Cell Tissue Res (2007) 328:639–650
DOI 10.1007/s00441-007-0385-7

REGULAR ARTICLE

Intra-epithelial spicules in a homosclerophorid sponge

Manuel Maldonado · Ana Riesgo

Received: 22 February 2006 / Accepted: 22 January 2007 / Published online: 6 March 2007

Abstract Attempts to understand the intricacies of biosilicification in sponges are hampered by difficulties in isolating and culturing their sclerocytes, which are specialized cells that wander at low density within the sponge body, and which are considered as being solely responsible for the secretion of siliceous skeletal structures (spicules). By investigating the homosclerophorid *Corticium candelabrum*, traditionally included in the class Demospongiae, we show that two abundant cell types of the epithelia (pinacocytes), in addition to sclerocytes, contain spicules intracellularly. The small size of these intracellular spicules, together with the ultrastructure of their silica layers, indicates that their silicification is unfinished and supports the idea that they are produced "in situ" by the epithelial cells rather than being incorporated from the intercellular mesohyl. The origin of small spicules that also occur (though rarely) within the nucleus of sclerocytes and the cytoplasm of choanocytes is more uncertain. Not only the location, but also the structure of spicules are unconventional in this sponge. Cross-sectioned spicules show a subcircular axial filament externally enveloped by a silica layer, followed by two concentric extra-axial organic layers, each being in turn surrounded by a silica ring. We interpret this structural pattern as the result of a distinctive three-step process, consisting of an initial (axial) silicification wave around the axial filament and two subsequent (extra-axial) silicification waves. These findings indicate that the cellular mechanisms of spicule production vary across sponges and reveal the need for a careful re-examination of the hitherto monophyletic state attributed to biosilicification within the phylum Porifera.

Keywords Biosilicification · Homosclerophorida · Pinacocytes · Sclerocytes · Silica · Spicules · *Corticium candelabrum* (Porifera, Demospongiae)

Introduction

Sponges are sessile filter-feeding animals, their bodies being permeated by numerous aquiferous canals along which ambient water flows transporting dissolved and particulate nutrients, oxygen, excreta, and reproductive products. Their simple anatomical structure comprises an external epithelium of flattened cells (exopinacocytes) and an internal epithelium of both flattened cells (endopinacocytes; lining the aquiferous canals) and non-flattened collar cells (choanocytes; lining the expansions of these canals, viz., the feeding chambers). Sponges also have a collagen-rich inter-epithelial mesenchyme (the mesohyl), which harbors populations of amoeboid cells, including the spicule-secreting sclerocytes.

Because of the silicifying activity of their sclerocytes, sponges, together with diatoms, are major silica producers (Maldonado et al. 2005). Sponges of the class Calcarea (about 5% of all known sponges) produce calcium carbonate spicules, whereas most representatives of the Demospongiae and all representatives of the Hexactinellida (syncytial sponges) classes secrete siliceous spicules. Although little is known about the production of these

This work was supported by grants from the Cellular and Molecular Program (BMC2002-01228) and the Natural Resources Program (CTM2005-05366-MAR) of the Spanish Ministry of Sciences and Education (MEC).

M. Maldonado (✉) · A. Riesgo
Centro de Estudios Avanzados de Blanes (CSIC),
Acceso Cala St. Francesc 14,
17300 Blanes, Spain
e-mail: maldonado@ceab.csic.es

spicules in Hexactinellida, the process in Demospongiae is thought to be reasonably well understood. To date, spicule formation has been described as occurring exclusively in the sponge mesohyl. The process begins intracellularly within a vacuole of the sclerocyte cytoplasm, in which a protein (principally silicatein) filament is produced to act as a scaffold, with the enzymatic polymerization of silicic acid being directed around it (e.g., Simpson 1984; Shimizu et al. 1998; Cha et al. 1999; Weaver and Morse 2003). The sclerocytes of demosponges are present as one or two distinct cell subtypes, i.e., microsclerocytes and/or megasclerocytes, depending on the species. Megasclerocytes are large nucleolated cells, ontogenetically developed from totipotent mesenchymal cells (archeocytes); they secrete large spicules (from approximately 100 μm to several millimeters in length; Simpson 1984; Garrone et al. 1981). Microsclerocytes are smaller anucleolate cells of unclear origin; they secrete small spicules (approxiamately <100 μm; Jørgensen 1944; Garrone et al. 1981; Simpson 1984; Custodio et al. 2002). Microsclerocytes and megasclerocytes also react differentially to the ambient availability of silicic acid, and marked differences have been reported in the number and/or size of the spicules that they produce at different threshold concentrations of silicic acid (Jørgensen 1944; Simpson 1984; Maldonado et al. 1999). Several groups have postulated that, during the secretion of large spicules by megasclerocytes, either the bare axial filament or an early spicule stage may be exocytosed, silicification continuing extracellularly within transient membrane invaginations of one or more sclerocytes (Simpson 1984; Uriz et al. 2000; Müller et al. 2005). However, the way that silicic acid is transported from the ambient seawater through the sponge mesohyl to the sclerocytes for polymerization remains a matter of conjecture.

Ultrastructural investigations conducted on the homosclerophorid *Corticium candelabrum* suggest that spicule deposition in this sponge follows more than one pathway and departs in several aspects from the general pattern known in other siliceous sponges.

Fig. 2 Spicules in exopinacoytes. **a** External epithelium showing two exopinacocytes (*xp*), each containing an intracellular siliceous spicule (*is*). Free intercellular spicules (*es*) occur below the epithelium. Sclerocytes (*sc*) can be seen secreting spicules (*is*) intracellularly deeper in the mesohyl. **b**, **c** Details of spicules within a membrane-bounded vacuole (*mv*) in the exopinacocytes. Note the large nucleolus (*n*) in the exopinacocyte nucleus

Materials and methods

Histological observations by light microscopy were conducted on tissue samples collected monthly from five adult sponges of a local population off the Blanes Coast (2° 48,12′ N, 41° 40,33′ E; Spain, Mediterranean coast) between November 2003 and October 2005, by scuba diving. Fixation for conventional transmission electron microscopy (TEM) and scanning electron microscopy (SEM) was carried out about 1 h after sample collection. Primary fixation for TEM and SEM was performed in 2.5% glutaraldehyde in 0.2 M Millonig's phosphate buffer (MPB) and 0.14 M sodium chloride for 1 h, followed by rinsing with MPB for 40 min. TEM samples were post-fixed in 2% osmium tetroxide in MPB, dehydrated in a graded acetone series, and embedded in Spurr's resin. Ultrathin sections obtained with an Ultracut Reichert-Jung ultramicrotome were mounted on gold grids and stained first with 2% uranyl acetate for 30 min and then with lead citrate for 10 min. TEM observations were conducted with a JEOL 1010 microscope operating at 80 kV and fitted with a Gatan module to obtain digital images. Fixed tissue samples for SEM were dehydrated in a graded alcohol series, placed on metal stubs, dried to the critical point, and coated with a gold layer in a Polaron SC-500. Spicules were acid cleaned prior to dehydration and sputtering. Observations were conducted with a Hitachi S-570 microscope.

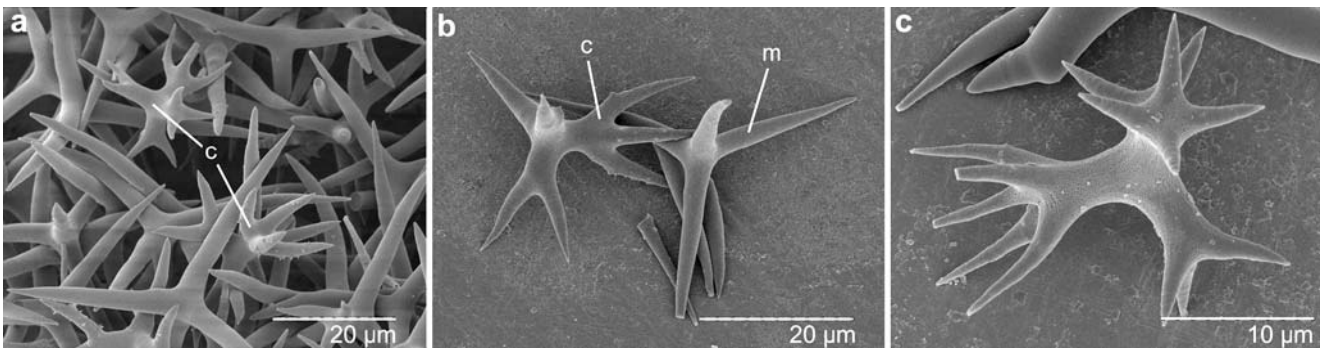
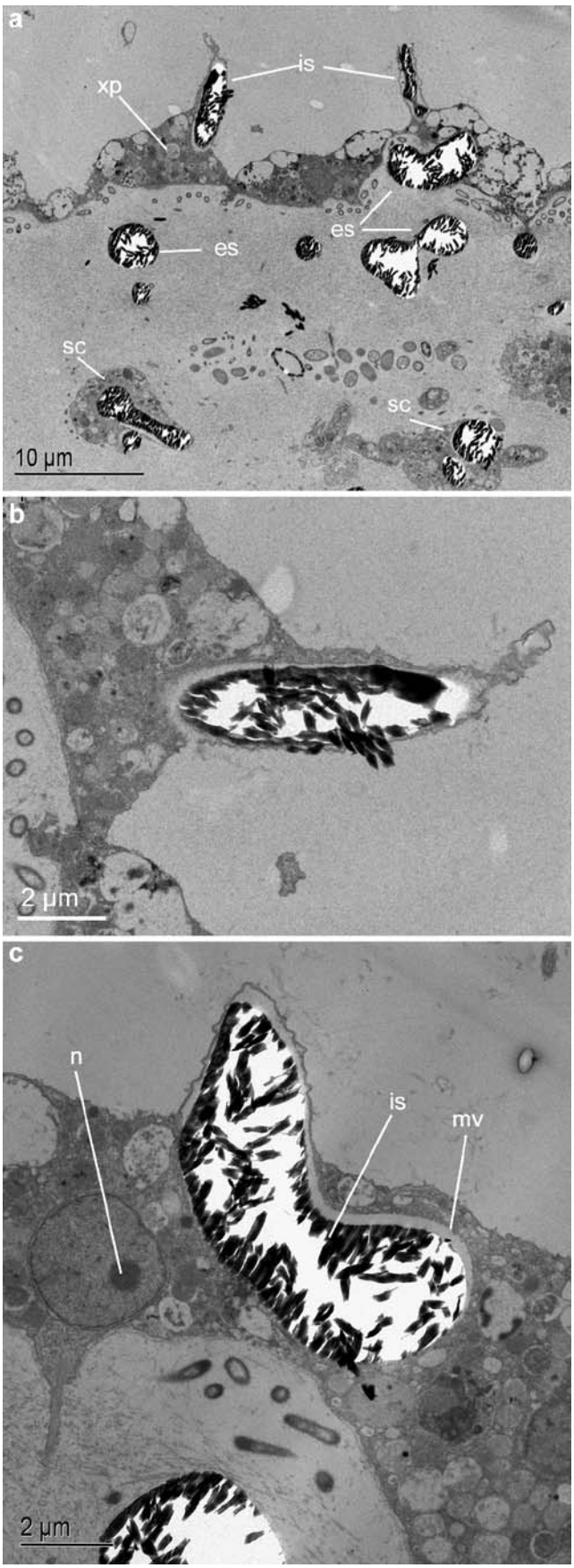


Fig. 1 Spicules of *Corticium candelabrum*. **a** Abundance of candelabra (*c*) relative to other microcalthrop types. **b** Comparative view of a candelabrum (*c*) with two branched rays and a microcalthrop (*m*). **c** Candelabrum with all rays being sub-branched



Results

The demosponge *C. candelabrum* was skeletally characterized by the production of a variety of microcalthrops, i.e., four-rayed spicules with diversely sub-branched rays of 25–100 µm in length (Fig. 1a,b). The four rays in one of the microcalthrop types, known as candelabrum, were all sub-branched and ornamented with microspines (Fig. 1a–c). Microcalthrops appeared scattered throughout the mesohyl, but the candelabra were concentrated below the external epithelium and the aquiferous canals (see also Muricy and Díaz 2002).

The strengthening of delicate epithelia by the accumulation of spicules beneath the tissue is common in sponges (e.g., Simpson 1984). To date, epithelium-reinforcing spicules have been thought to be produced conventionally by sclerocytes, followed by exocytosis to the mesohyl and transportation by either sclerocytes or other mobile cells to their final subepithelial emplacement (Simpson 1984; Custodio et al. 2002). However, we have found that the candelabra of *C. candelabrum* are not subepithelial, but rather "intra-epithelial". These microcalthrops occur within the epithelial cells, both in the exopinacocytes that form the external epithelium and in the endopinacocytes that line the aquiferous canals (Figs. 2, 3, 4). A single spicule per cell is seen intracellularly, within a large membrane-bounded cytoplasmic vacuole (Fig. 3). Although only a few endopinacocytes are seen to contain spicules, most exopinacocytes produce spicules. SEM observations indicate that the candelabra remain mostly intracellularly (Fig. 5a,b) in the exopinaocytes, with just a few spicule rays (possibly accidentally) piercing the pinacocyte cell membrane (Fig. 5c). The handling of sponges by divers during collection and/or the slight tissue shrinkage during fixation and drying to the critical point may have caused some spicules to pierce the plasmalemma of the exopinacocytes in which they are contained.

Free spicules and symbiotic bacteria occur in the mesohyl immediately below the epithelia. These spicules are usually larger than those occurring within the epithelial cells (Figs. 2a, 3a, 4c, 6a,b). Occasionally, small unfinished spicules can be seen in intimate contact with the external side of the pinacocyte membrane (Fig. 7). In these cases, no sclerocyte occurs nearby to account for the recent exocytosis of these immature spicules (Fig. 7). Indeed, sclerocytes have never been found to contact the epithelia. Instead, they consistently occur deeper in the mesohyl, below the bacterial layer that develops at the internal side of the epithelia (Figs. 2a, 6a,b). The small size of both the intra-epithelial spicules and those still in contact with the pinacocyte membrane compared with the large size of the free subepithelial spicules, together with the absence of sclerocytes from the close vicinity of the epithelia (Fig. 7), suggest that the intra-epithelial candelabra have not been

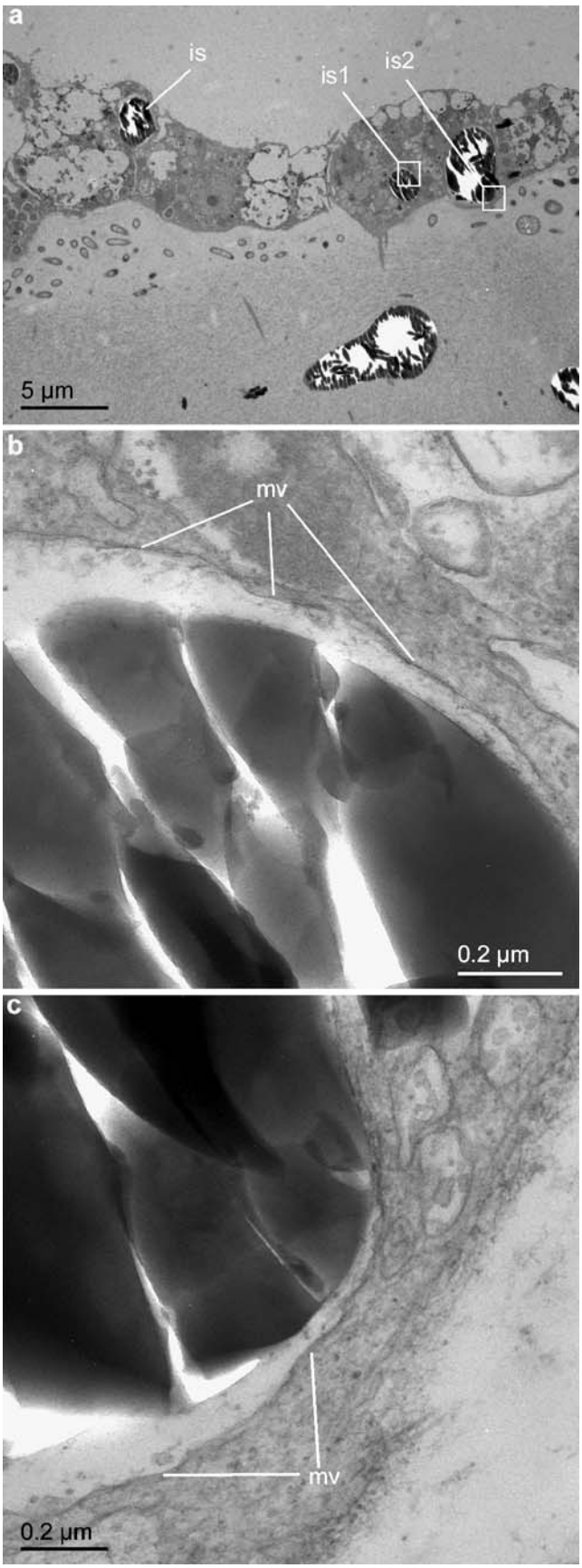


Fig. 3 Spicule in exopinacocytes. **a** General view of the exopinacoderm showing intra-epithelial spicules (*is*, *is1*, *is2*). **b**, **c** Enlarged views of *is1* and *is2* intra-epithelial spicules from **a** showing the relationship between the spicules and the membrane of the vacuole (*mv*) in which are contained

secreted by sclerocytes and engulfed from the adjacent mesohyl by the epithelial cells. Rather, these observations support the idea that candelabra are initially secreted within the epithelia cells and then exocytosed, but still as immature spicules. Large pseudopodia are never found in these pinacocytes (Fig. 7), providing further evidence supporting spicule exocytosis rather than endocytosis. Some images have also revealed that the pinacocyte membrane becomes disorganized at the contact point with the small spicules, producing numerous small vesicles (Fig. 7b). This may be a mechanism to avoid periodical exocytosis of large spicule-containing vacuoles leading to an undesirable increase of membrane surface in the pinacocytes.

Spicules of *C. candelabrum* also seem to have a distinct structure. In cross sections, the axial filament does not have the obvious usual hexagonal or triangular shape seen in most demosponges (Simpson 1984; Weaver and Morse 2003). Instead, in candelabra and other microcalthrops of *C. candelabrum*, both the filament and axial canal appear subcircular (Fig. 8a–e,g). More importantly, once a substantial silica layer has covered the axial filament, extra-axial ring-like filaments appear concentrically, each accumulating a thin layer of silica on the external side (Fig. 8a–e). SEM observations of early-stage spicules that have not yet fully completed this extra-axial silicification reveal an irregular, highly porous surface (Fig. 8f–h). We therefore assume that these peripheral filaments, which are 0.1–0.2 µm thick, participate in the control of extra-axial silicification steps to produce the sub-branching and ornamentation of the spicule surface in a second mineralization phase. Interestingly, when the structure of the small thin spicules associated with the external side of the pinacocyte membrane (Fig. 7b) is compared with that of spicules with thicker rays in later stages of silicification (Fig. 8d,e), it becomes evident that the former are unfinished, since their extra-axial silica deposits are either thin or lacking (Fig. 7b,c). Again, these observations support the idea that the secretion of the candelabra starts intracellularly within the pinacocytes, the candelabra being subsequently exocytosed to complete their extra-axial silicification exracellularly, although still apparently in contact with the pinacocyte membrane (Fig. 7). Of note, no axial canal is observed in sections of rays of fully grown spicules when examined by either TEM or SEM. The axial filament, which in some spicules shows a fuzzy peripheral area (Fig. 8b), appears to be completely "resorbed or degraded" in late-stage spicules, and the axial canal is

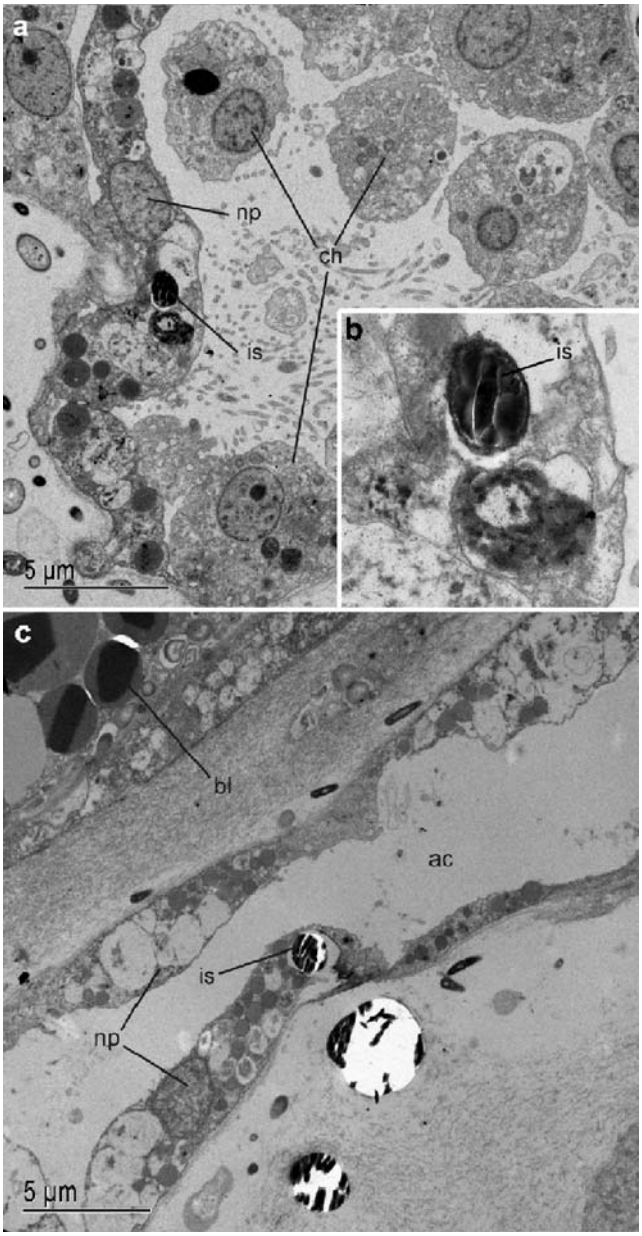


Fig. 4 Spicules in endopinacocytes. **a** Endopinacocyte (*np*) at the entrance of a choanochamber (*ch* choanocytes) containing an intracellular spicule (*is*). **b** Magnification of spicule (*is*) within a vacuole of the endopinacoyte of Fig. 4a. **c** Intracellular spicule (*is*) in one of the endopinacocytes (*np*) that line an aquiferous canal (*ac*). Note the vitellin platelets of an early blastula (*bl*) developing nearby

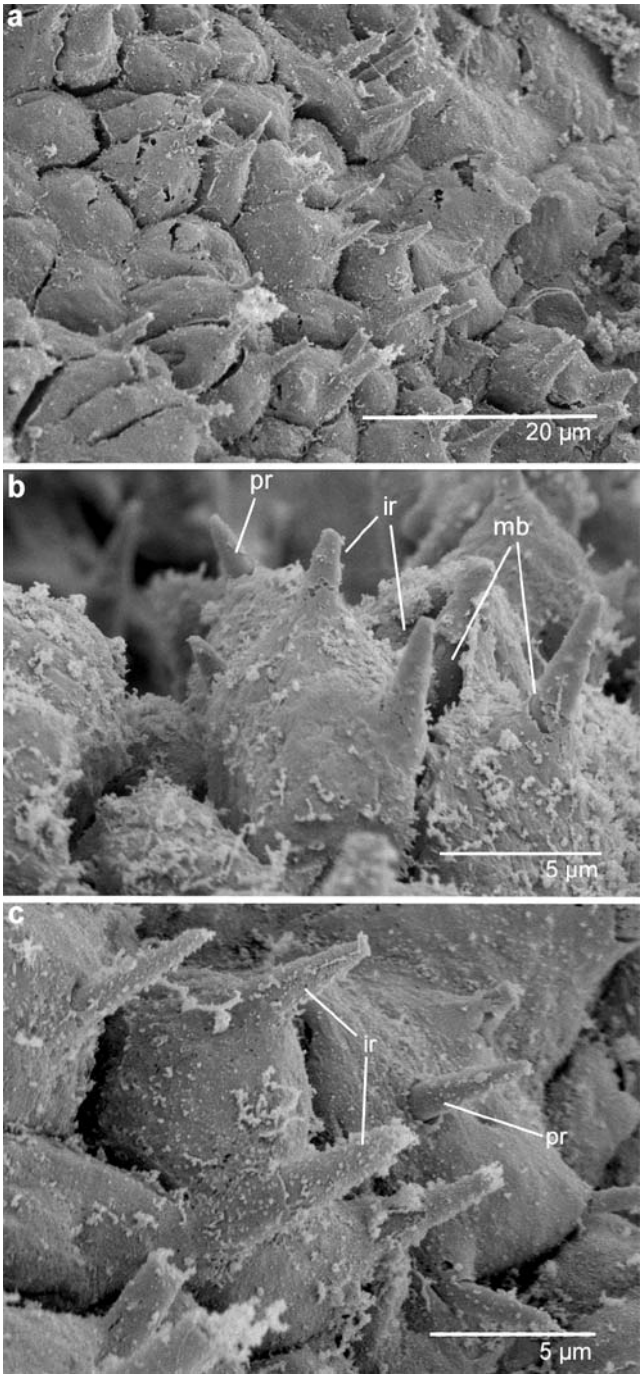


Fig. 5 External views of exopinacocytes. **a** General SEM view of the external surface of the sponge showing that most spicules contained in the cells protrude without piercing the plasmalemma of the exopinacocytes. **b** Detail of a small area of the sponge surface, showing both cells with spicules that remain intracellular, i.e., with rays (*ir*) that protrude but do not pierce the cell membrane (*mb*), and cells with spicules that pierce (possible artifactually) the membrane (*pr*). Note that intracellular spicules are seen at those areas where the cell membrane of the exopinacocytes is broken. **c** Group of exopinacocytes in which one of the intracellular spicule rays (*ir*) has pierced (*pr*) the cell membrane

obliterated by silica deposition (Fig. 8e,f). The mechanisms underlying these processes remain unascertained.

Finally, observations on spicule secretion by the sclerocytes of *C. candelabrum* have also revealed distinctive peculiarities when compared with that of other demosponges. These sclerocytes, like the pinacocytes, are nucleolated cells (Figs. 9a,f, 10a). Although they secrete small (<100 µm) spicules that may be interpreted as microscleres (Fig. 9a–e) intracellularly and release them to

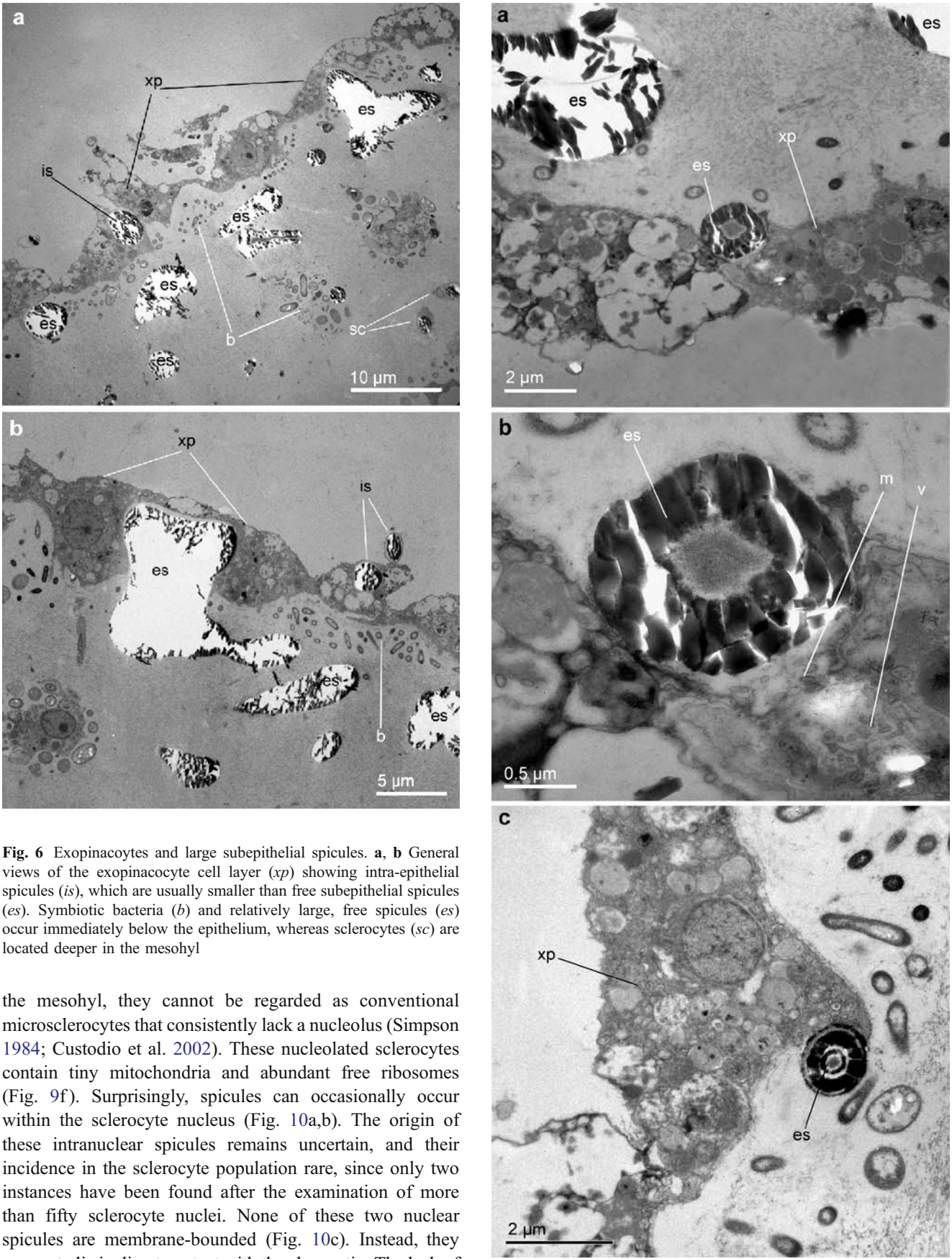


Fig. 6 Exopinacoytes and large subepithelial spicules. **a**, **b** General views of the exopinacocyte cell layer (*xp*) showing intra-epithelial spicules (*is*), which are usually smaller than free subepithelial spicules (*es*). Symbiotic bacteria (*b*) and relatively large, free spicules (*es*) occur immediately below the epithelium, whereas sclerocytes (*sc*) are located deeper in the mesohyl

the mesohyl, they cannot be regarded as conventional microsclerocytes that consistently lack a nucleolus (Simpson 1984; Custodio et al. 2002). These nucleolated sclerocytes contain tiny mitochondria and abundant free ribosomes (Fig. 9f). Surprisingly, spicules can occasionally occur within the sclerocyte nucleus (Fig. 10a,b). The origin of these intranuclear spicules remains uncertain, and their incidence in the sclerocyte population rare, since only two instances have been found after the examination of more than fifty sclerocyte nuclei. None of these two nuclear spicules are membrane-bounded (Fig. 10c). Instead, they appear to lie in direct contact with the chromatin. The lack of

◀ **Fig. 7** Exopinacocytes and small subepithelial spicules. **a** Exopinacocyte (*xp*) in contact with a small unfinished extracellular spicule (*es*) that lacks the rings of extra-axial silicification (cf. Fig. 8). **b** Enlargement of extracellular spicule (*es*) in **a** showing disorganization of the cell membrane at the contact point (*m*) between the spicule and the exopinacocytes and the production of numerous small vesicles (*v*). **c** Exopinacocyte (*xp*) in contact with a small extracellular spicule (*es*) in which the thin outer silica layer appears to be under construction

a membrane around these nuclear spicules excludes the possibility that the spicules have protruded through the nuclear membrane from either the cytoplasm or the intercellular space and suggests an intranuclear origin. The finding that small spicules occasionally occur in a membrane-bounded vacuole within the basal region of the cytoplasm of some choanocytes (Fig. 11a,b) is also intriguing. Again, this feature is uncommon (<3%) among the choanocyte cell population, since only three out of more than 100 examined choanocytes have been observed to contain spicules.

Discussion

Three hypotheses may explain the occurrence of spicules (i.e., candelabra) within sponge epithelial cells: (1) candelabra are secreted intracellularly by the pinacocytes; (2) candelabra are secreted intracellularly by the sclerocytes of the mesohyl, transported below the epithelia, exocytosed to the subepithelial mesohyl, and finally engulfed by the pinacocytes; (3) candelabra-bearing sclerocytes migrate from the mesohyl to enter the epithelia and become epithelial cells.

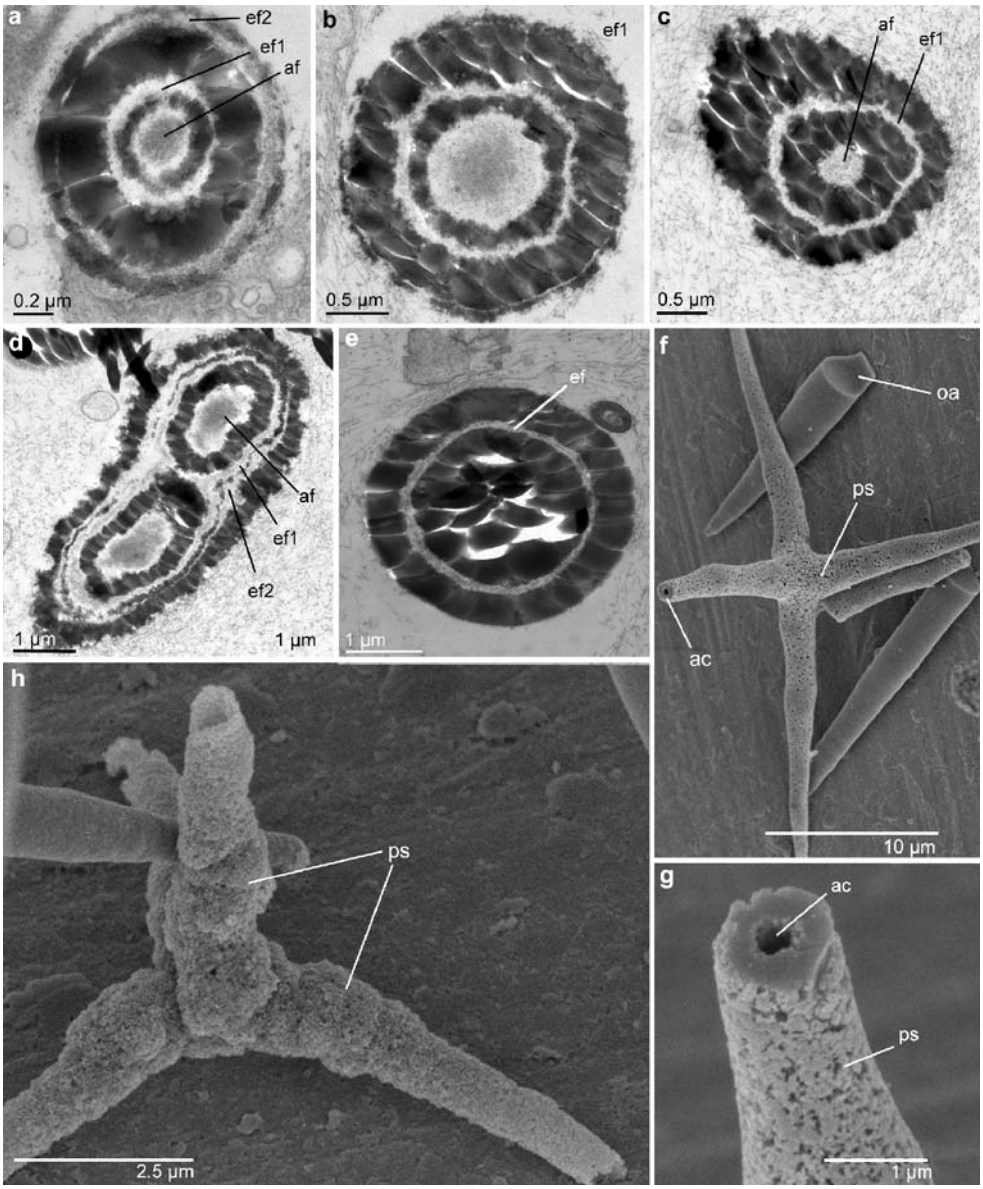


Fig. 8 Growing stages of spicules. **a–c** Cross sections of an early-stage spicule showing the axial filament (*af*), together with one (*ef1*) or two (*ef2*) extra-axial concentric rings that alternate with silica layers. **d** Early-stage spicule sectioned close to the point where a ray bifurcates. Note the axial filament (*af*) plus two additional extra-axial concentric rings (*ef1*, *ef2*) that alternate with thin silica layers. **e** Late-stage spicule showing that the axial filament has disappeared and the canal obliterated with silica. Only an extra-axial filament remains. **f–h** SEM images of early-stage spicules that have not completed the extra-axial silicification showing a highly irregular, porous surface (*ps*) and a wide axial canal (*ac*) at the tip of the rays. Note that the axial canal of the broken ray of a "fully-grown" spicule in **f** is obliterated with silica (*oa*)

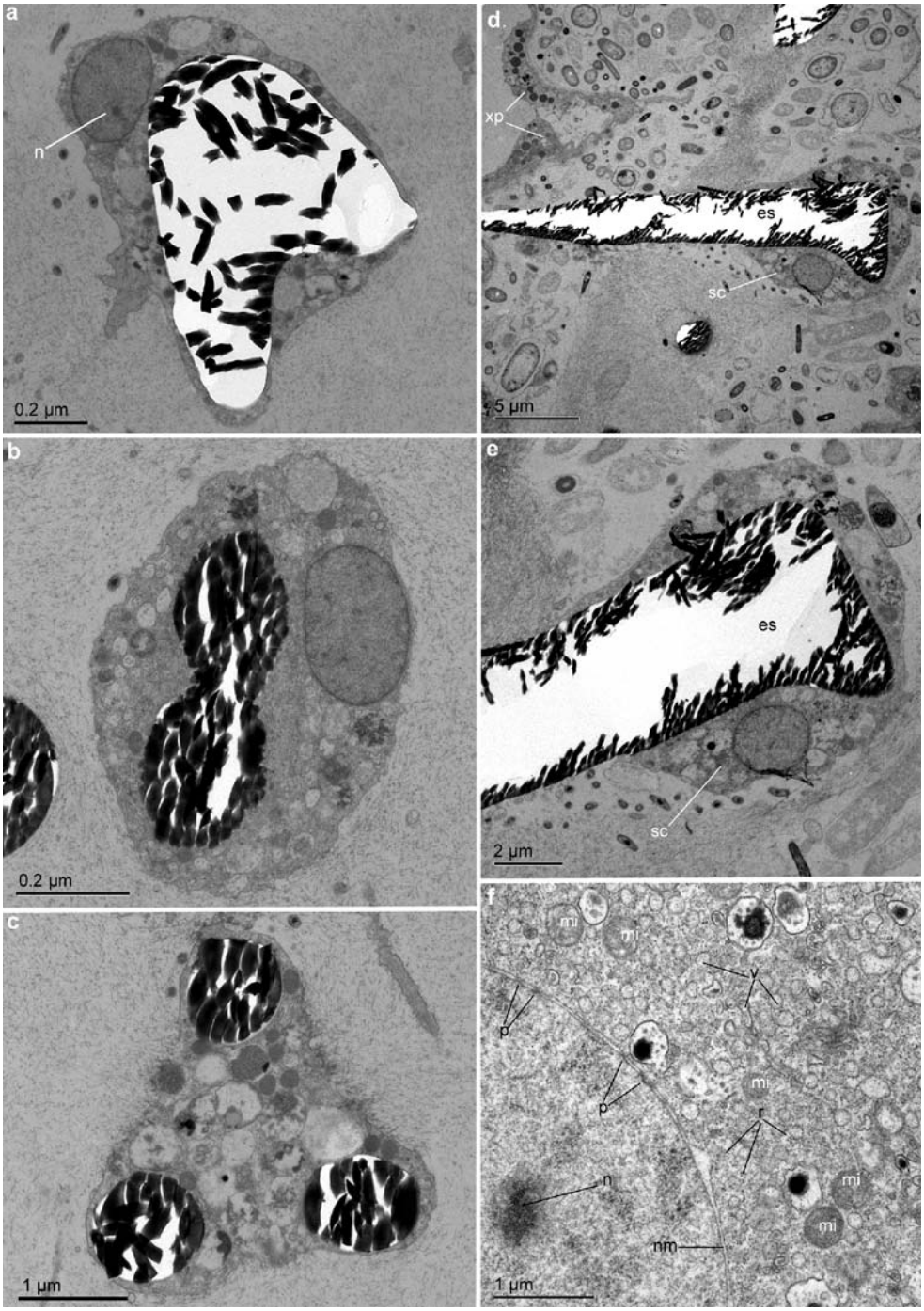


Fig. 9 Spicules in sclerocytes. **a** Nucleolated (*n*) sclerocyte secreting a spicule within a large cytoplasmic vacuole. **b**, **c** Cross sections of microcalthrop-secreting sclerocytes in which two or three spicule rays can be seen within the cytoplasm. **d**, **e** Sclerocyte (*sc*) exocytosing a spicule (*es*) in the vicinity of the exopinacocyte layer (*xp*). **f** Detail of sclerocyte showing the nucleolus (*n*) and the nuclear membrane (*nm*) with nuclear pores (*p*). Note also a portion of perinuclear cytoplasm characterized by abundant free ribosomes (*r*), tiny mitochondria (*mi*), and small electron-clear vacuoles (*v*)

Whereas a sclerocytic origin for the intra-epithelial spicules cannot definitively be discarded from our ultrastructural study (hypotheses 2 and 3 above), an epithelial origin (hypothesis 1) is favored for several reasons. Given that only candelabra (but not the remaining spicule types) are found in the epithelia, a sclerocytic origin for candelabra (hypotheses 2 and 3) would also require selective migration from the mesohyl toward the epithelia of only those sclerocytes containing candelabra. Hypothesis 2 would also involve an additional process of selective

Fig. 10 Spicules in sclerocytes. **a** Nucleolated (*n*) sclerocyte containing simultaneously a spicule (*is*) within a cytoplasmic vacuole and another within the nucleus (*ns*). **b**, **c** Enlargements of **a** showing that the cytoplasmic spicule is surrounded by a membrane (*sm* silicalemma), whereas the spicule (*ns*) within the nucleated (*n*) nucleus is not membrane-bounded. The lack of a membrane around the latter spicule suggests a true intranuclear origin, excluding the possibility that the spicule has penetrated the nuclear membrane (*nm*) from either the cytoplasm or the intercellular space ▶

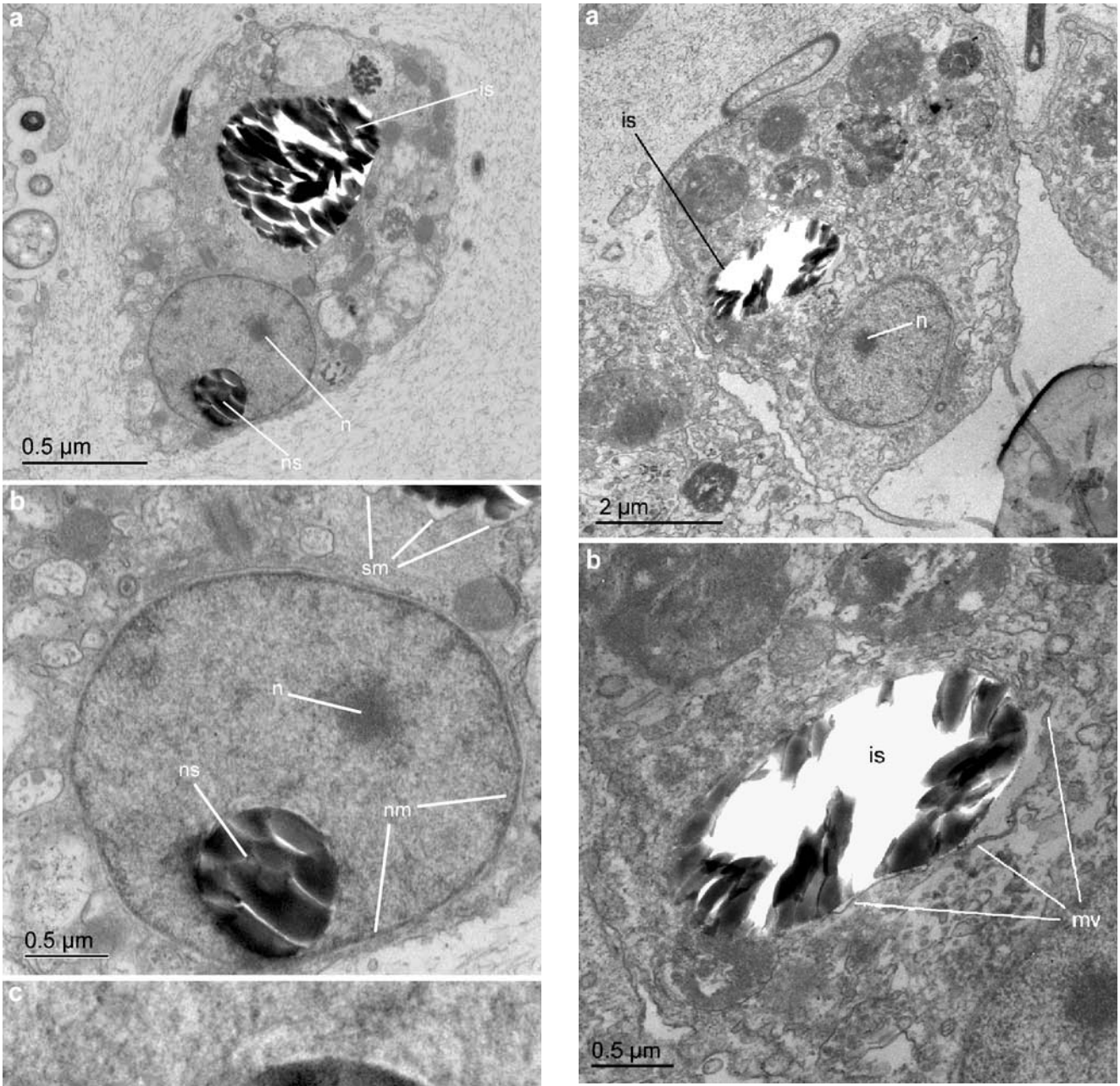


Fig. 11 Spicules in choanocytes. **a** Nucleolated (*n*) choanocyte containing a spicule (*is*) within the cytoplasm. **b** Enlargement of the spicule (*is*) in **a** showing that it lies within a membrane-bounded vacuole (*mv*)

engulfment of only candelabra by pinacocytes. Therefore, the hypotheses postulating a sclerocyte origin are far less parsimonious than the one postulating the "in situ" production by pinacocytes. An additional advantage may have selected for the epithelial secretion of epithelial spicules. Pinacocytes, which are in contact with ambient seawater, have immediate access to silicic acid, bypassing the need for silicic acid transportation through the mesohyl to reach the sclerocytes. More importantly, the hypotheses

for a sclerocyte origin are poorly supported by our observations, since we have found particular accumulations of neither sclerocytes nor small candelabra that could have theoretically been exocytosed by the sclerocytes below the epithelia. Spicules seen within the pinacocytes are usually smaller than those occurring free in the adjacent mesohyl (e.g., Figs. 2a, 3a, 4c, 6), suggesting that their formation starts within these cells, being subsequently exocytosed to complete their silicification intercellularly (Fig. 7). In addition, some TEM images of spicules being exocytosed by sclerocytes in the vicinity of the epithelia have revealed that, upon sclerocyte release, these spicules are too large to be subsequently phagocyted by pinacocytes (Fig. 9d,e).

If epithelial spicule secretion is the case in *C. candelabrum*, it may not be the only sponge with silica-secreting epithelia. This ability may well be present in other sponges. The exopinacocytes of early *S. domuncula* juveniles hatched from asexual buds have been found to produce silicatein (Schröder et al. 2004), the main enzymatic protein in the spicule axial filament. However, because these pinacocytes are derived ontogenetically from the totipotent mesohyl sponge cells that make the buds (Connes 1977), and as such could well comprise dedifferentiated sclerocytes, the true epithelial nature of these silicatein-producing exopinacocytes is debatable. Similarly, intra-epithelial secretion of spicules should not be confused with intracortical production of spicules. The latter one occurs within the microsclerocytes incorporated into the thin intermediate mesohyl layer sandwiched between the two pinacocyte monolayers that limit the thick cortex of some demosponges (e.g., astrophorids).

The extra-axial mineralization phase postulated for *C. candelabrum* from our structural observations is congruent with recent immunolabeling experiments in the demosponge *Suberites domuncula* indicating that silicatein molecules occur free in the mesohyl matrix of the sponge and are apparently able to self-organize into strings at the surface of "unfinished" spicules exocytosed by the sclerocytes (Müller et al. 2006). These silicatein strings are believed to direct enzymatically the final steps of extracellular silicification (Müller et al. 2006). This discovery and our findings suggest the extra-axial control, albeit still poorly understood, of late-stage silicification, which appears to occur mostly extracellularly. All sections of *C. candelabrum* microcalthrops in which the extra-axial silicification pattern was visible corresponded to spicules previously exocytosed to the mesohyl.

Until recently, the way that silica deposition was controlled beyond the initial stages of spicule formation and once the axial filament had become entirely surrounded by silica was unclear (Weaver and Morse 2003). We postulate that the extra-axial control of spicule deposition may be a more common process among demosponges than traditionally thought. Of note, micrographs published in 1981 (Garrone et al. 1981) illustrating the axial filament of HF-treated cross-sectioned spicules of the haplosclerid *Haliclona* also show the presence of an extra-axial ring-like filament similar to that reported here for *C. candelabrum*. At that time, no explanation was provided for the biological significance or function of such a structure, which was described as a "ring of organic matter". The extra-axial silicification steps may ultimately account for differences in the degree of either silica hydration (Schwab and Shore 1971; Weaver et al. 2003) or incorporation of organic matter (Bütschli 1901) between concentric silica layers, which have long been suspected as causing the stratifications seen in the cross sections of demosponge spicules. Likewise, the extra-axial control of spicule growth may account for the accretive structure found in hexactinellid spicules, in which concentric silica layers alternate with thin (nanometer-thick) organic layers of an unknown nature (Lévi et al. 1989; Aizenberg et al. 2005).

Whether the sclerocyte cell line and the putative silica-secreting pinacocytes are related ontogenetically in this sponge remains unclear. It seems unlikely, though not impossible, that pinacocytes have derived from sclerocytes. To postulate such an origin, one would also have to assume a remarkable ontogenetic transformation, given that sclerocytes constitute an amoeboid unciliated cell line, whereas all the endopinacocytes and some exopinacocytes of *C. candelabrum* are monociliated flat cells (Fig. 12a–c) joined by interdigitated junctions (not shown). Likewise, the silica-secreting cells of the pinacoderms of *C. candelabrum* are unlikely to be sclerocytes migrated from the mesohyl to the pinacoderms (hypothesis 3). Given that the number of spicule-bearing exopinacocytes is high (e.g., Fig. 5a), such a migration should be an obvious process, but we have found no evidence for it. Furthermore, no reports on homosclerophorids have been presented that would foster such a suspicion. According to the literature, the reverse process, i.e., the migration of cells from the pinacoderms and the choanoderm to transdifferentiate into mesohyl cells, seems to be more common in demosponges (e.g., Simpson 1984). The few available data on homosclerophorid development support the belief that pinacocytes do not arise from mesohyl cells but rather do so directly from a monociliated cell population of the larval epithelium (for a review, see Maldonado 2004). Thus, if there is a relationship between the sclerocytes and the pinacocytes, it seems more probable that the pinacocytes give rise to sclerocytes, as is known to be the case of the calcite-secreting sclerocytes of calcareous sponges (Ledger 1976; Simpson 1984). To date, the only reliable evidence regarding the origin of sclerocytes in demosponges concerns their megasclerocytes, which seem to be derived from totipotent archeocytes (Garrone et al. 1981; Simpson 1984).

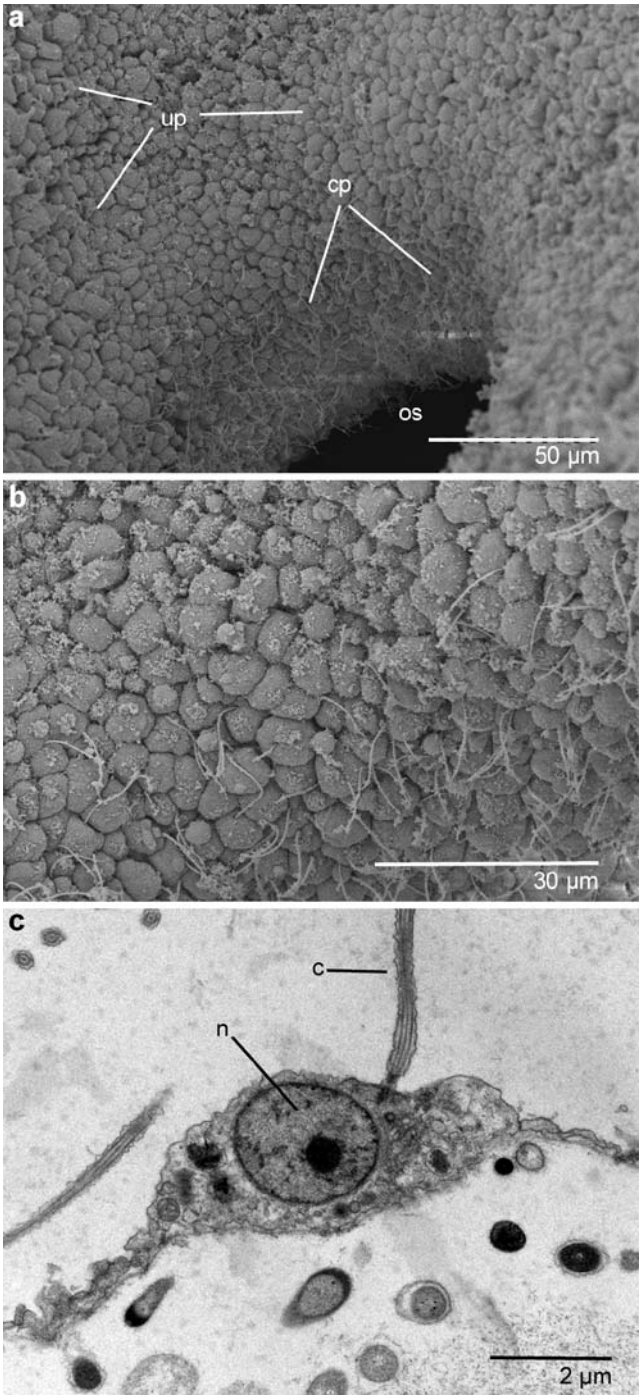


Fig. 12 Pinacocytes. **a**, **b** SEM images of the sponge surface showing an exhalant orifice or ostiole (*os*). Note that the pinacocytes that build the external epithelium (exopinacocytes) of the sponge are unciliated (*up*), whereas those that line the inhalant aquiferous canal (endopinacocytes) are monociliated (*cp*), with the cilia directed toward the canal lumen. **c** TEM section of an endopinacocyte showing a nucleolated (*n*) nucleus and a cilium (*c*) with its basal body

Although never demonstrated, microsclerocytes in some species have been suggested to arise from pinacocytes (Minchin 1909; Garrone et al. 1981; Simpson 1984; Schröder et al. 2004). Whereas this might be the case in *C. candelabrum*, there is no firm evidence to prove it. If we finally accept that pinacocytes are responsible for candelabra production, these cells might also be regarded as equivalent to microsclerocytes, because candelabra are small spicules that may well be interpreted as microscleres. However, unlike pinacocytes in most demosponges, and in agreement with pinacocytes in calcareous sponges, the pinacocytes of *C. candelabrum* are nucleolated cells (Figs. 2c, 3a), a feature that suggests they are more closely allied to the megasclerocyte line of demosponges. In addition, whether the sclerocytes of *C. candelabrum* are equivalent to either megasclerocytes or microsclerocytes is unsettled. In the absence of further evidence, the origin of the spicules within the nucleus of these atypical sclerocytes remains uncertain. However, these cells may have the ability to secrete spicules intranuclearly, a possibility that needs further investigation. The only related precedent in the literature is a report of the occurrence of tiny silica crystals of paracrystalline substructure (Garrone 1969), though not spicules of amorphous silica, with an unknown function within the sclerocyte nucleus of two fresh-water demosponges (Imsiecke and Müller 1995). The occurrence of small spicules within a membrane-bounded vacuole in the proximal cytoplasm of some (<3%) choanocytes is also intriguing. Our observations cannot resolve the origin of these spicules, which could have been either actively phagocytosed by the choanocytes or secreted by the choanocytes themselves (which may be derived from transdifferentiated sclerocytes).

All in all, our findings point to the need to reconsider spicule production in sponges. Silicification, it seems, is an even more complex process than traditionally recognized, involving a diversity of cellular mechanisms (i.e., occurring in sclerocytes and pinacocytes extracellularly, intracytoplasmically, and possibly intranuclearly) that could not have been anticipated a few years ago. This new perspective also revives the hypothesis that the ability to produce silica spicules may have evolved independently in more than one cell line and/or sponge group, an idea that should now be examined afresh.

For most of the 20th century, the microcalthrops of homosclerophorids were postulated to be the ancestral spicule type from which the remaining spicules of other siliceous sponges derived (Dendy 1912), a hypothesis that has been neither validated nor refuted to date. A recent molecular approach based on mitochondrial DNA suggests that the homosclerophorids are demosponges that have retained ancestral features lost in other demosponge groups (Wang and Lavrov 2007), whereas a recent ribosomal RNA study indicates that the homosclerophorids are so distinct that they should be set apart from the remaining demosponges, making a new clade within the phylum (Borchiellini et al. 2004). Our cytological findings regarding the secretion of

spicules in *C. candelabrum* clearly support the distinctive nature of the homosclerophorids, which can also be distinguished from the remaining demosponges by having a well-developed basement membrane under the choanoderm and/or the pinacoderms (Boute et al. 1996), sperm cells with a C-shaped acrosome (Baccetti et al. 1986; Riesgo et al. 2007), and a characteristic embryogenesis leading to the formation of a unique cinctogastrula larva (Boury-Esnault et al. 2003; Maldonado 2004). This body of available information, including our results herein, strongly suggests that the homosclerophorid sponges are a distinct group in which the ability for silicification may have evolved independently from the other demosponges.

Acknowledgements The authors thank Almudena García and Nuria Cortadellas (Electron Microscopy Unit, University of Barcelona) for their valuable help in processing TEM samples and three anonymous reviewers for their constructive comments.

References

Aizenberg J, Weaver JC, Thanawala MS, Sundar VC, Morse DE, Fratzl P (2005) Skeleton of *Euplectella* sp.: structural hierarchy from nanoscale to the macroscale. Science 309:275–278

Baccetti B, Gaino E, Sarà M (1986) A sponge with acrosome: *Oscarella lobularis*. J Ultrastruct Mol Struct Res 94:195–198

Borchiellini C, Chombard C, Manuel M, Alivon E, Vacelet J, Boury-Esnault N (2004) Molecular phylogeny of Demospongiae: implications for classification and scenarios of character evolution. Mol Phylogenet Evol 32:823–837

Boury-Esnault N, Ereskovsky A, Bézac C, Tokina D (2003) Larval development in the Homoscleromorpha (Porifera ; Demospongiae). Invert Biol 122:187–202

Boute N, Exposito JY, Boury-Esnault N, Vacelet J, Noro N, Miyazaki K, Yoshizato K, Garrone R (1996) Type IV collagen in sponges, the missing link in basement membrane ubiquity. Biol Cell 88:37–44

Bütschli O (1901) Einige Beobachtungen über Kiesel- und Kalknadeln von Spongien. Z Wissensch Zool 69:235–286

Cha JN, Shimizu K, Zhou Y, Christiansen SC, Chmelka BF, Stucky GD, Morse DE (1999) Silicatein filaments and subunits from a marine sponge direct the polymerization of silica and silicones in vitro. Proc Natl Acad Sci USA 96:361–365

Connes R (1977) Contribution à l'étude de la gemmulogenèse chez la Démosponge marine *Suberites domuncula* (Olivi) Nardo. Archs Zool Exp Gén 118:391–407

Custodio MR, Hajdu E, Muricy G (2002) In vivo study of microsclere formation in sponges of the genus *Mycale* (Demospongiae, Poecilosclerida). Zoomorphology 121:203–211

Dendy A (1912) The tetraxonid sponge spicule: a study in evolution. Acta Zool (Stockh) 2:95–152

Garrone R (1969) Une formation paracristalline d'ARN intranucléaire dans les choanocytes de l'Eponge *Haliclona rosea* O.S. (Démosponge, Haplosclérida). C R Acad Sci Paris 269:2219–2221

Garrone R, Simpson TL, Pottu-Boumendil J (1981) Ultrastructure and deposition of silica in sponges. In: Simpson TL, Volcani BE (eds) Silicon and siliceous structures in biological systems. Springer, New York, pp 495–525

Imsiecke G, Müller WE (1995) Unusual presence and intracellular storage of silica crystals in the freshwater sponges *Ephydatia muelleri* and *Spongilla lacustris* (Porifera: Spongillidae). Cell Mol Biol 41:827–832

Jørgensen CB (1944) On the spicule formation of *Spongilla lacustris* (L). I. The dependance of the spicule formation on the content of dissolved and solid silicic acid of the medium. D Klg Danske Vidensk Selskab Biol Medd 19:1–45

Ledger PW (1976) Aspects of the secretion and structure of calcareous sponge spicules. PhD thesis. University College, N. Wales

Lévi C, Barton JL, Guillemet C, Le Bras E, Lehuede P (1989) A remarkably strong natural glassy rod: the anchoring spicule of the monorhaphis sponge. J Mat Sci Lett 8:337–339

Maldonado M (2004) Choanoflagellates, choanocytes, and animal multicellularity. Invert Biol 123:231–242

Maldonado M, Carmona MC, Uriz MJ, Cruzado A (1999) Decline in Mesozoic reef-building sponges explained by silicon limitation. Nature 401:785–788

Maldonado M, Carmona MC, Velásquez Z, Puig A, Cruzado A, López A, Young CM (2005) Siliceous sponges as a silicon sink: an overlooked aspect of the benthopelagic coupling in the marine silicon cycle. Limnol Oceanogr 50:799–809

Minchin EA (1909) Sponge spicules. A summary of present knowledge. Ergeb Fortschr Zool 2:171–274

Müller WEG, Rothenberger M, Boreiko A, Tremel W, Reiber A, Schröder HC (2005) Formation of siliceous spicules in the marine demosponge *Suberites domuncula*. Cell Tissue Res 321:285–297

Müller WEG, Belikov SI, Tremel W, Perry CC, Gieskes WWC, Boreiko A, Schröder HC (2006) Siliceous spicules in marine demosponges (example *Suberites domuncula*. Micron 37:107–120

Muricy G, Díaz MC (2002) Order Homosclerophorida Dendy, 1905, Family Plakinidae Schulze, 1880. In: Hooper JNA, Van Soest RWM (eds) Systema Porifera. A guide to the classification of sponges. Kluwer Academic/Plenum, New York, pp 71–82

Riesgo A, Maldonado M, Durfort, M (2007) Dynamics of gametogenesis, embryogenesis, and larval release in a Mediterranean homosclerophorid demosponge. J Mar Freshwater Res (in press)

Schröder HC, Perovic-Ottstadt S, Wiens M, Batel R, Müller IM, Müller WEG (2004) Differentiation capacity of epithelial cells in the sponge *Suberites domuncula*. Cell Tissue Res 316:271–280

Schwab DW, Shore RE (1971) Mechanisms of internal stratification of siliceous sponge spicules. Nature 232:501–502

Shimizu K, Cha JN, Stucky GD, Morse DE (1998) Silicatein alpha: cathepsin L-like protein in sponge biosilica. Proc Natl Acad Sci USA 95:6234–6238

Simpson TL (1984) The cell biology of sponges. Springer, New York

Uriz MJ, Turon X, Becerro MA (2000) Silica deposition in Demosponges: spiculogenesis in *Crambe crambe*. Cell Tissue Res 301:299–309

Wang X, Lavrov DV (2007) Mitochondrial genome of the homoscleromorph *Oscarella carmela* (Porifera, Demospongiae) reveals unexpected complexity in the common ancestor of sponges and other animals. Mol Biol Evol (in press)

Weaver JC, Morse DE (2003) Molecular biology of demosponge axial filaments and their roles in biosilicification. Micro Res Tech 62:356–367

Weaver JC, Pietrasanta LI, Hedin N, Chmelka BF, Hansma PK, Morse DE (2003) Nanostructural features of demosponge biosilica. J Struct Biol 144:271–281