Developmental Cell
Spotlight

Developing Evolutionary Cell Biology

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Recent advances in both phylogenetic comparisons and the development of experimentally tractable organisms, in the growing field of evolutionary cell biology, pave the way for gaining a molecular understanding of the development of multicellularity in the animal lineage.

Evolutionary cell biology (ECB) is an emerging field of science that studies cells (the fundamental units of life) to gain insight into the processes of evolution and in parallel uses the tools and perspectives of evolutionary biology to gain insight into how cells work (Lynch et al., 2014). Two complementary recent papers from the group of Nicole King together highlight the scientific potential of evolutionary cell biology, as well as showcase the synergy between evolutionary cell biology and the related field of evolutionary developmental biology (EvoDevo). These studies deepen our understanding of how multicellularity emerged in the animal lineage. Moreover, by advancing approaches for phylogenetic comparisons and gene perturbation technologies associated with studying organisms relevant to ECB questions, they expand the set of experimentally tractable organisms to further dissect evolution on a cellular level.

A defining event in the evolution of animals (Metazoa) was the development of multicellularity, which exists in diverse branches of the tree of life (Knoll, 2011). A key question in the evolutionary cell biology field is what changes occurred in cells that led to their ability to come together in groups and ultimately form tissues. One approach to this problem is to identify gene families that are associated with the switch from a unicellular to multicellular life. Large-scale genome sequencing projects and phylogenetic analyses of diverse organisms that represent the transition to multicellularity or those immediately preceding this event have begun to address this fundamentally important problem in animals. In a recent study in *eLife*, Richter and colleagues (2018) compared the whole-genome sequences of 21 different choanoflagellates and performed phylogenetic comparisons to identify gene families that are signatures of the animal lineage. Choanoflagellates are considered to be the closest living relatives of animals and offer a powerful system for studying the evolution of multicellularity and cell differentiation (Hoffmeyer and Burkhardt, 2016). These simple organisms have a dual lifestyle and can exist in a unicellular state or a simple multicellular state as a colony or rosette. Richter et al. (2018) successfully cultured 21 different choanoflagellate species encompassing each of the major branches of this group and performed whole-genome sequencing. They then compared the choanoflagellate genomes with those from animals and unicellular organisms. The wide sampling of choanoflagellates species was critical to avoid being misled by the absence of gene families in selected genomes that occurred due to single gene loss events. In doing the genome comparisons, Richter et al. (2018) searched for three categories of genes: specific to choanoflagellates, shared between choanoflagellates and animals, and specific only to the animal lineage. This approach led to the identification of gene families unique to the animal lineage, some of which have the potential to have a pivotal role in the establishment of multicellularity.

Richter et al. (2018) reported that the basic animal lineage is distinguished by the presence of a core set of 39 gene families unique to animals. This group included known transcriptional regulators (e.g., MEX and TBX proteins), Wnt signaling proteins (α/β catenins, Dsh, Fzd), adhesion proteins (α integrin, vinculin), as well as a select number of cell cycle, cytoskeletal, and regulatory proteins (e.g., kinases, a small GTPase family). Thus, this analysis provides a global insight into the array of signaling and structural changes that are uniquely associated with the emergence of multicellularity in animals. Interestingly, this evolutionary transition was not simply a matter of acquiring or evolving gene families: a good deal of gene loss is also observed, including genes in biosynthetic pathways and osmosensing, providing a snapshot of the scope and scale of changes in gene families that occurred during this pivotal event.

A search for animal gene families in choanoflagellates also revealed to Richter and colleagues (2018) the presence of ancient proteins in these organisms that appear to have undergone domain shuffling in the lineages that gave rise to the animal descendants. An initial characterization of the innate immunity pathway identified in choanoflagellates supported this view. In the innate immune pathway, Toll-like receptors (TLRs) signal through adaptors (MyD88/Death kinase) to activate NF-κB. Richter et al. (2018) observed that a number of choanoflagellate species have an identifiable TLR and NF-κB but appear to lack several of the downstream adaptors and kinases. However, the choanoflagellate TLR is a fusion protein itself that possesses the typical extracellular leucine-rich repeat domain and then an intracellular kinase domain followed by the canonical TLR domain. In addition, another protein consisting of a kinase domain fused to a TLR region is present. Thus, it appears that the innate immunity signaling pathway in choanoflagellates is more direct than the one in animals, which may have arisen through the separate fusion of the TLR and kinase domains to a death domain. The significance of these different fusions is still unclear, but one possibility is that the more complex animal TLR signaling pathway could result in more fine-tuning or amplification of the signal.

A deeper understanding of the significance of changes in cellular function hypothesized from genome sequence analyses requires the ability to perturb the *in vivo* function of proteins from phylogenetically diverse organisms. Recent advances in the technologies for culturing and modifying diverse organisms have greatly expanded the array of possible experimental approaches and organisms to work in. Notably, methods for transforming other organisms near the base of the metazoan branch that have both unicellular and simple multicellular stages (e.g., the ichthyosporean *Creolimax fragrantissima* and the filasterean *Capsaspora owczarzaki*; see Figure 1) are now available (Parra-Acero et al., 2018; Suga and Ruiz-Trillo, 2013). Booth et al. (2018) now extend transformation tools to the choanoflagellate *Salpingoeca rosetta* and report in *Molecular Biology of the Cell* the development of a relatively efficient method for *S. rosetta* transformation by systematically varying a range of conditions that would make the cells accessible to exogenous DNA. The authors also generated a base set of expression plasmids for examining the *in vivo* localization of proteins of interest. Using this new method, Booth et al. (2018) investigated the localization of septins, a group of widely conserved cytoskeletal protein (Nishihama et al., 2011) with critical roles in cytokinesis in numerous organisms as well as cell polarity in animals. Booth et al. (2018) observed that a mTFP fusion of septin2 was localized to the basal region of individual *S. rosetta* as well as the sites of cell-cell contact. Interestingly, the septin was found in punctae intercalated between microtubules in this basal pole region in a pattern that is highly reminiscent of what is seen for septin and microtubules in polarized vertebrate epithelial cells. This work vividly illustrates how the ability to study key transitional organisms can reveal the first steps toward multicellularity.

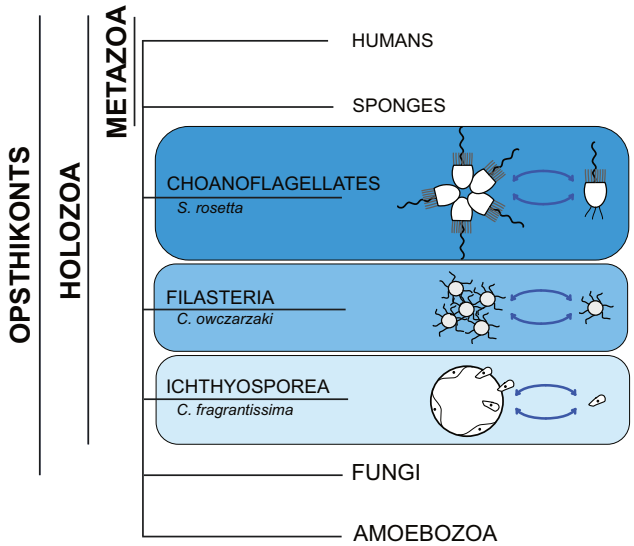

Figure 1. The Transition to Animal Multicellularity
A simplified phylogenetic tree illustrating the position of three key unicellular organisms that have simple multicellular stages and provide insights into the transition to multicellularity in the animal lineage.

The ability to generate transgenic choanoflagellates, *C. fragrantissima*, and *C. owczarzaki* holds the promise of being able to directly compare the functions of closely related proteins in these pivotal organisms. The next step is the development of methods to inactivate gene function. siRNA has been shown to be an effective tool in *C. fragrantissima* (Suga and Ruiz-Trillo, 2013), and it may also be useful to study gene function in those species of choanoflagellates that have the siRNA processing machinery (Richter et al., 2018), but perhaps not for *C. owczarzaki* that lack genes encoding these proteins (Bråte et al., 2018). Progress in studying gene function in *C. owczarzaki* and other organisms of interest may have to await the development of other tools such as CRISPR.

The availability of genome sequences for evolutionarily significant organisms is an important step toward understanding how cells and multicellular organisms evolved, but it is only a start. Methods for transfection and manipulation of key organisms are essential to allow experimental testing of ideas. New approaches such as those described here will undoubtedly now accelerate studies on the emergence of multicellularity in animals and enable vigorous growth of the broader field of evolutionary cell biology.

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