EVOLUTION

Ratcheting the evolution of multicellularity

Traits that entrench cells in a group lifestyle may pave the way for complexity

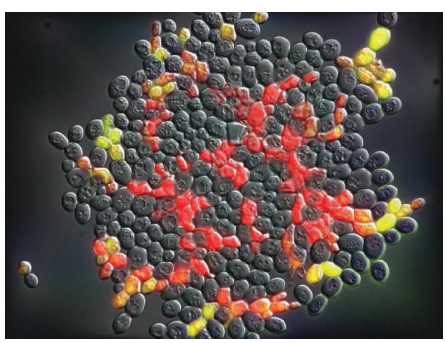
By **Eric Libby**[1] *and* **William C. Ratcliff**[2]

Multicellularity is one of the major transitions that allowed the evolution of large, complex organisms, fundamentally reshaping Earth's ecology (*1*). Early steps in this process remain poorly resolved, because known transitions occurred hundreds of millions of years ago (*2*) and few transitional forms persist. It is generally accepted that the first steps toward multicellularity were the formation of cellular clusters, followed by the success or failure of those clusters depending on their traits. As clusters of cells adapted, cells lost their evolutionary autonomy, becoming mutually reliant parts in a new higher-level whole (*1*, *3*). This transition may be facilitated by a "ratcheting" process in which cells adopt traits that entrench them in a group lifestyle, stabilizing the group and paving the way for the evolution of multicellular complexity.

The transition to multicellularity is thought to be stabilized by the evolution of traits that export fitness from cells to newly formed groups of cells (*3*, *4*). But conflicts between levels of selection can be problematic, as cell-level selection can easily overwhelm the generally slower process of group-level selection (*5*). For example, groups of microbes that produce costly extracellular metabolites can grow faster than those that do not, but within groups, free-riding cheats usually grow fastest of all (*6*). Such cell-level evolution can also lead to cancer and is a potential problem for all multicellular organisms.

Making matters more complicated, many primitive multicellular life cycles likely included both uni- and multicellular stages with heritable variation in traits that affect the fitness of each stage. Such dual-stage life cycles readily evolve de novo from unicellular starting points in the lab. For example, experiments selecting for multicellularity in the unicellular green alga *Chlamydomonas reinhardtii* produced a strain that formed sessile multicellular clusters that reproduced via motile unicellular propagules (*7*). Reliance on a unicellular stage, however, provides opportunity to forego group formation. As an example, the bacterium *Pseudomonas fluorescens* rapidly evolves to produce multicellular mats on the surface of static growth media to gain better access to oxygen. Once a mat has formed, selection then favors unicellular cheats that do not produce the glue responsible for mat formation, ultimately leading to the mat's destruction (*8*). The unicellular cheats that escape the mat's collapse may not evolve into mat formers again without suitable selective conditions. This experimental work reveals that a key problem in the transition to multicellularity is not how multicellular clusters evolve in the first place, but rather how multicellularity persists when the balance of selection tilts toward single cells.

One solution to stabilizing multicellularity is the evolution of traits that increase cell-level fitness in a group context, but come at a cost to free-living fitness. Accumulation of these traits would ratchet cells into a group lifestyle, ultimately preventing unicellular reversion. For example, in a yeast model of multicellularity (*9*), selection for fast sedimentation in a liquid culture of unicellular yeast results in the evolution of multicellular clusters composed of hundreds of clonal cells (see the figure). These clusters quickly evolve a secondary trait: elevated rates of programmed cell death (apoptosis) (*9*). At first glance, it is hard to see how cellular suicide could be beneficial, but mathematical modeling reveals that higher rates of apoptosis allow clusters to circumvent growth constraints imposed by volume and nutrient flow limitations (*10*). Specifically, apoptotic cells act as "weak links" in a chain of cells within a cluster. Breaking these links produces proportionally smaller, faster-growing propagules. Indeed, high rates of apoptosis repeatedly evolved in independent replicate populations of lineages that form large clusters (*9*). This contrasts with a unicellular context where apoptosis should be maladaptive. If any of the yeast that evolved high rates of apoptosis within clusters were to leave the group and revert to a unicellular lifestyle, they would find themselves at a competitive disadvantage relative to other, low-apoptosis unicellular strains. Moreover, if the balance of selection favored single cells over groups, then cells that leave the group would enjoy less of a fitness benefit due to the cost of apoptosis in free-living cells. Thus, the accumulation of ratcheting traits, such as apoptosis, makes the successful reversion to unicellularity a many-step process (i.e., the traits evolved in a group context would need to be counterbalanced). This stabilizes multicellularity by limiting evolutionary reversion, even when the environment favors unicellular growth.



Apoptosis as a ratchet. Apoptotic cells (green) and dead cells (red) act as weak links within a cluster of yeast cells. Breaking these links allows the cluster to dodge growth constraints (volume and nutrient flow limitations), producing smaller, faster-growing groups of cells.

CREDIT: W. C. RATCLIFF

For traits to act as ratchets, they must have opposite effects on fitness in multi- and unicellular contexts. This dichotomy is clear in traits such as apoptosis. Premature death of the organism is, after all, an almost universally costly trait. However, other traits that ratchet the evolution of multicellular complexity may be more subtle. For instance, a division of labor over the production of metabolites could provide a ratcheting function. Recent experiments with bacteria have shown that strains engineered to cooperatively cross-feed grow faster than the unicellular ancestor capable of autonomous growth, but this benefit requires coculture (*11*). In general, the more a trait makes cells in a cluster mutually reliant, the more it serves as a ratchet. This raises the intriguing hypothesis that division of labor might be common in multicellular organisms not only because of the adaptive benefits of specialization, but also because multicellular lineages with this ratcheting trait may be more evolutionarily persistent.

Ratcheting may also play a role in other major evolutionary transitions, leading to the establishment of new types of biological "individuals." For example, symbiosis between free-living prokaryotes led to the origin of eukaryotic cells—a new type of biological individual—capable of photosynthesis and oxidative metabolism. The evolution of host cell dependence on the metabolic utility of their symbionts acts as a ratchet

[1]Santa Fe Institute, Santa Fe, NM 87501, USA. [2]School of Biology, Georgia Institute of Technology, Atlanta, GA 30332, USA. E-mail: elibby@santafe.edu

by limiting opportunity for reversion. For example, *Paramecium* experimentally deprived of their photosymbiont *Chlorella* pay a substantial fitness cost when grown under bright conditions (*12*). Likewise, the symbiont's loss of genes required for life outside of the host cell acts as a ratchet (*13*). During the evolution of eusocial "superorganisms" from multicellular individuals, reproductive division of labor (*14*) acts as a ratchet, limiting the ability of individuals to return to an asocial, solitary state. Honey bee workers, for instance, are incapable of sexual reproduction and can only lay male eggs, making it difficult to start a solitary nest.

The ratcheting theory may also help solve a "chicken and egg" problem of multicellular evolution. It has been argued that a key step in the evolution of multicellularity is the export of fitness from cells to groups—"Darwinizing" groups while "deDarwinizing" cells (*3*, *15*). This would allow groups to adapt as individuals. But how do groups evolve multicellular traits when the traits that facilitate multicellular adaptation are themselves multicellular adaptations? For example, reproductive division of labor through germ cell–somatic cell differentiation resolves conflicts between cell and group fitness interests during the evolution of large, long-lived organisms like animals. But germ-soma differentiation is a complex multicellular adaptation whose origin requires that the balance of selection tilts firmly toward groups, not cells. By stabilizing life in early multicellular groups, ratcheting facilitates further multicellular adaptation and, in so doing, may play a key role in the evolution of multicellular complexity.

REFERENCES

1. J. Maynard Smith, E. Szathmáry, *The Major Transitions in Evolution* (Oxford Univ. Press, New York, 1995).
2. R. K. Grosberg, R. R. Strathmann, *Annu. Rev. Ecol. Evol. Syst.* **38**, 621 (2007).
3. R. E. Michod, *Proc. Natl. Acad. Sci. U.S.A.* **104** (suppl. 1), 8613 (2007).
4. H. J. Folse 3rd, J. Roughgarden, *Q. Rev. Biol.* **85**, 447 (2010).
5. G. C. Williams, *Adaptation and Natural Selection* (Princeton Univ. Press, Princeton, NJ, 1966).
6. K. M. Sandoz, S. M. Mitzimberg, M. Schuster, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 15876 (2007).
7. W. C. Ratcliff *et al.*, *Nat. Commun.* **4**, 2742 (2013).
8. P. B. Rainey, B. Kerr, *BioEssays* **32**, 872 (2010).
9. W. C. Ratcliff, R. F. Denison, M. Borrello, M. Travisano, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 1595 (2012).
10. E. Libby, W. C. Ratcliff, M. Travisano, B. Kerr, *PLOS Comput. Biol.* **1**, e1003803 (2014).
11. S. Pande *et al.*, *ISME J.* **8**, 953 (2014).
12. S. J. Karakashian, *Physiol. Zool.* **36**, 52 (1963).
13. J. P. McCutcheon, N. A. Moran, *Nat. Rev. Microbiol.* **10**, 13 (2012).
14. B. J. Crespi, D. Yanega, *Behav. Ecol.* **6**, 109 (1995).
15. P. Godfrey-Smith, *Darwinian Populations and Natural Selection* (Oxford Univ. Press, New York, 2009).

10.1126/science.1262053

VIROLOGY

Unanchored ubiquitin in virus uncoating

Components of a cellular degradation system are exploited by influenza virus during infection

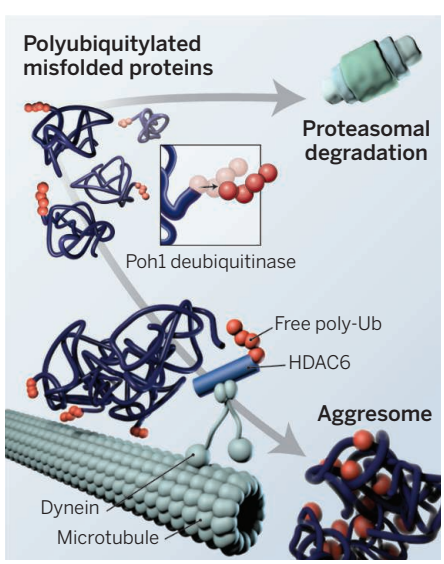
By **Ricardo Rajsbaum**[1] *and* **Adolfo García-Sastre**[2,3,4]

Misfolded proteins in eukaryotic cells can be modified with the small protein ubiquitin (Ub). The conjugation of a chain of Ub proteins [polyubiquitin (poly-Ub)] targets a substrate for destruction by complexes called proteasomes (*1*). But unanchored poly-Ub chains are emerging as key factors in multiple cellular responses, including innate antiviral pathways (*2*, *3*). Such free chains can also activate the aggresome pathway, another mechanism that degrades unwanted proteins when the proteasome system is overwhelmed or inhibited (*4*). On page 473 of this issue, Banerjee *et al.* (*5*) report that influenza virus engages the host cell's aggresome system by carrying unanchored poly-Ub chains. The strategy allows the virus to "uncoat" and replicate after its escape from the endosome during entry into the host cell.

Polyubiquitylation of proteins occurs post-translationally. The free carboxyl-terminal glycine residue of Ub can be conjugated to lysine residues of specific substrates. Ub itself can be covalently attached to other Ub proteins through one of its seven lysines (K6, K11, K27, K29, K33, K48, and K63) to form poly-Ub chains (*1*). Proteins modified with poly-Ub at K48 are usually targeted for the proteasome. In addition, a deubiquitylating enzyme, Poh1, releases K63-linked poly-Ub chains from protein aggregates (*6*). These freed chains are recognized by the enzyme histone deacetylase 6 (HDAC6), a component of the aggresome pathway. HDAC6 is a predominantly cytoplasmic protein deacetylase, but acts as an adaptor protein between unanchored Ub chains and dynein motor complexes in the aggresome-autophagy pathway (*7*–*9*). Thus, these unanchored poly-Ub chains function to recruit protein aggregates to the aggresome for degradation (*4*) (see the first figure).

Unanchored poly-Ub chains also have antiviral functions. Unanchored K63-linked poly-Ub chains bind to and activate retinoic acid–inducible gene I (RIG-I), a protein that induces the expression of type I interferons, potent antiviral cytokines (*3*). Additionally, unanchored K48-linked poly-Ub chains bind and activate the enzyme inhibitor of κB kinase ε (IKKε) and a downstream signaling cascade that stimulates the expression of antiviral genes (*2*).

Influenza A viruses (IAVs) are highly infectious pathogens that have caused major pandemics and annual epidemics. Successful virus infection requires crossing the host cell membrane. IAV is an enveloped virus that attaches to a target cell and gets internalized by the endocytosis pathway. Acidification of the endosome causes a conformational change in the viral surface protein hemagglutinin (HA), which triggers fusion of the viral and endosomal membranes. As a result, viral ribonucleoproteins (RNPs), consisting of the viral RNAs, nucleoprotein (NP), and polymerase, are released into the cytoplasm. RNPs are then transported into the nucleus where viral RNA replication takes place (*10*). But for this passage to occur, RNPs must lose an associated viral matrix M1 protein. This process, called uncoating, is facilitated by the viral M2 protein, a small ion channel in the viral membrane (*11*) (see the second figure).



Aggresome pathway. Misfolded proteins that are tagged with poly-Ub, but are not destroyed in the proteasome, are recognized by HDAC6. HDAC6 binds to free ubiquitin chains and to dynein motor complexes in the aggresome-autophagy degradation pathway.

CREDIT: V. ALTOUNIAN/*SCIENCE*