LETTER

Biomineralization toolkit: The importance of sample cleaning prior to the characterization of biomineral proteomes

In an interesting work published recently in PNAS, Drake et al. (1) presented a proteomic study of the skeleton from the stony coral *Stylophora pistillata*. This study identified proteins that are associated to the mineral phase (i.e., that potentially contribute to shape the skeleton). In other words, this set of proteins is supposed to represent the so-called "biomineralization toolkit." Although some of the 36 proteins reported in Drake et al. (1) appear as genuine extracellular matrix (ECM) proteins related to biomineralization, such as coral acid-rich proteins or carbonic anhydrase, some others are obvious intracellular contaminants that should not be considered as skeletal organic matrix proteins (SOMPs).

Indeed, Drake et al. (1) observed proteins from the cytoskeleton, such as actins, tubulins, and myosin. These proteins are intracellular components and should not be named SOMPs: as far as we know, there is no scientific evidence that they interact directly with the growing biomineral. We consider that the integration of intracellular components to the growing list of calcifying-matrix proteins is misleading and detrimental to our understanding of biocalcification mechanisms and to the elaboration of molecular models, and this problem needs to be carefully appreciated.

In our hands, when similarly investigating SOMPs from the coral *Acropora millepora*, we observed cytoskeletal proteins that were contaminants from calicoblastic cellular debris (Fig. 1 and Table 1). These contaminants could be simply removed by extensive and appropriate cleaning of the biomineral (Fig. 1). By using two types of sample treatment, we demonstrated convincingly that the presence of cytoskeletal proteins indicates an inadequate cleaning of the biomineral structures, which typically hold superficial contamination from skeleton-neighboring tissues (Table 1).

According to the most commonly accepted view, the formation of metazoan calcified skeletons results from the secretion of an acellular matrix that remains occluded within the biomineral phase once precipitated. During this extracellular process, cellular contaminants can be entrapped in void structures (such as the microcavities present inside all the aragonitic skeleton of stony corals), and need to be removed by thorough incubation of skeleton fine powder (< 200 μm) in concentrated sodium hypochlorite [10% (vol/vol), 5 h] before extraction and further proteomic analysis of the biomineralization proteins. This simple treatment removes most—if not all—cellular debris, leaving intact the skeleton-associated proteins, the true SOMPs that are part of the biomineralization toolkit.

We are convinced by our previous experiments (2–4)—which are reproducible and coherent with the current understanding of biomineralization processes—that a careful and appropriate cleaning of biominerals is crucial for generating accurate proteomic data and further correctly interpreting the results.

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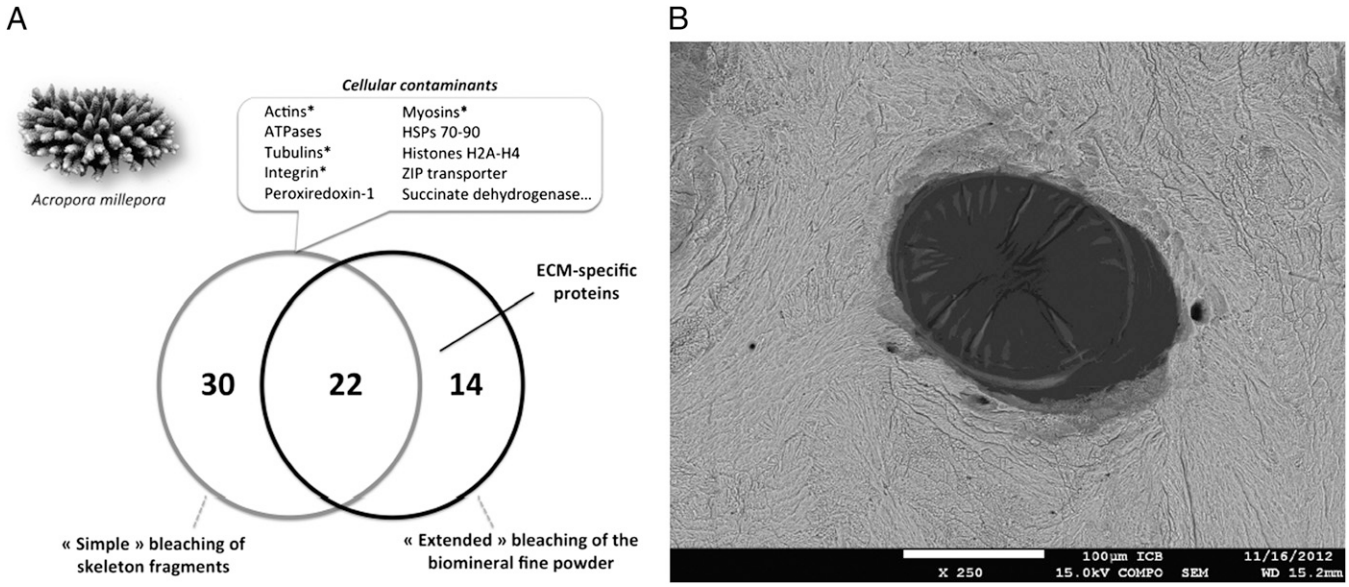

Fig. 1. Removal of organic contamination of *A. millepora*'s skeleton. (*A*) Comparison of the proteins identified by proteomics on the skeletal organic matrix of *A. millepora* in two different conditions. "Simple bleaching" consisted in treating the skeletal fragments with sodium hypochlorite solution once [5% (vol/vol), 72 h], and "extended bleaching" consisted in the simple bleaching followed by cleaning the skeletal sieved powder (< 200 µm) with sodium hypochlorite solution [10% (vol/vol) 5 h]. The asterisk represents similar proteins to those reported as ECM proteins in Drake et al.'s study (1). (*B*) SEM image from polished transversal section of *A. millepora* skeleton with focusing a pore covered with residual soft tissue that remained after cleaning the fragments by simple bleaching.

Table 1. List of the 30 proteins identified in the samples from coral skeleton treated by simple bleaching, which were further removed by extended bleaching

	Transcript references	BLASTP (above) and SwissProt reference (below)	E value
1*	>gi|379118176|gb|JT015846.1|	Actin sp|P12716.1|ACTC_PISOC	0.0
2	>gi|379125045|gb|JT022715.1|	Tubulin alpha-1C chain sp|P68365.1|TBA1C_CRIGR	0.0
3*	>gi|379099717|gb|JR997386.1|	Tubulin beta-4 sp|P30883.1|TBB4_XENLA	0.0
4	>gi|379084254|gb|JR981923.1|	Tubulin alpha-1C sp|Q9BQE3.1|TBA1C_HUMAN	0.0
5	>gi|379076599|gb|JR974268.1|	Tubulin alpha sp|P10872.1|TBA_TETPY	6e-85
6	>gi|379089391|gb|JR987060.1|	Tubulin alpha sp|P41351.1|TBA_TETTH	4e-161
7*	>gi|379122351|gb|JT020021.1|	Tubulin beta-4B sp|P68371.1|TBB4B_HUMAN	0.0
8	>mf105_rep_c206	ATP synthase beta Ssp|Q4FP38.1|ATPB_PELUB	0.0
9	>gi|379098186|gb|JR995855.1|	ATP synthase alpha sp|Q5R546.1|ATPA_PONAB	0.0
10*	>gi|379075456|gb|JR973125.1|	Myosin heavy chain sp|P24733.1|MYS_AEQIR	4e-06
11	>gi|379082904|gb|JR980573.1|	Myocilin Ssp|O70624.1|MYOC_MOUSE	7e-29
12	>gi|222798399|gb|EZ026787.1|	Histone H2A sp|P35061.2|H2A_ACRFO	1e-26
13	>gi|379114242|gb|JT011912.1|	Histone H2B sp|P35067.1|H2B_ACRFO	2e-76
14	>gi|379095792|gb|JR993461.1|	Histone H4; sp|P35059.2|H4_ACRFO	2e-65
15	>kb8_rep_c51392	Heat shock protein 90; sp|O44001.1|HSP90_EIMTE	0.0
16	>kb8_rep_c29387	Heat shock protein 90 Ssp|O44001.1|HSP90_EIMTE	0.0
17	>gi|379104815|gb|JT002485.1|	Heat shock protein 90 sp|O57521.2|HS90B_DANRE	0.0
18	>kb8_rep_c63048	Heat shock protein 70 sp|Q9S9N1.1|HSP7E_ARATH	3e-66
19	>gi|379073448|gb|JR971117.1|	Heat shock protein 70 sp|P63018.1|HSP7C_RAT	0.0
20	>kb8_c48899	Heat shock protein 70 sp|P11144.2|HSP70_PLAFA	0.0
21	>gi|379105500|gb|JT003170.1|	Zinc transporter ZIP14 sp|Q75N73.1|S39AE_MOUSE	1e-75
22	>gi|379096620|gb|JR994289.1|	Calpain-9 sp|O35920.1|CAN9_RAT	0.0
23	>gi|379108785|gb|JT006455.1|	Photosystem II precursor sp|P49472.1|PSBC_ODOSI	0.0
24	>gi|222803727|gb|EZ032115.1|	Voltage-dep. channel protein 2 Ssp|P81004.1|VDAC2_XENLA	5e-122
25	>gi|379104892|gb|JT002562.1|	Peroxiredoxin-1 sp|P0CB50.1|PRDX1_CHICK	9e-100
26	>gi|222782586|gb|EZ011257.1|	Succinate Dehydrogenase sp|Q7ZVF3.2|DHSA_DANRE	2e-65
27	>gi|379122454|gb|JT020124.1|	Endoplasmin sp|Q66HD0.2|ENPL_RAT	0.0
28*	>gi|379079965|gb|JR977634.1|	Integrin sp|P16144.5|ITB4_HUMAN	0.49
29	>gi|222799407|gb|EZ027795.1|	Transaldolase sp|B6JNZ3.1|TAL_HELP2	5.4
30	>kb8_c30860_frame-3	No hit	—

*Similar proteins to those reported as ECM proteins in Drake et al.'s study (1).