NANOFOSSILS AND THE SIZE LIMITS OF LIFE. H. Vali[1], S.K. Sears[2], N. Çiftçioglu[3], and E.O. Kajander[3]. [1]Electron Microscopy Centre, McGill University, 3640 University Street, Montreal, QC H3A 2B2 (vali@geosci.lan.mcgill.ca); [2]Department of Earth & Planetary Sciences, McGill University, Montreal, QC H3A 2A7; [3]Department of Biochemistry & Biotechnology, University of Kuopio, Kuopio, Finland

Fossils with well preserved morphological features, which resemble the remains of modern microorganisms, are used to trace evolutionary paths and events in biotic history[1]. Fossilization of microorganisms, however, cannot occur if suitable conditions for their preservation are not attained. Folk[2] suggested, however, that nano-scale particles, which are commonly observed in terrestrial sedimentary rocks, are fossilized "nannobacteria". McKay *et al.*[3] proposed, therefore, that the similarity in morphology between the nanometer ovoid and elongate features observed in the fracture surfaces of the martian meteorite ALH84001 and the "nannobacteria" of Folk was evidence of the existence of microbial life on Mars. Without structural or molecular data, however, many researchers have argued that a biological origin for these "nannobacteria" is speculative. An additional controversy within this debate is the theoretical and experimental limits of size of microorganisms[4].

Therefore, in the absence of well preserved fossilized microorganisms[1], the nano-scale features observed on mineral surfaces such as biominerals, and dissolution and alteration patterns that result from biogenic and inorganic interaction, should be used as evidence of biological activity in ancient non-sedimentary and post-depositional environments. We propose that these nano-scale features be termed "nanofossils." Magnetofossils, which are the preserved magnetsomes (chain of single domain magnetite crystals) formed intracellularly by magnetotactic bacteria, represent an ideal example of "nanofossils". The characteristic morphology of these nanofossils has been used as evidence for biological activity on Earth and Mars[3,5,6,7,8]. Although active magnetotactic bacteria generally range in size from 1 to 10 µm, nano-sized bacteria also have been observed (Fig. 1a, b).

Another example that confirms the existence of nanofossils is the "nanobacteria" induced formation of agglomerates of hydroxyapatite and a protein-containing organic matrix (see below). Although the existence of nanobacteria is currently being debated by researchers in a variety of fields[4], Kajander and co-workers have published convincing microbiological and molecular data for a unique culturable type of nanobacteria isolated from mammalian blood[9]. In vitro experiments showed nanobacterially induced mineralization (nanofossils) (Fig. 1c, d) and biogenic-specific dissolution of mineral surfaces (Fig. 1e, f). Although the "nanobacteria" are not initially visible under the optical light microscope, mineralized agglomerates can be observed easily, depending on culture conditions, after several days of incubation. The mode of formation of minerals within the nanofossils seems to be distinct from known biologically mediated or biologically controlled mineralization. In HRTEM, the internal structure of the nanofossils shows the ubiquitous presence of fine-grained hydroxyapatite.

The overall morphology and texture of the crystals (*e.g.,* shape, size, orientation and growth pattern) within the nanofossils resemble features observed in pre-dentin and pre-bone matrices. Although the mechanisms of mineralization are not well understood in bone and dentin, there is general agreement that glycosylated phosphoproteins are responsible. And though the morphological characteristics of nanofossils are distinctive, and the presence of glycosylated protein uncertain, a similar protein-based process of mineral formation in nano-organisms is conceivable.

Although the existence of organisms at the nano-scale range remains controversial, the formation of nanofossils shows that the concern for the limitations of size of living organisms and their fossil remains can be neglected. It should be emphasized, however. that not all nanofossils can be used as evidence for biological activity. Magnetite that is crystallized extracellularly by thermophilic bacteria[10] cannot be distinguished from magnetite formed by inorganic processes. However, biogenic siderite, formed by the same bacteria, has a distinct biogenic specific structure[11]. It is clear that morphology alone cannot be taken as evidence for the existence of nanofossils. The processes of nucleation and growth characteristics also must be considered.

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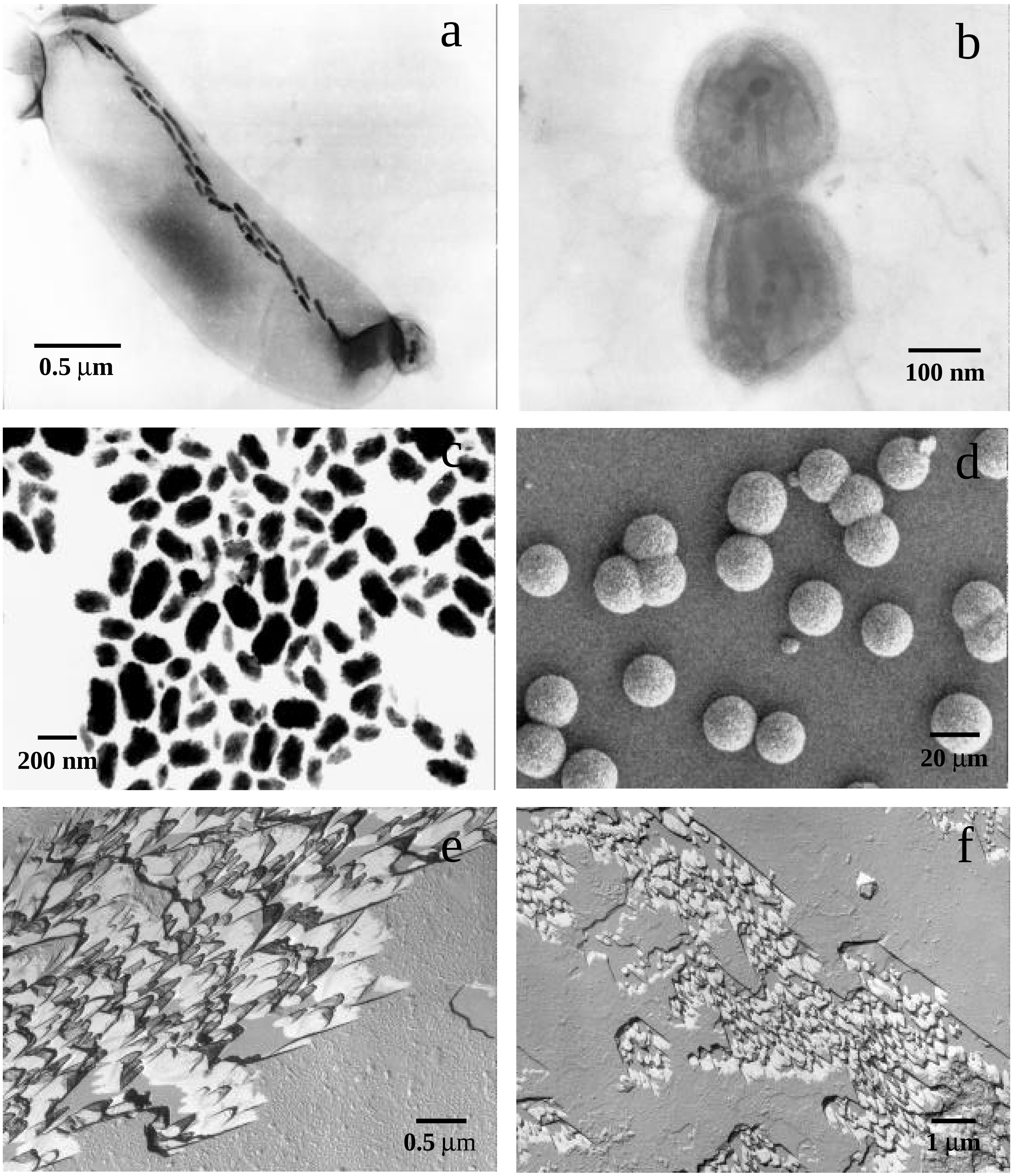

Figure 1. EM images of (a) magnetotactic bacteria showing normal size and nano-size (bottom right corner) bacteria, (b) Nano-size bacteria undergoing division(?), (c) agglomerated hydroxyapatite (nanofossils) isolated from serum, (d) same as (c) except grown in serum-free culture (SEM), and (e) (f) Pt-C replicas of dissolved mineral surfaces after exposure to nanobacteria containing medium.