



General Palaeontology (Palaeobiochemistry)

Biomineralisation in reef-building corals: from molecular mechanisms to environmental control

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Received 7 October 2003; accepted after revision 12 July 2004

Available online 30 September 2004

Written on invitation of the Editorial Board

Abstract

Coral reefs constitute real oasis sheltering for about one third of the identified fishes, representing a major advantage for the economy and tourism of many tropical countries. However it is paradoxical to notice that their formation at the cellular level or even at the scale of the organism is still poorly known. Effectively, biomineralisation, the process that is at the basis of their edification, is always the subject of numerous researches. Two combined mechanisms lead to the formation of a biomineral, the synthesis/secretion of macromolecules referred to as ‘organic matrix’, and the transport of ions (calcium, bicarbonates and protons in the case of calcification) to the mineralising site. This review shows a view of the works carried out on biomineralisation in scleractinian corals, including some aspects on the control of calcification by environmental parameters. It also gives insights into the biological basis of the use of coral skeletons as environmental archives in palaeo-oceanography. *To cite this article: D. Allemand et al., C. R. Palevol 3 (2004).*

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Résumé

La biominéralisation chez les coraux constructeurs de récifs : des mécanismes moléculaires à la formation du récif.

Alors que les récifs coralliens forment de véritables oasis hébergeant environ un tiers des poissons décrits et constituent un atout majeur pour l'économie et le tourisme de nombreux pays tropicaux, il est curieux de constater que leur formation à l'échelle cellulaire ou de l'organisme est encore peu connue. Le processus à la base de leur édification, la biominéralisation, est, il est vrai, peu compris, même s'il fait actuellement l'objet de nombreuses recherches. Deux mécanismes sont associés pour la formation d'un biominéral : la synthèse et la sécrétion de macromolécules, appelées « matrice organique », et le transport sur le site de

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minéralisation d'ions (calcium, bicarbonate et protons dans le cas de la calcification). Cet article présente une synthèse des travaux concernant la biominéralisation chez les coraux scléactiniaires ainsi que quelques aspects plus appliqués sur le contrôle de la calcification par les paramètres environnementaux. Il donne aussi un aperçu des bases biologiques de l'utilisation des squelettes coralliens comme archives environnementales en paléocéanographie. *Pour citer cet article : D. Allemand et al., C. R. Palevol 3 (2004).*

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Keywords: Coral; Calcification; Biomineralisation; Ion carrier; Calcium channel; Ca²⁺-ATPase; Organic matrix; Symbiosis

Mots clés : Corail ; Calcification ; Biominéralisation ; Transporteur ionique ; Canal calcium ; Ca²⁺-ATPase ; Matrice organique ; Symbiose

1. Introduction

“The organic forces separate the atoms of carbonate of lime, one by one, from the foaming breakers, and unite them into a symmetrical structure. Let the hurricane tear up its thousand huge fragments; yet what will that tell against the accumulated labour of myriads of architects at work night and day, month after month. Thus do we see the soft and gelatinous body of polypus, through the agency of the vital laws, conquering the great mechanical power of the waves of an ocean, which neither the art of man nor the inanimate works of nature could successfully resist.”

Charles Darwin,

in: *Journal of researches into the natural history and geology of the countries visited during the voyage round the world of H.M.S. Beagle under command of Captain Fitz Roy London*, John Murray Eds, Albemarle Street, 1845.

Coral reefs constitute the most important bioconstruction of the world with a calcification rate of about 2–6 kg_{CaCO₃} m⁻² yr⁻¹ [5] and a distribution over about 284 300 km² [80]. However, who knows that this huge construction results from the work of small colonial organisms related to hydra, medusa or sea anemone, the reef-building corals? These reefs are of major importance, not only for marine ecosystems and biodiversity (tropical reefs are the most productive marine ecosystem and would host almost a third of all world fishes), but also for the economy of numerous countries (tourism, fishing...), for palaeoclimatology or geology studies... [7,65,94]. Several applications of coral reefs are known, from drugs isolated from organisms [32] to the use of coral skeleton as bio-implants for human surgery [24,30]. However, in light of global change, a decline of coral reefs has been described worldwide [12,52,68,79].

In spite of this major importance, reef formation remains poorly known. Pioneering work by Darwin [21] was followed by finer description of coral calcification (see [64,69] and later [9]). Nevertheless, real physiological studies started after 1950 with the works of Muscatine (see review in [6]). At the dawn of the 21st century, molecular mechanisms underlying coral calcification are still almost unknown. As other biominerals, coral skeleton formation needs simultaneously a supply of ions and a control by an organic framework, the organic matrix, present inside the skeleton. After a brief description of coral anatomy, we present in this review recent data regarding control of calcification by ions and organic matrix and some data explaining relationships between light and calcification. Environmental control of coral calcification, as well as the experimental bases of the use of coral skeletons as environmental archives, will also be presented. This review is mainly based upon results obtained at the ‘Centre scientifique de Monaco’, which develops studies on coral biology from the ecosystem to the colony, the microcolony, the cell, and the molecule (for more details, see other recent reviews [1,2,36]).

2. Definitions

If the term ‘coral’ is broadly used, paradoxically it has no valid taxonomic definition. ‘Coral’ initially referred to the Mediterranean coral, *Corallium rubrum* (Anthozoa: Octocorallia) with a calcitic skeleton. Afterwards, it was used to name other Octocorallians, like the blue coral (*Heliopora*), but also Hexacorallians, like the black coral with horny skeleton, *Antipathes*, or reef-building corals (Scleractinians or Madreporaria) with aragonitic skeleton, or even Hydrozoans like the fire coral (*Millepora*). To throw people into confusion,

‘coral’ also designates Octocorallia without hard skeleton, the Alcyonarians (soft corals!). Scleractinian corals are known for their skeleton and are, in this case, called ‘reef-building corals’ or hermatypic corals (herm = reef). Hermatypic corals host in their tissues unicellular Dinoflagellates symbionts, commonly called zooxanthellae (*Symbiodinium* sp.) [76]. But all Scleractinians are not reef builders (ahermatypic corals), even if they are characterized by their aragonitic skeleton.

3. Morpho-functional anatomy

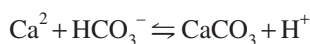
The coral skeleton is extracellular, located at the base of coral tissue, like a finger covered by a glove. Hermatypic corals are modular animals, constituted of similar units, the polyps resulting from a clonal reproduction (asexual reproduction by budding). Each polyp looks like a bag whose walls are made by two layers of cells, an ectodermal layer, and an endodermal layer, separated by a network of collagen, called mesoglea. The endodermal cell layer delimits a cavity, the coelenteron, with only one opening, the mouth, surrounded by tentacles (of a multiple of 6 = Hexacorallia). This cavity acts simultaneously as a gastric and circulatory cavity between polyps, and is therefore also called gastro-vascular cavity.

Polyps are linked together by the coenosarc made by an oral (facing sea water) and an aboral (facing skeleton) epithelium, each of them being composed of an ectodermal and an endodermal cell layer (Fig. 1). Ectoderm and endoderm are differentiated between oral and aboral layers: most of zooxanthellae are located within the oral endoderm, whereas only a few are found in the aboral endoderm. The oral ectoderm is thick (10 to 20 μm) and rich in cnidocytes, whereas the aboral ectoderm, also called calicoblastic epithelium, is flat (0.5 to 3 μm) and does not possess cnidocytes. Calicoblastic epithelium is responsible for the formation of the aragonitic skeleton. Calicoblastic cells are long (10 to 100 μm), highly interdigitated and overlap each other. They contain numerous mitochondria, suggesting an active metabolic role. Skeletogenic cells are connected by desmosome junctions [48, Tambutté and Allemand, unpublished data]. They are attached to the exoskeleton by specialized cells, called desmocytes [62]. Cells are often separated by large intercellular

spaces that may contain circular vesicles (50–70 nm in diameter) thought to function in exocytotic transport of organic matrix [48]. Consequently, the skeletogenic process occurs in a ‘biologically-controlled medium’ and is separated from the external seawater by four layers of cells, i.e. at least 40–50 μm thick.

4. Biomineralisation: ions and organic molecules

Composition of biominerals includes two fractions, one mineral and one organic, called organic matrix, the amount of each varying among species. In Scleractinian corals, skeleton is made of calcium carbonate (CaCO_3) crystallized in aragonite (orthorhombic system). In order to build their skeleton, corals have to supply calcium and inorganic carbon from ambient seawater to the calcification site but also to eliminate protons resulting from the mineralising process:



Epithelial transport of molecules can be achieved by two mechanisms: a paracellular pathway, between cells (in this case, transport of the molecule is only driven by its chemical gradient, transport is diffusional and passive), or a transcellular pathway through cells. In this last case, the molecule needs to enter the cell, cross the cell and then exit. Thus the transport is active, against a gradient with energy supply. Transport of charged species across the hydrophobic cell membrane requires specific carrier proteins.

Study of coral calcification was for a long time limited due to methodological problems linked with the use of radioisotopes, particularly ^{45}Ca [4]. Before 1990, most of coral scientists made their experiences with freshly-cut branches of corals (like *Acropora* or *Stylophora*), leading to a naked skeleton whose porosity facilitated the unspecific adsorption of ^{45}Ca . Furthermore, the presence of a large volume inside the coelenteric cavity led to errors, because the amount of radioactive calcium in this cavity was about 53% of total amount absorbed by the colony after 30 min of incubation with ^{45}Ca -labelled seawater [83]. Therefore, the lack of rinsing this cavity induced an important error. In order to cope with this problem, researchers from the ‘Centre scientifique de Monaco’ have set up two important technological improvements: (i) a specific preparation of a 1-cm-long coral colony (frag-

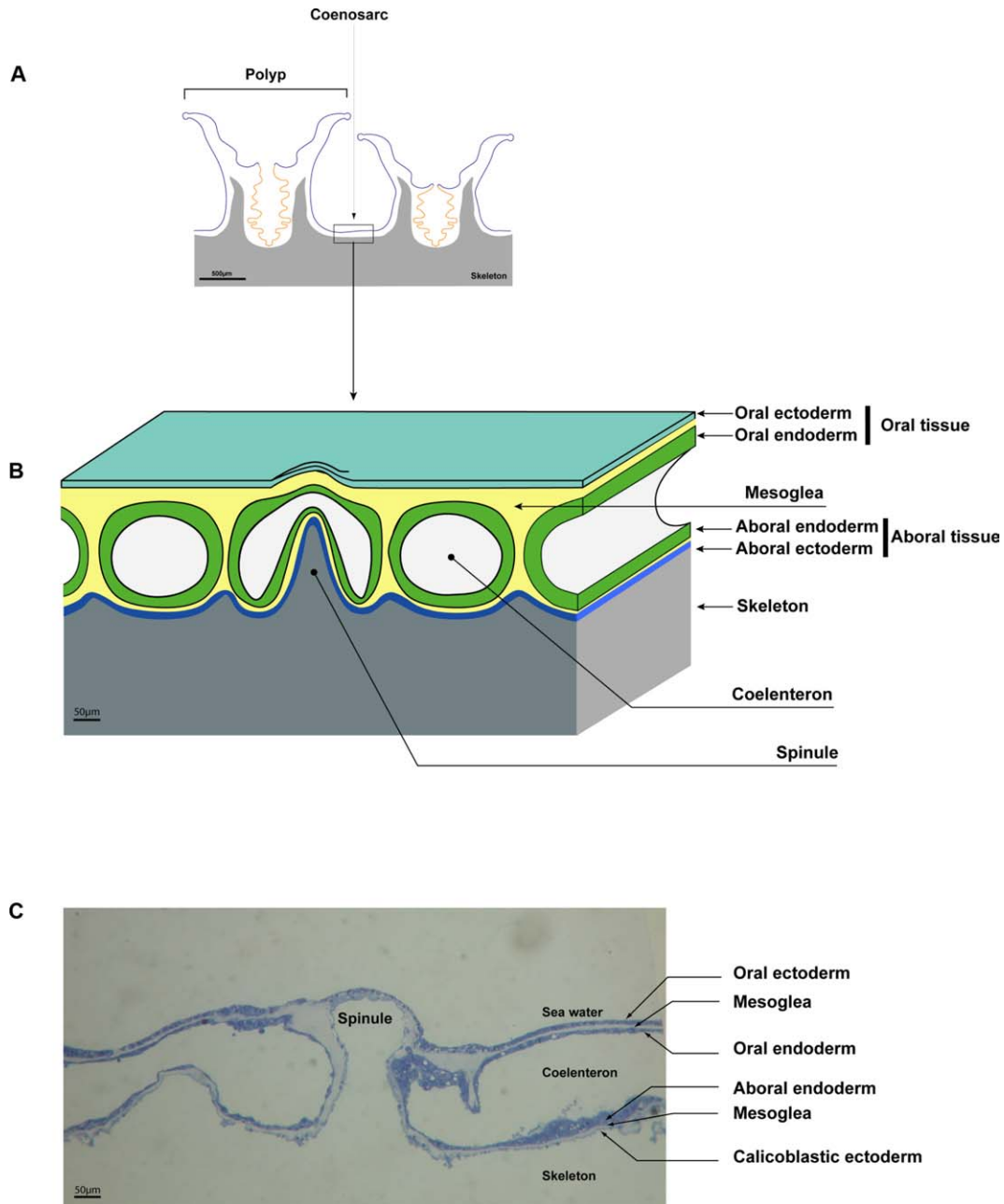


Fig. 1. Anatomy and histology of coral polyps. (A) Schema showing polyps linked together by the coenosarc tissue. (B) Scheme showing structure of coenosarc covering the skeleton. (C) Histological section of coenosarc showing oral and aboral tissues each composed of ectoderm and endoderm separated by mesoglea.

Fig. 1. Anatomie et histologie de polypes coralliens. (A) Schéma représentant des polypes réunis par le tissu du coenosarc. (B) Schéma de la structure du coenosarc recouvrant le squelette. (C) Coupe histologique du coenosarc montrant les tissus oraux et aboraux, chacun d'eux constitué d'un endoderme et d'un ectoderme séparés par la mésoglée.

ment of branched coral like *Stylophora* or *Acropora*, or isolated polyps like *Galaxea*), totally covered by coral tissue and called microcolony [3,83,84], (ii) a protocol adapted to rinse the coelenteric cavity (half-life time of filling, 4 min; [83]).

4.1. Ion supply

4.1.1. Calcium

Using a kinetic isotopic approach, it has been shown that radioactive calcium is incorporated into the skeleton of *Stylophora pistillata* only 2–4 min after its addition in the surrounding seawater [85], suggesting that transepithelial transport must be huge and very efficient. In this species, calcium flux is about $140 \text{ nmol h}^{-1} \text{ cm}^{-2}$, but values as high as $1700 \text{ nmol h}^{-1} \text{ cm}^{-2}$ have been measured in *Acropora palmata* [6]. Calcium fluxes measured in mammalian kidney or intestine are much lower (about 50 to $250 \text{ nmol h}^{-1} \text{ cm}^{-2}$, [11]). Within the microcolony, the calcium pool available for calcification is small and presents a very high turnover rate (i.e. 2 min, [85]).

By a pharmacological approach, using calcium channel inhibitors, two authors [54,85] demonstrated that transepithelial calcium transport involves at least one transcellular pathway. In the coral *Stylophora pistillata*, Tambutté et al. [85] showed that transport across oral layers was passive, the diffusional calcium flux between external sea water and the coelenteric fluid being 13-fold higher than the rate of calcification (i.e. respectively $12.6 \text{ nmol}_{\text{Ca}} \text{ min}^{-1} \text{ mg}^{-1}_{\text{protein}}$ and $0.98 \text{ nmol}_{\text{Ca}} \text{ min}^{-1} \text{ mg}^{-1}_{\text{protein}}$), suggesting that this step is not limiting. This result has been confirmed by Bénazet-Tambutté et al. [85] using other approaches as Ussing chambers. These authors showed that transepithelial ion transport across oral layers in the coral *Heliofungia actiniformis* and the sea anemone *Anemonia viridis* is passive and diffusional. Nevertheless, other data obtained in the coral *Galaxea fascicularis* showed that an active transepithelial transport might occur within oral layers [95], suggesting species specificity.

As the major transepithelial step does not occur at the oral level in *Stylophora pistillata*, we investigated where it could be located. Combining pharmacological and isotopic approaches, Tambutté et al. [85] showed that the calciblastic epithelium is the site of active transport. This transcellular pathway involves L-type

Ca^{2+} channel proteins [85]. The $\alpha 1$ subunit of this channel has been cloned and immuno-localized on the calciblastic epithelium [99]. This protein, of a calculated molecular mass of 213.2 kDa (accession number, Genbank database: U64465) shares with the rabbit $\alpha 1\text{C}$ subunit 52.5% and 86% identities and conservative substitutions, respectively. Opening of L-type Ca channel is normally regulated by membrane depolarisation, and its presence is generally restricted to excitable cells. Therefore, its presence within a transporting epithelium carrying out large and permanent Ca^{2+} fluxes appears surprising. This paradox may be explained by preliminary electrophysiological experiments (Zoccola et al., unpublished results) showing that, in spite of a strong homology to L-type Ca channel, the *Stylophora* calcium channel is not regulated by voltage.

Isa et al. [47] and Ip et al. [44] demonstrated the existence of a Ca^{2+} -ATPase activity in the homogenates of coral tissues. Since it has been demonstrated that this ATPase functions as an obligatory $\text{Ca}^{2+}/\text{H}^{+}$ exchanger with a probable stoichiometry of 1 to 1 [75], McConnaughey [57] (see also [58]) hypothesizes that the H^{+} generated by CaCO_3 precipitation is removed by Ca^{2+} -ATPase-mediated $\text{Ca}^{2+}/\text{H}^{+}$ exchange. Preliminary results using a molecular biology approach demonstrated the presence of this Ca^{2+} -ATPase within the calciblastic cells [100]. Fig. 2A presents a schematic diagram showing the pathway of entry and exit of calcium through the calciblastic cells during calcification in hermatypic corals.

At present, no data is available concerning the pathway of calcium transfer within the calciblastic cells. In order to maintain the likely intracellular free calcium concentration of $0.1 \mu\text{M}$, Ca^{2+} must be buffered either by Ca^{2+} -binding proteins (CaBP, whose presence remains to be demonstrated) or sequestered in vesicles (nevertheless not observed in the calciblastic cells).

4.1.2. Carbon

Study of source and supply of dissolved inorganic carbon (DIC) is far more complex than calcium study, since DIC can be derived either from seawater or from host respiration. Moreover, it has several chemically interconvertible species, a non-ionic form (CO_2 present in sea water at a concentration of about $12 \mu\text{M}$), freely diffusible across lipid membranes according to

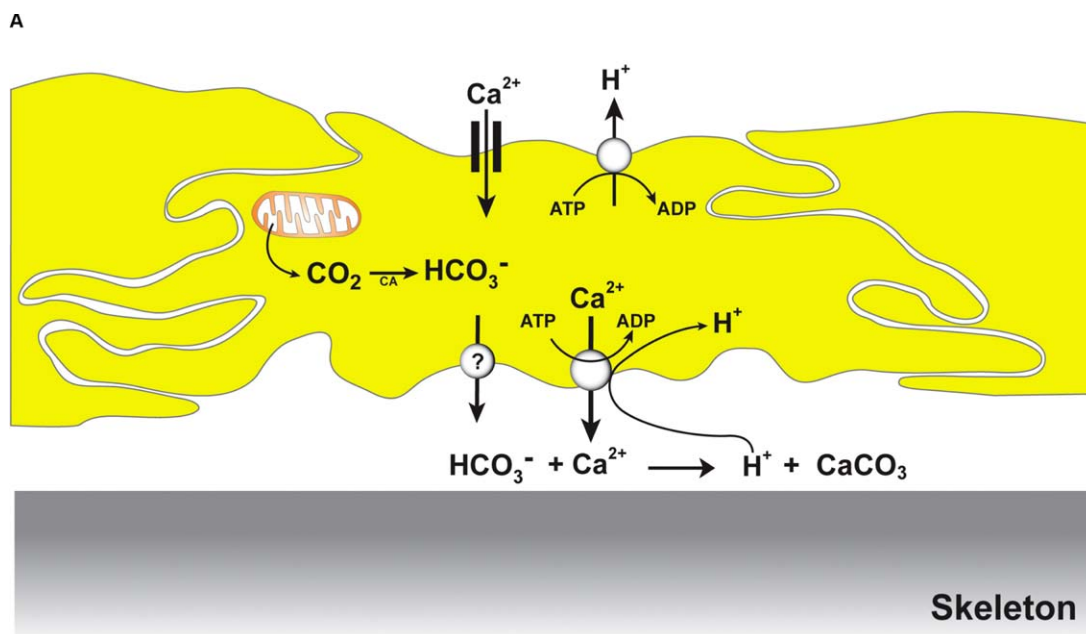


Fig. 2. (A) Schematic diagram showing the pathway of entry and exit of calcium through the calcicoblastic cells during calcification in hermatypic corals. This pathway is based on the data of Zoccola et al. [100] for Ca^{2+} -ATPase, and Bénazet-Tambutté et al. [8], Tambutté et al. [83,85] and Zoccola et al. [99] for the calcium channel. (B) Histogram showing the effects of a carbonic anhydrase inhibitor (ethoxzolamide, EZ) and an anion transport inhibitor (4-4'-diisothiocyanatostilbene-2,2'-disulphonic acid, DIDS) on the calcification rate of *Stylophora pistillata*.

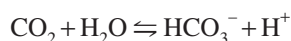
Fig. 2. (A) Diagramme schématique montrant les cheminements d'entrée et de sortie du calcium à travers la cellule calcicoblastique pendant la calcification d'un corail hermatypique. Ces voies sont fondées sur les données de Zoccola et al. [100] pour la Ca^{2+} -ATPase, et de Bénazet-Tambutté et al. [8], Tambutté et al. [84,85] et Zoccola et al. [99] pour les canaux calcium. (B) Histogramme montrant les effets de l'inhibiteur de l'anhydrase carbonique (éthoxazolamide, EZ) et d'un inhibiteur du transport d'anions (acide 4-4'-diisothiocyanatostilbène-2,2'-disulfonique, DIDS) sur le taux de calcification de *Stylophora pistillata*.

its gradient, and two ionic forms (HCO_3^- , CO_3^{2-}) requiring specific carrier proteins. The concentration of these ions is much higher, 2 mM for HCO_3^- and 0.4 mM for CO_3^{2-} . Their equilibrium depends on seawater pH [15,36]. As DIC is used both for calcification and photosynthesis, the study becomes even more

complex and shows the interrelation between calcification and photosynthesis. This complexity leads to a lack of data concerning DIC transport in corals.

Recent experiments using a double-labelling technique with ^{45}Ca and H^{14}CO_3 showed that ^{14}C is incorporated into the coral skeleton at the same rate as Ca^{2+} ,

suggesting that (i) both transports are very efficient, and (ii) the carbon pool for calcification, as for calcium, is small and presents a high turnover rate [35]. However, the rate of carbon incorporation underestimates the rate of calcification, compared to ^{45}Ca incorporation, by 40–60%. This suggests, as previously shown by Goreau [41] and Erez [25], that part (about 60–70%) of the carbon used for CaCO_3 formation may originate from respired CO_2 . Carbonic anhydrase, which plays a key role in numerous physiological processes in vertebrates and invertebrates [10], is also involved in the calcification process in corals. This enzyme speeds up the following equilibrium:



Carbonic anhydrase has been histochemically localized within the calcicoblastic epithelium [46]. Its inhibition by ethoxycarbonylamilofen, a permeant inhibitor, reduces by 80% the rate of calcification, suggesting that carbonic anhydrase increases the rate of equilibration between CO_2 produced by calcicoblastic cell respiration and HCO_3^- used as a substrate for CaCO_3 precipitation [85] (see Fig. 2B). It is significant that calcicoblastic cells are particularly rich in mitochondria [48] and that respiration of *Stylophora pistillata* microcolonies in the dark releases at least $240 \text{ nmol}_{\text{CO}_2} \text{ h}^{-1} \text{ mg}_{\text{protein}}^{-1}$ [35], which is 5-fold higher than the calcification rate measured in the same species (i.e., $50 \text{ nmol h}^{-1} \text{ mg}_{\text{protein}}^{-1}$ [85]).

Calcification has been shown to be completely inhibited by the anion channel inhibitor, DIDS [85] (Fig. 2B), suggesting that an anion carrier is involved in the transport of DIC across the calcicoblastic cells. This anion carrier is likely located on the calcicoblastic plasma membrane adjacent to the skeleton. Consequently, sources of DIC are (1) metabolic CO_2 and (2) HCO_3^- directly absorbed by the calcicoblastic cells by a mechanism remaining totally unknown (HCO_3^- channel as reported in the sea anemone by [34]). The cytoplasmic carbonic anhydrase would carry out the equilibrium between these two forms. An anion exchanger (perhaps the $\text{Cl}^-/\text{HCO}_3^-$ antiport known for its sensitivity to DIDS, [27]) could then transfer HCO_3^- to the site of calcification (secondary active transport). This raises the question of the composition of the fluids within the sub-calicoblastic space. Nothing is known about the composition of this fluid in corals, and little is known in other calcifying animals. It seems, how-

ever, that the inorganic carbon content of this fluid is higher than that of the surrounding medium in order to reach the solubility product. In molluscs, it has been shown that the dissolved inorganic carbon content of the extrapallial fluid is at least 2-fold higher than that of seawater [16]. Among the vertebrates, the fluid surrounding the otolith in fishes (i.e. the otolymph) contains about 2–3 fold more HCO_3^- than the blood [66] and concentrations of HCO_3^- as high as 130 mM have been reported in the uterine fluid of chickens during eggshell formation [63]. These data strongly suggest that an active mechanism of transport is involved to supply carbon to the site of calcification.

4.1.3. pH regulation

Nothing is known about pH regulation during calcification process. As stated above, CaCO_3 production leads to H^+ production. These H^+ may be removed from the site of calcification by the antiport $\text{Ca}^{2+}/\text{H}^+$ -ATPase. Preliminary results (Zoccola et al., in prep.) suggest that regulation of pH in the calcicoblastic cells may involve an H^+ -ATPase rather than classic systems such as Na^+/H^+ antiport (Fig. 2).

4.2. Organic matrix

It is now firmly established that organic matrix (OM) plays a major role in biomineralisation: crystal nucleation, micro- and macro-regulation of crystal morphology, mineral characteristics... [26,90,91]. As in all other biominerals, coral skeleton contains an organic matrix that represents less than 0.01% of the total weight of dry skeleton [14]. While small in quantity, this OM is nevertheless essential for controlling the formation of CaCO_3 crystals, since inhibition of the organic matrix synthesis by emetin or cycloheximide, or inhibition of the post-translational glycosylation of the proteins by tunicamycin, almost instantaneously stops the calcification process [1]. It should be noted that these inhibitors do not simultaneously affect photosynthesis and respiration.

Biochemical composition of OM in corals is poorly known, but the presence of proteins, polysaccharides, glycosaminoglycans [14] as well as of lipids [98] and chitin [88] has been reported in some species. While this notion is presently questioned (see [67]), OM is usually separated into soluble and insoluble matrices. The soluble fraction of this organic matrix is very rich

in acidic amino acids that can represent almost half of the proteins [14,18,19,59,97]. These proteins may also be sulphated or glycosylated [14,22,23].

While the proteins of OM of some invertebrates have been characterized (see papers by Luquet, Marin or Nys in this volume), only one protein has been sequenced in corals, galaxin, isolated from the skeleton of *Galaxea fascicularis* by Fukuda et al. [31]. This protein, of a molecular weight of 53 kDa, contains 298 amino acids, arranged in tandem repeats of about 30 amino acids rich in cysteine, serine and glycine. There are only few (less than 10%) acidic amino acids. This protein shows no homology with known proteins in databases.

Very few data exist on the metabolism of organic matrix. Using ^{14}C -labelled aspartic acid as a precursor of organic matrix, Allemand et al. [1] showed that this amino acid is rapidly incorporated into tissue proteins from the external medium, suggesting (i) that the biosynthetic pathway is very efficient and (ii) that no intracellular pool of aspartic acid is present in coral tissue. After a lag phase of 20 min, organic matrix proteins are incorporated within the skeleton. This lag time likely represents the exocytosis of organic matrix and its incorporation into the CaCO_3 crystal. Thus depositions of organic and inorganic fractions of the skeleton are performed simultaneously and not sequentially, as suggested in other biominerals, such as fish otoliths [60].

Therefore, calcification in hermatypic corals is controlled both by the cellular supply of ions (Ca^{2+} , HCO_3^-) and the organic matrix. The rate of ion transport and organic matrix synthesis and exocytosis is very high (< 2 min). Consequently, calcification is a highly biologically controlled mechanism.

5. Interactions between photosynthesis and calcification

Major calcifying organisms are also phototrophs, either directly (coccolithophorids) or indirectly through symbiosis (foraminifera, reef-building corals). All these organisms are responsible for the major part of the production of CaCO_3 of the earth. In these organisms, calcification is highly dependent on light, and values of light-enhanced calcification up to 127 have been reported, even if the mean stimulation value

is around 3 (review by [36]). However, mechanisms underlying these interactions are not known and even the terminology is discussed between light-enhanced or dark-repressed calcification [54] (see discussion in [36]). Several mechanisms have been hypothesized to explain these interactions:

- modification of the DIC equilibrium within coral tissues caused by CO_2 uptake for photosynthesis [40,58];
- production of energy by photosynthesis [13];
- production of O_2 by photosynthesis [74];
- removal of inhibiting substances [77];
- synthesis by symbionts of organic matrix molecules or precursors [61].

Modification of DIC equilibrium may involve either photosynthetic stimulation of calcification by lowering the CO_2 partial pressure in the coral tissue, thus favouring carbonate precipitation [39,40] (see Fig. 3A) or dehydration of HCO_3^- to CO_2 by H^+ produced during calcification, in turn stimulating photosynthesis [57,58] (see Fig. 3B). Both hypotheses assume that HCO_3^- freely diffuses into the coelenteric cavity, which, as shown above, is unlikely to be the case. Furthermore, Furla et al. [34] showed that light-enhanced calcification is a late process, occurring only 10 min after the onset of the photosynthetic process.

An alternative mechanism may involve photosynthesis-induced OH^- secretion within the coelenteric cavity (Fig. 3C). This light-dependent OH^- secretion may neutralize H^+ produced by CaCO_3 precipitation, thus allowing calcification to proceed. The net rate of OH^- efflux under saturating irradiance is $3.55 \text{ nequiv min}^{-1} \text{ mg}^{-1}_{\text{protein}}$ [33], while calcification in the scleractinian coral *Stylophora pistillata* occurs at a rate of $0.98 \text{ nequiv min}^{-1} \text{ mg}^{-1}_{\text{protein}}$ [85], i.e. at a rate 3.6-fold higher. Notwithstanding the fact that such a comparison between two phylogenetically distinct organisms may not be valid, it can be tentatively suggested that OH^- efflux may be sufficient to buffer calcification-induced H^+ production. In this way, calcification and photosynthesis may proceed independently, which is in agreement with data showing that calcification may be inhibited without altering photosynthesis [1,96]. In the dark, acidification of the coelenteric cavity caused by increased CO_2 together with low O_2 tension [42] and reduced ATP, causes inhibition of calcification. In the light, high O_2 tension increases the respiratory rate and thus CO_2 production

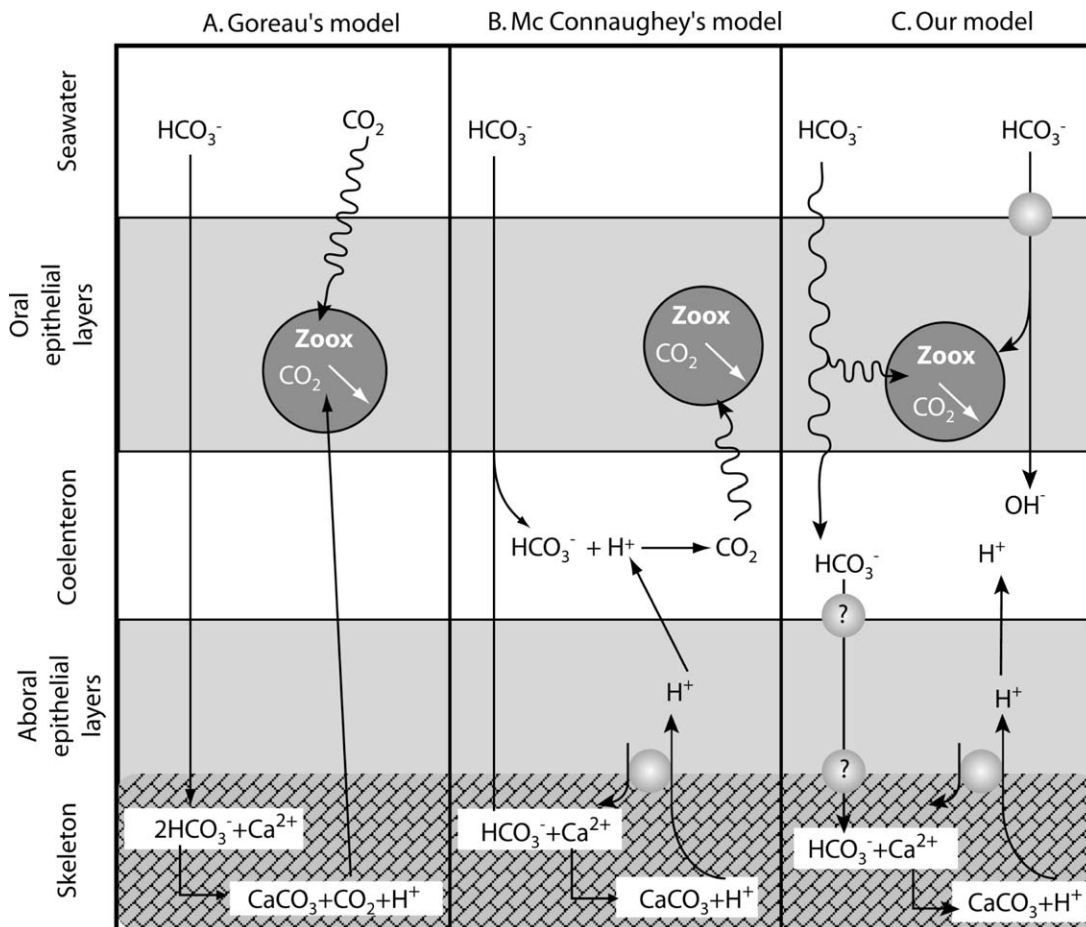


Fig. 3. Light-enhanced calcification: schematic diagram showing different hypotheses on the relationship between photosynthesis and calcification (see text for details). Zoox: zooxanthellae.

Fig. 3. Calcification stimulée par la lumière : schéma montrant différentes hypothèses sur la relation entre la photosynthèse et la calcification (voir le texte). Zoox : zooxanthelles.

as a substrate for calcification and production of ATP for energy-dependent carriers, in conjunction with high pH within the coelenteric cavity, which helps to neutralize H^+ produced by calcification.

Another hypothesis has been recently suggested by the group of J.-P. Cuif [20,38]. By analysing the composition in amino acids of OM from 13 symbiotic and 11 non-symbiotic corals, they showed that this composition is dependent upon the presence of symbionts. If aspartic acid remains the major amino acid in both groups of corals, the amount of glutamic acid is always higher in symbiotic vs. non-symbiotic corals. Similarly, threonine is high in symbiotic corals and low in non-symbiotic ones, while the opposite is observed for threonine. OM content of sugars (mannose, galactose,

galactosamine, glucosamine) is also significantly different in the two groups of corals. All these results suggest that symbionts may affect OM precursor synthesis, or may provide these precursors. This hypothesis is not exclusive with that of DIC equilibrium.

6. Environmental control of coral calcification

One goal of coral-reef ecologists is to predict the effects of environmental conditions (natural or anthropogenic) on the integrity of scleractinian coral communities. It is now well established that a change in most of the environmental parameters such as light, temperature, CO_2 partial pressure (p_{CO_2}) and nutrients di-

rectly affects coral growth rates and thus coral calcification. It has become patently clear that coral reefs around the world are coming under increasing pressures and their status is declining rapidly [92]. One prominent example of stress is the increased frequency of mass coral bleaching events (loss of chlorophyll, zooxanthellae and reduced calcification) since the early 1980s [43]. Corals are subject to stresses at the local, regional, and global scales. At the local and regional scale, reefs deteriorate in all areas where human activities are concentrated, due to an increase in seawater pollution (heavy metals, eutrophication, bacterial and viral diseases), and human pressures (diving, fishing, tourism...). In addition to these stresses, corals also experience the effect of the ‘global change’ [45], i.e. Increased seawater temperature, ultra-violet radiations, and p_{CO_2} as well as increased sediment loading.

Therefore, major actions were undertaken to better understand the effects of a change in the main environmental parameters on the survival of reef corals, and especially the effects on their rates of calcification. Effects of eutrophication, of increased p_{CO_2} levels and seawater temperature on coral calcification have attracted scientists’ attention. Eutrophication corresponds to an increase in nutrient concentrations, in sediment load and in a development of algae [50], all of them reducing light available to the corals and necessary for their calcification. Laboratory and in situ experiments have also reported a decrease in calcification under nutrient-enriched conditions. At the community level, Kinsey and Davies [49] measured a pronounced decrease (50%) in the overall calcification following an 8-month fertilization period of a patch reef system in the Great Barrier Reef. Tomascik [86] and Tomascik and Sanders [87] found a correlation between anthropogenic disturbances and decreased skeletal growth in the coral *Montastraea annularis*. At the organism level, several studies have monitored a lower calcification under different enrichments in ammonium, nitrate, and phosphorus [28,29,55,82], up to 50%. An iron enrichment, often seen during El Niño periods, also decreases by 20% the growth of scleractinian corals [29]. The way by which nutrients interact with calcification is still poorly known. They generally highly enhance the growth of the algal component, and this might lead to a disruption of the symbiosis, and especially the coupling between photosynthesis and calcification. Calcification is also affected by an increase in

the p_{CO_2} , which has risen from 280 to 370 μatm since the last century, as a result of human activities and could reach 705 μatm in year 2100 [45]. An increase in seawater p_{CO_2} induces a change in the other carbon components such as bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) and corresponds to a decrease in CO_3^{2-} . Coral calcification seems however intimately linked to the calcium-carbonate saturation state (ω), which is the ratio of the ion concentration product ($[\text{Ca}^{2+}] \times [\text{CO}_3^{2-}]$) to the solubility product of the mineral deposited. Therefore, a decrease in the concentration of CO_3^{2-} in seawater has been related to a sharp decrease in coral calcification, at the organism or community level [37,51,53,56]. Together with the change in p_{CO_2} , the global warming also corresponds to an increase in the mean sea surface temperature, which is expected to rise by 1–3°C over the coming century [68]. This is leading to the frequent El Niño events [93]. The bleaching and death of the coral colonies after an El Niño event is generally followed by a strong coral bioerosion, reducing the 3-D reef structure [43]. When both higher level of p_{CO_2} and temperatures are combined, coral calcification is lowered by 50% [73].

In conclusion, coral reef ecosystems are under the threats of local direct anthropogenic stresses, and of the more extended stresses of global change, which include, in addition to a raise in temperature and p_{CO_2} , an elevation of the sea level and in the ultraviolet radiations.

7. Corals as paleoenvironmental archives

“There has never been any doubt that corals write valuable information into their skeletons; it is their language that has remained blurry and ambiguous” [7].

Anticipation of future environmental and climatic change depends upon knowing and, hopefully, understanding what has happened in the past and is now happening. However, instrumental records of weather and climate go back only about several centuries and that time span is insufficient to judge whether recently observed rises in global temperature are normal or not. Corals can provide climate and environmental records for the world’s shallow-water tropical ocean regions, since these areas are poorly represented by other proxies (tree rings, ice cores, sediments, pollen). Moreover,

massive species can provide information both for the last several centuries (from living corals) and for well-dated windows of the more distant past (from well-preserved dead and fossil corals).

Corals build their skeletons from calcium and carbonate extracted from seawater, so their skeleton contains isotopes of oxygen, carbon, as well as trace metals (Sr, Mg, U, Th...). Thus, proxy climate and environmental information are stored in coral skeletons as growth characteristics (e.g., skeletal extension, density and calcification). Examples of information stored in coral skeletons include sea-surface temperatures (SSTs), river flow, rainfall, upwelling, salinity and anthropogenic influences.

The great majority of corals used for palaeo-reconstructions are massive genus (as *Porites* or *Diploastrea*). Corals are drilled and the sampling skeleton is cut in order to obtain a slice (1 cm width). Coral skeleton is characterized by annual density bands visible on X-ray radiographies (Fig. 4). The sum of a dark (high-density) and a light (low-density) band represents one year [7]. This characteristic provides a built-in chronometer that enables first-order dating as well as identification of particular years and specific seasons. Once years are located, very small samples of skeleton are taken on a line along the coral growth axis, and isotopic composition of oxygen ($\delta^{18}\text{O}$) and carbon ($\delta^{13}\text{C}$) are analysed with a mass spectrometer.

The skeletal $\delta^{18}\text{O}$ varies with temperature and salinity of the water where the coral grew in. The isotopic composition is expressed in the conventional delta notation relative to a standard, which is PDB for carbonates:

$$\delta^{18}\text{O} = \left[\frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}}}{(^{18}\text{O}/^{16}\text{O})_{\text{standard}}} - 1 \right] \times 10^3$$

However, the promise of coral density bands to yield similar fabulous records to those obtained from tree rings has not been yet realized despite extensive works. One problem is that such proxies need to be calibrated before their use. The great majority of calibrations have been carried out in the field (see for example [17,89]); however, calibrations carried out in the laboratory under controlled conditions seem necessary to decipher the effect of each environmental parameter (temperature, salinity, light...). Such an approach has been used successfully with foraminifera (e.g., [81]) but difficulties encountered with culture techniques have precluded the development of experimental calibrations of proxy records in corals. However, recently several works have been done concerning the effects of several parameters (temperature, light, nutrition, $p\text{CO}_2$) on coral isotopic composition [70–72].

Recently, it has been shown that isotopic record is profoundly influenced by biology (so-called ‘Vital Effect’): different coral colonies cultured in the laboratory under exactly the same temperature conditions have yielded $\delta^{18}\text{O}$ measurements that present a huge variability (2‰) (Fig. 5, *Stylophora pistillata*). However, when $\delta^{18}\text{O}$ measurements are done on nubbins from the same parent colony, there is no such variability (Fig. 5, *Acropora* sp.). This suggests that a calibration curve is specific for each colony considered.

We thus concluded that the $\delta^{18}\text{O}$ recorded in coral skeleton is affected not only by temperature and salinity of the seawater, but also by the biological activity.

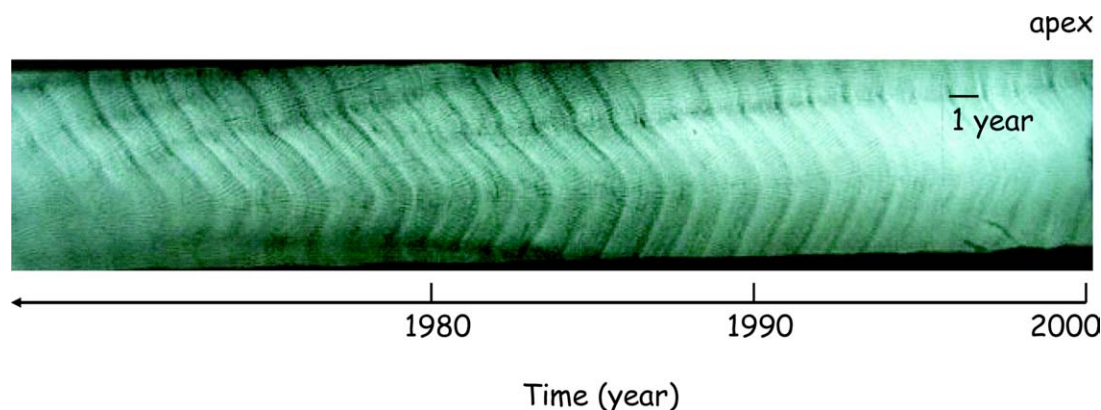


Fig. 4. X-ray radiography of a slice of the *Porites* skeleton. Annual bands are easily visible to date the samples.

Fig. 4. Radiographie d'une tranche dans le squelette de *Porites*. Les bandes annuelles permettent une datation facile de l'échantillon.

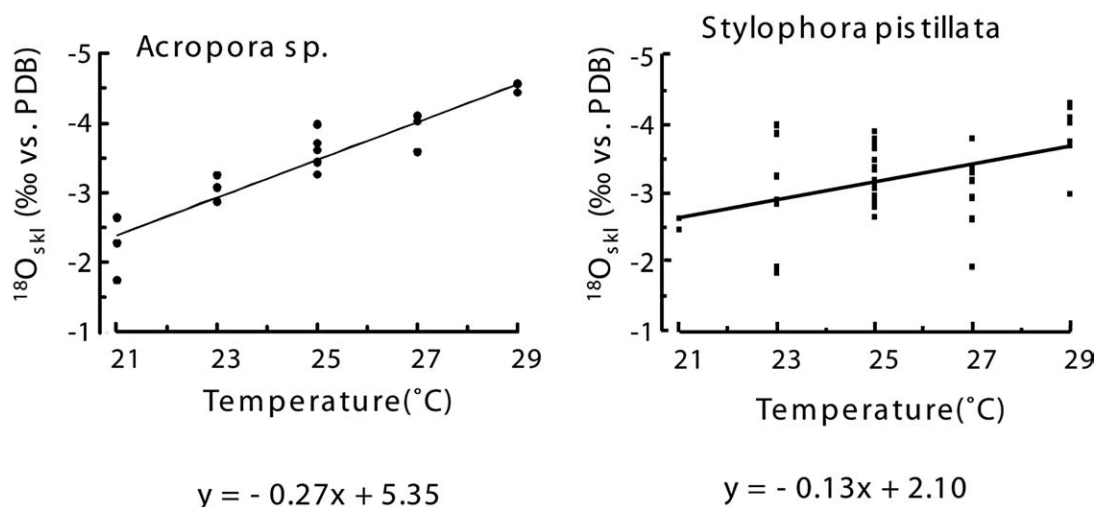


Fig. 5. Relationship between skeletal $\delta^{18}\text{O}$ and temperature in two different coral genus cultured in laboratory under controlled conditions.
 Fig. 5. Relations entre le $\delta^{18}\text{O}$ et la température chez deux genres différents de coraux cultivés en laboratoire sous conditions contrôlées.

This last point must to be taken into account in future palaeo-reconstructions.

8. Conclusions

This review highlights the complexity, but also the weakness of our knowledge concerning biomineralisation mechanisms in corals. Formation of a coral skeleton is the result of complex, biologically regulated phenomenon, largely unknown. Due to the fact that coral reefs are presently disturbed by human activities, it is urgent to develop research on coral biology, associating physiologists, molecular and genome biologists, geneticists, ecologists... Beyond the benefit for our environment, a better knowledge of coral calcification will help to understand biomineralisation mechanisms by themselves, which remain poorly understood, even in mammals: “Bone physiology is undoubtedly the source of many concepts of biomineralization, but it remains as one of the few physiological processes for which there is a unified theory and one of the few medical interests that have not benefited from comparative studies” [78, page 292].

Acknowledgements

Work reviewed in this paper has been funded by the Centre scientifique de Monaco (Principality of Mo-

naco). Palaeoclimatological studies benefited from a post-doctoral grant to S.R. from Prof. Richard Fairbanks (Lamont Doherty Earth Observatory, USA). We are grateful to Prof. P. Payan (UNSA), Prof. J.-P. Cuif (Orsay), Dr Anne-Juillet Leclerc (CNRS/CEA) for fruitful discussions and to Profs A. de Ricqlès and J. Livage (Collège de France) for their invitation to participate to this symposium.

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