Published in final edited form as:

Curr Opin Microbiol. 2015 August ; 26: vii–ix. doi:10.1016/j.mib.2015.07.003.

Editorial Overview: Heterogeneity in fungal cells, populations, and communities

Deborah A. Hogan and

Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, 03755, United States of America

Amy S. Gladfelter

Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire, 03755 United States of America

In ecology, many studies support the notion that community strength positively correlates with diversity. A diverse community has a reservoir of varying genotypes and phenotypes allowing responses to fluctuating environments and exploitation of specific niches in the shared habitat. While most of the studies on the benefits on diversity have been performed on complex communities comprised of different species, it is becoming increasingly clear that functionally relevant diversity or heterogeneity also exists even at the scale of genetically identical single cells that coexist in the same niche. With the advent of new microscopic approaches involving fluorescent protein reporters, flow cytometry-based methods, and advances in high through-put sequencing technology, we are gaining new insight into heterogeneity of microbes including the world of fungi. Our contributors have reviewed many different aspects of functional heterogeneity in fungi at the scale of single cells or even within a single cell. Together, these contributions emphasize how heterogeneity exists on variable temporal and spatial scales. The pieces in this issue all highlight the need for future studies that will examine the mechanisms that drive variability in behavior and the function of diverse behaviors within populations of microbes.

Two contributions by Wang and Lin [1] and Scaduto and Bennett [2] highlight some of the exciting advances in the field of heterogeneity in cell identities in two different fungal pathogens, *Cryptococcus neoformans*, a basidiomycete, and *Candida albicans*, an ascomycete. Both fungi grow as different morphotypes that have distinct morphologies, physiologies and behaviors. Wang and Lin [1] suggest a compelling model in which *C. neoformans*, an environmental saprophytic fungus with the potential to cause devastating human disease, has morphotypic states can be advantageous in one setting but less fit in other contexts. For example, hypha formation is linked with mating but is not well suited for growth in vivo at body temperature. In contrast, in the human lung, giant or titan cryptococcal cells are observed, though their role in disease is not yet known. In biofilms, there can be environmental and chemical cues that either homogenize the population (such

as quorum sensing) or that promote heterogeneity (environmental cues such as low oxygen or nutrient limitation). In addition, the production of specific matrix materials within the biofilm can feedback and impact gene expression in cells that are in contact with matrix components [3]. Thus, in *C. neoformans*, it is clear the same organism samples many different forms or states depending on the environmental context. It is still not yet clear, however, the functional advantages of this sampling nor the degree or function of variability within a given state or context.

Scaduto and Bennett [2] discuss the different states of *C. albicans*, including the canonical white state, the opaque, mating competent form, and more recently discovered grey and gut phenotypes, and the links between these cellular states and environmental cues. In this review, the authors discuss how cells in these different states can interact within one another in the context of biofilms. For example, pheromone produced by opaque cells is capable of influencing both white and opaque cell behavior [4]. The authors also compare mating pathways in *C. albicans* to those in other *Candida* species indicating a broad trend in which different phenotypes are linked to morphology. As described for *Cryptococcus* above, the behaviors of *C. albicans* reviewed here highlight the roles of varied forms of single cells within a single species population and shows that the coexistence of variable forms leads to different outcomes for the community.

Future single cell genomics and transcriptomics will likely enumerate new distinct states that are present but may not yet be associated with clear phenotypic characteristics. Such studies are critically needed both to identify new sources of variable behavior and to understand the mechanisms controlling phenotypic switching processes. Additional challenges lie in determining the degree of phenotypic heterogeneity within a given state such as within a population of seemingly similar filamentous cells. While there may not be easily monitored changes in morphology, there is likely variation among cells in terms of metabolism, stress resistance and cell wall characteristics in the filamentous state as there are in the yeast forms. There are likely reservoirs of phenotypic plasticity still awaiting discovery. Nearly everyone who has gazed down a microscope realizes the heterogeneity between cells that is almost always detectable no matter what protein, process or cell type is being studied. A charge for future fungal biology study is that the variation is not thrown out in determining the population “average” but begins to be quantified and analyzed in its own right.

Gernstein and Berman [5] describe another type of heterogeneity that is important to acknowledge and understand: karyotype variation as manifested in ploidy differences within a population. From yeast to man, it has become recognized that within a population derived from a common ancestor, there can be rapid expansion in heterogeneity due to mitotic missegregation or polyploidization. Heterogeneity in ploidy has long been underestimated due to the elimination of the variability within populations once they are propagated ex vivo. While ploidy variation has been shown to be common in both *Candida albicans* and *Cryptococcus* and to impact adaptation in these fungi ([6] for review), there is only recently a growing understanding on the generation and stability of changes in ploidy or the consequences of karyotype variation in different morphotypes in these species.

Many fungi spend substantial periods of their life cycles as syncytia-multinucleated mycelia. In this setting many nuclei cohabitate in the same cytoplasm but remarkably there is even variable behavior seen between different territories within one of these large mycelia. Roper and colleagues [7] highlight how cytoplasmic flow and factors that restrict movement of molecules and organelles within the cytoplasm contribute to heterogeneity within fungi syncytia. The authors nicely contrast the movement of macromolecular structures by active transport, diffusion or by flow, and highlight the reasons why it is useful to understand the processes by which movement occurs. For instance, while active microtubule-driven active transport is involved in the movement of polarity associated transcripts and proteins towards the growing hyphal tip, in some cases movement of the microtubules themselves occurs by flow. Some highly regulated processes for the control of flow have been described. For example, flow in ascomycetes can be modulated by Woronin Bodies which control the movement of cytoplasm through pores within the septa thus limiting the movement of cytoplasm into older cells. Woronin bodies generally allow flow through pores between cells near the actively growing hyphal tips and the state of the pores can also create local turbulence and inhomogeneities in cytoplasm flow that generates heterogeneous cytoplasm even within a single compartment. Thus, even within a fungal filament, there is heterogeneity in terms of the properties of cytoplasm between different regions of the hyphae. One can imagine that these different regions may have distinct reactions to or interactions with host cells or other microbial cells. Additionally, even within the cytoplasm of a multinucleate cell, different nuclei can have different dynamics which can influence fungal cell biology in terms of where septa or branches form leading to different morphologies. Thus it is interesting to consider how fungal pathogens can meet their needs of strict spatial control of effector release or host invasion in the context of a dynamic cytoplasm. In fungal species that shift between yeast and filamentous forms, the different cellular geometries likely create different cytoplasm dynamics. It is interesting to consider how cell shape itself influences the kinetics or outputs of pathways that involve diffusible proteins and small molecules, and how these differences relate to important biological phenotypes.

Oliveira-Garcia and Valent [8] describe the fascinating process by which fungi prevent detection by plant hosts by remodeling their cell wall in a variety of different ways in order to avoid detection by the plant. One of the interesting topics discussed in this review is the fact that multiple fungal plant pathogens mask cell wall chitin and sequester released chitin monomers (N-acetylglucosamine or GlcNAc) to avoid detection by plant hosts. The presence of such processes suggest that there is significant release of these molecules from the fungus. One wonders if this may provide insight into why GlcNAc is a potent inducer of phase variation and changes in morphology in other fungi like *C. albicans* [9, 10] or *Histoplasma capsulatum* [11]. Perhaps changes in cell wall dynamics either preceding or concomitant with a change in cellular state, generates a particularly strong GlcNAc signal. Because of the opportunities for in vivo live-cell imaging, plant-fungus interactions create an important opportunity to monitor the spectrum of phenotypes within a population to determine if there are subpopulations with respect to some phenotypes, such as metabolism, but uniformity in other properties such as cell wall remodeling to avoid detection.

Xu and Dongari-Bagtzoglou [12] address the mycobiome in the oral environment, and describe the oral mycoflora as being the most complex in the human body. The complexity may in fact increase or decrease as the spatial heterogeneity is assessed as it has been for bacterial species, such as *Rothia*, in different habitats within the mouth [13]. Spatial analysis of the microbial communities within oral biofilms makes it possible to examine the behavior of individual fungal cells in the context of other species. Gene fusions to reported genes or mRNA FISH approaches can then be used to measure phenotypic properties in the different contexts. One of the important take home messages in the analysis of the mycobiome was that there is abundant fungal diversity in the mouth, more than previously recognized, and that we have many opportunities to understand their biological roles in this accessible environment and capture heterogeneity in individual cell behavior in the context of a complex community.

Together, these reviews illustrate the complexity that microbiologists must acknowledge in studying microbes in the context of microbial communities, eukaryotic hosts and in different environmental contexts. There are surely more types of heterogeneity in fungal populations that we have yet to recognize and a challenge that lays ahead is to acknowledge and study the uses of heterogeneity. Together, these reviews will aid microbiologists in deciding how to measure and monitor the activity of populations and how to assess whether heterogeneity-generating mechanisms impact the processes that they study. In addition, these reviews highlight some of the processes that influence the microbe's response to the host environment. Of course, we must also acknowledge that microbes have not cornered the market on heterogeneity within populations-the mammalian and plant hosts have their own heterogeneity on the cellular and subcellular level that will ultimately need to be integrated with variable behavior in the microbe. Heterogeneity on the host side is highlighted in the many flow cytometry based assays used to examine the immune response of different populations. In the vast majority of cases, only subpopulations are able to respond to added stimuli or pathogens. Future studies will unite these two lines of work by determining if there are specific in vivo cues that accentuate variation within the population of either host or microbe. The discovery of specific regulatory pathways the influence variation will aid in our understanding of if variability is selected for and the importance of heterogeneity mechanisms in nature.

Biographies

Deborah A. Hogan is an Associate Professor of Microbiology and Immunology in the Geisel School of Medicine at Dartmouth. Her research focuses on intra and interspecies interactions in the context of opportunistic infections caused by *Pseudomonas aeruginosa* and the fungus *Candida albicans*. Her recent work focuses on the fungi within the human-associated microbial communities.

Amy Gladfelter is an Associate Professor of Biology at Dartmouth. Her research focuses on understanding how fungal cells are spatially organized and control their shape. She blends quantitative imaging with modeling in a variety of fungal species to identify mechanisms of cellular compartmentalization and sources of variability in cell behavior.

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