doi:10.1017/S1431927616012101
Microsc. Microanal. 22 (Suppl 5), 2016

Tracking the Early Events of Mineral Formation during Coral Development

Anat Akiva[1, #], Maayan Neder[2] and Tali Mass[2]

[1.] Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel.
[2.] Marine Biology Department, The Leon H. Charney School of Marine Sciences, University of Haifa, Mt. Carmel, Haifa 31905, Israel.
[#.] Present address: Laboratory of Materials and Interface Chemistry and Center of Multiscale Electron Microscopy, Department of Chemical Engineering and Chemistry, Eindhoven University of Technology, The Netherlands.

In the tropical and subtropical oceans, corals provide a literal and figurative ecological framework that retains nutrients, supports high rates of primary production, and permits extensive biological diversity. These fragile ecosystems are threatened with extinction in the coming century, in part due to the acidification of the ocean [1-2]. The change in the acidity of the ocean may affect the coral skeleton formation, which is composed of calcium carbonate mineral. Elucidating the mechanism of calcium carbonate mineral formation in corals may help us to understand how environmental changes affect the process of coral biomineralization. Although various aspects of biomineralization in corals have been studied for decades, mostly on adult corals, the basic mechanism responsible for the precipitation of calcium carbonate in the form of aragonite remains enigmatic [reviewed by 3].

Corals have a biphasic life cycle with planktonic larval stages and benthic adults. These two phases are separated by settlement and metamorphosis, which are critical stages in coral development during which planulae undergo intense changes in morphology, building of new tissues, initiation of calcium carbonate precipitation, and in some cases uptake of symbionts. The planktonic free-swimming planulae deposit minerals almost immediately after settlement [4]. This suggests that immature mineral phases (presumably amorphous calcium carbonate - ACC) is present in pre-settled planulae.

To elucidate the key mechanism that facilitates the initial, rapid calcification in the early stages of the coral development, we correlate cryo-scanning electron microscopy (SEM) with cryo-energy-dispersive X-ray spectroscopy (EDS) and cryo-fluorescence techniques on coral planulae frozen samples [5]. Using this approach and thus, avoiding the process of chemical fixation, dehydration and chemical staining with heavy metals, each planula is rapidly frozen at high pressure. As a result, the water is vitrified while keeping the sample as close as possible to its native state. We observed freeze-fractured surfaces of the sample using the cryo-SEM with both secondary electron and backscattered electron (BSE) detectors. The elements that are present in the mineralized regions are detected by EDS under cryogenic conditions. The cryo-fluorescence platform helps to identify auto-fluorescent symbionts and to detect mineralized regions in the developing planula via calcein blue staining.

Our results show that first mineral deposition in corals already starts at the pre-settlement (Fig. 1). Immature minerals can vary in shape, Mg content and crystallinity. After settling, the aboral epidermis is attached to the substrate and begins skeleton formation. The first calcareous elements after settling are circular platelets and rod-shaped granules, which can also vary in Mg and Ca content and aggregate to form the primary septa, followed by the formation of the basal disk [6,7].

References:

[1] T Hughes *et al*, Science **301** (2003), pp. 929-933.
[2] IPCC, Climate Change (2013).
[3] D Allemand *et al* in "Coral reefs: An Ecosystem in Transition", ed. Z Dubinsky and N Stambler, (Springer, New York) pp. 119-150.
[4] JS Jell Acta Palaeontologica Polonica **25** (1980), pp. 311-320.
[5] GM Khalifa *et al*, Journal of Structural Biology (2016), available online.
[6] J Vandermeulen and N Watabe, Marine Biology **23** (1973), pp. 47-57.
[7] The authors acknowledge funding from the Israel Science Foundation (Grant 312/15) and from the United States-Israel Binational Science Foundation (BSF; Grant # 2014035).

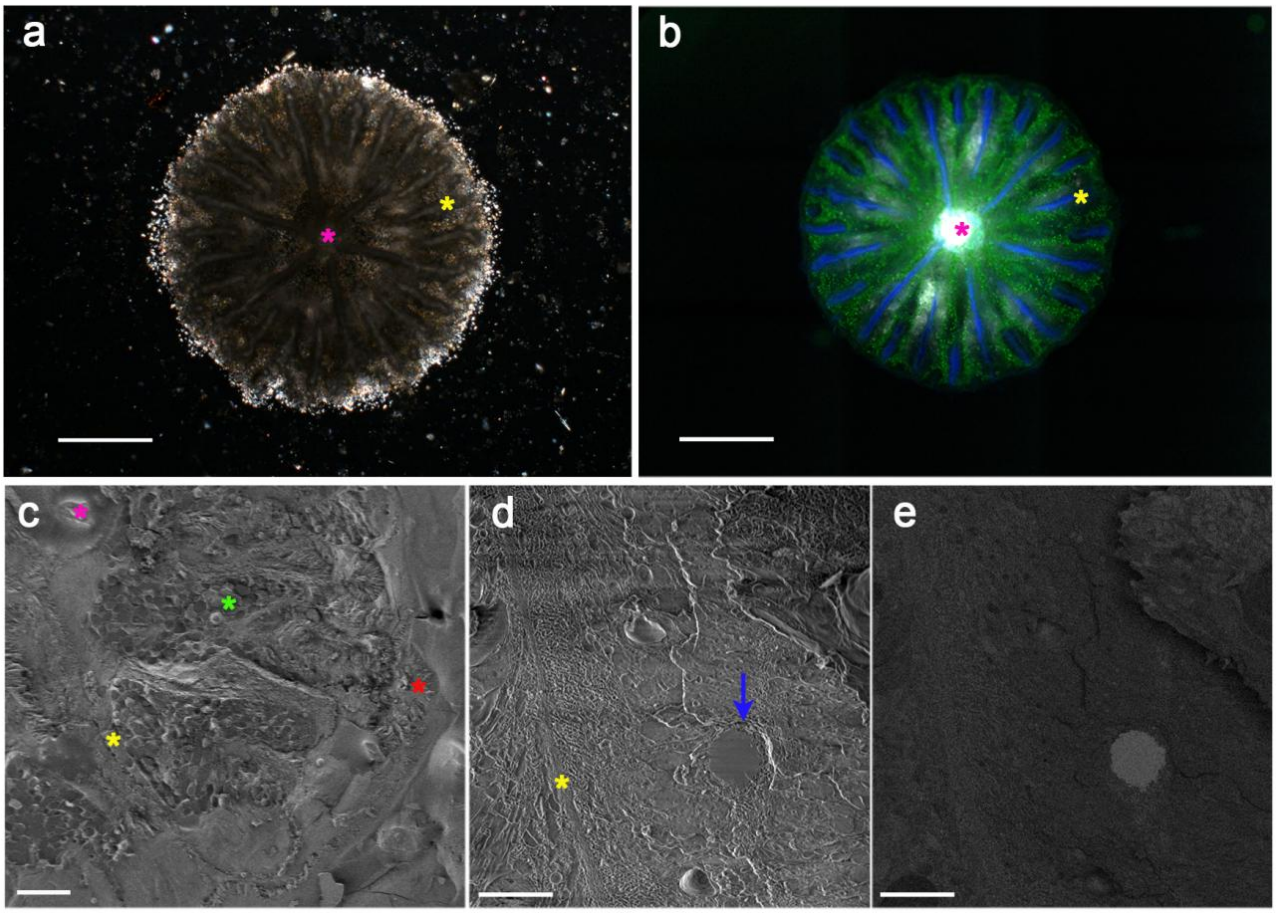

Figure 1. Coral planula morphology: a-b) at early stage of settlement, c-e) at the free swimming stage: a) polarized light shows the mineral distribution in the coral planula body. (b) Confocal fluorescence image of a coral planula. Green and white fluorescence are generated from autofluorescent proteins in the planula body. The mineral is stained by calcein blue. c-e) Cryo-SEM images of freeze fracture coral planula. c) Secondary electron image shows the coral morphology. d) Secondary electron image shows early event of mineralization (blue arrow). e) Back scattered electron image of the same region as in d) shows electron-dense material that corresponds to the mineralized area. Asterisk color code: oral disk (pink), primary septum (yellow), stinging cells (red), lipid-containing cells (green) Scale bar: a-b: 500 µm, c: 100µm, d-e: 10 µm.

https://doi.org/10.1017/S1431927616012101 Published online by Cambridge University Press