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MINIREVIEW

The Unique Metabolism of SAR11 Aquatic Bacteria

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The deeply branching clade of abundant, globally distributed aquatic α-Proteobacteria known as "SAR11", are adapted to nutrient-poor environments such as the surface waters of the open ocean. Unknown prior to 1990, uncultured until 2002, members of the SAR11 clade can now be cultured in artificial, defined media to densities three orders of magnitude higher than in unamended natural media. Cultivation in natural and defined media has confirmed genomic and metagenomic predictions such as an inability to reduce sulfate to sulfide, a requirement for pyruvate, an ability to oxidize a wide variety of methylated and one-carbon compounds for energy, and an unusual form of conditional glycine auxotrophy. Here we describe the metabolism of the SAR11 type strain *Candidatus* "Pelagibacter ubique" str. HTCC1062, as revealed by genome-assisted studies of laboratory cultures. We also describe the discovery of SAR11 and field studies that have been done on natural populations.

Keywords: SAR11, Pelagibacter, metabolism, genomics, oligotrophy, culturability

Introduction

In 1946 Claude ZoBell vigorously defended his medium #2216 as the one best suited to growing marine bacteria (ZoBell, 1946). He confessed that it was not much different from media used by Fischer in 1894. It consisted mainly of aged or "rotted" seawater with a complex mixture of enzymatically digested animal meat and milk known as peptone. While Fischer had found that extracts of phytoplankton and fish were more beneficial amendments to aged seawater than peptone, ZoBell saw the reverse. He attributed the superiority of his medium to the superiority of "modern" Bacto-Peptone over the cruder peptones available in the late 19th century. Decades of growing marine bacteria on ZoBell's medium gave comfortingly consistent, reproducible, results: each milliliter of seawater on average yielded hundreds of colonies on culturing plates. Thus, seawater held hundreds of live, viable bacteria per milliliter. Some protested that far more bacteria, up to millions per milliliter, could be seen under the microscope. But ZoBell brushed their criticism aside: one cannot tell if a cell is alive by looking at it in a microscope. One had to grow it in medium #2216 to be sure.

As it turns out, the microscopists were correct. Each milliliter of even the most oligotrophic seawater contains hundreds of thousands of bacterial cells known as SAR11 (Morris *et al*., 2002). These cannot grow on a mixture of peptone added to natural seawater, but they can grow on natural seawater with no additions other than highly dilute ammonium and phosphate (Rappe *et al*., 2002). We now know how to enhance growth: give them only the specific compounds that genome analysis tells us they must have. They must have a source of reduced sulfur, preferably methionine or dimethylsulfoniopropionate (DMSP) (Tripp *et al*., 2008). For maximum growth, they must have glycine or a glycine precursor in their growth media (Tripp *et al*., 2009; Carini *et al*., 2012). Although glycine and methionine contain carbon, their carbon cannot be used as a carbon source. For maximum growth, they must have an additional carbon source. For some, but not all, strains that additional source can be glucose (Schwalbach *et al*., 2010). The universal carbon source seems to be pyruvate, or compounds that can form pyruvate for gluconeogenesis, like oxaloacetic acid (Carini *et al*., 2012).

In addition to knowing what they must have, we know of unusual resources they can use. They can obtain energy from the oxidation of a variety of C1-moieties derived from one-carbon or methylated compounds, and may be able to use a small amount for biomass (Sun *et al*., 2011). They also can make ATP from sunlight (Giovannoni *et al*., 2005a), in order to increase their longevity in carbon-limited stationary phase (Steindler *et al*., 2011). These energy sources are unusual for a heterotrophic bacterium, and no doubt lessen the demand for high-value carbon sources, which are scarce in the environments where SAR11 strains live.

This review recounts the discovery, cultivation, and genome sequencing of SAR11 members, along with the unraveling of their unique metabolism by genome analysis and experimentation. It also touches on selected culture-independent studies.

Discovery of the SAR11 clade

The SAR11 clade was discovered in 1990 using 16S rRNA PCR amplification of environmental DNA collected from the Sargasso Sea (Giovannoni *et al*., 1990). Phylogenetic

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analysis placed the clade deep within the α-Proteobacteria, with no known cultured representatives. Statistical inferences from clone libraries and rRNA hybridizations indicated that SAR11 members comprised at least 12.5% of the heterotrophic bacteria in the Sargasso Sea. Later studies found SAR11 members throughout the world's sunlit, oligotrophic ocean waters at abundances up to 50% of all heterotrophic bacteria, implying a global population of 10^{28} cells (Morris *et al*., 2002).

Soon after the discovery of marine SAR11 bacteria in the Sargasso Sea, a freshwater clade was discovered in an Antarctic Lake (Bahr *et al*., 1996). The freshwater clade was eventually known as "LD12", for the Lake District in which it was found (Zwart *et al*., 2002). A brackish water clade has been proposed based on a study of the Chesapeake Bay estuary in the Eastern US (Kan *et al*., 2008), but this result was not confirmed in a wider, global study (Logares *et al*., 2010). The global study did however confirm the clear distinction between marine-brackish clades and freshwater clades (Logares *et al*., 2010). Additional SAR11 clades and subclades have been discovered or proposed very recently (Brown *et al*., 2012; Grote *et al*., 2012), as workers continue to probe the evolution, diversity, abundance, and importance of the SAR11 group.

Culture-independent studies of SAR11 members

The biogeography and diversity of the SAR11 group has been studied using a variety of 16S-based techniques. Different SAR11 populations were detected in clone libraries from the top and the bottom of the euphotic zone in the Atlantic and Pacific oceans (Field *et al*., 1997). One 16S-based study using T-RFLP showed periodic blooms of SAR11 members in response to deep mixing events (Morris *et al*., 2005) while another showed regular seasonal patterns of abundance over annual cycles (Carlson *et al*., 2009). The global distribution and abundance of SAR11 populations have been studied with FISH (fluorescent *in-situ* hybridization) (Morris *et al*., 2002). SAR11 diversity has also been studied with ARISA (automated ribosomal intergenic spacer analysis) (Brown *et al*., 2012) and ITS (intergenic spacer) analysis (Brown *et al*., 2012). A global study of 16S sequences revealed less diversity in geographically separated freshwater environments than in geographically separated saline environments, leading to the conclusion that saline and freshwater colonization happened early in the evolution of the SAR11 group, with few additional transitions between freshwater and marine environments (Logares *et al*., 2010). In the permanently stratified waters of the subtropical Pacific, qPCR of 16S sequences sampled over time revealed that surface SAR11 population abundance was positively correlated to diatom and *Synechococcus* abundance and negatively correlated to nitrate, nitrite, and phosphorus concentrations (Eiler *et al*., 2009). Interestingly, although the waters in the subtropical Pacific are permanently stratified, SAR11 members were found down to a depth of 4,000 m. Collectively, these 16S studies show the SAR11 group to be exceptionally diverse, globally distributed, and highly abundant, with periodic blooms.

Culture independent studies of the SAR11 group have gone beyond 16S analysis. Microradiography combined with FISH (MAR-FISH) has been used to show that at least some members of the marine SAR11 groups compete strongly for glucose (Alonso and Pernthaler, 2006) and amino acids (Malmstrom *et al*., 2005). In contrast, members of the freshwater group LD12 did not compete strongly for glucose, representing only a tenth of the cells taking up glucose (Salcher *et al*., 2011). LD12 members competed strongly for a mix of amino acids, and showed particular success in competing for glutamine, glutamate, and glycine, representing 58% to 76% of all bacterial uptake at concentrations less than 100 nM, and measureable uptake down to 0.1 nM. Measurements of the uptake of bromodeoxyuridine (BrdU), a proxy for "metabolic activity", have indicated positive responses to additions of dimethylsulfoniopropionate (DMSP), glycine betaine, para-hydroxybenzoic acid, and vannilic acid (Mou *et al*., 2007). This ready response of SAR11 members to nutrient addition is in accord with a proteomic study showing only modest differences in gene expression between stationary and exponential phase (Sowell *et al*., 2008), indicating that SAR11 cells remain poised to resume growth, even in stationary phase. This makes it unlikely that they reconfigure themselves into a spore-like "viable but nonculturable" (VBNC) state, as some other aquatic bacteria are thought to do (Huq and Colwell, 1996). Metaproteomic studies in the Sargasso Sea and a coastal upwelling region revealed high expression of SAR11 transporters, particularly for amino acids, carboxylates, polyamines, and sugars. High expression of phosphate and phosphonate transporters was notable in the Sargasso Sea, but not in the nutrient rich upwelling region (Sowell *et al*., 2009, 2011). In sum, culture-independent studies have revealed something of the ecophysiology of the SAR11 group.

Culture-independent studies of marine SAR11 members have shown a high rate of intraspecific recombination, more characteristic of a sexually reproducing population than a clonal population, at least by some measures (Vergin *et al*., 2007). Recombination was revealed in the incongruity between the phylogenetic trees of housekeeping genes and 16S sequences and in the patterns of intragenic nucleotide substitutions across strains (Vergin *et al*., 2007). It was speculated that pili similar to those that allow *E. coli* to use DNA as a sole carbon source might be the means by which SAR11 cells acquire exogenous DNA (Vergin *et al*., 2007). Interestingly, energy starved cells grown in the dark were seen to develop numerous connecting pili (Steindler *et al*., 2011), calling to mind the long-known fact that competence in uptake of DNA is sometimes triggered by starvation in bacteria (Archer and Landman, 1969).

Despite evidence that SAR11 genes recombine, there is evidence that their synteny is well preserved. A metagenomic study showed that neighboring genes on very short environmental contigs (typically two genes) were often within five open reading frames in the closed genome of the type strain HTCC1062 (Wilhelm *et al*., 2007). Gene synteny was also seen in comparison of the gene order in several closed genomes sequenced after HTCC1062 (Grote *et al*., 2012). Together these facts indicate that broad metabolic themes are implemented across the diverse members of the SAR11 group, but there is much variation in the gene sequences implementing those themes, offering many opportunities for adaptation.

Culture independent studies have provided much information on the biogeography, phylogeny, and diversity of SAR11 members, and a glimpse into their metabolism. It took genomics, metagenomics, and culturing to gain deep insight into the unique metabolism of what has come to be the type strain for the SAR11 group, HTCC1062.

Cultivation of SAR11 members in oligotrophic natural seawater

Soon after SAR11 was discovered in 1990, Button used dilution to extinction techniques in oligotrophic seawater to grow tiny bacteria (reportedly as low as 0.002 μm^3 in size and 1 fg C dry weight) that grew very slowly (doubling only daily or weekly) (Button *et al*., 1993). He did not characterize the cells phylogenetically, being more interested in the overall phenomenon of "oligotrophy", the paradoxical appearance of a requirement for nutrient poor conditions to allow growth. The Giovannoni Lab scaled up Button's culturing techniques using miniaturized culturing plates, liquid handling robots, and a specially designed vacuum manifold that transferred an array of 48 DAPI-stained culture samples at a time onto a membrane for cell counting (Connon and Giovannoni, 2002). These improved techniques eventually resulted in the successful cultivation of several coastal Pacific SAR11 strains, including strain HTCC1062, for which the taxonomic status *Candidatus* 'Pelagibacter ubique' was proposed (Rappe *et al*., 2002).

Additional SAR11 isolates were brought into culture from a wider variety of locations as a result of further methodological refinements. Teflon culturing plates were used to isolate several Sargasso Sea strains of SAR11, including the sequenced isolate HTCC7211 (Stingl *et al*., 2007). Longer incubation times at lower temperatures were used to cultivate strains in the Northwestern Pacific (Song *et al*., 2009; Oh *et al*., 2011). Hawaiian strains were also isolated using these techniques (Grote *et al*., 2012).

The morphology and growth kinetics of most strains in natural seawater media amended only with ammonium and phosphate is quite similar: very small, curved rods double every 24 to 48 h at optimum temperature until they reach approximately 10^5 to 10^6 cells/ml (Rappe *et al*., 2002). There is evidence that viability in carbon-limited stationary phase can be extended if cells are put on a light/dark cycle (Steindler *et al*., 2011). This is presumed to be due to nutrient savings afforded by light-driven energy generation using proteorhodopsin.

Attempts to improve cell yield with nutrient additions, including peptone, typically slowed or stopped growth (Rappe *et al*., 2002). In order to explain this "oligotrophic" behavior, the genome of HTCC1062 was sequenced (Giovannoni *et al*., 2005b). Annotation of the genome and metabolic reconstruction of biosynthetic pathways provided hypotheses for culture-dependent studies aimed at explaining the behavior of HTCC1062 in culture.

Reduced sulfur requirement

The first genome analysis of HTCC1062 did not report an incomplete pathway for assimilatory sulfate reduction (Giovannoni *et al*., 2005b). Because partial evidence of the pathway was seen, it was presumed to be complete, as in all known free-living bacteria inhabiting oxygenated marine environments. However, *in vitro* experimentation revealed a linear increase in maximum cell yield when nanomolar titrations of methionine and DMSP were added to natural seawater amended with excess carbon (Tripp *et al*., 2008). This demonstrated a requirement for reduced sulfur, presumably due to loss of assimilatory sulfate reduction. This was an astonishing result, given that the concentration of reduced sulfur in the ocean is barely detectable while the concentration of sulfate is at saturation, 10^6 to 10^7 times higher. It seemed counter-intuitive that a heterotrophic marine bacterium as abundant as SAR11 could afford to risk sulfur starvation by losing the few genes required for assimilatory sulfate reduction. However, the savings in nitrogen and phosphorus afforded by gene loss, multiplied by the huge effective population size of SAR11, was proposed to create enough advantage to "streamline" SAR11 genomes under selective pressure (Giovannoni *et al*., 2005b).

Metabolic reconstruction *in silico* has noted the absence of assimilatory sulfate reduction in two other clades of abundant marine bacteria, SAR86 (Dupont *et al*., 2012) and Archaeal Marine Group II (MG-II) (Iverson *et al*., 2012). The widespread dependence of abundant, free-living bacteria on reduced sulfur, present at barely measureable concentrations in their environment, was completely unexpected by marine microbiologists, and gives a new picture of the ocean's sulfur cycle.

Central carbon metabolism: gluconeogenesis, not glycolysis

HTCC1062 has a novel variant of the Enter-Doudoroff (ED) pathway, allowing it to utilize either glucose or pyruvate as a central carbon source in natural seawater (Schwalbach *et al*., 2010) or in defined artificial media (Carini *et al*., 2012). However, in thinking of the Oregon coastal HTCC1062 strain as a model for the SAR11 group, it is more appropriate to think of pyruvate as the central carbon compound for SAR11, with glucose as its precursor. One reason for conceptualizing it this way is that not all strains of SAR11, notably HTCC7211, have the ED pathway, and strain HTCC7211 was shown to be able to use pyruvate, but not glucose, as an amendment in natural seawater (Schwalbach *et al*., 2010). Analysis of multiple SAR11 genomes also supports the interpretation that glucose utilization is not conserved within the SAR11 clade (Grote *et al*., 2012). There is a low, but continuous flux of pyruvate in the sea resulting from the photolysis of dissolved organic matter that may, in part, support SAR11's pyruvate needs (Kieber *et al*., 1989). For these reasons, it is appropriate to conceptualize pyruvate as the essential central carbon of SAR11 strains, not glucose. Therefore, pyruvate is shown in bold on Fig. 1.

There are compounds that can substitute for pyruvate as a central carbon source. In natural seawater media, acetate and taurine were shown to be substitutes for pyruvate (Schwalbach *et al*., 2010). These results imply the presence of an active glyoxylate bypass to the TCA cycle (red carbons shown in Fig. 1). Substitution experiments done in artificial seawater confirm the substitution of pyruvate by oxaloacetate, but not by taurine or acetate (Carini *et al*., 2012). The presence of compounds in natural seawater that may act as glyox-

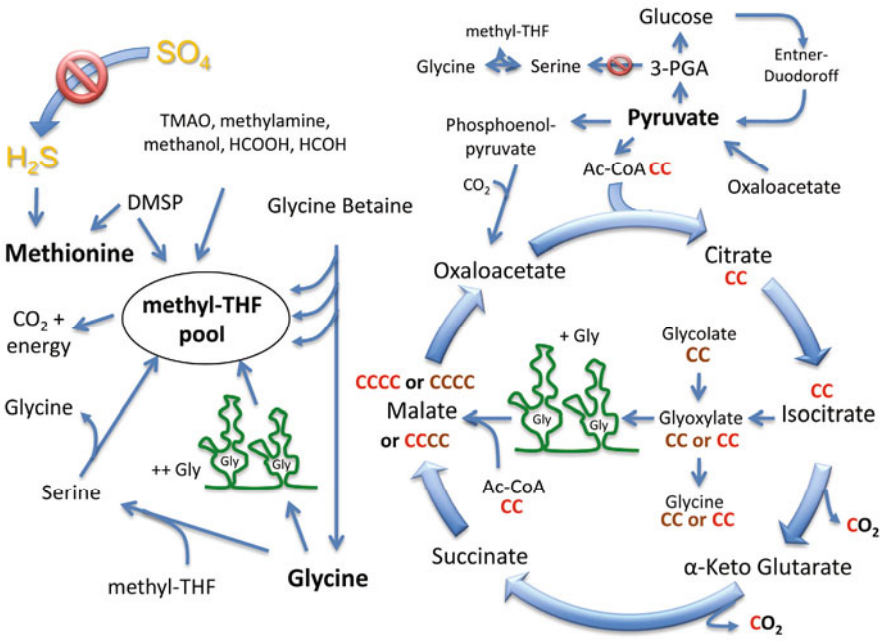

Fig. 1. Schematic of central carbon, sulfur, and glycine-serine metabolism in SAR11. The red circles with slashes indicate functions that are missing from SAR11 members. The green structures represent glycine-activated riboswitches that enable expression of the enzyme they control. The + Gly and ++ Gly labels on riboswitches indicate the relative intracellular level of glycine required to activate them at 50% efficiency. Red carbon atoms show carbon flow through the glyoxylate bypass to the TCA cycle. Brown carbon atoms show introduction of carbon into the middle of the glyoxylate bypass by glycolate. Ac-CoA, acetyl CoA; DMSP, dimethylsulfoniopropionate; Gly, glycine; HCOH, formaldehyde; HCOOH, formate; 3-PGA, 3-phosphoglyceric acid; THF, terahydrofolate; TMAO, trimethylamine oxide.

ylate cycle activators could potentially explain these results.

Conditional glycine auxotrophy

There is a lesion in the canonical pathway of glycine biosynthesis from serine in HTCC1062 (upper right of Fig. 1). Note that the reversible methylation/demethylation reaction interconverting glycine and serine was retained. Originally, it was thought that this lesion was compensated for by degradation of threonine to glycine, followed by glycine cleavage to form the methyl groups required to methylate glycine and make serine. However, natural seawater experiments showed that titrations of glycine additions above natural background improved maximum yield, just as methionine titrations did (Tripp *et al.*, 2009). This indicated glycine auxotrophy, at least in order to support maximum yield. However, it left open the possibility of glycine prototrophy at environmentally relevant concentrations. One puzzling result was that threonine did not replace glycine as an amendment that would support maximum growth. This cast doubt on threonine aldolase as a fully functional replacement for glycine biosynthesis. Additional doubt was cast on threonine aldolase under controlled conditions in artificial media. Methionine and pyruvate were not sufficient for growth, but methionine, glycine, and pyruvate were (Carini *et al.*, 2012). If threonine aldolase were a viable source of even some glycine biosynthesis, one would have suspected at least some growth with methionine and pyruvate alone, since there is a path from pyruvate to threonine. Still, this leaves open the possibility of unknown regulatory activation of threonine aldolase.

Yet another interesting result was seen in the same study using defined media (Carini *et al.*, 2012). Glycolate could partially replace glycine, revealing a new pathway to glycine biosynthesis that was detected in natural seawater experiments: amination of glyoxylate. Confounding this already complex situation is a glycine-activated riboswitch on the glyoxylate cycle that could potentially limit the use of glyoxylate as a glycine replacement. At the same time, as discussion below will reveal it could make glycolate a potential central carbon source at the same time. Yet another layer of complexity is added in the form of a second glycine-activated riboswitch on the glycine cleavage complex, which could play a role in the generation of methyl groups for serine biosynthesis from glycine.

Obviously, complex regulatory factors are at work in SAR11 glycine-serine metabolism, and more research will be needed to explain the full dynamics of glycine-serine metabolism. All that can be said now is that although metabolic reconstruction has so far suggested a potential for some glycine biosynthesis, experimental conditions have so far not found a single central carbon source that can support maximum growth without added glycine in the medium.

There are glycine precursors that can substitute for glycine in HTCC1062. Notable among them are serine, glycine betaine, and glycolate. Serine substitutes for glycine through the removal of a hydroxymethyl group to form glycine. Glycine betaine substitutes by the presence of demethylation enzymes that degrade it to glycine (Sun *et al.*, 2011). Glycolate putatively substitutes for glycine by oxidation to glyoxylate, which is aminated to form glycine (Carini *et al.*, 2012). This last partial substitution could not be detected in natural seawater media (Tripp *et al.*, 2009), but was detected in artificial media (Carini *et al.*, 2012). It is postulated that the partial substitution is due to a novel, conserved, glycine-activated riboswitch on malate synthase (Tripp *et al.*, 2009). At low glycine concentrations, the riboswitch is closed, diverting glycolate-derived glyoxylate to glycine biosynthesis. As intracellular glycine concentrations rise, the riboswitch gradually opens, diverting more and more glycolate away from glycine biosynthesis. This gradual diversion of glycolate away from glycine biosynthesis offers a potential explanation of why, mole for mole, glycolate cannot substitute for glycine.

It is worth considering where the methyl groups for serine biosynthesis might come from, depending on the glycine source. If the source is glycine betaine, methyl groups can come from demethylation of the compound itself. If the source is glycine or glycolate, methyl groups can come from the glycine cleavage complex. Notice in Fig. 1 that glycine cleavage is triggered by a second glycine-activated riboswitch, which has a high average activation potential. It is easy to see how this riboswitch is activated if the glycine source is a high concentration of glycine itself. It is not so easy to see how this riboswitch can be activated if the glycine source is glycolate. That is because the riboswitch on malate synthase diverts glycolate away from glycine biosynthesis, at generally low intracellular concentrations of glycine. How then can glycolate generate sufficient glycine to trigger the glycine cleavage complex? One possible answer is that the activation curves for the malate synthase and glycine cleavage riboswitches overlap (Tripp *et al.*, 2009). In other words, there are intermediate glycine concentrations that could potentially allow both riboswitches to be open at the same time. When both riboswitches are open, it would be possible for glycolate to generate methyl groups for serine biosynthesis via glycine degradation.

Use of C1 compounds and methyl groups

While the use of C1 and methylated compounds by methylotrophs has been extensively studied, their use by other bacteria has received less attention. A recent comparative study of multiple SAR11 genomes showed a capacity to oxidize methylated tetrahydrofolate (methyl-THF) and confirmed this with wet lab studies (Sun *et al.*, 2011). The methyl-THF pool can be used for at least limited biosynthesis (Fig. 1), and approximately six percent of labeled carbon tracer added was found in biomass, under the conditions tested. Given that the genes putatively responsible for the oxidation and biosynthetic use of C1 and methylated compounds were also found in non-SAR11 genomes, the point has been made that this is perhaps an under explored feature of bacteria in general (Sun *et al.*, 2011).

Strong transcriptional and translational response to Iron starvation

When HTCC1062 cells growing exponentially in enriched natural seawater media were starved for iron by the introduction of a high concentration of iron chelator, transcription of the periplasmic iron binding protein *sfuC* increased 16-fold and translation of the same protein increased 27-fold (Smith *et al.*, 2010). These increases in *sfuC* expression were seen despite a decrease in global gene expression, no doubt in response to the induced starvation. The expression pattern of other genes in response to iron limitation was different from the pattern seen in cells entering stationary phase, demonstrating a specific response to iron limitation (Smith *et al.*, 2010). This aggressive, specific response of SAR11 cells to iron starvation could be one of their selective advantages in aquatic environments where iron is sometimes thought to be limiting (Martin and Fitzwater, 1988).

Adaptive gene loss: The "Black Queen Hypothesis"

Recently, the "Black Queen Hypothesis" (BQH) has been offered as an explanation of how it is possible for free-living populations to lose seemingly essential functions, such as glycine and methionine biosynthesis as described here for SAR11 (Morris *et al.*, 2012). Loss of function under genetic drift is known in organisms that abandon a free-living lifestyle to associate with a host. Conversely, the BQH says that it is possible under the right conditions for loss of function to occur under selective pressure in free-living organisms. It says that if a costly, but universally required resource leaks into the environment, selection pressures can develop in com-

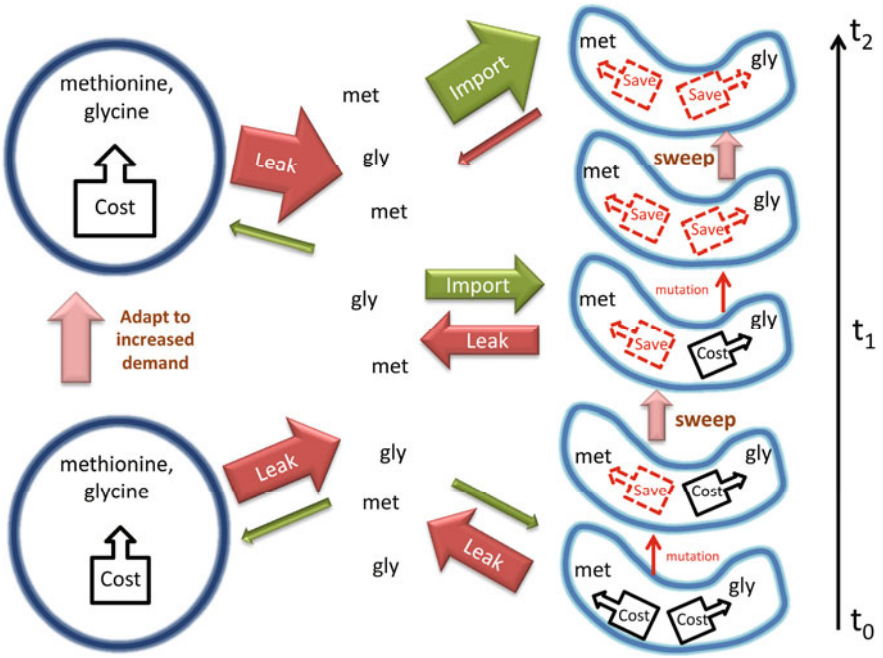

Fig. 2. Schematic of the Black Queen Hypothesis. The blue circles represent a population of glycine and methionine producers who are able to adapt to a slight increase in the cost of production, due to consumption of leaked glycine and methionine by other community members. The cyan vibroids represent a population that can dispense with glycine and methionine production by increasing importation. Time progresses from bottom to top, resulting in adaptive gene loss in the population of blue vibroids. See text for full discussion.

munity sub-populations that ultimately lead to auxotrophy for that resource, so long as the producers of the resource can meet the demand for production.

Figure 2 shows a BQH scenario for loss of glycine and methionine biosynthesis in SAR11. At t_0 (bottom), SAR11 and other community members are all producing glycine and methionine, which leak into the environment, becoming community resources through import. Next, a loss of function mutation arises in SAR11 methionine biosynthesis. The associated benefit due to resource savings initiates a sweep through the SAR11 population. The sweep pressures remaining community members to compensate and adapt. When the remaining producers cannot contribute any additional methionine to support further loss of function in additional sub-populations, the sweep ends (at t_1 in Fig. 2). The process of mutation, sweep, and compensation repeats for glycine and ends at t_2. At this point, SAR11 is an abundant, yet dependent, free-living community member.

Conclusion

Why don't the majority of marine microbes visible under a microscope grow in standard laboratory media? The emerging answer for members of the dominant marine group SAR11 is that their genomes have undergone adaptive gene loss and streamlining under selection pressure. A clearer picture of the specific adaptive losses in SAR11 will come from experiments that are now possible by cultivation in defined media. Experiments so far have challenged the intuitive assumption that it is unlikely for a dominant, free-living clade of bacteria to lose essential functions and become dependent upon other community members. They have also challenged the notion of "oligotrophy", the hypothesis that cells adapted to nutrient poor conditions are either impossible to culture or can only grow to their naturally low density in exceptionally dilute media. Finally, they have invited a re-examination of the presumption that externally acquired methyl groups are important only to methylotrophs.

These changing perceptions have come from examining only the sulfur and central carbon metabolism of SAR11 strains. The rest of their metabolism is still largely undescribed, with new tools now in place to enable its exploration.

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