Silica Deposition in Demosponges

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1 Introduction

Demosponges are the most widespread class of the phylum Porifera. They secrete siliceous (spicules) and proteinaceous (fibers) elements, which constitute the skeleton that shapes the sponge growth and allow cell organization and establishment of the aquiferous system through which the surrounding water accesses the internal cells.

The siliceous spicules of demosponges display very different shapes and sizes, ranging from micrometers to millimeters. They are widespread in the sponge mesohyl or arranged in bundles, tracts or networks tending to form three-dimensional structures as the sponge grows massive, erect or branching (Fig. 1). Spongin, a collagen-like protein, accompanies the siliceous skeletons to a variable extent and may even embody the spicules totally (Fig. 2).

The high diversity of spicule shapes was early shown by sponge paleontologists (Hinde and Holmes 1892) and taxonomists and, more recently, has been illustrated by scanning electron microscope images (e.g., Hartman 1981; Simpson 1984) and drawings (Wiedenmayer 1977; Boury-Esnault and Rützler 1997). In short, spicules are conventionally divided into microscleres and megascleres (Fig. 3) according to their shape, size or skeletal function (but see Uriz et al. 2003). Demosponge megascleres show one (monaxons) or four (tetraxons) symmetry axes (Fig. 4). Yet instances of incipient polyaxonid symmetry have been reported in some apparently monaxonid spicules (Rützler and Smith 1993; Uriz and Maldonado 1995), which supports a relationship between the subclasses Tetractinomorpha (with tetraxon spicules) and Ceratinomorpha (with monaxon spicules; Lévi 1973) within demosponges. Microscleres can also range from monaxons to polyaxons (star-like forms; Fig. 5). Hypersilified representatives of both spicule categories, which are named desmas, are present in several genera belonging to different orders of demosponges. Moreover, some genera and families of desma-bearing sponges are conventionally

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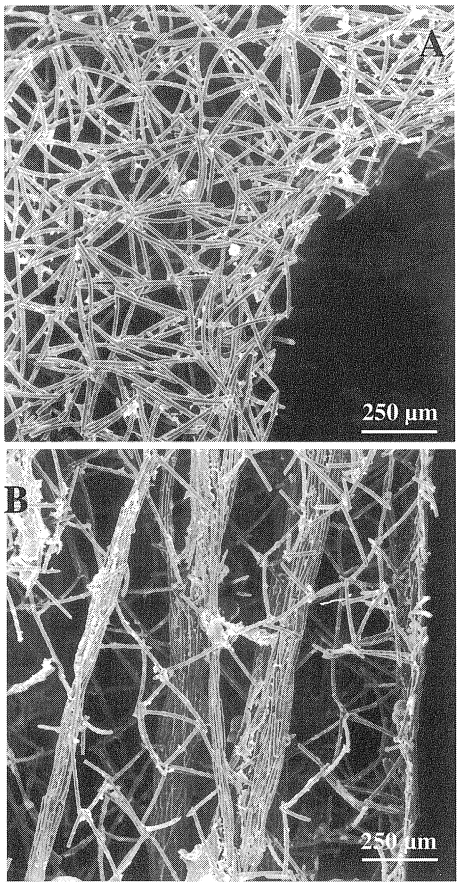

Fig. 1A,B. *Haliclona submonilifera* (Uriz 1988). A Spicule network at the sponge surface. B Combination of spicule tracks and uni-spiculated networks at the sponge choanosome. (Modified from Uriz 1988)

included in the "lithistids", a group with no phylogenetic meaning (Pisera and Lévi 2002).

Megascleres and microscleres appear to play several functions in sponges. Megascleres have a clear supportive function (Uriz et al. 2003), whereas the role of microscleres is difficult to ascertain (Fig. 6). Only the anisocheloid microscleres of the carnivorous sponge *Abestopluma hypogea* (Vacelet and Boury-Esnault 1996), have been shown experimentally to play a role in capturing micropreys (Vacelet and Boury-Esnault 1995).

The polymerization of the silicic acid around the axial filament to build a sponge spicule was reviewed ca. 20 years ago (Garrone et al. 1981; Simpson 1984). However, our knowledge of this process has currently improved. Demosponge spicules usually have a central core, the axial canal (but see Rützler and Macyntyre 1978), which in intact forms is occupied by an organic filament (the

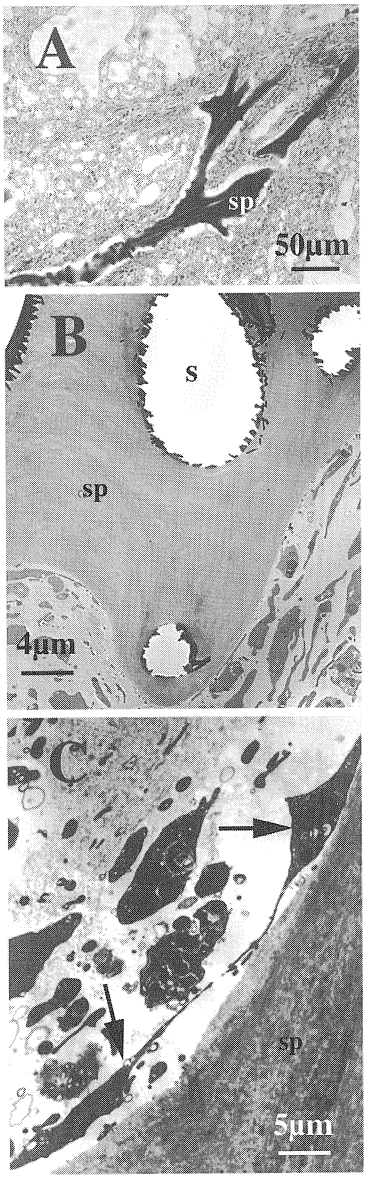

Fig. 2A–C. Instances of spongin surrounding spicules in *Crambe crambe*. A Light microscope image of a plumose spicule tract completely surrounded by spongin (sp). B TEM image of a transversal section of a spicule bundle. See the empty rounded spaces, which correspond to the spicules (*s*) and the fibrillar aspect of the surrounding spongin (*sp*). C TEM image of the spongocytes (*arrows*) that secrete the perispicular spongine (*s*). (A, B modified after Uriz et al. 2000)

axial filament; Fig. 7). A clear relationship between branching axial filaments and polyaxonid spicules has been shown in TEM studies of three-dimensional carbon replicas (Drum 1968), in SEM observation of corrosion casts of mature spicules (Rützler and Muzik 1993; Rützler and Smith 1993) and in SEM images

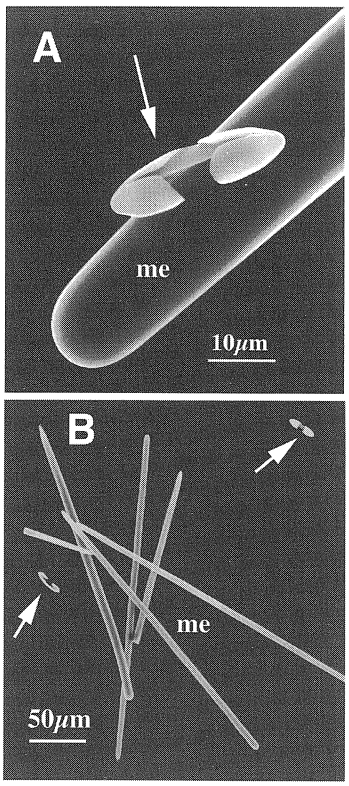

Fig. 3A,B. Scanning electron micrographs showing the comparative size of typical megascleres and microscleres from the same species (*Esperiopsis lesliei*, Uriz 1988). *me* Megasclere (style), *arrows* indicate microsclera (*palmate isochelae*). (Modified after Uriz 1988)

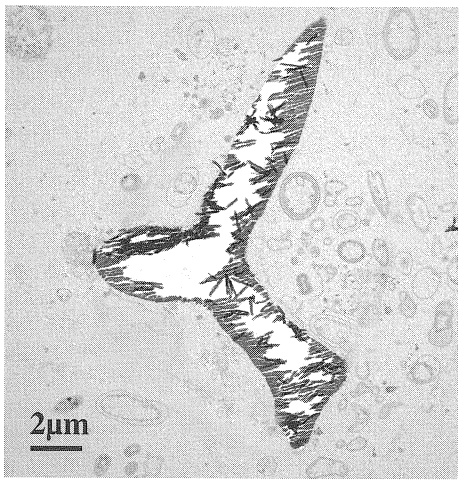

Fig. 4. Transmission electron micrograph of three actines of a tetraxon spicule of *Corticium candelabrum*

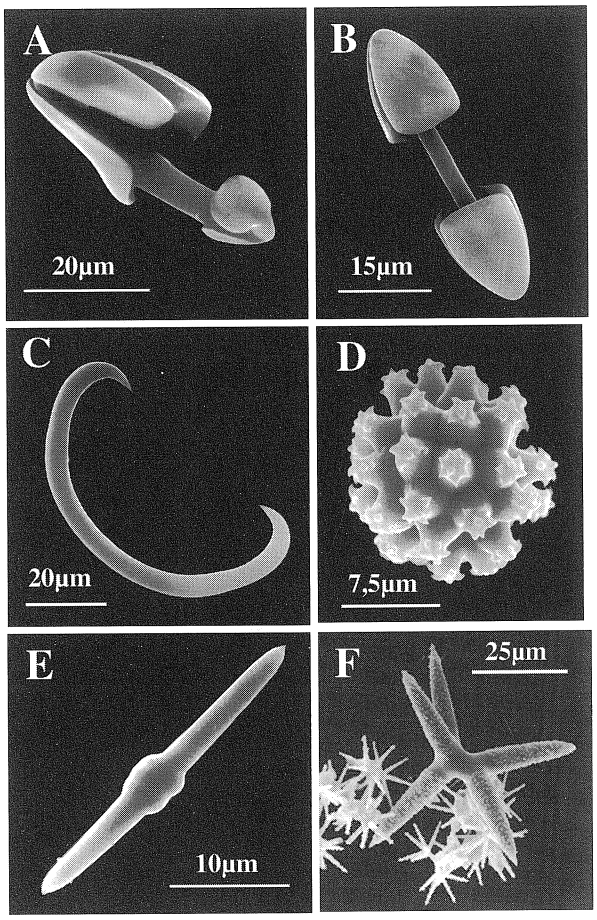

Fig. 5A–F. Various shapes of microsclere. A–C, E Monaxons: A anisochela, B palmate isochela, C sigma, E microxea. D, F Polyaxons: D spheraster, F metaster and spirasters. (A–C Modified after Uriz 1988; D, F modified after Boury-Esnault et al. 1994)

of young spicules (Uriz and Maldonado 1995). Although a relevant role of the axial filament in determining the spicule symmetry was early proposed (e.g., Reiswig 1971), and its organic (proteinaceous) nature was reported long ago (e.g., Schwab and Shore 1971a; Shore 1972), only the molecular studies performed at the end of the 1990s (Shimizu et al. 1998; Cha et al. 1999; Kraso et al. 2000), revealed the true nature and function of this filament in governing silica deposition. Notwithstanding, other related topics such as the role of the membranes, which surround the growing spicules, in determining the final spicule shape or how organic molecules, which do not constitute a discrete filament induce silica polymerization (Pisera 2003), remain poorly understood.

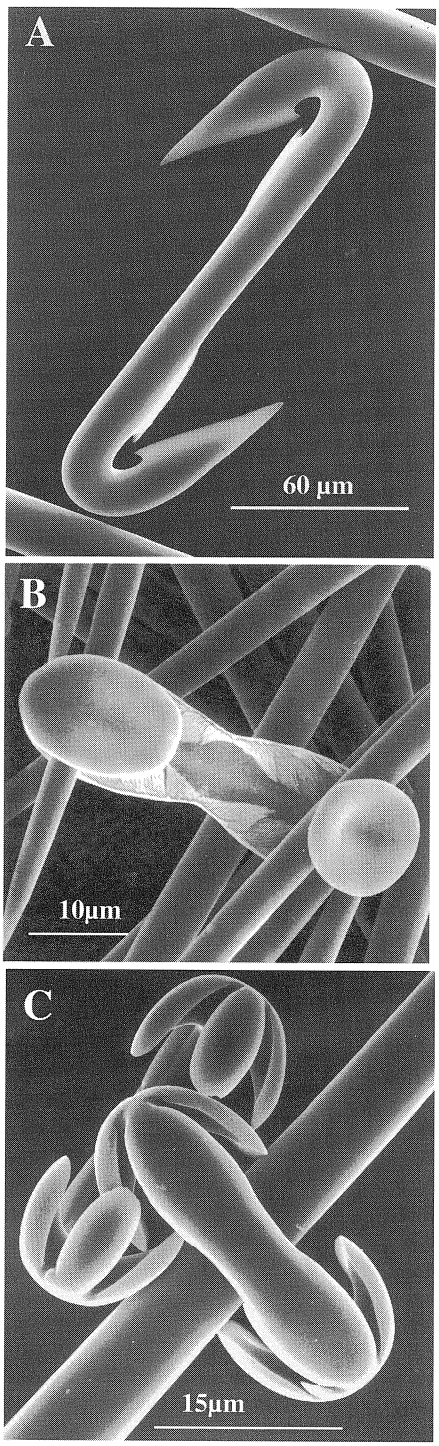

Fig. 6A–C. Instances of microscleres, which might play a role in framing the skeleton by joining monactine megascleres. **A** Diancister of *Hamacantha esperioides* (Ridley and Dendy) between two monactine megascleres (modified after Uriz 1988). **B** Plachochela of *Guitarra* sp. **C** Isochelae of *Crambe acuata* (Lévi). (Modified after Uriz and Rützler 1993)

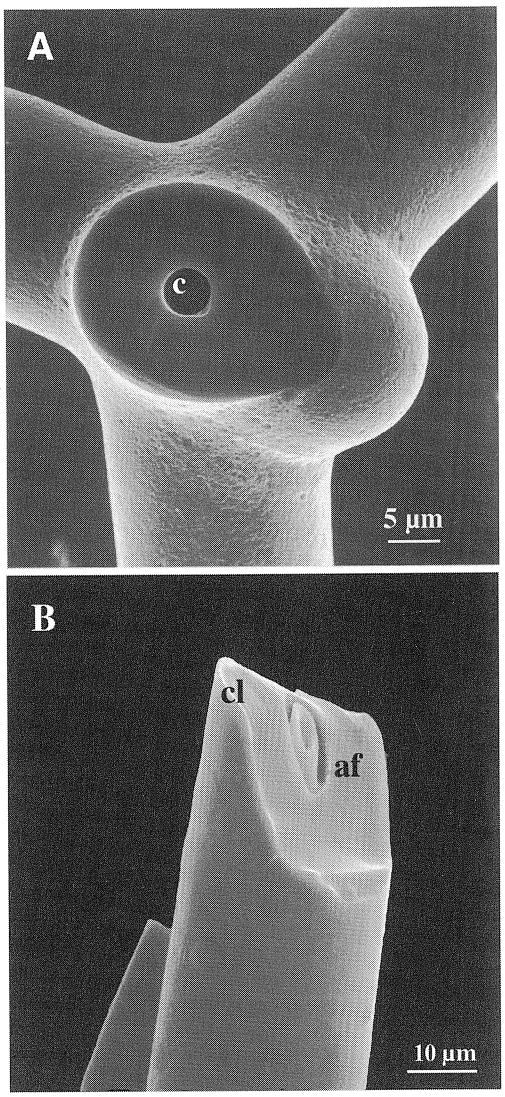

Fig. 7. A Scanning electron micrograph of a tetraxon spicule showing the axial canal (*c*). **B** SEM image of an axial filament in a monaxon spicule

Spicule formation is a complex process controlled by numerous variables. Chemical, physiological, biological, and environmental issues may all modulate at one or several stages spicule formation. We review the current knowledge on silification and spicule formation in demosponges, and we specifically examine the cells involved and the role of the axial filament, membranes, and environmental factors in modulating silica deposition. Although recent

advances in molecular and genetic mechanisms have significantly contributed to better understand the process of spicule formation, many other questions outlined several decades ago are still unresolved. Spiculogenesis in demosponges faces both new and traditional challenges that reveal a promising future.

2 The Cells Involved

Sclerocytes, the cells that secrete spicules, are conventionally divided into megasclerocytes and microsclerocytes, depending on whether they secrete megascleres or microscleres. Megasclerocytes are large ameboid cells with a nucleolate nucleus, easily distinguishable from archeocytes in both adults and larvae (Fig. 8) thanks to their abundant small clear vesicles, microtubules,

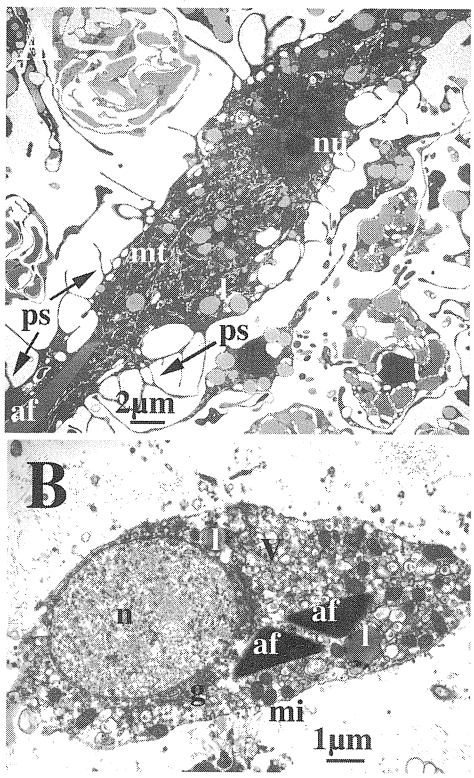

Fig. 8A,B. Larval sclerocytes containing axial filaments. A Megasclerocyte from *Scopalina lophyropoda* larva. B Megasclerocyte of *Crambe crambe* larva (modified after Uriz et al. 2000). *af* Axial filament, *g* Golgi apparatus, *l* lipidic inclusions, *mi* mitochondria, *n* nucleus, *nu* nucleolus, *ps* pseudopodia, *v* vesicles

mitochondria, and conspicuous Golgi complex. Ultrastructural differences between megasclerocytes and microsclerocytes, as outlined in several papers, rely mainly on cell and nuclei sizes, which are notably smaller in microsclerocytes, and on the absence of a clear nucleolus in the latter (Wilkinson and Garrone 1980).

Yourassowsky and Rasmont (1983) demonstrated that at Si concentrations as low as 15 µg/l, sclerocytes of the freshwater sponge *Ephydatia fluviatilis* differentiate and axial filaments are secreted, although spicules are not produced. However, these authors were not able to assess whether traces of silica are necessary for sclerocyte differentiation since total elimination of Si from the experimental cultures was not possible due to methodological constraints. Later, Kraso et al. (2000) found that the presence of silicate in the water contributes to regulate the expression of the silicatein genes and thus, appears to be crucial for the formation of the axial filament and the spicule.

Maldonado et al. (1999) showed that different spicule types are produced, depending on the experimental silicon concentration in the water, and suggested that each spicule type may be secreted by a specific sclerocyte type, which would be activated by a particular $Si(OH)_4$ threshold. According to the experimental results of Maldonado et al., sclerocytes involved in desma production would require higher Si concentrations to secrete spicules than isochelae-producing microsclerocytes, and these in turn would require higher concentrations than the megasclerocytes that secrete the styles. There are several field studies reporting variation in the spicule complement (i.e., types of spicules present) in individuals of the same species from different habitats (e.g., Carballo and Uriz 1998; Cristobo 1998), but the instances are notably scarcer for megascleres than for microscleres. However, although some degree of environmentally determined spicule variation is now widely recognized, new species continue to be described on the basis of the presence or absence of a particular microsclere (e.g., *Hymedesmia* spp.).

Microsclerocytes responded differently than megasclerocytes to changes in silicic acid concentration in experimental cultures of *Spongilla lacustris* (Jorgensen 1944). Microscleres of *Spongilla lacustris* also showed different features than megascleres when germanium was added to the culture medium (Simpson 1990). Simpson's results showed that germanium induced the formation of two terminal bulbs in the birotules of *E. fluviatilis* while it produced a central swelling in oxeas. Simpson interpreted these bulbs as centers for $Si(OH)_4$ processing, which would be placed at the extremities of the microscleres and at the center of the megascleres of *E. fluviatilis*, respectively. All the above mentioned investigations indicate that higher concentrations of Si are necessary for microsclerocytes than for megasclerocytes to secrete their respective spicules, which in the species *C. crambe* has been related to the extracellular (megascleres) or intracellular (microscleres) spicule secretion (Uriz et al. 2000).

The question remains as to whether clusters of sclerocytes develop simultaneously in a particular zone of the sponge and thus spicules are formed in

their definite place or spicules are secreted anywhere in the sponge mesohyl and then transported to the appropriate site to form the skeletal framework (see Uriz et al. 2003). The second option appears to be more reliable for megasclerocytes according to the video images that recorded sclerocytes actively transporting megascleres (Bond and Harris 1988; Bond 1992; TokyoCinema 1996). However, the process may be different in the case of microsclerocytes. Instances of microscleres forming particular structures (e.g., rosette formations of *Mycale anisochelae*) suggest that several microsclerocytes should be in close contact to secrete these microscleres. Microsclerocytes secreting sterrasters have been found in their definite position within the sponge cortex of *Stelletta* (Simpson et al. 1985). Moreover, numerous side-by-side placed sclerocytes elaborating a bundle of raphids have been reported by Wilkinson and Garrone (1980) in an ultrastructural study of the poecilosclerid *Neofibularia irata.*

The formation of the desmoid (hypersilified) spicules appears as a special case (Fig. 9). These spicules are reported to have an axial filament (Fig. 9A) which does not reach the final part of the spicule arms (Pisera and Lévi 2002). The terminal arms of these spicules interlock each other without fusing (Fig. 10) in such a way that a simultaneous formation of several spicules with secreting sclerocytes in close contact must be envisaged. Silification of the terminal arms of desmas, and spines or other ornamentation of nondesmoid spicules is likely modulated by the sclerocyte membrane (see below).

3
The Axial Filament

As stated above, the axial filament has been assigned an important role in determining spicule morphology in sponges (e.g., Reiswig 1971; Simpson et al. 1985). Although several amino acids were identified from the axial filaments of the species *Acarnus erithacus* (Shore 1972), the molecular structure and functioning of the axial filament remained elusive until recently (Shimizu et al. 1998; Cha et al. 1999; Kraso et al. 2000). Cha et al. (1999) demonstrated that the axial filament of *Tethya aurantium* guides Si polymerization along its length. This finding represents the first experimental demonstration of a role in determining the spicule shape for the axial filament.

Filaments triangular in cross section have been reported in tetraxon megascleres of astrophorids and spirophorids (Bütschli 1901; Reiswig 1971; Simpson et al. 1985) and hadromerids (Bütschli 1901), all them belonging to the subclass Tetractinomorpha. More recently, Uriz et al. (2000) showed that the axial filaments of the styles of the poecilosclerid *Crambe crambe* are always triangular before silica deposition starts and in young spicules (Fig. 11A), but that the filament is retracted in mature spicules and the angles of the triangle may become cut off in parallel with a decrease in Si content, given rise to hexago-

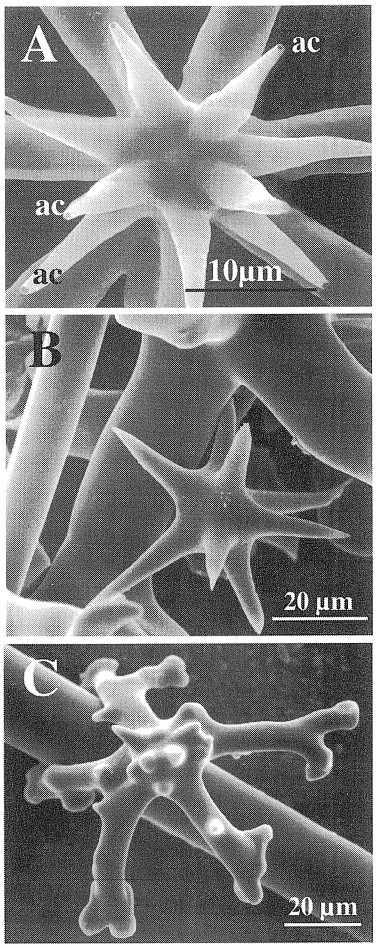

Fig. 9A–C. Scanning electron micrographs of different steps in the formation of a desma. A A very young desma showing the actines, which have a terminal orifice corresponding to the end of an axial canal (modified after Uriz and Maldonado 1995). B Desma of *C. acuata* in an intermediate stage of silification. C A completely formed desma of *C. tuberosa*. (Modified after Uriz and Maldonado 1995)

nal shapes. Filament retraction may account for the "false" hexagonal shapes occasionally reported (e.g., Simpson et al. 1985). Yet, true hexagonal shapes in cross section are reported for filaments of haplosclerid megascleres (Garrone 1969), which are thinner than those triangular shapes and were not resolvable through light microscope (Reiswig 1971; Wilkinson and Garrone 1980). Amazingly, only in hexagonal filaments a paracrystalline structure has been conspicuously observed (Garrone 1969; Wilkinson and Garrone 1980; Donadey et al. 1990).

A particular case has been observed in a representative of the subclass Homosclerophora with tetractinal spicules of a size intermediate between megascleres and microscleres. Cross sections of spicules of *Corticium candelabrum* show an irregular, narrow axial filament (Fig. 11B–D) which appear to

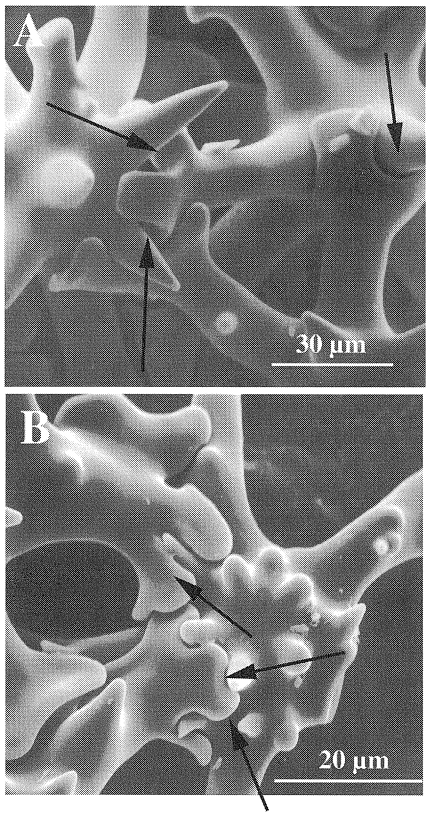

Fig. 10A,B. Scanning electron micrographs of interlocked desmas: see the complementary shape of their terminal arms called zygomes (*arrows*). **A** *C. acuata* (modified after Uriz 1988). **B** *C. tuberosa*. (Modified after Uriz and Maldonado 1995)

be embedded within the silica of the spicule walls instead of being discrete (Fig. 9D).

Although the tetraxon spicule has traditionally been considered ancestral to those diactinal and monactinal (e.g., Dendy 1921; Reiswig 1971), the fossil record shows evidence that the monaxonid type appeared first and may represent the origin for other demosponge spicule shapes (Finks 1970). However, a polyaxonid origin has been demonstrated for some apparently monaxonid spicules (Fig. 12) such as the tylostyles of *Terpios* spp. (Rützler and Muzic 1993; Rützler and Smith 1993) and the tylostyles-subtylostyles of the poecilosclerids *Discorhabdella* and *Crambe* (Uriz and Maldonado 1995). In the latter genera, mature tylostyles are tuberose (Fig. 13A), but young forms have a clear appearance of acanthostyles with conical spines at the proximal end. However, at higher magnifications the spines showed an axial canal, which proves the existence of an axial filament and that the "spines" were in fact short spicule rays, and thus the spicules were polyaxones instead of true monaxones (Fig. 13B). Consequently, polyaxonid spicules are present in representatives of the subclasses Tetractinomorpha and Ceractinomorpha (Lévi 1973) and the

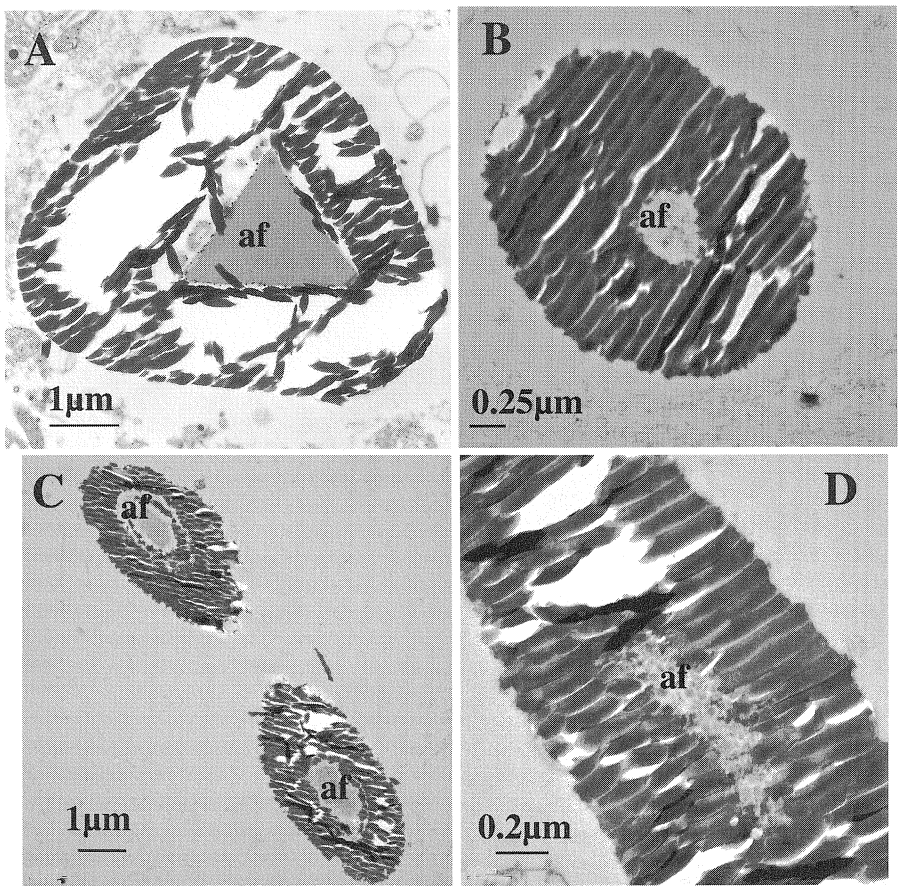

Fig. 11A–D. Transmission electron micrographs of axial filaments in demosponges. **A** Cross section of the axial filaments (*af*) of a *C. crambe* spicule, clearly triangular (modified after Uriz et al. 2000). **B, C** Transversal sections of spicules of *Corticium candelabrum* showing an irregular axial filament (*af*). **D** Longitudinal section of a spicule of *C. candelabrum* showing a narrow, very irregular filament

polyaxial symmetry might be the plesiomorphic condition within the Demospongiae

In contrast to monactinal forms (styles to tylostyles), all the diactinal spicules (oxeas and strongyles) so far examined have axial filaments unequivocally hexagonal in cross sections (Garrone 1969; Simpson and Vaccaro 1974; Wilkinson and Garrone 1980; Weissenfels and Langenbruch 1985). This fact has been reported for both marine (*Haliclona* spp.) and freshwater (*Ephydatia* spp.) haplosclerids, the poecilosclerid *Neofibularia irata* (Wilkinson and Garrone 1980) and the rhaphids of the axinellid *Axinella polypoides* (Donadey et al. 1990). Simpson et al. (1985) assumed that both oxeas and triaenes of

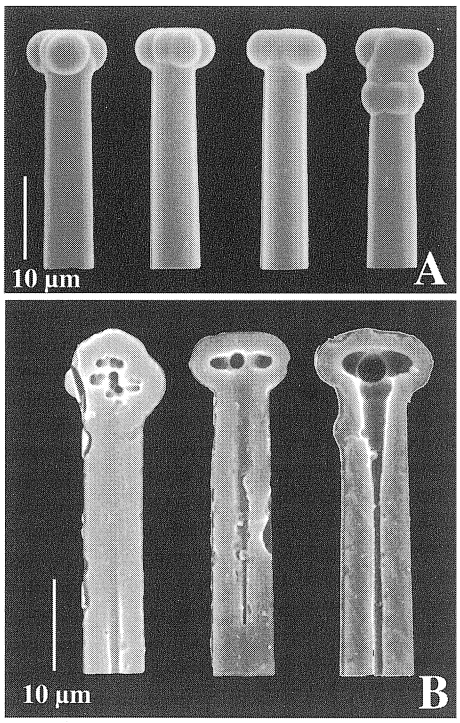

Fig. 12A,B. Polyaxial tylostyles of *Terpios* spp. A Scanning electron micrographs of the polytylote basal zone. B Scanning electron micrographs of corrosion casts of tylostyles showing the internal ramified axial canals (previously filled by the axial filament). (Modified after Rützler and Smith 1993)

Stelletta grubii had triangular axial filaments because triangular forms were abundant in TEM sections of the sponge internal zone, but they recognized that their observations do not allow them to ensure whether these filaments belonged to one or both spicule types. The shape of axial filaments in halichondrids (sponges also bearing diactines) has not yet been examined through TEM, but their shape, although unresolved through light microscopy, was suspected not to be triangular in cross section (Reiswig 1971). On the other hand, homosclerophorid spicules appear to have an irregular, poorly defined axial filament.

The two cross-sectional shapes of the axial filaments, triangular and hexagonal, appear to correlate with the two different models of spicule secretion postulated by Simpson (1990) for monactines and diactines. Oxeas of *E. fluviatilis* cultured in a synthetic medium containing high concentrations of silicon (1.0 mM) produced central bulbous structures (Simpson 1981) similar to those produced in oxeas of *Spongilla lacustris*, cultured in the presence of germanium (Simpson 1990) and in natural populations of *Dendroxea* spp. (Fig. 14; e.g., Corriero et al. 1996). In contrast, monactines start silification at the proximal end (Simpson 1990), which in tylostyles and subtylostyles present a terminal or subterminal swelling, respectively. Pulsed exposures to germanium

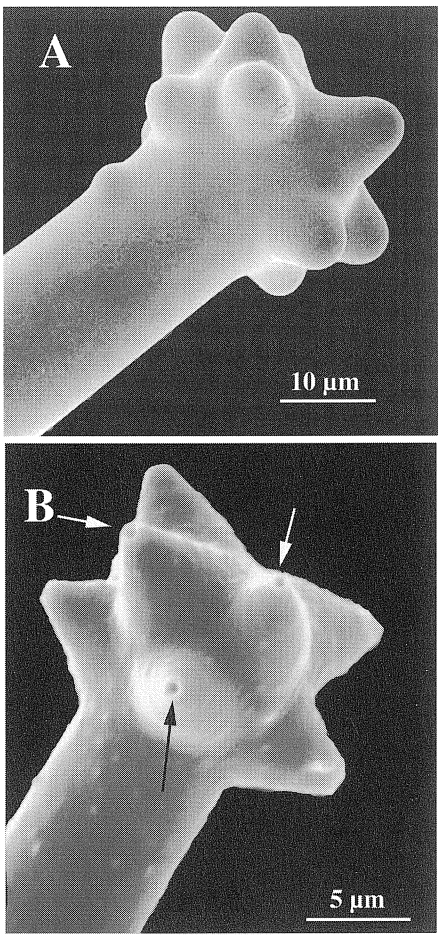

Fig. 13A,B. Tylostyles of *Crambe tuberosa* (SEM). **A** Mature tylostyle with a tuberose tyle. **B** Young polyaxial tylostyle showing the origin of the tubercles, which consists in short actines containing an axial filament (*arrows* point to the orifices corresponding to the axial canals). (Modified after Uriz and Maldonado 1995)

allowed Simpson (1990) to demonstrate that diactines of *E. muelleri*, *E. fluviatilis* and *Spongilla lacustris* grow bidirectionally from a central $Si(OH)_4$ processing region, whereas the growth of the monactines of *Suberites domuncula* (tylostyles) is unidirectional from the spicule head. This fact is supported by the many instances of very young tylostyles with a marked tyle and a thin shaft observed in this species (Uriz 1983). However, the existence of true silification centered along the axial filament has not been unambiguously demonstrated. Likewise, the formation of silica bulbs seems to be regulated by the unit-type membrane that surrounds the growing spicule (Simpson 1981).

A particularly appealing research topic is the formation of the articulate framework of desmas, and more concretely the final part of the desmoid arms, which are devoid of axial filament (Pisera and Lévi 2002). It has been demonstrated that silica polymerization in sponges requires the presence of enzymes

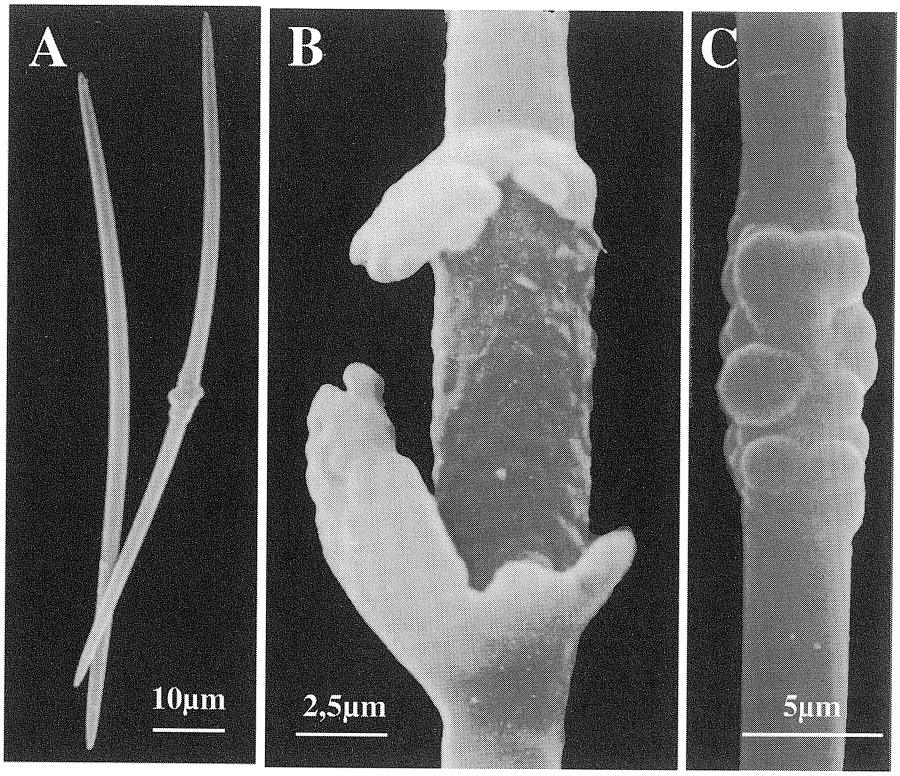

Fig. 14A–C. Central swelling in oxeas of *Dendroxea* spp. (SEM). **A, C** *D. adumbrata* (Corriero et al. 1996). **B** *D. pseudodiscoides.* (Modified after Corriero et al. 1996)

(Shimizu et al. 1998), which first catalyze the hydrolysis of Si compounds and then their polymerization. Organic molecules (proteins), which do not form a concrete macrofilament, may be responsible for silification in the terminal arms of desmas, according to the outstanding observations by Pisera (2003).

4 Extracellular Versus Intracellular Silica Deposition: the Role of Membranes

A never-ending discussion on whether the process of silica polymerization around the axial filament is intracellular or extracellular can be found in most papers dealing with spicule secretion of demosponges (see Simpson 1984 for a review). This discussion was mainly due to the attempt of extrapolating particular observations to the whole demosponge spicules and species. Most microscleres observed are secreted intracellularly and only in one case (toxas

of *Microciona*) the secretion appeared to be extracellular at a light microscope level (Simpson 1978), although this instance would need verification through electron microscopy. Similarly, most previous studies showed an intracellular secretion of megascleres (Garrone 1969; Simpson and Vaccaro 1974; Garrone et al. 1981; Hartman 1981). An extracellular secretion of megascleres has been documented only once (Uriz et al. 2000), although it was previously envisaged due to the physical impossibility of a sclerocyte to completely surround a spicule several milimeters long, such as those of many astrophorid sponges. Extracellular filaments in early stages of silification surrounded by the sclerocyte pseudopodia (Fig. 15) have been reported in the demosponge *Crambe crambe* (Uriz et al. 2000), which indicates that filaments are secreted before silification starts and shows that megasclere silification proceeds extracellularly in this species. The extracellular silica secretion questions the hypothesis that filaments continue to grow even after the onset of silification (Simpson 1990) and supports Reiswig's (1971) statement that the axial filament is formed in full length and breadth before silica deposition started.

With the information at hand, it can be concluded that most microscleres are secreted intracellularly in a space surrounded by a unit-type membrane (called silicalemma) and that inter- and intra-ellular secretion processes occur in different types of megascleres and/or species. Consequently, both membrane types (the internal silicalemma and the external plasmalemma) appear to play similar roles in silica deposition and may even have the same origin since fusion between them appears likely (Uriz et al. 2000). However, freeze-fracture images (Garrone and Lethias 1990) of the internal surfaces of those membranes look very different, the silicalemma being covered by a higher density of "intra-membrane particles", although the different magnification at which the pictures of the two membranes were taken makes comparisons imprecise.

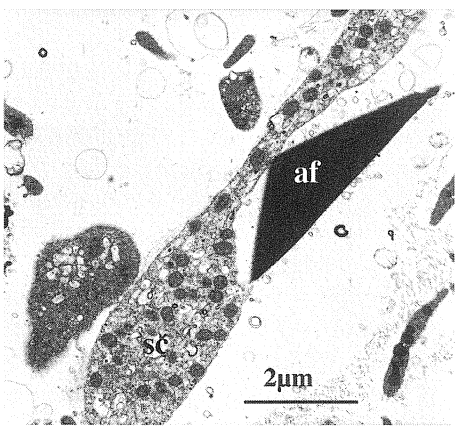

Fig. 15. Sclerocyte (*sc*) of *Crambe crambe*, which surrounds an axial filament (*af*) that has been released to the mesohyl (TEM)

It can be highlighted that all the instances of an intracellular secretion of megascleres reported up to now correspond to small diactines from species of the order Haplosclerida. It has been assumed that the diactines of the poecilosclerid *Neofibularia* are intracellular, but this assumption remains to be demonstrated (Wilkinson and Garrone 1980). Moreover, the secretion of long megascleres of astrophorids or spirophorids has never been examined through TEM.

5
The Process of Silica Polymerization

Silica polymerization occurs and is crucial in many forms of living beings from diatoms to mammals (Volcani 1981). This process is accomplished in organisms at low (ambient) temperatures, whereas silica polymerization under non-living conditions requires extreme temperature, pressure and pH (Kröger et al. 1999).

There is a great deal of speculation as to the possible pathways followed by the silicic acid after it enters the sponge until it polymerizes around the axial filament to form the spicules. There is no current evidence of cellular transport of Si from the pseudoepithelial cells (pinacocytes and choanocytes) to mesohyl cells such as the sclerocytes (Uriz et al. 2000). As a consequence, the hypothesis that suggests transport of silicic acid by simple diffusion through the ground substance to the sclerocyte (Simpson 1981) appears to be more likely.

Recent research on silica content in the different compartments of a sclerocyte secreting a spicule and its surroundings (Uriz et al. 2000) showed that mitochondria, vesicles, dense inclusions, and the cytoplasm of the sclerocyte far from the growing spicule contained less than 10% Si (relative to that contained in the spicule wall). In contrast, cytoplasm close to the growing spicule contained up to 50%, and the extracellular space between the sclerocyte and the growing spicule contained 50–65% (Fig. 16). These results proved that there is a clear concentration of Si in the surroundings of the axial filament both within and outside the sclerocyte. These data do not support the Si transport through vesicles suggested by previous hypothesis (Schröeder 1936).

A relevant role has been attributed to the unit-type membranes of the sclerocyte (plasmalemma or cell membrane and silicalemma) in actively transporting silicic acid first to the sclerocyte, and then to the space which surrounds the axial filament (Simpson 1989). Silicic acid super-saturation in the membrane-bound compartment containing the axial filament may be achieved by active molecular transporting. Hence, the local regulation and control over physico-chemical factors may be done through selectivity in ion and molecular transport. However, little is known about how cell transporters proceed and manage silicon in sponges. However, Hildebrand et al. (1997) have recently isolated and functionally characterized cDNAs encoding proteins that

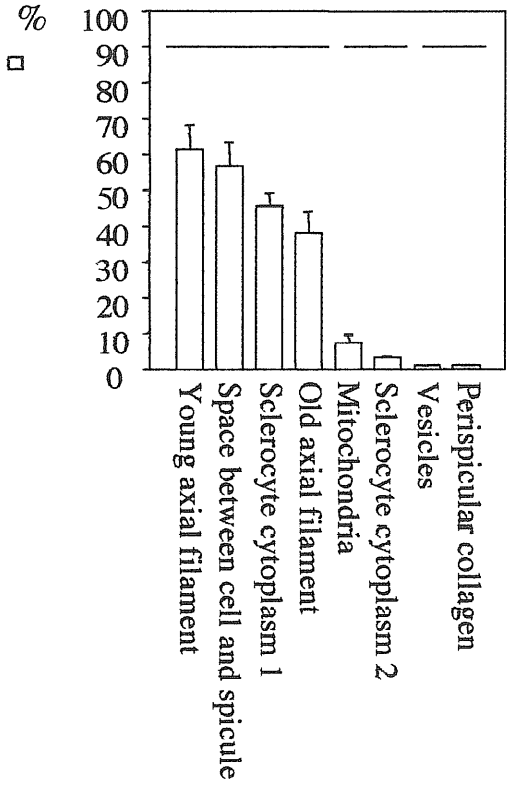

Fig. 16. Percentage of Si content in the different compartments of a sclerocyte and its surroundings. (Modified after Uriz et al. 2000)

interact specifically with silicon and transport it across biological membranes of the diatom *Cylindrotheca fusiformis*. The mechanism may be comparable in sponges, but silicon transporters still remain elusive in these animals.

Experiments with germanium addition to freshwater sponges have demonstrated that the membrane that surrounds the growing spicule plays a role in modulating the final spicule shape, which is initially determined by the axial filament. This would explain the cases of spines without any protein core (De Pomar 1973) or those of complicated microscleres (Simpson 1984) and, particularly the zygosis (interlocked arms) of the desmoid spicules (Fig. 10).

It has been shown that the membrane that embodies the axial filament in a sclerocyte of *Spongilla lacustris* cultured in a Ge-rich medium (Ge/Si concentration ratio of 14) forms a bulbous vacuole (Fig. 17) at the place where there will be a swelling in the mature spicule (Simpson 1981). As Simpson suggested, uncoupling of the axial filament and the silicalemma due to an excess of the silicalemma synthesis appears to account for the abnormal silica deposition and may also be responsible for the formation of spines and other spicule orna-

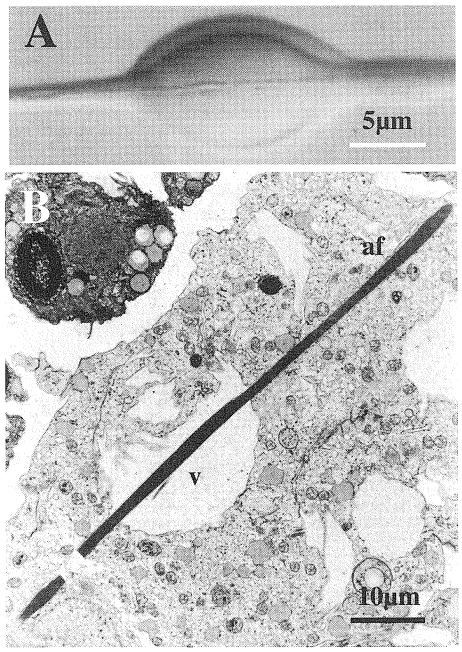

Fig. 17A,B. *Ephydatia fluviatilis.* A Central swelling of an oxea from specimens grown in a Ge-enriched medium. **B** Transmission electron micrograph of a sclerocyte showing a central vacuole (*v*) around the axial filament (*af*), which resulted in the central spicule swelling. (Modified after Simpson 1981)

mentation. An increase of the membrane surface may end in an accumulation of silica in the space delimited by the membrane.

Shimizu et al. (1998) demonstrated that the main proteins that constitute the axial filament of sponge spicules (called silicateins α, β, and γ) are enzymes highly similar to members of the papain family of proteases, and they can catalyze the hydrolysis and polycondensation of silicon alkoxides. Cha et al. (1999) showed that silicatein induced very fast silica polymerization in vitro as compared to silica polymerization without this protein. A dependence on the three-dimensional conformation of the silicatein units isolated from the axial filaments of *Tethya aurantium* has been assumed since the activity of the protein was abolished by thermal denaturation (Cha et al. 1999).

Si polymerization both in vitro (Cha et al. 1999, 2000; Kröger et al. 1999) and in vivo (e.g., Volcani 1981) produces rounded small particles, which tend to fuse to each other giving rise to a network of micro- or nanospheres. The size of spheres resulting from silicic acid polycondensation has been reported to range from 1 nm in laboratory experiments in presence of cellulose (Perry and Lu 1992) to 1200 nm in naturally occurring tylostyles of the sponge *Cliona schmidti.*

In diatoms, Kröger et al. (1999) have isolated several proteins (called silaffins), which generate networks of silica nanospheres within seconds at ambient temperature when added to a solution of silicic acid. The size of these nanospheres varied depending on the protein used: by using silaffin-1, the

nanospheres reached 500–700 nm in diameter while they were <50 nm when a mixture of silaffins was utilized. Cha et al. (2000) have recently synthesized cysteine-lysine block polypeptides that mimic the properties of silicatein, by controlling at the same time hydrolysis and condensation as well as structural templating of tetraethoxysilane (TEOS). Polymerization by these polypeptides results in silica spheres ca. 100 µm in size.

Spherical silica units less than 20 nm in diameter that further coalesce can also be observed in TEM sections of higher plants (Sangster and Parry 1981) and silica particles of 40–50 nm have been shown in SEM images of diatoms (Volcani 1981).

Nanosphere formation has also been reported in early silification stages of sponge spicules (Fig. 18A; Schönberg 2001; Rosell and Uriz 2002) and the nanospheres increase their size and tend to fuse (Rosell and Uriz 2002; Pisera 2003) as late silification proceeds (Fig. 18B). In sponges inhabiting silicon-poor environments, instances of growing spicules showing an uneven surface (Fig. 19A) made of rounded protuberances as a result of incomplete silification have often been reported and are described as "malformations" (e.g., Uriz and Maldonado 1993; Fig. 19B). SEM pictures of spicule surfaces from sponges reveal nanospheres ranging from ca. 17 nm in *Spongilla lacustris* (Simpson 1981) to 100–1200 nm in growing tylostyles of several Clionidae species (Schönberg 2001; Rosell and Uriz 2002). Nanospheres from 100–120 nm in maximum diameter can be shown in TEM images of styles of *Crambe crambe* at early stages of silification (Fig. 20; Uriz et al. 2000).

Although completely different proteins appear to be involved in silification processes in diatoms (Kröger et al. 1999) and sponges (Shimizu et al. 1998; Cha et al. 1999), the resulting silica nanospheres appear to be quite similar. Consequently, nanosphere formation is the first observable feature of silica polymerization both in in vitro and in organisms; they increase in size and fuse with each other forming a network as the silification proceeds. Fusion of nanospheres by addition of silica results in an even spicule surface.

The spicules of sponges are formed by successive concentric silica layers as has been repeatedly shown in transverse sections of megascleres (e.g., Bütschli 1901; Garrone 1969; Uriz et al. 2000). Several mechanisms responsible for silica stratification have been proposed. They have been interpreted by Schwab and Shore (1971b) as the result of pauses in silification, which would allow the siliceous surface to become highly hydroxylated. It has been suggested that these pauses might be coincident with a replacement of the sclerocyte that deposits the silica (Uriz et al. 2000), although the involvement of several sclerocytes in secreting a single spicule, although conceivable in the secretion of large spicules, has not been verified.

The concentric silica layers are particularly conspicuous in spicules of hexactinellid sponges where, conversely to demosponge spicules, layers appear not to be completely adhered, according to their irregular fracture in cross section (e.g., Lévi 1989).

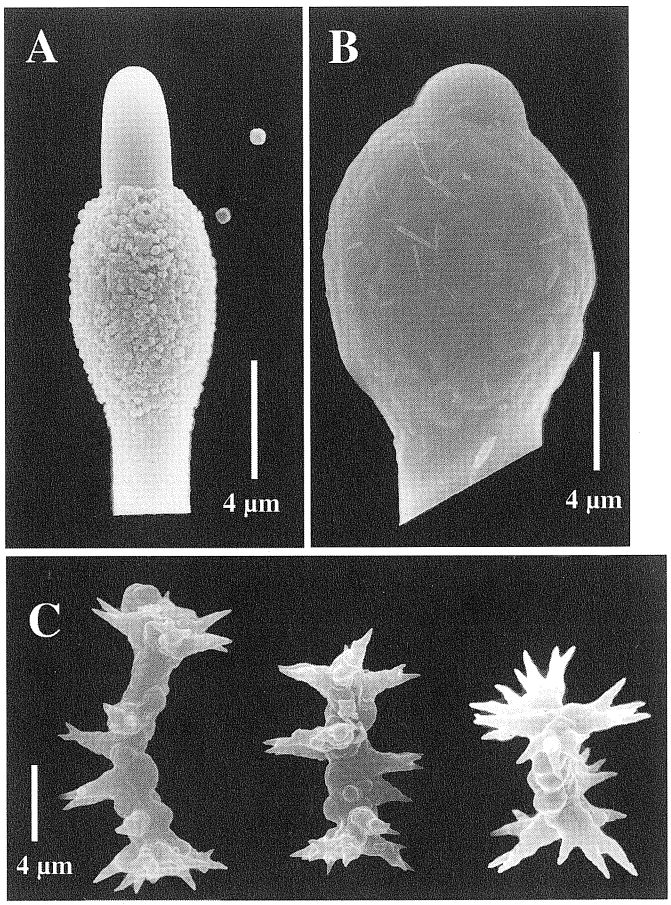

Fig. 18A–C. Different stages of silification of *Cliona schmidti* spicules. A Young tylostyle showing the surface of the tyle formed by silica nanospheres. B Tylostyle tyle in a subsequent stage of silification. C Irregularly silified amphiasters. (Modified after Rosell and Uriz 2002)

6
Environmental Factors Modulating Silica Deposition

There are several environmental factors which influence the final spicule size or even the expression or absence of one or several spicule types (particularly microscleres). Species growing in upwelling zones (e.g., Uriz 1988), in the Antarctic or at great depths are reported to have larger spicules than their conspecifics living in oligotrophic seas (e.g., Bibiloni 1990; Bavestrello et al. 1993). Spicules from eutrophic environments show a well-defined shape with conspicuous tyles, spines, points or any ornamentation typical of the spicule (Uriz

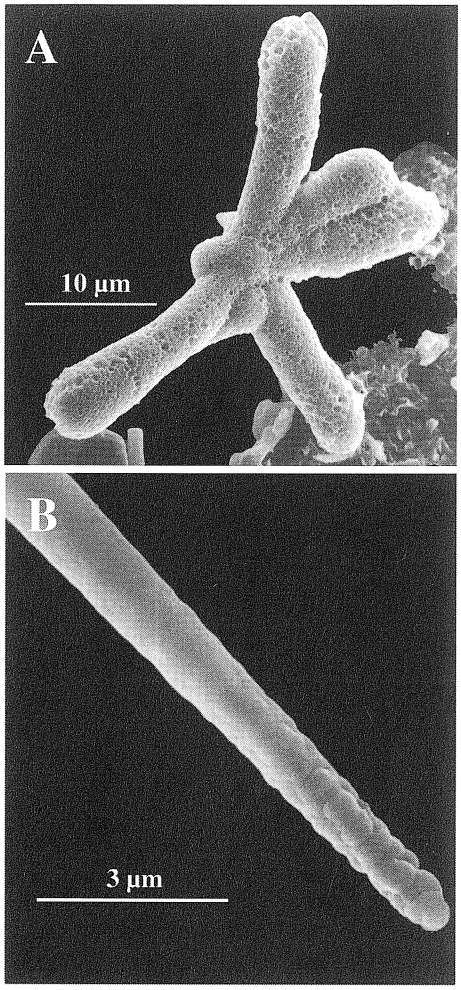

Fig. 19. A An incompletely silified desma of *Crambe crambe* showing the surface irregular as a result of incomplete nanosphere fusion (modified after Maldonado and Uriz 1996). B Distal zone of a monactine megasclere of *Clathria oxistyla* Ferrer-Hernàndez from a silica-poor environment. See the tuberose aspect of the spicule surface. (Modified after Uriz and Maldonado 1993)

1988). Among the environmental factors that are responsible for differences in spicule conformation, silicic acid concentration appears to be particularly important. However, low temperatures and high concentrations of particulate organic matter, which are usually associated with high amounts of silicates in upwelling areas, are also to be considered.

Manipulative experimentation in the laboratory (e.g., Pé 1973; Yourassowsky and Rasmont 1983; Maldonado et al. 1999; Le Pennec et al. 2002) has been decisive in understanding the role of silicates and other substances in the silica deposition process. Yourassowsky and Rasmont (1983) first demonstrated that silicic acid limitation drastically affects spicule secretion.

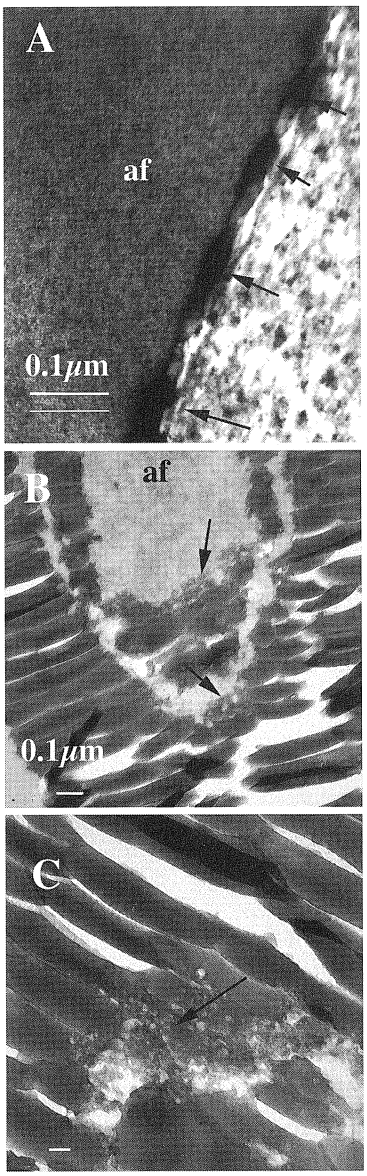

Fig. 20A–C. Nanospheres formation (*arrows*) in close contact to the axial filament (*af*) (TEM). A Early silification pattern in a spicule of *Crambe crambe* . B, C Nanospheres formation at the central zone of *Corticium candelabrum* spicules

When these authors cultivated freshwater sponges (*Ephydatia fluviatilis*) from gemmules in a mineral medium containing as little silicic acid as possible (i.e., less than 15 µg/l), no spicules at all were secreted, although sclerocytes differentiated and expressed the axial filament. By recording the growing sponges through time-lapse video under a light microscope, they reported that when silica was added to the medium, the sclerocytes that contained an axial fila-

ment produced normal spicules. In contrast, when the silica-poor medium was maintained, the sclerocytes degenerated and shed their filaments.

More recently, Maldonado et al. (1999) have performed the opposite experiment by growing larvae of a marine sponge in silica-enriched medium. Individuals of the sponge *Crambe crambe* inhabiting some regions of the western and central Mediterranean lack one or more of the spicule types (e.g., Boury-Esnault 1971; Bibiloni 1990; Uriz et al. 2000). In contrast, specimens from the Adriatic coasts produced the whole spicule complement of the species, including desmas and isochelae (Thiele 1899). Silica concentration in the water column was thought to be responsible for these differences in the sponge skeleton and this was experimentally tested. These authors examined the production of spicules by newly settled sponges maintained during 4.5 months under three concentrations of silicic acid (<4.5 µM, ca. 30 µM and ca. 100 µM). All the sponges cultured in the silica-poor medium (Si concentration similar to that of most Mediterranean waters) only produced one spicule type (small styles), while sponges cultured under ca. 30 µM produced small styles, large styles and isochelae. A further spicule category, the asterose desmas, appeared when the sponges were cultured under ca. 100 µM $Si(OH)_4$. These results show that *C. crambe* is genetically capable of producing several spicule types that are normally absent from natural populations inhabiting Si-poor environments.

Increasing silicic acid concentration resulted in longer and wider megascleres in cultured freshwater sponges (Pé 1973). Furthermore, high silicic acid concentrations produce bulbous expansions in both megascleres (Pé 1973) and microscleres (Jorgersen 1944) of freshwater sponges and other abnormalities such as tuberose ornamentation in megascleres (styles) and supplementary spines or teeth in isochelae of the marine sponge *Crambe crambe* (Maldonado et al. 1999). Other contrasting effects such as a decrease in the growth rate of the spicules length have also been described in *Ephydatia fluviatilis* cultured under 25 µM $Si(OH)_4$ (Elvin 1971). This may be explained by possible saturation of the sites of Si polymerization, which is in concordance with the decrease in Si uptake observed in the sponge *C. crambe* when $Si(OH)_4$ increased from 30 µM to 100 µM (Maldonado et al. 1999).

Other mineral elements besides silicon have also been reported to influence spicule secretion in demosponges. The addition of a Fe^{2+} chelator (2,2′-Bipyridin) to the culture medium at concentrations lower than 4 µg/ml affected skeletogenesis of freshwater sponges, which slowed down silification, produced bulbous shorter spicules and reduced the total production of spicules by 50% compared to the controls (Holvoet and Van der Vyver 1990). These results indicate that Fe^{2+} is necessary for silica polymerization and allowed these authors to suggest that enzymes with Fe^{2+} cofactors appeared to be directly involved in the biosilification of the axial filament. More recently, it has been suggested (Le Pennec et al. 2002; Müller et al. 2003) that Fe^{2+} in the water would be taken up by sponge cells and transferred after oxidation to Fe^{3+} to the axial filament where they would possibly activate the enzymes that catalyze silica polymerization to form the spicules.

Studies dealing with the effects of physical parameters on silica deposition are scarce, particularly for marine sponges. However, they have shown that temperature also affects silica deposition even when it is maintained within the range found in natural sponge habitats. In *Haliclona oculata*, spicules are larger in specimens inhabiting colder regions (Hartman 1958). Simpson (1978) reported that *Microciona prolifera* produced wider megascleres at lower temperatures. However, experimental cultures of *Ephydatia muelleri* (Lieberkühn) from asexual gemmules under temperatures ranging from 10 and 25 °C and different silicic acid concentrations (Elvin 1971) showed that the rate of growth in spicule length, and the rate of silica deposition increased with increasing temperatures. Low temperatures may induce a decrease in the silification rate as a consequence of a slow-down of the sponge metabolism, but a slower spicule growth appears to result in the formation of larger spicules.

7 The Future

Although studies on demosponge spiculogenesis have been scarce in the last decade, the few available instances have greatly contributed to the progress of our knowledge on the whole process. Several unresolved questions were outlined in ca. 20-year-old revisions (Garrone et al. 1981; Simpson 1984). Some of those questions have been resolved, but others still remain unanswered.

The identification and characterization of protein subunits in the axial filament of *T. aurantium* (Shimizu et al. 1998; Cha et al. 1999) and the identification of the genes that encode these proteins (Kraso et al. 2000) have represented an extraordinary advance in our knowledge of the silification process. Knowledge of the role of chemical elements and physical factors in the silica deposition of demosponges has experienced important progress in the last decade. The effects of silicate concentration and other inorganic ions (i.e., Ge and Fe^{2+}) on gene and spicule expression has allowed the identification of a different physiological behavior in microsclerocytes and megasclerocytes. The extracellular secretion of a potentially polyaxonid megasclere has reinforced the hypothesis that large tetraxonid megascleres may be accomplished extracellularly and that both intracellular and extracellular megasclere secretion occurs in demosponges.

Other questions such as how silica polymerization proceeds in the spicules or part of spicules without any trace of axial filaments are partially resolved. Pisera (2003) has shown images that suggest the presence of organic molecules which would induce silica polymerization without forming discrete filaments. However, this issue remains to be verified by molecular techniques.

Several of the pending questions are to be fixed by ultrastructural studies. Yet, these studies face the technical difficulty of catching a dynamic complex process by means of microscale "snapshots". What is the fate of sclerocytes once they have finished the spicule secretion? Is the same sclerocyte able to

secrete several spicules or does it degenerate after the spicule secretion. How many sclerocytes participate in the complete spicule secretion when it is performed extracellularly?

The identification and quantification of the molecules that transport Si through the unit-type membranes, already identified for diatoms (Perry and Lu 1992), would help to confirm the role of membranes in defining the spicule shape, particularly in the zones devoid of axial filament.

There is still an important gap in the knowledge about how spicules are arranged to form the various skeletal frameworks that we can find in Demosponges. The video images recorded by Bond and Harris (1988), Bond (1992) and TokyoCinema (1996) gave precious information on continuous cell rearrangement in sponges that fixed SEM and TEM images cannot produce. Thanks to these videos, we know that sclerocytes are continuously moving while secreting spicules, following the general sponge cell flow, and that at least not all the cells that transport spicules are spongocytes as was previously assumed (Simpson 1984).

Morphogenesis, however, still remains enigmatic for spicule arrangement in the different zones of the sponge body. Several homeobox genes that confer positional information during development have been identified in demosponges (e.g., Coutinho et al. 1994; Seimyia et al. 1994; Degnan et al. 1995; Richelle-Maurer et al. 1998; Richelle-Maurer and Van der Vyver 1999). Possibly, they have a role in the differentiation and arrangement of sclerocytes and the various spicule types, but how they are expressed to form the sponge skeleton still requires a great deal of investigation.

The application of the panoply of new molecular and ultrastructural methods to old unresolved questions such as those outlined may produce a blossoming of new and interesting information about the complex process of silica deposition in sponges in the forthcoming years.

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