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By Jef Akst

From Simple To Complex

The switch from single-celled organisms to ones made up of many cells has evolved independently more than two dozen times. What can this transition teach us about the origin of complex organisms such as animals and plants?

Sean McCabe

Given the complexity of most organisms—sophisticated embryogenesis, differentiation of multiple tissue types, intricate coordination among millions of cells—the emergence of multicellularity was ostensibly a major evolutionary leap. Indeed, most biologists consider it one of the most significant transitions in the evolutionary history of Earth's inhabitants. But single-celled organisms have stuck together or assembled to spawn multicellular descendants more than two dozen times, suggesting that maybe it's not such a big leap after all.

"The transition from unicellularity to multicellularity is critical for explaining the diversity of life on Earth," says evolutionary biologist Casey Dunn of Brown University. "We tend to think of it as quite special, but maybe it's not. Maybe this is an easier transition than we think."

To understand how and why it happened, scientists are utilizing the recent explosion in genomics data to assemble more accurate phylogenies and piece together each step in the transition to multicellular life. Despite their efforts, however, the origins of this intriguing phenomenon remain shrouded in

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mystery. Evolution and extinction over hundreds of millions of years have blurred the details of the transition, and the answers provided by genome sequencing only lead to more questions.

Surprises in Sea Anemone Genome

"New studies are always pushing the envelope on our thinking," says evolutionary biologist Mansi Srivastava of the Whitehead Institute for Biomedical Research in Massachusetts. Since scientists began studying a much wider array of animals, far afield from the classic model systems of fruit flies and mice, "our thinking about what having certain kinds of genes means to being an animal has shifted."

Sean McCabe

Conventional thought on evolutionary change has led researchers to believe that genetic innovations underlie the transition. Advances in genomics research, however, are revealing that more and more of the genes associated with complex processes also exist in simpler animals and even in their unicellular cousins. This suggests that the appearance of new genes cannot fully explain the appearance of new traits that are key to multicellularity. Sponges are commonly considered the most basal of all the metazoan (animal) lineages, yet a recently published sponge genome revealed genes known to be involved in the development of a neuromuscular system, which sponges lack.[1] "These genes that we previously thought were associated with complex multicellular animals really have to do with basic multicellular functions—to get the simplest multicellular animals, you have to have these genes present," says Srivastava, who coauthored the analysis.

As some of the most ancient animals, sponges can provide information regarding the evolution of the metazoan lineage, but for true insights about the origin of multicellularity, scientists must look even further back on the evolutionary tree. Choanoflagellates, unicellular organisms that look remarkably similar to the feeding structures of sponges, are the closest living relatives of metazoans. It turns out that they also share a number of genes once thought to be unique to multicellular animals. Tyrosine kinases (TK), for example, enzymes that function in cell-cell interactions and regulation of development in animals, were identified in the choanoflagellates in the early part of this decade, and the first sequenced choanoflagellate genome, published in 2008, revealed that they have more TK genes than any animal—and many other components of the TK signaling pathway as well.[2]

"So this gene family that was thought to be essentially a trigger that unleashed animal origins, we can now say with great confidence evolved long before the origin of animals," says evolutionary biologist Nicole King of the University of California, Berkeley, who has been studying choanoflagellate biology for over 10 years.

Scientists have also identified choanoflagellate homologs of cadherins, known to be involved in cell-cell adhesion and signaling in animals. And more recently, a widespread search for genes associated with integrin-mediated adhesion and signaling pathways revealed that the integrin adhesion complex originated much earlier than even the choanoflagellates, dating back to the common ancestor of

animals and fungi.[3]

"It's pretty surprising to find these adhesion genes in far-flung species," says Srivastava. "We would have thought that integrin signaling has to do with cells sticking together, but it goes much further back in time than our most recent unicellular cousins."

The genomic exploration of the evolution of multicellularity is really just beginning, but already, a trend is emerging. "Almost every month now we are seeing genes that were supposed to be exclusive to metazoans that are already present in their single-cell relatives," says evolutionary biologist Iñaki Ruiz-Trillo of the University of Barcelona. "I think that means co-option of ancestral genes into new functions is important for evolutionary innovations like the origin of multicellularity."

"Probably the more data we collect, the fewer and fewer animal-specific genes there are going to be," agrees Dunn. "And we're going to have to explain the origins of multicellularity in terms of changes in the way these gene products interact with each other."

Unfortunately, because the genomics data are so new, experimental data regarding the functions of these genes in single-celled organisms remains limited. Research by biochemist Todd Miller of Stony Brook University in New York and his colleagues, for example, demonstrated that while tyrosine kinases exist in great numbers in choanoflagellates, they lack the tight regulation found in animal signaling pathways, suggesting regulatory elements may have been key to the evolution of multicellularity. But this idea remains speculative, Miller says, as the targets of these enzymes in the unicellular relatives of animals and the details of their activation are still unknown.

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—Casey Dunn, Brown University

"What we'd really like to be able to do is compare signaling pathways overall and see how they evolved," Miller says. "We know a lot about the proteins themselves, but it would be great to have a glimpse into a simple pathway where we can begin to unravel what the core elements of the pathway are before layers and layers of additional regulatory elements were added, as we see in metazoan cells."

To confuse matters more, the vast stretches of time that separate most multicellular organisms from their unicellular cousins—more than half a billion years, in most cases—make for a lot of uncertainty. And as sequencing studies raise more questions, phylogenetic studies are also throwing a shadow of doubt on the animal tree. For example, are sponges really the most basal animals, as has long been thought? A recent phylogenetic study performed by Dunn and his colleagues suggested that perhaps ctenophores (comb jellies) are the earliest diverging extant multicellular animals.[4] "Either sponges or ctenophores are sister to all other animals," Dunn says. "The answer you get depends still on the organisms you include in the analysis, the analysis methods you use, and what genes you look at."

"In order to understand evolutionary transitions, you need to have a robust

phylogenetic framework," says Ruiz-Trillo. The more genomes are sequenced, the better the phylogenies get, and the more similarities and differences are recognized between multicellular organisms and their unicellular cousins. "It's a really exciting time" for studying the evolution of multicellularity, King says. With so many open questions and more and more sequenced genomes available each year, "there's a lot of low-hanging fruit."

A multicellular model?

Animals aren't the only multicellular organisms, of course, and thus not the only system applicable to the study of multicellularity's origins. In fact, multicellularity is believed to have evolved as many as 25 different times among living species. So while the search for metazoan origins may be riddled with uncertainty, perhaps scientists can draw inferences from the study of multicellularity in other lineages.

Comparing brown algae to their unicellular diatom relatives, for example, researchers saw an increase in membrane-spanning receptor kinases, a protein family known to play a role in cellular differentiation and patterning in both animals and green plants.[5] The independent evolution of more kinase genes in each of these lineages suggests that this family of proteins may have been key to this transition.

Of all the multicellular lineages, however, the volvocine green algae represent the best-studied and most tractable system for teasing out the evolution of multicellularity. In contrast to most other origins of multicellularity, which likely arose close to a billion years ago, the change to multicellularity in these algae may have occurred as little as 200 million years ago—possibly limiting evolution's mark on their genomes. Furthermore, between the unicellular *Chlamydomonas* species and the most-derived multicellular *Volvox* there are several extant intermediate species, some of which appear to have changed little since their divergence from their unicellular ancestors. While recent evidence indicates a complicated evolutionary history, including multiple origins of some traits and reversals,[6] this lineage nonetheless presents a phylogenetic road map by which step-by-step

transitions can be inferred. (See illustration)

Volvocine algae are aquatic, flagellated eukaryotes that range in complexity from unicellular species to a variety of colonial forms to multicellular *Volvox*, some of which boast up to 50,000 cells. This transition involved a series of key innovations, including cell-cell adhesion, inversion, and differentiation of somatic and germ cell lines. Two species in particular have become models for the evolution of multicellularity—the single-celled *Chlamydomonas reinhardtii* and the 2,000-or-so-celled *Volvox carteri*.

As with animals, comparisons of their recently sequenced genomes have revealed that there are few striking differences among the genetic codes of these organisms that could explain the drastic differences in their morphology. "It was pretty disappointing at first," admits developmental biologist Stephen Miller of the University of Maryland, Baltimore County, who helped to analyze the *Volvox* genome (the sequence was published last summer). "We were hoping to see differences that would point to explanations for why *Volvox* is so much more developmentally complex than *Chlamydomonas*, but that certainly wasn't the case."

Not only do the genes exist in *Chlamydomonas*, they are so similar to the *Volvox* versions that they appear to be able to stand in for missing or mutant copies in their multicellular cousins. *Volvox*'s *glsA* gene, for example, codes for an essential component of asymmetric division; *glsA* mutants can only divide symmetrically, resulting in adults comprised entirely of small somatic cells, with none of the large germ cells, known as gonidia, that normally give rise to the next generation. While the homologous protein in *Chlamydomonas* is only about 70 percent identical to *glsA*'s protein, it can restore asymmetric cell division when the gene is transformed into *glsA* mutants. "Its ortholog in *Chlamydomonas* is perfectly capable of carrying out the same function," Miller says.

Similarly, *invA* is essential to the process known as inversion, which gives adult *Volvox* their spherical shape, with the gonidia on the inside and the small, flagellated somatic cells around the exterior. In *invA* mutants, inversion fails to occur due to the cells' inability to move relative to the cytoplasmic bridges that connect them, and the gonidia are exposed on the surface of the spheroid. Just like *glsA* mutants, however, this phenotype can be rescued by the transformation of the *Chlamydomonas* ortholog, known as *IAR1*.

There are exceptions to this pattern, however, such as the appearance in *Volvox* of many new genes that encode cell wall or extracellular matrix (ECM) proteins, with a dramatic increase in the number and variety of *Volvox* genes in two major ECM protein families, as compared with *Chlamydomonas*. While *Volvox carteri* have only a couple thousand times as many cells as *Chlamydomonas*, they can grow to more than 100,000 times larger thanks to a dramatic increase in the amount of ECM, which constitutes more than 99 percent of the volume of a mature *Volvox*.

Another significant genetic change in *Volvox* becomes evident when examining the mating locus—a region on one chromosome containing sex-specific genes that dictate whether the organism will be male or female during the sexual part of the volvocine life cycle. A notable difference in the sexual strategies of *Chlamydomonas* and *Volvox* is the size of their gametes. While the sperm and the egg of *Chlamydomonas* are nearly indistinguishable and are produced in similar quantities, *Volvox* eggs are significantly larger than its sperm, and there are far fewer of them. This transition to oogamy, as it's called, appears to be a hallmark

of multicellularity.

"It's a remarkably conserved trait," says cell and evolutionary biologist James Umen of The Salk Institute. "When you look at any lineage that becomes multicellular and has sex, it almost invariably goes from having mating types where the gametes are same size to having a sperm/egg system of some sort." And in contrast to most of the innovations associated with the asexual reproductive phase of the *Volvox* life cycle, new genes do seem to be a big part of the evolution of dimorphic gametes. The mating locus of *Volvox* is greatly expanded to more than 500 percent of the size of *Chlamydomonas'* 200–300 kilobase mating locus, and contains many genes that fall outside the mating locus in *Chlamydomonas*, as well as at least 13 new gender-specific genes.[7]

"Overall the two genomes are very similar, but the mating locus of *Volvox* kind of exploded in terms of size and context," says Umen. "In general, things related to sex don't follow the normal rules regarding evolution; innovation seems to be a really important part of sex."

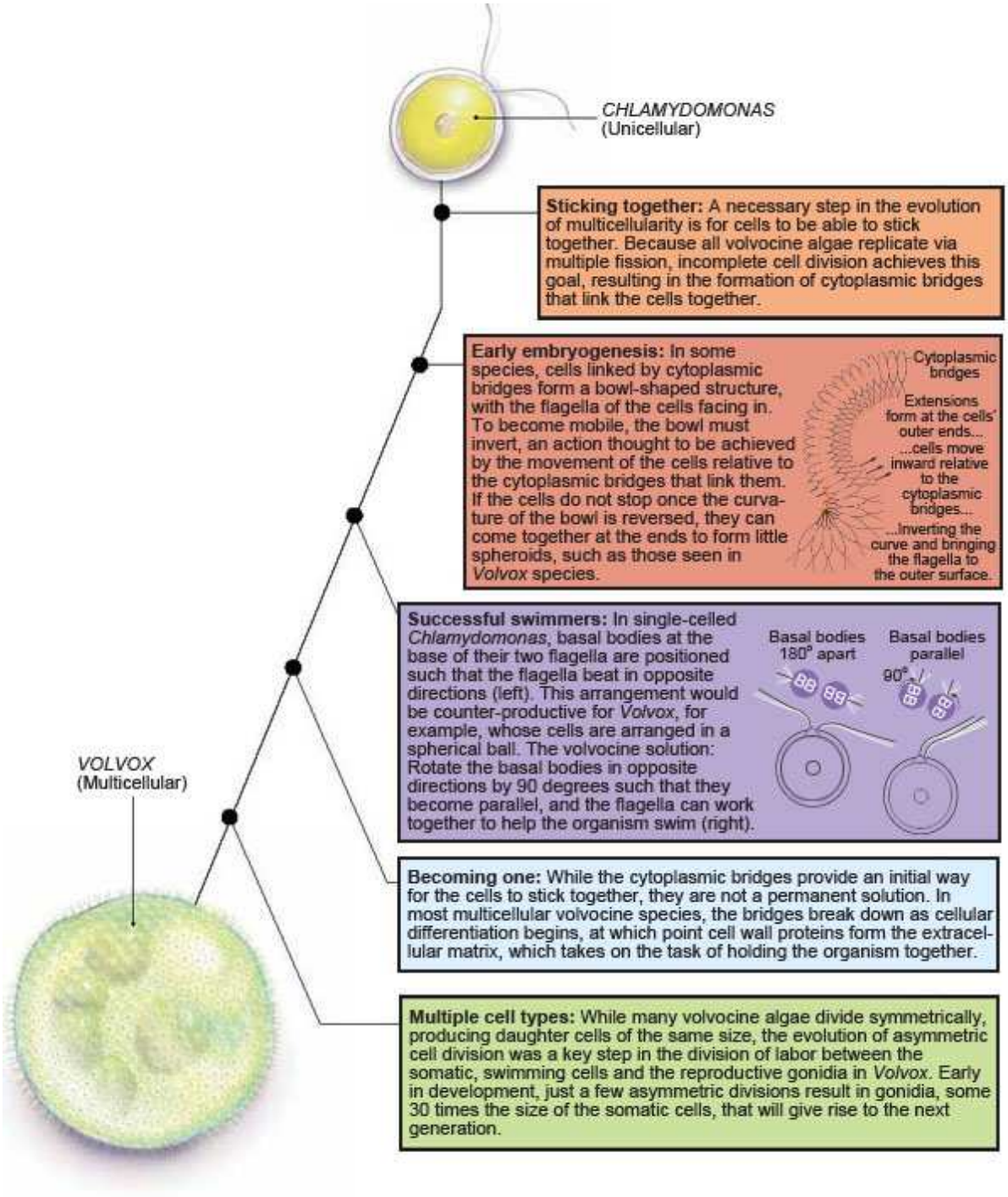
But how much can scientists learn about the evolution of the complex multicellularity exhibited by animals and other lineages from studying the volvocine algae? According to some, not much. *Volvox* represents a relatively simple form of multicellularity, with only two cell types and no organized tissues or organs.

"I think it's dangerous to generalize too much," says Stephen Miller. "Because [multicellularity] has evolved independently in each of these cases, there don't have to be similarities in how it evolved. But I would guess there might end up being some common themes."

One emerging idea is that complex multicellularity, such as that of animals, plants, and fungi, may have evolved only a handful of times, and that it almost always resulted from the division of a single cell into the components of the larger organism, King says. In contrast to slime molds, for example, which form via aggregation of neighboring cells, the earliest multicellular animals were likely to have evolved by failing to disperse after the mother cell divided.

Evidence of this comes from a recent study out of King's lab that found choanoflagellates fail to form colonies when cell division is inhibited.[8] If the earliest ancestors of animals were anything like modern-day choanoflagellates, this suggests that animal development from a single-celled embryo is core to our evolution, and not a secondary development.

Similarly, the volvocine algae all divide via multiple fission, where the nucleus divides many times before the cytoplasm splits to generate that number of daughter cells. "It's a way of producing a large number of genetically identical cells all at once," says evolutionary biologist Matthew Herron of the University of British Columbia. "The only thing you need to do to produce an eight-cell colony [is have] them to stick together."



The evolution of *Volvox*

The volvocine algae are a model system for studying the evolution of multicellularity, as the group contains extant species ranging from the unicellular *Chlamydomonas* to a variety of colonial species and the full-fledged multicellular *Volvox* varieties. Comparing the biology of these organisms, evolutionary developmental biologist David Kirk of the Washington University in St. Louis proposed 12 steps that were key to this transition. Here are some highlights that were key to this transition (*BioEssays*, 27:299-310, 2005).

Lucy Reading-Ikkanda (diagrams and cells); SOURCE: David L. Kirk

Complexity breeds cooperation

Beyond the molecular and developmental logistics of evolving multicellularity, there is the added complication of genetic conflict. An incredible amount of cooperation is required for individual cells to come together and function as one, and with natural selection acting at the level of the individual cell, there will be significant evolutionary pressure to cheat the system and sabotage the success of

the multicellular whole.

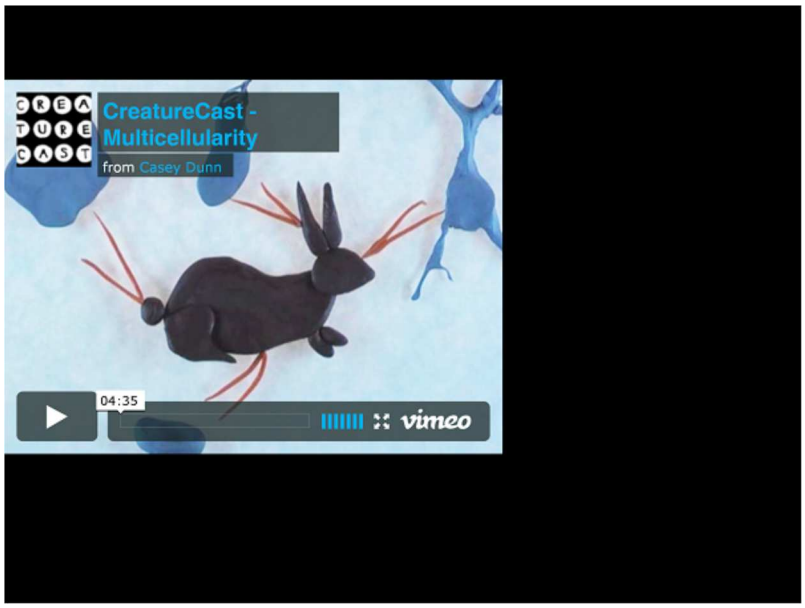
Sean McCabe

The collaboration of first a few, then millions of cells to create an entirely new kind of "individual" thus requires a shift in the level of biological organization upon which natural selection acts. In this way, the evolution of multicellularity can be considered what has been termed an "evolutionary transition in individuality" (ETI), where the unit of selection changes from a single cell to a group of cells—the newly evolved multicellular individual. Other ETIs include the congregation of replicating molecules to yield the first prokaryotic cells, the associations of prokaryotic cells to create eukaryotic cells with organelles such as chloroplasts and mitochondria, and the establishment of cooperative societies composed of discrete multicellular individuals, like eusocial insect colonies.

"The general principle is, in any of these kinds of transitions there's always some form of cooperation that's needed," says Herron. "In the example of the ants and bees, it's the workers that are being cooperative in the sense that they're sacrificing their own reproduction in order to help the queen reproduce. And in multicellular organisms like us and *Volvox*, the somatic cells are cooperating in the sense that they're sacrificing their own reproduction in order to help the reproductive cells reproduce."

Sean McCabe

But such transitions are not always smooth, as conflict can arise when selfish mutations result in cheaters that attempt to benefit from the group without contributing their fair share. One of the first cooperative steps required for the evolution of multicellularity in the volvocine algae was the development of the ECM from cell wall components, which can be metabolically costly to produce. The ECM can thus be thought of as a shared resource, and cells that do not contribute to its production may still benefit from its existence, thus gaining a growth or reproductive advantage.



CreatureCast - Multicellularity from Casey Dunn on Vimeo.

To defend themselves against such cheating, these new kinds of individuals must evolve mechanisms of conflict mediation. One proposed theory for how the volovcine algae defend themselves against ECM cheaters is the evolution of genetic control of cell number. In unicellular *Chlamydomonas*, the number of cells produced depends on the size of the parent cell, which in turn is contingent on the amount of resources available. Under these circumstances, it is conceivable that a cell in a multicellular organism could benefit from not contributing to ECM production by putting that saved energy to use making more offspring cells. But all volvocine algae that have evolved an ECM have also switched to genetic control over cell number. As a result, cheaters have less to gain because the total number of daughter cells produced by the group is limited.

The differentiation of somatic and germ cells to yield a division of labor between viability and reproduction represents another potential conflict. In essence, somatic cells are giving up their own reproductive output to support the success of the entire colony of cells, presumably by providing enhanced motility. They don't always cooperate willingly, however, Herron says; mutations still arise that cause some somatic cells in *Volvox* to try to reproduce on their own rather than support the entire organism's reproductive success as sterile swimmers.

There are lots and lots of transitions from single cells to organisms that have more than one cell, but as far as evolving into complex multicellular organisms with cellular differentiation—that only happened a handful of times.
—Matthew Herron, University of British Columbia

The somatic regenerator, or *regA*, gene appears to be one important factor in keeping somatic cells from defecting. In *regA* mutants, somatic cells develop normally at first, but then they enlarge and develop into gonidia that can divide to yield *Volvox* offspring. "What it causes is a dysfunctional colony," Herron says. "In the lab, we can keep these mutant colonies alive, but they sink to the bottom of the test tube. We assume that they would not last long in nature."

One proposed mechanism of conflict mediation following this transition is the early segregation of the germ line. The *Volvox* gonidia that will produce the next generation are formed by just a few rounds of asymmetric cell division very early in development, so there is little time for mutations to accumulate in these cells. While somatic cells may still accumulate mutations, these defects will not be passed on. "They are evolutionary dead ends," Herron says.

These recurrent mutations in *Volvox* suggest that "the conflict between the individual cells and the interest of colony may still be going on," he adds. Such conflict may limit the organism's complexity, as selection on individual cells battles with the whole organism's attempt to survive and reproduce, suggesting that perhaps the evolution of advanced multicellularity wasn't so easy after all. "There are lots and lots of transitions from single cells to organisms that have more than one cell, but there are a lot fewer transitions that go as far as evolving into *complex* multicellular organisms with cellular differentiation," Herron says. "That only happened a handful of times."

References:

1. M. Srivastava et al., "The *Amphimedon queenslandica* genome and the evolution of animal complexity," *Nature*, 466:720-26, 2010. **Free F1000 Evaluation**
2. N. King et al., "The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans," *Nature*, 451:783-88, 2008. **Free F1000 Evaluation**
3. A. Sebé-Pedrós et al., "Ancient origin of the integrin-mediated adhesion and signaling machinery," PNAS, 107:10142-47, 2010.
4. C.W. Dunn et al., "Broad phylogenomic sampling improves resolution of the animal tree of life," *Nature*, 452:745-49, 2008. **Free F1000 Evaluation**
5. J.M. Cock et al., "The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae," *Nature*, 465:617-21, 2010. **Free F1000 Evaluation**
6. M.D. Herron et al., "Triassic origin and early radiation of multicellular volvocine algae," *PNAS*,106:3254-58. 2009.
7. P. Ferris et al., "Evolution of an expanded sex-determining locus in *Volvox*," *Science*, 328:351-54, 2010.
8. S.R. Fairclough et al., "Multicellular development in a choanoflagellate," *Current Biology*, 20:R875-76, 2010.