SCT, suggesting that these structures are more differentiated than the RICT and BCT cellular territories, possibly in relation with their specific functions. For example in the rostellar pad and suckers the basal lamina is associated with the attachment of muscle fibers and thus it may be related with contraction. On the contrary, in the tegument the basal lamina may act as in epithelial tissues of vertebrates, to maintain the structure integrity of that structure. In accordance, the first differentiated cells that can be recognized in the nascent protoscolex that are still joined to the germinal layer of the hydatid cyst (other than the hooks) correspond to the sucker cellular territory (Galindo et al., 2002, 2003). Interestingly, the rostellar cone, the rostellar pad and the sucker territories are also separated from the tegument. Thus, cells from these territories are not a structural part of the syncytium which forms the tegument.

Considering our results, it will be now possible to assign production or secretion of specific proteins to a particular cellular territory, or territories of the protoscolex. Thus, antigens 5 and B that were found as part of the interstitial material (Sanchez et al., 1993) or along the interface of the tegumentary syncytium (Jones et al., 1996) may now be assigned to particular cellular territories of protoscoleces.

The presence of different cellular territories in the mature protoscolex is further supported by studies of gene expression in different regions of this form and of the adult form of the parasite. Thus, the homeobox-containing gene EgHbx3 is expressed in the stalk (Martinez et al., 1997), an antioxidant protein is mainly expressed in the tegument, subtegumental and calcareous corpuscles forming cells (Hou et al., 2007), a protein involved in binding of fatty acids is present in the tegument (Esteves et al., 1993) and the expression of a paramyosin gene in adults worms was located in the tegument and subtegumental parenchyma as well as in the suckers (Fu et al., 1999). Contrarily, other genes such as those encoding a non-integrin laminin-binding protein (Zhang et al., 1997), calreticulin (Cabezon et al., 2008) and RAD9 mRNA (Cabrera, 2007) are expressed in all cellular territories of the protoscolex.

Finally, a regionalization of the DNA synthetic activity in the mature protoscolex has been shown (Galindo et al., 2003). Thus, while the rostellar cone and rostellar pad cellular territories do not present cells in DNA synthesis, all other cellular territories show different degrees of DNA replication, the body being the most active. Following these results and those of Gustafsson (1976), it was proposed that the body of the protoscolex is the cellular territory from which growth occurs first followed by strobilation (Galindo et al., 2003). If this is the case, the body of the protoscolex should give origin to the neck of the adult worm. Again, these results and proposals are in agreement with the existence of defined cellular territories in the protoscolex.

Our structural description of the protoscolex and the reported functional data support the proposal of cellular territories with anatomical structures and specific functional activities. We propose that cellular territories in the protoscolex should be used for the unambiguous assignment of specific functions in this parasitic form.

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