

UNDERSTANDING THE ORIGINS OF ANIMAL MULTICELLULARITY THROUGH THE STUDIES OF CHOANOFLAGELLATES

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*ABSTRACT: Choanoflagellates are the closest living relative of animals (Alegado, 2014; King, 2015; King 2016; Rokas, 2008). This has been confirmed through multiple lines of phylogenetic analyses, comparative genomics, and similarities in cell biology (Alegado, 2014; King, 2001). Previous studies of the choanoflagellate *Salpingoeca rosetta* suggest that bacteria may have played an important role in the early origins of animals (Alegado, 2014; King, 2016; Levin, 2011). One such study showed that the Bacterioidetes *Algoriphagus machinoponensis* can release lipid signaling molecules that induces choanoflagellates to grow into multicellular colonies known as rosettes (Alegado, 2016; Beemelmans, 2014). Another study showed that when grown in the presence of *Vibrio fischerii*, choanoflagellates will exhibit swarming behavior and sexually reproduce (Levin, 2013). Choanoflagellates have been repeatedly proven to be an experimentally tractable, phylogenetically relevant model system for investigating the unicellular ancestry of animals. Through the use of molecular and comparative genomic approaches, we can possibly study the origins and evolution of animals through choanoflagellates.*

Multicellularity may be defined as the differentiation and organization of cells into functioning tissues within an organism (Anderson, et al., 2016; Rokas, 2008). Multicellularity arose independently several times in life's history (Abedin, 2010; Alberts, 2002; King, 2016). The morphological diversity of multicellular organisms such as animals, fungi, and plants stem from the fact that each lineage obtained multicellularity in different ways (Abedin, 2010; Alberts, 2002; King, 2016). One way each lineage varies is in their method of adhesion or cell-cell connections (Abedin, 2010). Another property crucial to all multicellular organisms is their method of intercellular communications (Alberts, 2002). How each eukaryotic lineage achieves these unique multicellular challenges is partly what distinguishes them from each other (Abedin, 2010). Surprisingly, the origin and evolution of animal multicellularity is still largely unknown (Anderson, et al., 2016; King, 2016). However, by studying closely related extant species, we

may be able to learn more about the current state of life today (Anderson, 2016; King & Carroll, 2001; King, 2016).

For animals, the closest living relative is considered to be the choanoflagellate, a single-celled eukaryote with a complex lifestyle (Alberts, 2002; King & Carroll, 2001; King, 2016; Rokas, 2008). There are multiple lines of evidence that support the idea of animals sharing a common ancestor with choanoflagellates, including several independent phylogenetic analyses, comparative genomics, and similarities in cell biology (Alegado, et al., 2014; King & Carroll, 2001).

Choanoflagellates are also characterized by their complex life cycle, which consists of a single celled form, a chain form, and a multicellular spherical form known as a rosette (Alegado, et al., 2014; King, 2016). Rosette formation is induced by its prey, *Algoriphagus*, which releases lipid based signaling molecules that induce incomplete cytokinesis to promote rosette formation (Beemelmans, et al., 2014).

By identifying the molecules responsible for rosette formation, it is becoming possible to create defined media to use choanoflagellates as a model system for studying the origin of animals (King, 2016). Furthermore, choanoflagellates have demonstrated interesting behaviors in the presence of different species of bacteria, namely *A. machipongonensis* and *Vibrio fischeri* (King, 2016; Levin & King, 2013). These bacterial interactions may hold some clues to the origin of animals.

Choanoflagellate's phylogenetic relevance and similarities to modern animal cell structures

Many animal genes not found anywhere else in the tree of life have been found in choanoflagellates. For example, the first tyrosine kinase receptor found outside the animal kingdom was found in the choanoflagellate *Monosiga brevicollis* (King & Carroll, 2001). Another example of an animal gene shared with choanoflagellates is the GK protein interaction domain (GKPID), which enables animals to orient their mitotic spindles to maintain organized tissues (Anderson, et al., 2016). Studies like these suggest that the last common ancestor of animals and choanoflagellates probably possessed many of the genes necessary for multicellularity (King & Carroll, 2001; King, 2016; Rokas, 2008). Thus, by studying the genes of choanoflagellates and comparing them to the genes of animals today, we may learn something about the evolution and origins of animal multicellularity (Anderson, et al., 2016; King, 2016).

Not only do choanoflagellates share genes with animals that are not found anywhere else, they also share similarities to modern animal structures. One similarity is between the single-celled form of choanoflagellates and the eukaryotic sperm cell. Just like a sperm cell, the main parts of a choanoflagellate are the ovoid head and a singular flagellum (King, 2016; King, 2015; Alegado & King, 2014). The second similarity is when a single cell undergoes a series of incomplete cytokinesis

to form a ball shaped rosette which bears resemblance to morula stage embryos (Alegado & King, 2014; Beemelmans, et al. 2014). Finally, when linearly arranged as chain colonies, they resemble and function similarly to the epithelial cells of the gastrointestinal tract (Beemelmans, et al., 2014). Through the undulations of the flagella, choanoflagellates can trap prey within its microvilli before engulfing it (Alegado & King, 2014). In fact, sponges—choanoflagellate's closest related Metazoan relative—use a form of epithelia that has several hallmarks of eumetazoan epithelia, including a basement membrane and proteinaceous intercellular junctions (Alegado & King, 2014; Dayel & King, 2014). The conserved roles and phylogenetic distributions of epithelia suggest that Metazoans shared a common ancestor with choanoflagellates.

Bacterial influence on choanoflagellate behavior

One of the most fascinating aspects of choanoflagellates is their interaction with bacteria. It has been shown that rosette formation is actually induced by the presence of its prey, *Algoriphagus* (Beemelmans, et al., 2014). *Algoriphagus* is a Bacterioidetes similar to ones found in the human intestinal microbiome, and produces a novel class of signaling molecules related to sphingolipids that promotes rosette formation in choanoflagellates (Alegado, et al., 2012). Furthermore, electron microscopy has imaged *Algoriphagus* producing outer membrane vesicles composed of these important signaling molecules (King, 2016). This has fascinating implications on possible methods of interactions between animals and bacteria.

The main signaling molecule produced by *Algoriphagus* is Rosette Inducing Factor 1 (RIF-1) (Beemelmans, et al., 2014). By itself, however, it is only able to induce 1% of the cells into rosettes (King, 2016). For complete rosette formation, it also needs a second lipid based signaling molecule lysophosphatidylethanolamine (LPE) (King,

2016). Together, they can recapitulate full rosette development. This is because LPE promotes proper development and maturation (King, 2016). The presence of these two lipid based signaling molecules enables the choanoflagellate to form robust rosettes, which may increase its efficiency at capturing and consuming prey (Alegado & King, 2014).

To prevent rosette formation, *Algoriphagus* also produces IOR-1 (Inhibitor of Rosettes-1) to decrease choanoflagellate's predatory capabilities (Cantley, 2016). IOR-1 looks similar to the chemical structure RIF-1 if it were to be cleaved down its symmetrical axis (Cantley, 2016; King, 2016). This suggests that there may be coevolution between the two species that may resemble the Red Queen Hypothesis, with the prey constantly trying to change its lipid composition to avoid its predator's relentless adaptations.

Bacteria can also induce choanoflagellates to exhibit strong, unmistakable swarming behavior (King, 2016). This behavior is promoted when choanoflagellate cultures are grown in the presence of *Vibrio fischeri*. In addition to swarming, *V. fischeri* encourages sexual reproduction between the single-celled choanoflagellates (King, 2016; Levin & King, 2013). Although the molecular signal that induces this phenomenon is still unknown, there is strong evidence that choanoflagellates are reproducing sexually. Under a light microscope, it is possible to see videos of a larger "female" choanoflagellate engulfing a smaller "male" choanoflagellate (King, 2016). Furthermore, it is possible to obtain Punnett squares that exhibit Mendelian inheritance (King, 2016). Additionally, just like sexual reproduction in animals, choanoflagellates experience a change in ploidy from diploid to haploid during this process (Levin & King, 2013).

As mentioned before, in the presence of *Algoriphagus*, choanoflagellates will grow into multicellular rosettes. Grown in the presence of *V. fischeri*, they will exhibit swarming behavior and reproduce sexually. Thus, it may come as no

surprise that when grown in a culture with both *V. fischeri* and *Algoriphagus*, choanoflagellates will grow as colonies of rosettes exhibiting strong swarming behavior and sexual reproduction (King, 2016). In other words, simple multicellular structures are reproducing sexually due to the presence and influence of bacteria. This has strong implications on the importance of bacteria during the early evolution of animals.

Discussion

Studies of choanoflagellates have shown that these voracious bacteriovores are a promising model system for understanding the early evolution of animals. These studies have shown much evidence to demonstrate the close relationship between choanoflagellates and animals. By studying this closely related extant species, we may learn how animals first evolved (King & Carroll, 2001). Furthermore, these studies suggest that bacteria may have played an important role in animal origins (Alegado, et al., 2012). Understanding the regulation and mechanisms behind these processes may also serve to elucidate current biological mysteries. For example, it has been shown that the gut microbiome has a significant impact on the development of children, the appetite in mice, and the shaping of the immune system (Nikoopour, 2014; Vijay-Kumar, 2010). However, the exact mechanisms of interaction between host and microbiome is still unknown. Coincidentally, *Algoriphagus* is a Bacteroidetes similar to those found in the human gut microbiome (Alegado, et al., 2012). By studying the lipid based signaling molecules between choanoflagellates and *Algoriphagus*, we may learn something about the communication between humans and their gut microbiota.

Still more work has to be done to definitively conclude that these lipid based signaling molecules are causing behavioral changes in choanoflagellates. Although a recent study has identified the gene necessary for rosette formation,

we have yet to uncover a receptor for the signaling molecules (Levin, Greaney, Wetzel, & King, 2014). Identifying this receptor and outlining its molecular effects may help us understand the gene regulation of early animal evolution (Alegado & King, 2014; Levin & King, 2014).

Other experiments may look to see if the multicellular rosettes gain a fitness advantage over its single-celled form. Previous studies in other model organisms have shown that multicellular forms can increase resistance to predation, UV tolerance, and ability to grow in new ecological niches in relation to their unicellular counterparts (Boraas, Seale, & Boxhorn, 1998; Goldman & Trivisano, 2011; Koschwanez, Foster, & Murray 2011). It would be interesting to see what kind of fitness advantage (if any) multicellular colonies of choanoflagellates gain.

Another interesting experiment would be to study the coevolution between choanoflagellates and bacteria. The current state of knowledge of these interactions is still nebulous and it is unclear why bacteria would produce the RIF-1 and LPE. At first glance it would appear that bacteria are expending energy for the benefit of choanoflagellates. However, it is extremely rare in biology, if not impossible, for one species to be completely altruistic for the sake of another. One hypothesis may be that RIF-1 and LPE are waste products that choanoflagellates use to sense the presence of *Algoriphagus*. By identifying and defining the symbiotic relationship between bacteria and choanoflagellates, we may learn more about our own relationship with bacteria and how bacteria can influence us.

Because of the identification of the signaling molecules for rosette formation, it is becoming possible to create defined media to grow robust colonies of rosettes. This will make studying questions of mutualism, fitness advantage, and gene regulation in choanoflaellates easier and more accessible. In conclusion, there are several lines of evidence to support the fact that choanoflagellates are the closest living ancestor to animals. These similarities include but are not limited to similarities in cell biology, phylogenetic

analyses, and genomic analyses. By studying this organism, we may learn something about the early evolution of animals and the role of bacterial influence.

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