Chemical clues to the earliest animal fossils

Steroid biomarkers show that the Ediacaran fossil *Dickinsonia* was an animal

By Roger E. Summons and Douglas H. Erwin

The Ediacara biota are a diverse assemblage of macroscopic body forms that appear in the sedimentary rock record between 570 and 541 million years ago. First recognized in Namibia and Australia, these remarkable organisms have since been found in Russia, China, Canada, Great Britain, and other regions. Although they immediately preceded the rapid appearance and diversification of animals in the Cambrian (541 million to 485 million years ago), their position within the tree of life has long been a puzzle. Some Ediacaran fossils appear segmented, but most lack obvious characters such as appendages, a mouth, or a gut that might link them to animal clades. On page 1246, Bobrovskiy *et al.* (1) use biomarkers to confidently assign at least one Ediacaran group, *Dickinsonia* and related taxa, to the Metazoa (animals).

The Ediacara biota were first described at a time when paleontologists relied on overall similarities in form to establish phylogenetic affinities. Various Ediacaran groups were allied with later animal clades on the basis of such similarities. Thus, *Dickinsonia* and other apparently segmented taxa were considered annelids, *Rangea* a pennatulacean cnidarian, and *Parvancorina* a likely arthropod (2). In 1992, Seilacher instead proposed that most of these organisms were part of a single clade that formed a separate, independent kingdom of complex multicellular organisms, the Vendozoa (3). After this, scientists considered a range of novel solutions, and the fossils were allied with a wide array of clades.

More recent systematic inquiry has focused on identifying discrete morphological characters. There has been growing consensus that the Ediacaran biota does not represent a single clade but a diversity of taxa, mostly of Metazoa (4, 5). On the basis of a detailed analysis of the features of *Dickinsonia* fossils, Gold *et al.* have suggested that they were at least bilaterian-grade animals (6). Thus, we can identify discrete groups provided that they have not been highly altered by subsequent metamorphism. Sterols and steranes can be particularly useful as biomarkers because some aspects of sterol structure are specific to particular source organisms (7). For example, Love *et al.* identified 24-isopropylcholestan, which is prevalent in several sequences of rocks between 660 and 535 million years ago, as a steroid characteristic of demosponges (8).

Bobrovskiy *et al.* used an approach designed to identify diagnostic hydrocarbon biomarkers associated with individual fossils preserved in remarkably unaltered, fine-grained sediments of the White Sea region in Russia. In earlier work, they isolated an organic biofilm associated with circular impressions known as *Beltanelliformis*, analyzed the entrained hydrocarbons, and assigned a colonial cyanobacterial origin (9). For the current study, the authors sampled thin layers of organic material associated with fossils of *Dickinsonia* and *Andiva* from the same sequence of rocks for biomarkers. They also analyzed samples of adjacent layers of sediment to control for more broadly distributed biomarkers.

The surrounding sediments contained diverse steranes, most of which comprised compounds with 29 carbon atoms (stigmasteroids) that are diagnostic of green algae. By contrast, the steroids in the *Dickinsonia* fossil biofilms were almost exclusively composed of compounds with 27 carbon atoms (cholesteroids), a signature of all animal phyla other than sponges and a few mollusk taxa. The steroid assemblages from the smaller *Andiva* fossils were confounded by background signals and harder to interpret.

The cholesterol predominance from *Dickinsonia* allowed Bobrovskiy *et al.* to refute alternative hypotheses of their affinities to lichens or large protists. Nevertheless, the White Sea *Dickinsonia* organic remains are enigmatic in another, biologically striking way. The sterols of living organisms are produced with a single three-dimensional...
configuration, or stereochemistry, but the steroids preserved in sedimentary rocks are typically mixtures that include more reduced and thermodynamically stable forms, as a result of chemical transformations during burial and subsequent heating. By contrast, the Dickinsonia cholesteroids mostly have the same 5β(H) stereochemistry. The only known pathway to this steroid, informally termed coprostone, is via the steroid coprostanol, which is produced in the gut of higher mammals (10–12). Coprostone is thought to be unstable on geological time scales (13). Bobrovskiy et al. attribute the presence of these unusual steroids to reduction of Dickinsonia cholesterol by bacteria during the original decomposition of the animal. Yet, coprostanones are absent in much younger, exceptionally preserved animal fossils, where the dominant steranes are 5α(H)-cholestanes (14). The association of unusual steroids associated with Dickinsonia suggests that it may have had a distinct metabolic physiology (see the photo).

Molecular clock evidence suggests that animals originated before 720 million years ago, although the pattern of their divergence during the Cryogenian (720 million to 635 million years ago) and Ediacaran (635 million to 541 million years ago) remains unresolved (15). Because molecular clock estimates and morphological characters from fossils offer limited resolution, our best hope for unraveling the early history of animals and the affinities of the Ediacara biota lies with identification of biomarkers that allow us to differentiate specific metazoan clades, particularly among the bilaterians. Further refining the phylogenetic signals from biomarkers may also help to resolve the early history of animals during the Cryogenian and early Ediacaran. Moreover, the fossil-specific biomarker approach taken by Bobrovskiy et al. promises to yield many new insights into the fossilization processes that led to soft-tissue preservation across the animal kingdom and throughout geological time.

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SYNTHETIC BIOLOGY

Programming cells and tissues

New toolkits of biological parts allow powerful cell programming by synthetic biologists

By David S. Glass and Uri Alon

The field of synthetic biology envisions designing genetic circuits to program cells and tissues. These circuits will enable cells to detect disease states and act to remedy them, directly controlling processes and materials, and even allow cells to self-assemble into new, user-defined tissues (1). Starting from circuits comprised of a few components (2, 3), registries of biological parts have been curated, and increasingly larger circuits have been engineered in cells (4), but the size and capabilities of these circuits have been limited. A key challenge to engineering larger systems is composability, the ability to connect any two parts and achieve predictable behavior. On page 1252 of this issue, Gao et al. (5) describe a composable protein-based system for building circuits, and on page 1217, Andrews et al. (6) describe a sequential logic system with many states. Recently, Toda et al. (7) used synthetic cell-cell signaling to drive differentiation and adhesion to form prototype tissues. These studies demonstrate that careful attention to composability can expand synthetic biology beyond its traditional limits.

Composability is a relatively subtle concept, and it is important to keep in mind several related—but distinct—concepts. Orthogonality in synthetic biology refers to parts that do not interfere or that minimally interfere with one another. This amounts to a lack of cross-talk between the parts. For example, two transcription factors are orthogonal if they do not regulate each other’s promoters. Modularity refers to a system that can be divided into subsystems, each with a defined function. These concepts can apply at multiple levels—within a protein, in circuits, and in multicellular systems (see the figure).

Composability is a more stringent criterion than either orthogonality or modularity. Parts that are composable are modular units that have matching inputs and outputs and are designed so that any two parts can be connected to each other and yield predictable behavior. Standardization of parts and their interfaces is one way to develop such “plug-and-play” capability (1). Even electronic circuits, often considered easy to engineer compared to biological ones, are not necessarily composable, and combining parts can fail if input and output impedances are not designed appropriately; a similar concept in biological circuits has been termed retroactivity (8). Natural systems show modularity and orthogonality to a first approximation but do not automatically provide parts that are composable enough for engineering purposes. Thus, synthetic biologists must carefully engineer natural parts to gain composability.

One important goal in synthetic biology has been to bring circuit design from the level of gene regulation, which takes hours owing to the slow process of making proteins, to the level of protein-protein interactions, which can occur within seconds to minutes. Protein circuits are not only faster but also provide powerful capabilities, including interfacing directly with cellular pathways and operating at distinct subcellular sites (9). The problem in producing protein-based circuits has been the lack of a toolkit of composable proteins that can regulate one another in arbitrary and predictable ways. Gao et al. solved this issue using molecular scissors called viral proteases (10). They engineered these proteases to specifically regulate each other in a programmable way using binding domains, degradation tags, and orthogonal cleavage sites to define sites of protease activity. Because of the attention paid to composability, the authors were able to produce a family of two-input Boolean logic gates encoded strictly at the protein level. They also formed more complex circuits, including a pulse generator, which benefited from the rapidity of protein-protein interactions. They even used the protease system to implement circuits that selectively kill cells harboring mutant and...