How nontraditional model systems can save us

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ABSTRACT **In this essay I would like to highlight how work in nontraditional model systems is an imperative for our society to prepare for problems we do not even know exist. I present examples of how discovery in nontraditional systems has been critical for fundamental advancement in cell biology. I also discuss how as a collective we might harvest both new questions and new solutions to old problems from the underexplored reservoir of diversity in the biosphere. With advancements in genomics, proteomics, and genome editing, it is now technically feasible for even a single research group to introduce a new model system. I aim here to inspire people to think beyond their familiar model systems and to press funding agencies to support the establishment of new model systems.**

My career as a biologist began in the orange groves and lake waters of central Florida. An unstructured childhood was spent learning to observe and wonder. Without realizing it at the time, my training began with the mantra, "Study nature, not books," the familiar entreaty of Louis Agassiz, a founder of what would become the Marine Biological Lab (MBL) in Woods Hole, Massachusetts. In that humid air, listening to cicadas click, I subconsciously practiced asking basic questions about the structure of the natural world. I suspect many of us began our careers this way, even though we ended up thinking about systems of molecules from behind the black curtains of the microscope room, immersed in the frosty air of the cold room, or bathed in the glow of a computer screen. Before the grown-up challenges of funding, publishing, and progressing in a career, we easily marveled at the complexity and surprises of the living world. How can we recapture the joy that comes from curiosity-driven inquiry? This being an essay for the mid-career award, it seems appropriate to blend material for a midlife with a plan for how we as a cell biology community can identify big new questions. So instead of a fancy car, a new microscope, or a dangerous (professional) liaison, try developing a new model system as an outlet for your midlife crisis!

Photo Credit: Rob Strong

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NONTRADITIONAL SYSTEMS PRODUCE PUZZLES AND PARADOXES

I begin with my own experiences in working with a nontraditional model fungus called *Ashbya gossypii*. As a postdoc in Peter Philippsen's group in UniBasel, Switzerland, I started work with *Ashbya*, which is relatively closely related on the genome scale to the budding yeast *Saccharomyces cerevisiae* but has some strikingly different cell biology (Dietrich *et al.*, 2004). As a postdoc, I began studying the perplexing ability of nuclei in *Ashbya*'s multinucleate cells to divide asynchronously despite being in a common cytoplasm (Gladfelter *et al.*, 2006). This was a paradox, because decades of classic work demonstrated that cytosolic factors (CDK/cyclins) control the cell cycle and should synchronize nuclei in a syncytium (Johnson and Rao, 1970). This observation has fueled many experiments in my lab for the past decade, and we have been led to new models for compartmentalizing cytosol, have gained insight

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into how ploidy can vary in syncytia, and have been continually surprised and inspired (Anderson *et al.*, 2013, 2015; Lee *et al.*, 2013, 2015). For example, one set of experiments led us to realize functional uses for polyQ-tract proteins in localizing the mRNAs encoding cyclins (Lee *et al.*, 2013). This discovery was an early example of a now growing list of functional uses for protein aggregation outside pathologies. Importantly, as a junior faculty member, I had enough of an autonomous niche that my students and I could ask big questions without concerns about being scooped by bigger labs.

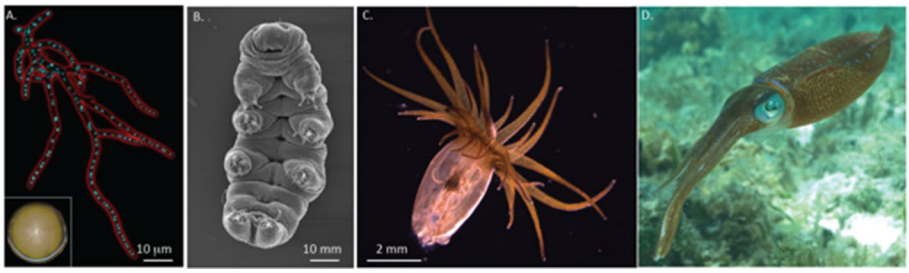

FIGURE 1: Images of nontraditional model systems discussed in this essay. (A) *Ashbya* (fungus), image provided by Hanspeter Helfer. (B) Tardigrade (water bear), image provided by Bob Goldstein (University of North Carolina, Chapel Hill). (C) *Aiptasia* (sea anemone), image provided by John Pringle (Stanford University). (D) *Sepioteuthis sepioidea* (reef squid), image provided by Roger Hanlon (Marine Biological Laboratory).

WHAT ARE THE CHALLENGES OF WORKING IN A NONTRADITIONAL SYSTEM?

While there is a tremendous amount of stimulation and freedom that comes from work on a less-studied system, there are also challenges. For example, there is not a large community of users, so I sought out the yeast, filamentous fungus, and cell biology communities—all of whom have been open to *Ashbya* as an alternative system. It also means most reagents are not simply an email away but instead typically have to be developed in the lab. This is slow, but again, if you are not in a race to publish, it is less of a concern. A molecular geneticist extraordinaire, such as Patricia Occhipinti, a long-term technician in my lab, makes it possible to generate any kind of new strain. Investing in a person like this as a lab constant is critical for establishing a new system. Also, if a system is somewhat related to an established model system, some tools may be transportable or at least adaptable, and so we have borrowed many tricks from the yeast genetics world. Finally, one has to convince funding agencies of the value of the problems that can be addressed by this system. (More on this later.) If the system is conducive to studying a fundamental problem, brings contrasting mechanisms into our current thinking about a key process, and is tractable, it should be possible to make a compelling case for support. There are truly unknown problems in biology waiting in the wings.

SUCCESS STORIES OF ESTABLISHING NONTRADITIONAL MODEL SYSTEMS

In the most successful new system launches, there is either new biology or a new handle on an old problem that make the systems especially suited for investment. The entry point to a new experimental system tends to be either through old descriptive literature that captures some fascinating phenomena or through a genomic approach that reveals a surprising manifestation of, or absence of, genes thought to be critical for a process. For example, Bungo Akiyoshi, who recently established his own lab at Oxford, comes from a PhD working on kinetochores in yeast with Sue Biggins. Akiyoshi noticed a complete absence of typical kinetochore proteins in the genome of *Trypanosoma brucei*, a protozoan parasite (Akiyoshi and Gull, 2013, 2014; Figure 1). He went on to identify a novel mechanism of chromosome segregation for eukaryotes, and, because of the unique identity of the proteins, they are likely to be drug-able targets for treatment of African sleeping sickness disease, which is caused by infection with *T. brucei*. In seeking out basic biology in an understudied system, Akiyoshi's lab is finding new mechanisms to old cell biological problems that could lead to a potential disease cure.

Adoptions of alternative systems can also stem from a lab with a principal investigator familiar with established systems and willing to take risks. Wallace Marshall at the University of California, San Francisco, for example, long a student of flagella in *Chlamydomonas*, has recently established work in *Stentor*, a ciliate among the largest single-celled organisms known. Marshall's lab has established genetic techniques, proteomics, and transcriptomics to study cell polarity, cytoplasmic flow, and regeneration in these simple and intriguing pond dwellers (Slabodnick *et al.*, 2013, 2014; Slabodnick and Marshall, 2014). Similarly, Bob Goldstein at the University of North Carolina, Chapel Hill, an established leader in *Caenorhabditis elegans* developmental cell biology, invested in establishing another microscopic animal—the Tardigrade or aptly named "water bear" (Figure 1). The water bear is fascinating in terms of evolutionary development but also in its ability to withstand prolonged desiccation (Gabriel and Goldstein, 2007; Gabriel *et al.*, 2007; Tenlen *et al.*, 2013). The water bear has already journeyed into space for experiments with the aim of understanding how these tiny animals that normally reside in moss are so resilient. Both water bears and *Stentor* are proving to be tractable, rich sources of new biology and may provide important insight into stress responses and regeneration.

There are also many "forgotten" systems that were once widely studied but fell out of favor in the recombinant DNA revolution at the end of the 20th century. Many of these creatures happen to reside in the sea or other aquatic habitats and boast deep literatures of physiology, behavior, and, in some cases, cell biology (Figure 1). The squid is a great example, showing us microtubule motors and the action potential, yet the squid research community has shrunk dramatically, in part because squid cannot be readily cultured, thus requiring a steady supply from a marine lab such as the MBL in Woods Hole. Yet there is still tremendous biology being discovered in squid and other cephalopods. These organisms widely use RNA editing to generate variation in protein sequences posttranscriptionally, as is being studied intensively by Josh Rosenthal at the University of Puerto Rico and the MBL (Alon *et al.*, 2015). RNA editing is dependent on temperature and, likely, other environmental conditions (Garrett and Rosenthal, 2012). Investment in cephalopodomics is promising to bring these traditional systems back in vogue. Finally, a hero of yeast geneticists, John Pringle, almost completely switched model systems to a simple sea anemone about 10 years ago. He has rejuvenated his program with new questions relevant to coral ecology and symbiosis (Lehnert *et al.*, 2012, 2014). The sea is vast, and we have tapped into an extremely small amount of the biodiversity, especially at the cell and molecular level.

HOW CAN WE MAKE IT MORE FEASIBLE FOR LABS TO ESTABLISH NEW SYSTEMS?

Millions of years of evolution has done the hard work of creating vast biodiversity on the planet, so how can we start feasting intellectually on this biology at the level of molecules, cells, and tissues? There is no question that, in this century, we will face problems arising out of climate change, habitat destruction, and expanding and aging human populations that are highly mobile, allowing rapid spread of infectious diseases. How can we best prepare to solve the challenges that will arise from these monumental changes on the planet? I would argue that the track record of basic science solving problems relevant to human health and society is sparkling. Why should we stop here when the technology is in our reach to readily tame many systems?

The question is who can pay for the risk of attempting to establish a new system. For *Ashbya*, Peter Philippsen had the drive and ingenuity to fund sequencing the *Ashbya* genome more than 15 years ago. He was willing to take the risk and had the creativity to invest in a completely new system after decades of work in budding yeast. He had enough funding flexibility at the time, being in a European institution with industry support, to see it through to complete establishment. What could be done to make it more feasible for even small and new labs to find a parallel alternative model? It could be argued that this is for the realm of the National Science Foundation (NSF), as the work will likely initially result in new science but may not immediately be translated to human health. However, the National Institute of General Medical Sciences at the National Institutes of Health (NIH) has a stellar history in funding basic science that leads to Nobel Prizes and cures for diseases with time. What if everyone who had an R01 and the interest could apply for a "model organism development" supplement to perform foundational experiments to establish a new system in parallel with their conventional system?

The advantages and joys of work in an unchartered system revolve around the intellectual challenges, the breathing room of broad problems, which is especially useful for junior scientists, and the potential to discover truly new solutions in biology and for society. Each summer I move part of my lab to the MBL in Woods Hole, and one future for this hallowed place may well be to facilitate the development of new systems—if we can convince funding agencies and our deans to allow us this freedom. My children, ages seven and nine, eagerly attend the Children's School of Science in Woods Hole while we are there, and each day they head to the ponds, the surf, and the fields to observe nature. For their generation, who are currently learning to gaze at diverse creatures, I hope they have a menagerie of choices of systems in which to study biology.

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REFERENCE

Akiyoshi B, Gull K (2013). Evolutionary cell biology of chromosome segregation: insights from trypanosomes. Open Biol 3, 130023.

Akiyoshi B, Gull K (2014). Discovery of unconventional kinetochores in kinetoplastids. Cell 156, 1247–1258.

Alon S, Garrett SC, Levanon EY, Olson S, Graveley BR, Rosenthal JJ, Eisenberg E (2015). The majority of transcripts in the squid nervous system are extensively recoded by A-to-I RNA editing. eLife 4, 05198.

Anderson CA, Eser U, Korndorf T, Borsuk ME, Skotheim JM, Gladfelter AS (2013). Nuclear repulsion enables division autonomy in a single cytoplasm. Curr Biol 23, 1999–2010.

Anderson CA, Roberts S, Zhang H, Kelly CM, Kendall A, Lee C, Gerstenberger J, Koenig AB, Kabeche R, Gladfelter AS (2015). Ploidy variation in multinucleate cells changes under stress. Mol Biol Cell 26, 1129–1140.

Dietrich FS, Voegeli S, Brachat S, Lerch A, Gates K, Steiner S, Mohr C, Pöhlmann R, Luedi P, Choi S, *et al*. (2004). The *Ashbya gossypii* genome as a tool for mapping the ancient *Saccharomyces cerevisiae* genome. Science 304, 304–307.

Gabriel WN, Goldstein B (2007). Segmental expression of Pax3/7 and engrailed homologs in Tardigrade development. Dev Genes Evol 217, 421–433.

Gabriel WN, McNuff R, Patel SK, Gregory TR, Jeck WR, Jones CD, Goldstein B (2007). The Tardigrade *Hypsibius dujardini*, a new model for studying the evolution of development. Dev Biol 312, 545–559.

Garrett SC, Rosenthal JJ (2012). A role for A-to-I RNA editing in temperature adaptation. Physiology 27, 362–369.

Gladfelter AS, Hungerbuehler AK, Philippsen P (2006). Asynchronous nuclear division cycles in multinucleated cells. J Cell Biol 172, 347–362.

Johnson RT, Rao PN (1970). Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. Nature 226, 717–722.

Lee C, Occhipinti P, Gladfelter AS (2015). PolyQ-dependent RNA-protein assemblies control symmetry breaking. J Cell Biol 208, 533–544.

Lee C, Zhang H, Baker AE, Occhipinti P, Borsuk ME, Gladfelter AS (2013). Protein aggregation behavior regulates cyclin transcript localization and cell-cycle control. Dev Cell 25, 572–584.

Lehnert EM, Burriesci MS, Pringle JR (2012). Developing the anemone *Aiptasia* as a tractable model for cnidarian-dinoflagellate symbiosis: the transcriptome of aposymbiotic *A. pallida*. BMC Genom 13, 271.

Lehnert EM, Mouchka ME, Burriesci MS, Gallo ND, Schwarz JA, Pringle JR (2014). Extensive differences in gene expression between symbiotic and aposymbiotic cnidarians. G3 (Bethesda) 4, 277–295.

Slabodnick M, Prevo B, Gross P, Sheung J, Marshall W (2013). Visualizing cytoplasmic flow during single-cell wound healing in *Stentor coeruleus*. J Vis Exp, e50848.

Slabodnick MM, Marshall WF (2014). *Stentor coeruleus*. Curr Biol 24, R783–R784.

Slabodnick MM, Ruby JG, Dunn JG, Feldman JL, DeRisi JL, Marshall WF (2014). The kinase regulator mob1 acts as a patterning protein for *Stentor* morphogenesis. PLoS Biol 12, e1001861.

Tenlen JR, McCaskill S, Goldstein B (2013). RNA interference can be used to disrupt gene function in tardigrades. Dev Genes Evol 223, 171–181.