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Review

Bend or break: how biochemically versatile molecules enable metabolic division of labor in clonal microbial communities

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Abstract

In fluctuating nutrient environments, isogenic microbial cells transition into "multicellular" communities composed of phenotypically heterogeneous cells, showing functional specialization. In fungi (such as budding yeast), phenotypic heterogeneity is often described in the context of cells switching between different morphotypes (*e.g.*, yeast to hyphae/pseudohyphae or white/opaque transitions in *Candida albicans*). However, more fundamental forms of metabolic heterogeneity are seen in clonal *Saccharomyces cerevisiae* communities growing in nutrient-limited conditions. Cells within such communities exhibit contrasting, specialized metabolic states, and are arranged in distinct, spatially organized groups. In this study, we explain how such an organization can stem from self-organizing biochemical reactions that depend on special metabolites. These metabolites exhibit plasticity in function, wherein the same metabolites are metabolized and utilized for distinct purposes by different cells. This in turn allows cell groups to function as specialized, interdependent cross-feeding systems which support distinct metabolic processes. Exemplifying a system where cells exhibit either gluconeogenic or glycolytic states, we highlight how available metabolites can drive favored biochemical pathways to produce new, limiting resources. These new resources can themselves be consumed or utilized distinctly by cells in different metabolic states. This thereby enables cell groups to sustain contrasting, even apparently impossible metabolic states with stable transcriptional and metabolic signatures for a given environment, and divide labor in order to increase community fitness or survival. We speculate on possible evolutionary implications of such metabolic specialization and division of labor in isogenic microbial communities.

Keywords: phenotypic heterogeneity; cross-feeding systems; gluconeogenesis; glycolysis; metabolic specialization; division of labor

Introduction

Unicellular organisms seldom naturally exist as individuals. Rather, they live in communities with varying degrees of complexity (Stahl *et al.* 2006; Callieri *et al.* 2018). This community lifestyle provides many advantages to the individuals within, including enhanced growth/proliferation, better survival in fluctuating environments, and resilience against invaders/cheaters (Shade *et al.* 2012; Dos Santos *et al.* 2018). Microbial communities can be comprised of individuals from (a) different domains of life (lichens), (b) different species within the same kingdom of life (polymicrobial bacterial/fungal communities), or (c) arise from the clonal expansion of a single cell. In all these communities, there exists a diversity among the individuals due to their genetic makeup or variations in the environment they live in (Elowitz *et al.* 2002; Raj and van Oudenaarden 2008; Balázsi *et al.* 2011; Ackermann 2013, 2015). Interestingly, even communities formed by genetically identical cells growing in the same microenvironment, can exhibit substantial phenotypic variation with respect to gene expression patterns, metabolic states, physical properties, response to nutrients and external stresses (Stockholm *et al.* 2007; Ackermann 2015; Takhaveev and Heinemann 2018). Collectively, cellular phenotypic heterogeneity includes all these variations among the individual cells of the community, and this functionally impacts the overall fate of the community (Ackermann 2013, 2015). An unanswered question in the field of microbial phenotypic heterogeneity is, how do genetically identical cells/clonal cells achieve phenotypic heterogeneity within communities, and what does that imply? Here, we highlight emerging ideas that address these questions, focusing primarily on metabolic heterogeneity. We illustrate how given biochemical processes lead to metabolic constraints that enable distinct phenotypic states to sustain themselves within a clonal community.

What might phenotypic heterogeneity mean to a clonal community?

Phenotypic heterogeneity in clonal cell populations can confer multiple benefits to the individuals of the community. Variations amongst individuals ensure that a subset of the population survives sudden changes in the environment and can thereby allow genotypes to persist in fluctuating conditions. This collective bet-hedging increases the survivability of individuals in the community (López-Maury *et al.* 2008; Beaumont *et al.* 2009; Grimbergen

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et al. 2015). Phenotypic heterogeneity also can lead to division of labor between individuals of the community, wherein different individual cells perform distinct functions. This can enable challenging biochemical processes to operate (van Gestel et al. 2015; West and Cooper 2016; Giri et al. 2019). Much like other microbial species discussed in other reviews (Süel et al. 2007; Stewart et al. 2011), several fungi exhibit incredible heterogeneity within clonal communities (Hogan and Gladfelter 2015; Hewitt et al. 2016). Well-studied examples include the filamentous fungi *Aspergillus niger* which exhibits heterogeneity at multiple levels: differences between individual spores (*e.g.*, during spore formation, quiescence, or germination), differences between individual hyphae of the same mycelium, or phenotypic variation between distinct, isogenic mycelia (Hewitt et al. 2016; Wosten et al. 1991; Vinck et al. 2005, 2011; de Bekker et al. 2011a, b).

Phenotypic heterogeneity in yeasts

Yeasts are a well-studied subset of fungi and comprise of species from two distinct phyla: the Ascomycota and the Basidiomycota (Kurtzman and Fell 2006; Suh et al. 2006). Similar to other fungi, yeasts exhibit a spectrum of phenotypic heterogeneity. For example, the opportunistic pathogen *Candida albicans* exhibits different types of heterogeneity (Marr et al. 2001; Sherry et al. 2014). Under standard laboratory conditions, these fungi exist in their yeast form. However, when subjected to conditions that mimic the mammalian host environment (temperature, pH, presence of serum, and so on), they undergo a dramatic transformation where a subset of the population switches their morphogenetic states from yeast cells to long hyphal (tube-like) cells (Noble et al. 2017; Kornitzer 2019). These hyphal cells perform nuanced functions including penetrating the host tissue, invading newer niches, and successfully evading the host immune response (Cheng et al. 2012; Dühring et al. 2015). Interestingly, biofilms formed by *C. albicans* are comprised of cells in both the yeast as well as the hyphal state, and this heterogeneity is critical for them to successfully infect a host (Gulati and Nobile 2016). Another well-studied type of phenotypic heterogeneity in *C. albicans* arises due to white/opaque switching (Soll 2014). *C. albicans* switch between two distinctive types of cells, white and opaque. Each cell type is heritable for multiple generations and switching occurs without a change in the genetic make-up of the microorganism. Distinct properties exhibited by white and opaque cell types result largely from the differential regulation of ~400 genes (Lohse and Johnson 2009). The metabolic state of cells (Lan et al. 2002; Ene et al. 2016), biofilm formation (Daniels et al. 2006), response to host immunity (Kolotila and Diamond 1990; Geiger et al. 2004; Lohse and Johnson 2008), and the ability to undergo mating (Miller and Johnson 2002) all show differences between white and opaque cells.

Saccharomyces cerevisiae, the model organism that has illuminated diverse aspects of eukaryotic biology, exhibits well-studied phenotypic heterogeneity like *C. albicans* (Palková and Váchová 2016). Under standard laboratory conditions, *S. cerevisiae* cells propagate as 'unicellular' yeasts. However, environmental and nutritional cues, including nitrogen starvation result in cells forming pseudohyphae, and eventually filamentous growth. During pseudohyphal development, *S. cerevisiae* cells become elongated and budding occurs synchronously in a unipolar fashion resulting in the production of chains of cells called pseudohyphae (Gancedo 2001). Some (predominantly haploid) *S. cerevisiae* strains also change their morphology after extended growth in standard laboratory medium and these filamentous cells invade into the solid agar medium on which they are growing (Gimeno et al. 1992; Roberts and Fink 1994). Pseudohyphal cells are distinct from yeast cells with respect to gene expression patterns, and multiple signaling pathways are required to elicit this switching in response to nitrogen starvation (Gancedo 2001; Cullen and Sprague 2012).

Protein-based elements of inheritance, such as prions, also drive phenotypic heterogeneity in clonal *S. cerevisiae* populations. Prions are ordered, self-assembled aggregates of proteins that can be inherited by daughter cells in *S. cerevisiae* (Uptain and Lindquist 2002; Wickner et al. 2007). When a yeast protein self-aggregates and forms prions, there is reduced normal cellular activity of this protein, and this often results in changes in cellular phenotypes (Halfmann et al. 2010, 2012). For example, Ure2 is a nitrogen catabolite repressor and when it is active, shuts down the machinery that allows *S. cerevisiae* to utilize poor nitrogen sources. However, when Ure2 self-aggregates and forms prions called [URE3], *S. cerevisiae* cells constitutively utilize poor nitrogen sources (Wickner 1994; Shorter and Lindquist 2005). Hypothetically, if a population of yeast cells were to be subjected to a sudden change in quality of nitrogen sources (rich to poor), the population that exhibits the [URE3] phenotype would have a better chance to survive this change as it is already primed to utilize poor quality nitrogen sources. Yeast cells spontaneously form prions at a frequency of $\sim 10^{-6}$. As a result, at any given time, a sizable population of yeast cells will contain a few [prion$^+$] cells exhibiting alternate phenotypes. If the environment is such that the [prion$^+$] state is beneficial, these cells would then have a greater chance of surviving and proliferating in that environment. If after a period of time, the environment changes to a state where [prion$^+$] no longer confers any advantage or causes a growth disadvantage, those cells that do not have the prion would now have a greater chance of surviving, and their percentage would increase in the overall population. Thus, prions can be bet-hedging devices that allow cells to spontaneously switch between phenotypes in a heritable fashion in fluctuating environments (Tuite 2016). In all these examples, phenotypic heterogeneity increases as specific nutrients become limiting in the environment.

Metabolic heterogeneity in yeast communities

Perhaps the most fundamental form of phenotypic heterogeneity exhibited by *S. cerevisiae* is observed during their development in response to glucose limitation. Early reports describing this phenomenon showed that colonies of *S. cerevisiae* on low-glucose medium formed structurally complex communities with hallmarks of microbial biofilms, including differential gene expression patterns (Mináriková et al. 2001; Reynolds and Fink 2001). Subsequent studies identified transcriptional networks and signaling pathways essential for complex community development in response to glucose limitation (Mináriková et al. 2001; Granek and Magwene 2010). Interestingly, mounting evidence suggests that these complex biofilm communities exhibit phenotypic heterogeneity within. For example, studies of structured yeast communities developing in glucose-limited environments, showed two populations of cells within, exhibiting high and low metabolic activity (Cáp et al. 2012; Palková and Váchová 2016). These groups of cells show heterogeneity with spatial organization with respect to mitochondrial activity, glycolytic activity, autophagy, and general starvation response (Čáp et al. 2015; Váchová and Palková 2018).

This raises a fundamental question: how does this phenotypic (particularly metabolic) heterogeneity arise within this clonal community, where the distinct states retain spatial organization? Emerging evidence suggests that the nature and organization of biochemical networks within these cells can explain how cells in

heterogeneous states organize and function within the community.

Self-organizing biochemical systems enabling metabolic heterogeneity in clonal yeast communities

Recent studies now address how this occurs (Varahan *et al.* 2019, 2020). In yeast communities developing in low-glucose environments, most cells are initially in a gluconeogenic state, which is expected in this environment. This state is maintained by stable transcriptional programs that drive gluconeogenesis and related pathways under glucose-limited conditions. Gluconeogenesis is an unavoidable, essential metabolic process for cells growing in low-glucose environments, where cells use available carbon substrates to synthesize the gluconeogenic precursor, oxaloacetate (OAA). Oxaloacetate is converted into phosphoenolpyruvate (PEP) using the PEP carboxykinase enzyme (Pck1) which represents the first committed step of gluconeogenesis. PEP is sequentially converted into fructose-1,6-bisphosphate predominantly using the same enzymes that are involved in glycolysis (in the reverse direction), and fructose-1,6-phosphate is converted into fructose-6-phosphate using the fructose-1,6-bisphosphatase (Fbp1) enzyme which is also gluconeogenesis specific. Fructose-6-phosphate is then converted into glucose-6-phosphate and this acts as a carbon precursor for the synthesis of complex sugars like trehalose and glycogen (Nelson and Cox 2017). However, as the colony develops, groups of cells with dramatically opposite metabolic states emerge. Surprisingly this new population of cells exhibits high glycolytic, along with high pentose phosphate pathway (PPP) activities, and this is fuelled by the break-down of trehalose obtained from the gluconeogenic cells which is converted into glucose-6-phosphate. This trehalose break-down is simultaneously utilized for the synthesis of ribose sugars using the PPP and sequentially oxidized to pyruvate to meet the energy demands of the cells. Despite the nutrient environment being glucose-depleted, these cells showed all hallmarks of yeast cells in glucose-replete environments (Varahan *et al.* 2019).

The explanation for the appearance and maintenance of these glycolytic cells came from emergent, self-organizing biochemical principles. Initially, within a colony, cells were highly gluconeogenic, and an outcome of gluconeogenesis is the production and accumulation of an originally limiting resource, the disaccharide trehalose - which is made of two glucose molecules. As trehalose amounts increase, this newly available resource becomes abundantly available to all cells. Some cells (likely stochastically) increase trehalose uptake and switch to consuming and breaking-down trehalose for glucose, and this results in global, stable transcriptional changes which allow them to sustain high rates of glycolysis (Varahan *et al.* 2019). As these cells switch to glycolysis and consume more trehalose, the amounts of available trehalose start depleting. Hence, the remaining cells can no longer switch, and remain trehalose 'producers' that continue in a gluconeogenic state (Figure 1). Simulated coarse-grained resource consumption models can remarkably recapitulate this phenomenon, at the level of both patterns forming as well as the organization of specialized cell groups within the colony (Varahan *et al.* 2019). In effect, this emergence of metabolic heterogeneity and organization of the population of cells with distinct metabolic states can be driven by a self-organizing biochemical network of gluconeogenic cells producing a resource and glycolytic cells consuming it. The outcome of this system is what appears to be a fully functional cross-feeding system within a clonal colony, with cells in one state (gluconeogenic) sustaining the cells in the other (glycolytic) (Figure 1).

How such specialization leads to metabolic division of labor?

For reproductive success and survival, cellular communities must perform multiple complex biological tasks. The metabolic burden on any single cell can be large if it is required to carry out all necessary biochemical reactions to enable growth and manage survival alone, and this can lead to fitness trade-offs. Therefore, a strategy deployed by many cell communities to overcome this problem is to break down complex processes into discrete sets of steps and divide tasks among different individuals of the community (Giri *et al.* 2019). Division of labor enhances the efficiency of biological processes that are executed within communities by eliminating the need to perform or switch between multiple tasks, leading to fitness advantages (van Gestel *et al.* 2015; West and Cooper 2016). Due to this, division of labor is widely prevalent across microbial communities and is found at different levels of biological organization (Michod 2007; Tarnita *et al.* 2013).

A key parameter dictating the success of any cellular community is the effective utilization of available nutrients. Depending on the type and quality of nutrients available, cells within communities often switch between metabolic states, to adapt to fluctuating availability of nutrients (Johnson *et al.* 2012; Campbell *et al.* 2015). However, some biochemical reactions are mutually incompatible, *i.e.*, the same cell cannot perform these reactions simultaneously. For example, mass-action driven metabolic fluxes ensure that the same cell cannot sustain high rates of glycolysis and gluconeogenesis at the same time. Therefore, dividing metabolic labor can become a strategy that allows microbial communities to survive fluctuating nutrient environments (Tsoi *et al.* 2018). Another classic example comes from cyanobacterial communities. Cyanobacterial species *Synechococcus elongatus* requires photosynthesis and nitrogen fixation for its survival and growth. However, these two biochemical processes are mutually incompatible, due to distinct oxygen requirements. Therefore, cells in these cyanobacterial communities segregate into photosynthetic cells and nitrogen-fixing cells and this metabolic division of labor allows these communities to perform both essential tasks simultaneously (Flores and Herrero 2010; Rossetti *et al.* 2010). Metabolic division of labor has typically been observed in mixed microbial communities. For example, the nitrification process in the soil exhibits metabolic division of labor wherein the ammonia-oxidizing bacteria convert the ammonia into nitrite and nitrite-oxidizing bacteria convert nitrite to nitrate (Beeckman *et al.* 2018). Cross-feeding observed in mixed microbial populations is another example of metabolic division of labor since each species within is responsible for producing distinct metabolites that are shared amongst the different members of the community (Schink 2002; Wintermute and Silver 2010).

Metabolic cross-feeding systems

In many naturally occurring microbial communities, the challenging task of breaking down very complex nutrients available in an external environment is performed collectively by multiple species. Molecules resulting from the metabolism within one strain are metabolized by other strains, and this phenomenon is called cross-feeding (Figure 2A). Cross-feeding has been explored in many heterogeneous microbial communities comprised of either different species of microbes in single community or

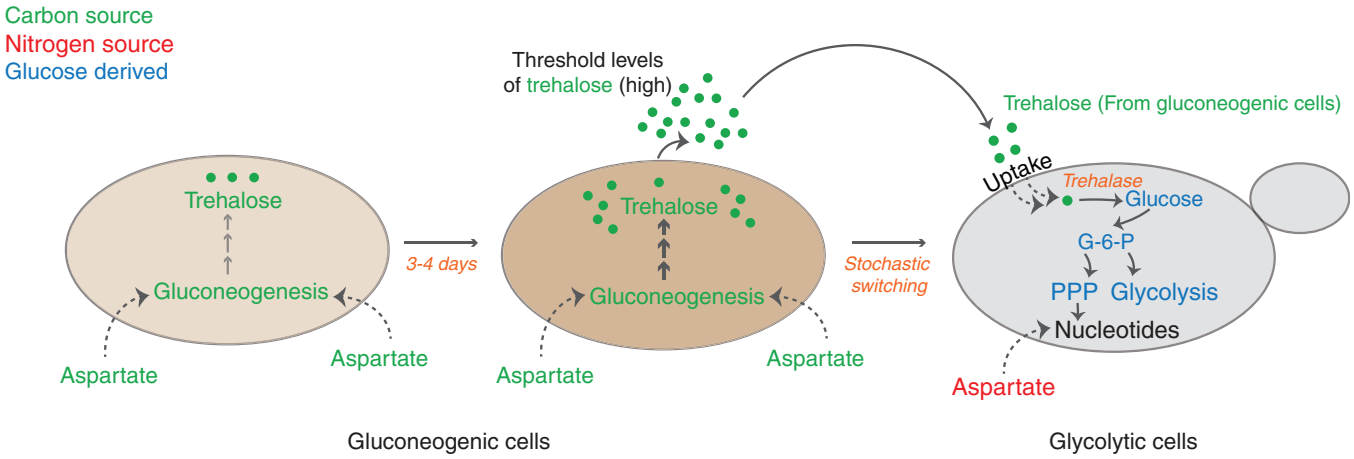

Figure 1 Emergence of metabolic heterogeneity in a clonal group of cells growing in glucose-limited conditions. Cells in glucose-limited environments perform gluconeogenesis. This is primarily fuelled by aspartate (nonlimiting resource) which acts as a carbon source. As the community develops, gluconeogenic reserves build-up and trehalose concentration in the extracellular environment increases. At a threshold concentration of extracellular trehalose, some cells stochastically switch to a high glycolytic, PPP state. This state is fuelled by the utilization of trehalose (as a carbon source), and aspartate (nonlimiting) is primarily used as a nitrogen source for the biosynthesis of nucleotides. This consumption of trehalose by the glycolytic cells results in decreased concentrations of external trehalose, bringing the concentration below the threshold. This in turn restricts the other, remaining cells in their original gluconeogenic state and they continue to synthesize trehalose. This results in a self-organized community that exhibits specialization of function and division of labor.

laboratory-engineered cell populations where metabolic interdependencies are generated via genetic manipulation that create nutrient auxotrophies (Schink 2002; Shou *et al.* 2007; Wintermute and Silver 2010; Mee *et al.* 2014; Campbell *et al.* 2015). These studies reveal how complex communities can achieve metabolic heterogeneity when each genotype (typically an auxotroph for a nutrient) divides labor to produce or receive a specific metabolite. This sharing of metabolic resources enables increased growth and fitness of the community. Cross-feeding systems typically consist of specialist strains that performs a restricted group of biochemical tasks and relies on another species for obtaining metabolites and other products (auxotrophies) needed for their growth (Figure 2A). Generally, species that complement each other's auxotrophies become an interdependent community. The resulting metabolic syntrophy allows the community as a whole to flourish in a given environment (Morris *et al.* 2013; Pande *et al.* 2014). Individuals in a cross-feeding community can have higher overall fitness compared to a community made of a single species of a generalist microbe that can perform all the biochemical tasks on its own. This growth advantage comes from the division of metabolic labor wherein a fitness cost of producing a resource needed for the growth of the complementary auxotroph is less than the benefit of not having to produce other resources when they are provided by their partner in the cross-feeding system (Axelrod and Hamilton 1981; Hillesland and Stahl 2010).

Interestingly, even clonal communities of microorganisms exhibit metabolic division of labor. For example, a recent study showed that a clonal population of *Bacillus subtilis* exhibit metabolic division of labor wherein a subpopulation of cells produce acetate which allows these bacteria to grow rapidly. But to mitigate the toxicity of acetate at higher concentrations, a distinct subpopulation of cells in these clonal communities start converting the acetate to acetoin (which is nontoxic) and this allows the community as a whole to grow in a detoxified environment (Rosenthal *et al.* 2018). In work in yeast, a general principle emerged of how threshold levels of specific metabolites drive self-organizing biochemical networks enabling the emergence of metabolic heterogeneity and subsequent metabolic division of labor in clonal yeast communities (Varahan *et al.* 2019, 2020).

Resource threshold-dependent metabolic cross-feeding within yeast communities

Strikingly, as introduced earlier, in clonal yeast communities, cells self-organize into effective cross-feeding, mutualistic groups within a community. In glucose-limited conditions, the prevalent metabolic pathway (gluconeogenesis) leads to the production and accumulation of trehalose. This new (formerly limiting) resource allows some cells to stochastically switch to a trehalose-consuming state, which is glycolytic, and these glycolytic cells effectively function as auxotrophs (for trehalose) (Varahan *et al.* 2019). The glycolytic cells obtain glucose via the uptake and breakdown of this trehalose, which they obtain from the gluconeogenic cells (Figure 1). This is remarkably efficient as a cross-feeding system. Importantly, this self-organized biochemical system that leads to a cross-feeding network is enabled by the build-up of a novel resource (trehalose) to sufficiently high levels (a "threshold"), which did not exist initially in the environment (Figure 2B). This leads to a deeper question, of how the high flux through gluconeogenesis which produces the resource (trehalose) itself is sustained. Underlying this finding was that the entire cross-feeding system and trehalose production is sustained by existing, nonlimiting resources of specific amino acids (Varahan *et al.* 2020). These relatively nonlimiting amino acids (primarily aspartate) act as carbon sources in gluconeogenic cells to drive gluconeogenesis (Figure 3A). But they also have alternate fates that are metabolically important in sustaining the cells that switch to a glycolytic state as well (Figure 1; Varahan *et al.* 2020).

This highlights the concept of biochemically versatile metabolites and how their metabolic flexibility can drive, enable, or control biochemical networks that set up cross-feeding systems of specialized cell states in clonal cell communities.

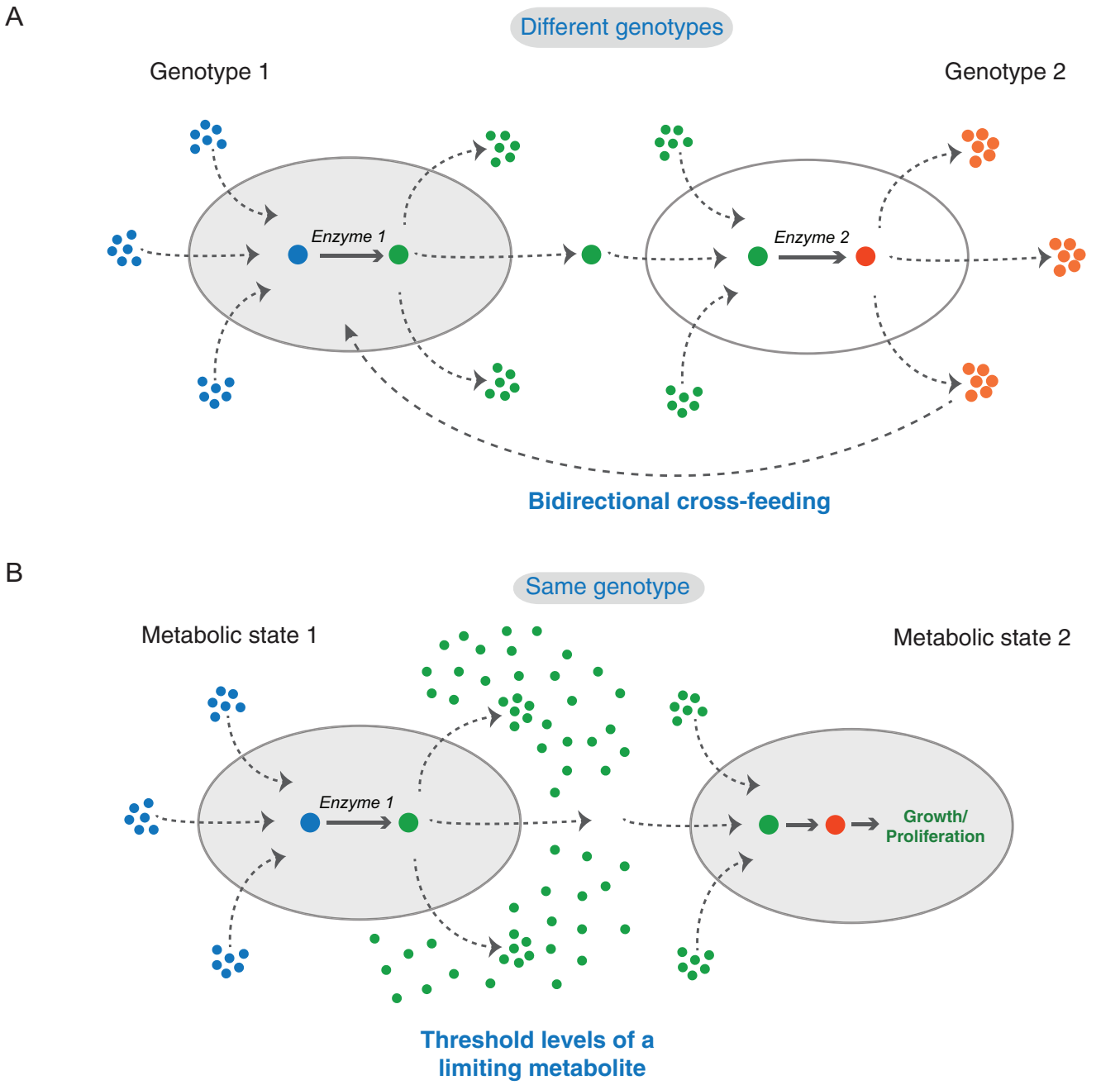

Figure Key:

- ● = Non-limiting metabolic resource available in the environment
- *Enzyme 1* = converts ● to ●. Specific to or expressed in only one genotype/metabolic state
- ● = Limiting resource 1 produced by genotype 1. Diffuses/Exported out of cells and consumed by neibhouring cells (genotype 2). Non-essential for growth of producer cells.
- ● = Limiting resource 2 that is essential for growth of genotype 2 (auxotrophic species) or cells in an alternate metabolic state
- *Enzyme 2* = converts ● to ●. Specific to or expressed in only one genotype/metabolic state
- ● = Resource produced by genotype 2 which is consumed by genotype 1 (in case of bidirectional cross-feeding)

Figure 2 Metabolic cross-feeding in microbial communities, either of different genotypes, or clonal. (A) Complex microbial communities are comprised of different species with distinct genotypes. In dual-species communities, a particular species within (genotype 1) consumes the nonlimiting resource available in the external environment and produces a limiting resource necessary for the growth of the second species (genotype 2). In specific scenarios, a second species can produce a limiting resource necessary for the growth of the other species in these communities. Complementary auxotrophies result in metabolic syntrophy and allows the community as a whole to flourish. (B) In clonal communities, when the levels of a limiting resource produced from nonlimiting resources reach a threshold concentration, it can result in the emergence of cells in a new metabolic state (which consumes this limiting resource to maintain this state). This switch to an alternate metabolic state is reversible and depends on the availability of the limiting resource.

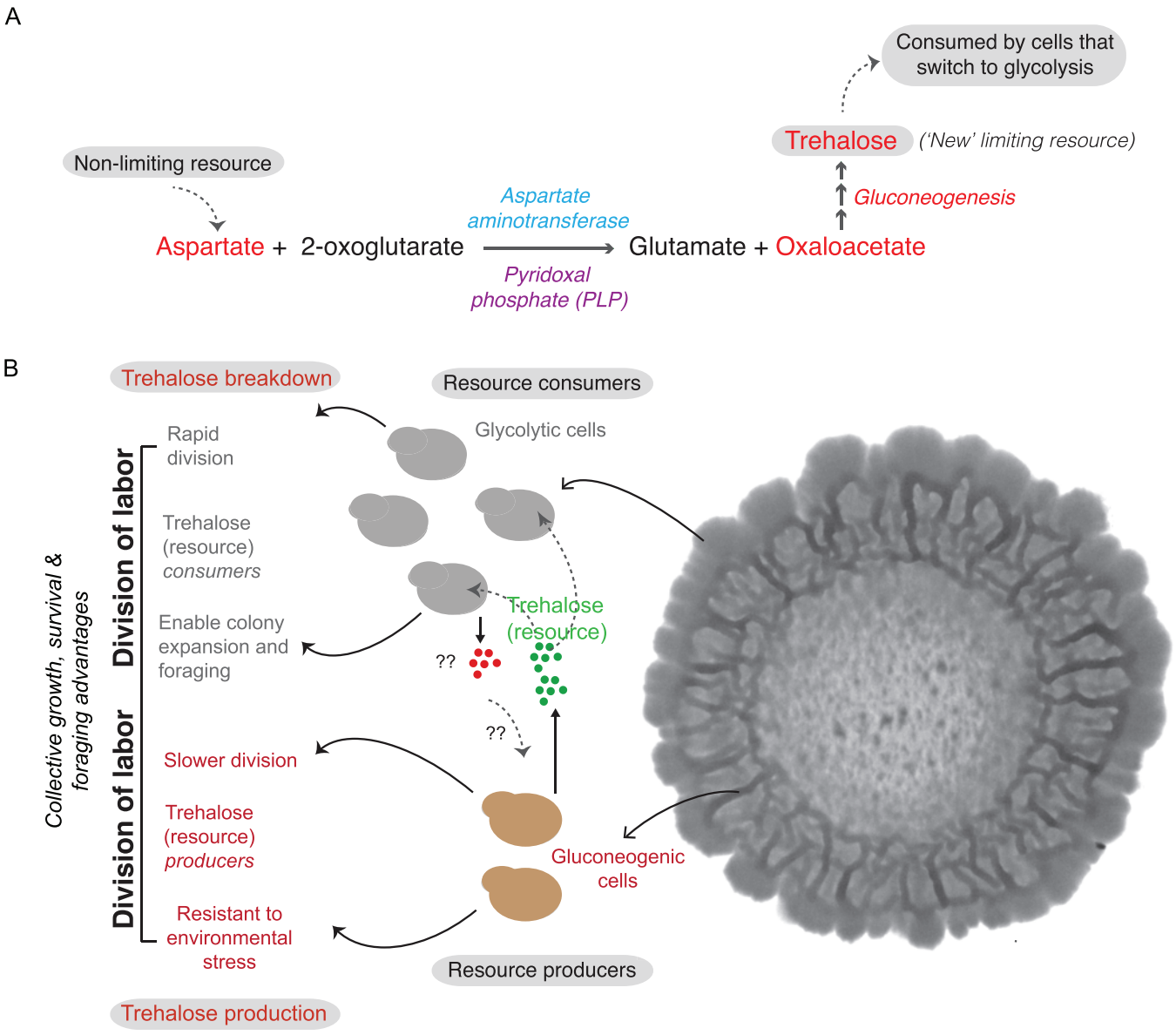

Figure 3 Biochemically versatile metabolites orchestrate the emergence and maintenance of metabolic heterogeneity resulting in co-operative division of labor in yeast. (A) Aspartate (nonlimiting resource) acts as a carbon substrate to produce the gluconeogenic precursor, oxaloacetate via a transamination reaction. Oxaloacetate is converted into trehalose (limiting resource) via gluconeogenesis and this results in the emergence of the glycolytic sub-population of cells that consumes this resource to fuel their metabolic state. (B) Cooperative division of labor exhibited by the gluconeogenic and glycolytic cells provides collective growth, survival, and foraging advantages to the community as a whole. Gluconeogenic cells accumulate trehalose and this protects them from various environmental stresses like desiccation, freeze/thaw cycles, and temperature fluctuations that cells in yeast communities often face in their natural environments. The glycolytic cells (that consume the trehalose) allow yeast colonies to rapidly expand over time, forage, and reach new territory. The glycolytic cells are ideally positioned spatially to allow the colony to expand and forage toward new territory. It is possible that the glycolytic cells produce a metabolic resource that is consumed by the gluconeogenic cells to maintain their metabolic state.

How cellular metabolic states and biochemically versatile metabolites orchestrate the emergence and maintenance of metabolic heterogeneity?

Cellular metabolism obviously enables many biological processes that are critical for the functioning of cells. It is now apparent that certain metabolites directly control cell growth and proliferation, functioning both as signaling molecules as well as molecules that can be utilized for different anabolic/catabolic processes. These include metabolites like ATP, NAD(P)H, and acetyl-CoA (Westheimer 1987; Cantó *et al.* 2015; Pietrocola *et al.* 2015). Other studies demonstrate that metabolites like s-adenosylmethionine (SAM), carbamoyl phosphate, and UDP-glucose also act as sentinel metabolites and drive biochemical pathways required for growth and proliferation (Walsh *et al.* 2018). These metabolites allow cells to efficiently perform methyl transfers (SAM), phosphoryl transfers (ATP), electron and ADP-ribosyl transfers (NAD(P)H/NAD(P)$^+$), acyl transfers (acetyl-CoA and carbamoyl phosphate), and glucosyl transfers (UDP-glucose), which in turn drive multiple transcriptional and metabolic transformations across primary biochemical pathways (Nelson and Cox 2017; Walsh *et al.* 2018). Depending on how much of these metabolites are present, they can direct overall metabolic programs toward growth, differentiation, or survival states.

However, there are also other metabolites, with biochemically versatile roles, that work "behind the scenes" to enable metabolically distinct states to sustain themselves. These molecules can have multiple uses, and may (depending on how they are utilized) change the availability of some of the sentinel metabolites described above. Studies from the organized, metabolically specialized cell groups within yeast colonies illustrate how this might be so. Here, the two molecules central to setting up the cross-feeding system are the disaccharide trehalose, and the amino acid aspartate (Figure 1; Varahan *et al.* 2019, 2020). Both molecules can function differently and drive completely distinct metabolic pathways depending on the metabolic state of the cells. When trehalose (which is produced by gluconeogenesis during glucose starvation) is consumed and broken down into glucose, it can fuel high rates of glycolysis and the PPP, as it does in the highly glycolytic cells within the colonies growing in glucose-limited conditions (Varahan *et al.* 2019). Indeed, the roles of trehalose as a critical requirement for cells to exit quiescence and enter rapidly dividing states are well documented (Silljé *et al.* 1999; Futcher 2006; Shi *et al.* 2010; Shi and Tu 2013). Yet, trehalose also has distinct, critical functions in the gluconeogenic cells, or cells entering quiescence. In these cells, it behaves as a membrane and protein protectant molecule, allowing them to survive multiple freeze/thaw cycles and extreme desiccation (Wiemken 1990; D'Amore *et al.* 1991; Shi *et al.* 2010; Erkut *et al.* 2011, 2016). This plasticity in its use itself therefore can directly determine cell state. Interestingly, in this gluconeogenic/glycolytic cell cross-feeding system observed in yeast, trehalose is initially a limiting resource that builds up to above a threshold, when it triggers some cells to switch to a consumer state (Varahan *et al.* 2019). The production of this resource is itself driven by a more abundant, and more metabolically versatile resource, the amino acid aspartate. In glucose-limited conditions, aspartate primarily functions as a carbon precursor for driving gluconeogenesis (Vengayil *et al.* 2019; Varahan *et al.* 2020). Aspartate is easily converted to the gluconeogenic precursor oxaloacetate, and therefore drives high flux through gluconeogenesis. This is sufficient to produce sufficient trehalose required to reach the levels that can then trigger a switch of some cells to a glycolytic (trehalose-consuming) state (Figure 3A; Varahan *et al.* 2019, 2020). Interestingly, aspartate can be important in glycolytic cells, which have solved their requirements of carbon for rapid growth, for very different reasons. The glucose from trehalose drives glycolysis and the PPP, leading to the production of ribose sugars required for nucleotide synthesis. But in order to make sufficient nucleotides, cells require aspartate to function as a *nitrogen* donor, and aspartate therefore plays a critical, distinct role in sustaining the glycolytic cell state (Figure 1; Varahan *et al.* 2020). Thus, this metabolic flexibility of trehalose and aspartate allows the existence of the glycolytic cell state in these communities while these metabolites simultaneously are essential for the maintenance of the gluconeogenic state.

What these studies describe are cross-feeding systems that are largely formed by self-organizing biochemical networks, which themselves have metabolically flexible molecules as the driving entities behind them. What this more broadly entails is that while sentinel metabolites power cell metabolism (Walsh *et al.* 2018), for the emergence of interdependent, specialized groups of cells within clonal communities, such metabolically flexible, versatile molecules play central roles. Identifying and understanding the spectrum of such metabolites and how they can enable organized phenotypic heterogeneity in clonal cell communities would be an exciting area of future study. Here, the many roles and fates of amino acids, and their ability to drive distinct arms of carbon and nitrogen metabolism (Amelio *et al.* 2014; Yang and Vousden 2016; Walvekar *et al.* 2018; Yoo *et al.* 2020), are likely to become increasingly apparent.

Implications of metabolic specialization and division of labor in clonal yeast communities

How might the separation of biochemical processes and division of labor occur within spatially restricted clonal yeast communities be advantageous to the community, and might this inform the roles of such systems in other microbes? For example, this begs the question, why should the cells growing in low-glucose conditions perform high amounts of glycolysis, when they can survive via gluconeogenesis? Interestingly, by maintaining cells in two metabolic states, with a spatial organization, a host of advantages can be conferred to the community as a whole (Figure 3B). Several studies have shown that cells use their metabolism to counter a variety of stresses that they encounter in their environments. For example, in response to oxidative stress, cells divert their metabolic flux from glycolysis toward the PPP to increase cytoplasmic NADPH, which provides the redox power for known antioxidant systems (Pollak *et al.* 2007; Ralser *et al.* 2007), or counter oxidative insults by harvesting lysine that is available in their extracellular environment. As a consequence, NADPH which would otherwise be utilized for the biosynthesis of lysine is channelled toward the synthesis of glutathione, which allows cells to counter the oxidative insults efficiently (Olin-Sandoval *et al.* 2019). In low-glucose environments, the gluconeogenic cells are very well adapted to survive environmental insults that yeast communities often face in natural environments like (a) desiccation, (b) freeze/thaw cycles, and (c) temperature fluctuations (Erkut *et al.* 2016; Varahan *et al.* 2020). This comes from the accumulation of trehalose, which as described earlier is a biochemical endpoint metabolite of gluconeogenesis, and is a versatile protectant (Wiemken 1990; D'Amore *et al.* 1991). Contrastingly, the emergent glycolytic cells provide new advantages to cells within the colony. These cells (which are themselves sustained by the gluconeogenic cells) proliferate rapidly, and allow the colonies to expand over time, forage, and reach new territory. The glycolytic cells are ideally positioned spatially (at the periphery of the colony) to allow the colony to expand and forage toward new territory (Varahan *et al.* 2020), but themselves cannot exist without the gluconeogenic cells. The ability to forage for nutrients is a key survival strategy deployed by many microbes growing in nutrient-limiting conditions, and thus this becomes possible within the yeast colonies only due to the division of labor that sustains distinct metabolic states (Figure 3B).

Possible evolutionary implications of the metabolic division of labor: sympatric speciation?

Metabolic specialization within a community confers growth and fitness advantages to the community as a whole. However, it is tempting to speculate on the possibility of a longer-term trajectory. When the environmental selection pressure (nutrient limitations) that drives metabolic heterogeneity in isogenic communities is sustained over very long periods (which yeast and most microbes experience in the wild), it could potentially lead to mutagenic events where subsets of the clonal population locked into a particular metabolic state evolve auxotrophies and subsequently perform restricted biochemical tasks. Such auxotrophs will exist only as long as they are supported by the

complementary population that forms the cross-feeding system. This is of course seen in other syntrophic microbial populations which have lost their ability to facultatively switch back to any alternate metabolic state. In other words, could prolonged metabolic heterogeneity as a consequence of constant environmental selective pressure therefore be imagined as an evolutionary precursor of sympatric speciation events in isogenic communities?

Speciation is the evolution of a new species from a surviving ancestral species (Wu 2001; Mallet 2008; Shapiro *et al.* 2016). Multiple factors contribute to speciation events and these include (a) gradual evolution of genetic incompatibilities, (b) specialization to an ecological niche, and (c) alterations to the chromosome via homologous recombination or horizontal gene transfer events (Presgraves 2010; Shapiro and Polz 2015; Shapiro *et al.* 2016). In yeasts, hybridization events have led to speciation by combining genomes that have evolved independently. These genomes, when combined, provide advantages to the hybrid individuals. New hybrids become a different species only when they are self-fertile and exhibit reproductive isolation from their parental strains (Greig *et al.* 2002; Dettman *et al.* 2007; Leducq *et al.* 2016). Several examples of the emergence of new species of yeast as a consequence of hybridization exist. A classic example is *Saccharomyces pastorianus*, the source of lager beer, which is a result of a hybridization event between *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*. This newly formed hybrid inherited properties from both parental strains, making it optimal for lager beer brewing (Libkind *et al.* 2011; van den Broek *et al.* 2015; Gorter De Vries *et al.* 2019).

However, hybridization may also dilute beneficial properties that exist in parental strains. This is particularly true when sympatric speciation is ecologically driven, and occurs in order to adapt to an environmental niche. This phenomenon is called hybrid dysfunction, wherein species that have evolved to adapt to a particular environmental niche (environment-specific adaptations) produce hybrids with reduced fitness in either parental habitat (Johnson 2008, 2010). We can apply this concept of ecologically driven hybrid dysfunction to clonal yeast colonies with division of labor and make plausible speculations. The metabolic heterogeneity in clonal yeast communities is an ecologically driven event in response to glucose limitation. Potentially, when this clonal community of yeast remains in glucose-limited conditions for extended periods of time, the two metabolically distinct population of cells could evolve into two populations that have diminished ability to switch back to an alternate metabolic state, and rather adapt to grow exclusively in a specific environment (gluconeogenic or glycolytic). Because the gluconeogenic and glycolytic cells exhibit diametrically opposite metabolic states, they would exhibit hybrid dysfunction or hybrid incompatibility as it is impossible to have a cell performing glycolysis and gluconeogenesis at the same time. Indeed, very recent studies suggest that this might be possible. In yeast continuously propagated in melibiose as a carbon source, which can break down to galactose and glucose, the release of these as a public good might allow adaptive diversification of a clonal population (Mahilkar *et al.* 2021).

It, therefore, is a plausible speculation that metabolic heterogeneity that occurs in isogenic communities as a consequence of sustained selective pressure may result in ecologically driven speciation events. Could multispecies communities that exhibit metabolic cross-feeding have originally started as isogenic groups of cells that carried out metabolic division of labor as an adaptation strategy to a specific environmental niche?

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Conflicts of interest

None declared.

Literature cited

Ackermann M. 2013. Microbial individuality in the natural environment. 7:465–467. doi:10.1038/ismej.2012.131.

Ackermann M. 2015. A functional perspective on phenotypic heterogeneity in microorganisms. Nat Rev Microbiol. 13:497–508. doi:10.1038/nrmicro3491.

Amelio I, Cutruzzolá F, Antonov A, Agostini M, Melino G. 2014. Serine and glycine metabolism in cancer. Trends Biochem Sci. 39:191–198. doi:10.1016/j.tibs.2014.02.004.

Axelrod R, Hamilton W. 1981. The evolution of cooperation. Science. 211:1390–1396. doi:10.1126/science.7466396.

Balázsi G, Van Oudenaarden A, Collins JJ. 2011. Cellular decision making and biological noise: from microbes to mammals. Cell. 144:910–925. doi:10.1016/j.cell.2011.01.030.

Beaumont HJE, Gallie J, Kost C, Ferguson GC, Rainey PB. 2009. Experimental evolution of bet hedging. Nature. 462:90–93. doi:10.1038/nature08504.

Beeckman F, Motte H, Beeckman T. 2018. Nitrification in agricultural soils: impact, actors and mitigation. Curr Opin Biotechnol. 50:166–173. doi:10.1016/j.copbio.2018.01.014.

Callieri C, Eckert EM, Cesare AD, Bertoni F. 2018. Microbial communities. Encyclopedia Ecol. 1:126–134.

Campbell K, Vowinckel J, Mülleder M, Malmsheimer S, Lawrence, N, *et al.* 2015. Self-establishing communities enable cooperative metabolite exchange in a eukaryote. eLife. 4:e09943. doi:10.7554/eLife. 09943.

Cantó C, Menzies KJ, Auwerx J. 2015. NAD+ metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. Cell Metab. 22:31–53. doi:10.1016/j.cmet.2015.05.023.

Cáp M, Stěpánek L, Harant K, Váchová L, Palková Z. 2012. Cell differentiation within a yeast colony: metabolic and regulatory parallels with a tumor-affected organism. Mol Cell. 46:436–448. doi:10.1016/j.molcel.2012.04.001.

Čáp M, Váchová L, Palková Z. 2015. Longevity of U cells of differentiated yeast colonies grown on respiratory medium depends on active glycolysis. Cell Cycle. 14:3488–3497. doi:10.1080/15384101.2015.1093706.

Cheng SC, Joosten LAB, Kullberg BJ, Netea MG. 2012. Interplay between *Candida albicans* and the mammalian innate host defense. Infect Immun. 80:1304–1313. doi:10.1128/IAI.06146-11.

Cullen PJ, Sprague GF. 2012. The regulation of filamentous growth in yeast. Genetics. 190:23–49. doi:10.1534/genetics.111.127456.

D'Amore T, Crumplen R, Stewart GG. 1991. The involvement of trehalose in yeast stress tolerance. J Ind Microbiol. 7:191–195. doi: 10.1007/BF01575882.

Daniels KJ, Srikantha T, Lockhart SR, Pujol C, Soll DR. 2006. Opaque cells signal white cells to form biofilms in *Candida albicans*. 25: 2240–2252. doi:10.1038/sj.emboj.760109.

de Bekker C, Bruning O, Jonker MJ, Breit TM, Wösten HAB. 2011a. Single cell transcriptomics of neighboring hyphae of *Aspergillus niger*. Genome Biol. 12:R71. doi:10.1186/gb-2011-12-8-r71.

de Bekker C, van Veluw GJ, Vinck A, Wiebenga LA, Wösten HAB. 2011b. Heterogeneity of *Aspergillus niger* microcolonies in liquid shaken cultures. Appl Environ Microbiol. 77:1263–1267. doi: 10.1128/AEM.02134-10.

Dettman JR, Sirjusingh C, Kohn LM, Anderson JB. 2007. Incipient speciation by divergent adaptation and antagonistic epistasis in yeast. Nature. 447:585–588. doi:10.1038/nature 05856.

Dühring S, Germerodt S, Skerka C, Zipfel PF, Dandekar T, *et al.* 2015. Host-pathogen interactions between the human innate immune system and *Candida albicans*-understanding and modeling defense and evasion strategies. Front Microbiol. 6:625. doi: 10.3389/fmicb.2015.00625.

Elowitz MB, Levine AJ, Siggia ED, Swain PS. 2002. Stochastic gene expression in a single cell. Science. 297:1183–1186. doi:10.1126/science.1070919.

Ene IV, Lohse MB, Vladu AV, Morschhäuser J, Johnson, AD, *et al.* 2016. Phenotypic profiling reveals that *Candida albicans* opaque cells represent a metabolically specialized cell state compared to default white cells. mBio. 7:e01269–16. doi:10.1128/mBio.01269-16.

Erkut C, Gade VR, Laxman S, Kurzchalia TV. 2016. The glyoxylate shunt is essential for desiccation tolerance in *C. elegans* and budding yeast. eLife. 5:e13614. doi:10.7554/eLife.13614.

Erkut C, Penkov S, Khesbak H, Vorkel D, Verbavatz, J-M, *et al.* 2011. Trehalose renders the dauer larva of *Caenorhabditis elegans* resistant to extreme desiccation. Curr Biol. 21:1331–1336. doi: 10.1016/j.cub.2011.06.064.

Flores E, Herrero A. 2010. Compartmentalized function through cell differentiation in filamentous cyanobacteria. Nat Rev Microbiol. 8:39–50. doi:10.1038/nrmicro2242.

Futcher B. 2006. Metabolic cycle, cell cycle, and the finishing kick to start. Genome Biol. 7:107. doi:10.1186/gb-2006-7-4-107.

Gancedo JM. 2001. Control of pseudohyphae formation in *Saccharomyces cerevisiae*. FEMS Microbiol Rev. 25:107–123. doi: 10.1111/j.1574-6976.2001.tb00573.x.

Geiger J, Wessels D, Lockhart SR, Soll DR. 2004. Release of a potent polymorphonuclear leukocyte chemoattractant is regulated by white-opaque switching in *Candida albicans*. Infect Immun. 72: 667–677. doi:10.1128/iai.72.2.667-677.2004.

Gimeno CJ, Ljungdahl PO, Styles CA, Fink GR. 1992. Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. Cell. 68:1077–1090. doi: 10.1016/0092-8674(92)90079-r.

Giri S, Waschina S, Kaleta C, Kost C. 2019. Defining division of labor in microbial communities. J Mol Biol. 431:4712–4731. doi: 10.1016/j.jmb.2019.06.023.

Gorter De Vries AR, Voskamp MA, Van Aalst ACA, Kristensen LH, Jansen L, *et al.* 2019. Laboratory evolution of a *Saccharomyces cerevisiae* × *S. eubayanus* hybrid under simulated lager-brewing conditions. Front Genet. 10:242. doi:10.3389/fgene.2019.00242.

Granek JA, Magwene PM. 2010. Environmental and genetic determinants of colony morphology in yeast. PLoS Genet. 6:e1000823. doi:10.1371/journal.pgen.1000823.

Greig D, Louis EJ, Borts RH, Travisano M. 2002. Hybrid speciation in experimental populations of yeast. Science. 298:1773–1775. doi: 10.1126/science.1076374.

Grimbergen AJ, Siebring J, Solopova A, Kuipers OP. 2015. Microbial bet-hedging: the power of being different. Curr Opin Microbiol. 25:67–72. doi:10.1016/j.mib.2015.04.008.

Gulati M, Nobile CJ. 2016. *Candida albicans* biofilms: development, regulation, and molecular mechanisms. Microbes Infect. 18: 310–321. doi:10.1016/j.micinf.2016.01.002.

Halfmann R, Alberti S, Lindquist S. 2010. Prions, protein homeostasis, and phenotypic diversity. Trends Cell Biol. 20:125–133. doi: 10.1016/j.tcb.2009.12.003.

Halfmann R, Jarosz DF, Jones SK, Chang A, Lancaster AK, *et al.* 2012. Prions are a common mechanism for phenotypic inheritance in wild yeasts. Nature. 482:363–368. doi:10.1038/nature10875.

Hewitt SK, Foster DS, Dyer PS, Avery SV. 2016. Phenotypic heterogeneity in fungi: Importance and methodology. Fungal Biol. Rev. 30: 176–184. doi:10.1016/ j.fbr.2016.09.002.

Hillesland KL, Stahl DA. 2010. Rapid evolution of stability and productivity at the origin of a microbial mutualism. Proc Natl Acad Sci USA. 107:2124–2129. doi:10.1073/pnas.0908456107.

Hogan DA, Gladfelter AS. 2015. Editorial overview: host-microbe interactions: fungi: heterogeneity in fungal cells, populations, and communities. Curr Opin Microbiol. 26:7–9. doi:10.1016/j.mib.2015.07.003.

Johnson NA. 2008. Hybrid incompatibility and speciation. Nat Educ. 1: 20.

Johnson NA. 2010. Hybrid incompatibility genes: remnants of a genomic battlefield? Trends Genet. 26:317–325. doi:10.1016/j.tig.2010.04.005.

Johnson DR, Goldschmidt F, Lilja EE, Ackermann M. 2012. Metabolic specialization and the assembly of microbial communities. 6: 1985–1991. doi:10.1038/ismej.2012.46.

Kolotila MP, Diamond RD. 1990. Effects of neutrophils and *in vitro* oxidants on survival and phenotypic switching of *Candida albicans* WO-1. Infect Immun. 58:1174–1179. doi:10.1128/IAI.58.5.1174-1179.1990.

Kornitzer D. 2019. Regulation of *Candida albicans* hyphal morphogenesis by endogenous signals. J Fungi. 5:21. doi:10.3390/jof5010021.

Kurtzman CP, Fell JW. 2006. Yeast systematics and phylogeny—implications of molecular identification methods for studies in ecology in biodiversity and ecophysiology of yeasts, the yeast handbook. Berlin, Heidelberg: Springer. doi:10.1007/3-540-30985-3_2.

Lan CY, Newport G, Murillo LA, Jones T, Scherer S, *et al.* 2002. Metabolic specialization associated with phenotypic switching in *Candida albicans*. Proc Natl Acad Sci USA. 99:14907–14912. doi: 10.1073/pnas.232566499.

Leducq JB, Nielly-Thibault L, Charron G, Eberlein C, Verta JP, *et al.* 2016. Speciation driven by hybridization and chromosomal plasticity in a wild yeast. Nat Microbiol. 1:15003. doi:10.1038/nmicrobiol.2015.3.

Libkind D, Hittinger CT, Valério E, Gonçalves C, Dover J, *et al.* 2011. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc Natl Acad Sci USA. 108: 14539–14544. doi:10.1073/pnas.1105430108.

Lohse MB, Johnson AD. 2009. White-opaque switching in *Candida albicans*. Curr Opin Microbiol. 12:650–654. doi: 10.1016/j.mib.2009.09.010 19853498

Lohse MB, Johnson AD. 2008. Differential phagocytosis of white versus opaque *Candida albicans* by Drosophila and mouse phagocytes. PLoS One. 3:e1473. doi:10.1371/journal.pone.0001473.

López-Maury L, Marguerat S, Bähler J. 2008. Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nat Rev Genet. 9:583–593. doi:10.1038/nrg2398.

Mahilkar A, Alugoju P, Kavatalkar V, Rajeshkannan E, Bhat PJ, *et al.* 2021. Public-good driven release of heterogeneous resources leads to genotypic diversification of an isogenic yeast population in melibiose. bioRxiv 2021.04.12.439421. doi: 10.1101/2021.04.12.439421.

Mallet J. 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. Philos Trans R Soc Lond B Biol Sci. 363:2971–2986. doi:10.1098/rstb.2008.0081.

Marr KA, Lyons CN, Ha K, Rustad TR, White TC. 2001. Inducible azole resistance associated with a heterogeneous phenotype in *Candida albicans*. Antimicrob Agents Chemother. 45:52–59. doi: 10.1128/AAC.45.1.52-59.2001.

Mee MT, Collins JJ, Church GM, Wang HH. 2014. Syntrophic exchange in synthetic microbial communities. Proc Natl Acad Sci USA. 111: 2149–2156. doi:10.1073/pnas.1405641111.

Michod RE. 2007. Evolution of individuality during the transition from unicellular to multicellular life. Proc Natl Acad Sci USA. 104:8613–8618. doi:10.1073/pnas.0701489104.

Miller MG, Johnson AD. 2002. White-opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell. 110:293–302. doi: 10.1016/s0092-8674(02)00837-1.

Mináriková L, Kuthan M, Ricicová M, Forstová J, Palková Z. 2001. Differentiated gene expression in cells within yeast colonies. Exp Cell Res. 271:296–304. doi:10.1006/excr. 2001.5379.

Morris BEL, Henneberger R, Huber H, Moissl-Eichinger C. 2013. Microbial syntrophy: interaction for the common good. FEMS Microbiol Rev. 37:384–406. doi:10.1111/1574-6976.12019.

Nelson DL, Cox MM. 2017. Lehninger Principles of Biochemistry. 7th ed. New York, NY: W.H. Freeman. ISBN: 9781319108243.

Noble SM, Gianetti BA, Witchley JN. 2017. *Candida albicans* cell-type switching and functional plasticity in the mammalian host. Nat Rev Microbiol. 15:96–108. doi:10.1038/nrmicro. 2016.157.

Olin-Sandoval V, Yu JSL, Miller-Fleming L, Alam MT, Kamrad S, *et al.* 2019. Lysine harvesting is an antioxidant strategy and triggers underground polyamine metabolism. Nature. 572:249–253. doi: 10.1038/s41586-019-1442-6.

Palková Z, Váchová L. 2016. Yeast cell differentiation: lessons from pathogenic and non-pathogenic yeasts. Semin Cell Dev Biol. 57: 110–119. doi:10.1016/j.semcdb.2016.04.006.

Pande S, Merker H, Bohl K, Reichelt M, Schuster S, *et al.* 2014. Fitness and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria. 8:953–962. doi:10.1038/ismej. 2013.211.

Pietrocola F, Galluzzi L, Bravo-San Pedro JM, Madeo F, Kroemer G. 2015. Acetyl coenzyme A: a central metabolite and second messenger. Cell Metab. 21:805–821. doi:10.1016/j.cmet.2015.05.014.

Pollak N, Dölle C, Ziegler M. 2007. The power to reduce: pyridine nucleotides–small molecules with a multitude of functions. Biochem J. 402:205–218. doi:10.1042/BJ20061638.

Presgraves DC. 2010. The molecular evolutionary basis of species formation. Nat Rev Genet. 11:175–180. doi:10.1038/nrg2718.

Raj A, van Oudenaarden A. 2008. Nature, nurture, or chance: stochastic gene expression and its consequences. Cell. 135:216–226. doi:10.1016/j.cell.2008.09.050.

Ralser M, Wamelink MM, Kowald A, Gerisch B, Heeren G, *et al.* 2007. Dynamic rerouting of the carbohydrate flux is key to counteracting oxidative stress. J Biol. 6:10. doi:10.1186/jbiol61.

Reynolds TB, Fink GR. 2001. Bakers' yeast, a model for fungal biofilm formation. Science. 291:878–881. doi:10.1126/science.291.5505.878.

Roberts RL, Fink GR. 1994. Elements of a single map kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. Genes Dev. 8: 2974–2985. doi:10.1101/gad.8.24.2974.

Rosenthal AZ, Qi Y, Hormoz S, Park J, Li SH-J, *et al.* 2018. Metabolic interactions between dynamic bacterial subpopulations. eLife. 7: e33099. doi:10.7554/eLife.33099.

Rossetti V, Schirrmeister BE, Bernasconi MV, Bagheri HC. 2010. The evolutionary path to terminal differentiation and division of labor in cyanobacteria. J Theor Biol. 262:23–34. doi: 10.1016/j.jtbi.2009.09.009.

Santos D, Galdino ACM, de Mello TP, de L, Ramos S, Branquinha MH, *et al.* 2018. What are the advantages of living in a community? A microbial biofilm perspective! Mem Inst Oswaldo Cruz. 113: e180212. doi:10.1590/0074-02760180212.

Schink B. 2002. Synergistic interactions in the microbial world. *Antonie van Leeuwenhoek*. Int J Gen Mol Microbiol. 81:257–261. doi: 10.1023/a:1020579004534.

Shade A, Peter H, Allison SD, Baho DL, Berga M, *et al.* 2012. Fundamentals of microbial community resistance and resilience. Front. Microbiol. 3:417. doi:10.3389/fmicb. 2012.00417.

Shapiro BJ, Leducq JB, Mallet J. 2016. What is speciation? PLoS Genet. 12:e1005860. doi:10.1371/journal.pgen.1005860.

Shapiro BJ, Polz MF. 2015. Microbial speciation. Cold Spring Harb Perspect Biol. 7:a018143. doi:10.1101/cshperspect.a018143.

Sherry L, Rajendran R, Lappin DF, Borghi E, Perdoni, F, *et al.* 2014. Biofilms formed by *Candida albicans* bloodstream isolates display phenotypic and transcriptional heterogeneity that are associated with resistance and pathogenicity. BMC Microbiol. 14:182. doi: 10.1186/1471-2180-14-182.

Shi L, Sutter BM, Ye X, Tu BP. 2010. Trehalose is a key determinant of the quiescent metabolic state that fuels cell cycle progression upon return to growth. Mol Biol Cell. 21:1982–1990. doi: 10.1091/mbc.e10-01-0056.

Shi L, Tu BP. 2013. Acetyl-CoA induces transcription of the key G1 cyclin *CLN3* to promote entry into the cell division cycle in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA. 110:7318–7323. doi:10.1073/pnas.1302490110.

Shorter J, Lindquist S. 2005. Prions as adaptive conduits of memory and inheritance. Nat Rev Genet. 6:435–450. doi:10.1038/nrg1616.

Shou W, Ram S, Vilar JMG. 2007. Synthetic cooperation in engineered yeast populations. Proc Natl Acad Sci USA. 104:1877–1882. doi: 10.1073/pnas.0610575104.

Silljé HHW, Paalman JWG, Ter Schure EG, Olsthoorn SQB, Verkleij AJ, *et al.* 1999. Function of trehalose and glycogen in cell cycle progression and cell viability in *Saccharomyces cerevisiae*. J Bacteriol. 181:396–400. doi:10.1128/JB.181.2.396-400.1999.

Soll DR. 2014. The role of phenotypic switching in the basic biology and pathogenesis of *Candida albicans*. J Oral Microbiol. 6:22993. doi:10.3402/jom.v6.22993.

Stahl DA, Hullar M, Davidson S. 2006. The structure and function of microbial communities. New York, NY: Springer New York. doi: 10.1007/978-3-642-30123-0_34.

Stewart MK, Cummings LA, Johnson ML, Berezow AB, Cookson BT. 2011. Regulation of phenotypic heterogeneity permits *Salmonella evasion* of the host caspase-1 inflammatory response. Proc Natl Acad Sci USA. 108:20742–20747. doi: 10.1073/pnas.1108963108.

Stockholm D, Benchaouir R, Picot J, Rameau P, Neildez TMA, *et al.* 2007. The origin of phenotypic heterogeneity in a clonal cell

population *in vitro*. PLoS One. 2:e394. doi:10.1371/journal.pone.0000394.

Süel GM, Kulkarni RP, Dworkin J, Garcia-Ojalvo J, Elowitz MB. 2007. Tunability and noise dependence in differentiation dynamics. Science. 315:1716–1719. doi:10.1126/science. 1137455.

Suh SO, Blackwell M, Kurtzman CP, Lachance MA. 2006. Phylogenetics of *Saccharomycetales*, the ascomycete yeasts. Mycologia. 98:1006–1017. doi:10.3852/mycologia.98.6.1006.

Takhaveev V, Heinemann M. 2018. Metabolic heterogeneity in clonal microbial populations. Curr Opin Microbiol. 45:30–38. doi:10.1016/j.mib.2018.02.004.

Tarnita CE, Taubes CH, Nowak MA. 2013. Evolutionary construction by staying together and coming together. J Theor Biol. 320:10–22. doi:10.1016/j.jtbi.2012.11.022.

Tsoi R, Wu F, Zhang C, Bewick S, Karig, D, *et al.* 2018. Metabolic division of labor in microbial systems. Proc Natl Acad Sci USA. 115:2526–2531. doi:10.1073/pnas.1716888115.

Tuite MF. 2016. Remembering the past: a new form of protein-based inheritance. Cell. 167:302–303. doi:10.1016/j.cell.2016.09.036.

Uptain SM, Lindquist S. 2002. Prions as protein-based genetic elements. Annu Rev Microbiol. 56:703–741. doi:10.1146/annurev.micro.56.013002.100603.

Váchová L, Palková Z. 2018. How structured yeast multicellular communities live, age and die? FEMS Yeast Res. 18: doi:10.1093/femsyr/foy033.

van den Broek M, Bolat I, Nijkamp JF, Ramos E, Luttik MAH, *et al.* 2015. Chromosomal copy number variation in *Saccharomyces pastorianus* is evidence for extensive genome dynamics in industrial lager brewing strains. Appl Environ Microbiol. 81:6253–6267. doi:10.1128/AEM.01263-15.

van Gestel J, Vlamakis H, Kolter R. 2015. Division of labor in biofilms: the ecology of cell differentiation. Microbiol Spectr. 3:MB-0002-2014. doi:10.1128/microbiolspec.MB-0002-2014.

Varahan S, Sinha V, Walvekar A, Krishna S, Laxman S. 2020. Resource plasticity-driven carbon-nitrogen budgeting enables specialization and division of labor in a clonal community. eLife. 9:e57609. doi:10.7554/eLife.57609.

Varahan S, Walvekar A, Sinha V, Krishna S, Laxman S. 2019. Metabolic constraints drive self-organization of specialized cell groups. eLife. 8:e46735. doi:10.7554/eLife.46735.

Vengayil V, Rashida Z, Laxman S. 2019. The E3 ubiquitin ligase Pib1 regulates effective gluconeogenic shutdown upon glucose availability. J Biol Chem. 294:17209–17223. doi:10.1074/jbc.RA119.009822.

Vinck A, De Bekker C, Ossin A, Ohm RA, De Vries, RP, *et al.* 2011. Heterogenic expression of genes encoding secreted proteins at the periphery of *Aspergillus niger* colonies. Environ Microbiol. 13:216–225. doi:10.1111/j.1462-2920.2010.02322.x.

Vinck A, Terlou M, Pestman WR, Martens EP, Ram AF, *et al.* 2005. Hyphal differentiation in the exploring mycelium of *Aspergillus niger*. Mol Microbiol. 58:693–699. doi:10.1111/j.1365-2958.2005.04869.x.

Walsh CT, Tu BP, Tang Y. 2018. Eight kinetically stable but thermodynamically activated molecules that power cell metabolism. Chem Rev. 118:1460–1494. doi:10.1021/acs.chemrev.7b00510.

Walvekar AS, Srinivasan R, Gupta R, Laxman S. 2018. Methionine coordinates a hierarchically organized anabolic program enabling proliferation. Mol Biol Cell. 29:3183–3200. doi:10.1091/mbc.E18-08-0515.

West SA, Cooper GA. 2016. Division of labour in microorganisms: an evolutionary perspective. Nat Rev Microbiol. 14:716–723. doi:10.1038/nrmicro.2016.111.

Westheimer FH. 1987. Why nature chose phosphates. Science. 235:1173–1178. doi:10.1126/science.2434996.

Wickner RB. 1994. [URE3] as an altered *URE2* protein: Evidence for a prion analog in *Saccharomyces cerevisiae*. Science. 264:566–569. doi:10.1126/science.7909170.

Wickner RB, Edskes HK, Shewmaker F, Nakayashiki T. 2007. Prions of fungi: inherited structures and biological roles. Nat Rev Microbiol. 5:611–618. doi:10.1038/nrmicro1708.

Wiemken A. 1990. Trehalose in yeast, stress protectant rather than reserve carbohydrate. Antonie Van Leeuwenhoek. 58:209–217. doi:10.1007/BF00548935.

Wintermute EH, Silver PA. 2010. Emergent cooperation in microbial metabolism. Mol Syst Biol. 6:407. doi:10.1038/msb.2010.66.

Wosten HAB, Moukha SM, Sietsma JH, Wessels JGH. 1991. Localization of growth and secretion of proteins in *Aspergillus niger*. J Gen Microbiol. 137:2017–2023. doi:10.1099/00221287-137-8-2017.

Wu CI. 2001. The genic view of the process of speciation. J. Evol. Biol. 14:851–865. doi:10.1046/j.1420-9101.2001.00335.x.

Yang M, Vousden KH. 2016. Serine and one-carbon metabolism in cancer. Nat Rev Cancer. 16:650–662. doi:10.1038/nrc.2016.81.

Yoo HC, Yu YC, Sung Y, Han JM. 2020. Glutamine reliance in cell metabolism. Exp Mol Med. 52:1496–1516. doi:10.1038/s12276-020-00504-8.

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