although, remarkably, the Cas proteins are not homologous to the proteins involved in RNAi (Makarova *et al*., 2006). The mechanistic details of CRISPR/Cas functioning are being investigated at a feverish pace in several laboratories. Important aspects of transcription of the CRISPR loci and processing of the transcripts have already been elucidated (van der Oost *et al*., 2009). The most notable finding is probably the discovery of the CASCADE complex that consists of several Cas proteins and is involved in the CRISPR transcript processing and possibly in the attack on the alien genomes as well (van der Oost *et al*., 2009). The stage of alien DNA integration into the CRISPR loci remains quite enigmatic, and few details are available on the virus abrogation stage either. We do not really need to turn to the crystal ball to predict that within the next few years a full molecular picture of CRISPR/Cas functioning will emerge, and we will learn of new, fascinating mechanisms. What is less clear, is whether this system has additional functions under 'normal' conditions such as regulation of bacterial gene expression or involvement in repair. My expectation is that such functions will be discovered.

Regardless of the molecular details, the CRISPR/Cas system is of extraordinary interest for understanding evolution because it realizes the Lamarckian principle of inheritance and evolution, until now a huge taboo in evolutionary biology. Indeed, CRISPR/Cas use an external cue (alien DNA) to modify a specific genomic locus and then express this locus to specifically react to the original cue, a classic Lamarckian scenario (Koonin and Wolf, 2009).

The CRISPR/Cas system is widespread and obviously important but it is only one of the diverse antivirus systems in bacterial and *Archaea*. The classic restriction–modification enzymes represent another well-characterized class of such systems, but there is much more to be discovered as suggested in particular by the identification of expansive 'defense islands' in bacterial and archaeal genomes (Makarova *et al*., 2009). These islands are significantly enriched for genes encoding components of known defence systems but many genes in the islands remain uncharacterized and are likely to represent novel defence mechanisms. I have no doubts that many such systems will be discovered over the next few years.

Combined with the investigation of new viruses on the scale of the entire biosphere, the study of defence systems will allow us to assess the full scale of the arms race between parasites and hosts that permeates the entire history of life and seems to be one of the key formative factors of evolution. To me, the beauty of these discoveries is that, along with the characterization of new, fascinating molecular mechanisms, they change our core ideas on evolution.

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Evolution as an experimental tool in microbiology: 'Bacterium, improve thyself!'

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> Evolutionary speculation constitutes a kind of metascience, which has the same intellectual fascination for some biologists that metaphysical speculation possessed for some mediaeval [*sic*] scholastics. It can be considered a relatively harmless habit, like eating peanuts, unless it assumes the form of an obsession; then it becomes a vice (Stanier, 1970).

I was introduced to this cutting phrase written by Roger Stanier due to it being quoted in Carl Woese's prescient 1987 review, 'Bacterial Evolution' (Woese, 1987). This paper colourfully recounted the history (of utter failure) of classical microbial taxonomy and prescribed how the then nascent field of molecular evolution would revolutionize thinking in microbiology. Indeed, it is hard to conceive of just how lost we would be without the historical context provided by molecular-based phylogeny.

Here I predict a second impact and that evolution will have upon microbiology beyond illuminating the past: as a powerful *genetic tool* for exploring the function of contemporary biological systems. Evolved isolates that emerge from natural selection provide fascinating information: which parts of a system allow *improvement* in a given environment. This is in contrast to traditional genetic approaches that generally identify mutations that made things *worse*. These two pieces of information can be quite complementary, as our body of knowledge obtained from identifying which gene products are necessary in a given condition (and their associated biochemical properties) has only rarely helped in guessing the targets of adaptation. Think of it this way: simply knowing what parts of a car are necessary for it to move is rather insufficient to identify which should be changed (and how) to make it run faster, or (for most manufacturers) more efficiently.

Experimental evolution is simply the serial (or continuous) transfer of microbial populations to allow new, beneficial variants to occur and rise in frequency due to natural selection. This approach was first employed 60 years ago (Atwood *et al*., 1951), but has become ever more common in the past couple decades. A whole other commentary could be, and has been (Elena and Lenski, 2003), written about how these experiments with microbes have been invaluable for exploring fundamental evolutionary processes due to being able to evolve replicate populations of billions of organisms for thousands of generations, measure fitness changes in competitions against their common ancestor, etc. Although experimental evolution may sound fancy (or fanciful), at its heart it is just a genetic screen for a particular phenotype: fitness (relative reproductive success). Most classical genetic screens require mutants of interest to be sufficiently extreme in phenotype to stand out from the crowd immediately: the one in a thousand (or a million or more) that is either antibiotic resistant, suddenly cranked-up for expression of a reporter gene, etc. In contrast, experimental evolution (and practitioners of it) could be variously described as patient, elegant and/or lazy. Through giving spontaneous mutations and natural selection time to work, rare variants with advantages as low as one percent (but often larger) can be identified as they come to dominate their population. Consider it like PCR for mutants of modest benefit.

Until very recently, even when blessed with evolved isolates with the exciting phenotype of improved growth, researches were cursed by having no means to uncover what had changed at the level of genotype. A senior colleague of mine quipped that this constituted 'population genetics without the genetics'. Fair critique, frankly, for neither traditional cloning approaches nor targeted sequencing of candidate genes had much success finding the mutations. Yearning for the genetic basis of adaptation was deciding to ingest an unhealthy dose of peanuts. Enter (drum roll, please) high-throughput pyrosequencing approaches and all has changed: today genome resequencing can routinely identify all new mutations that occurred in an isolate for approximately $500 (already a 100-fold price drop in 5 years).

Comforted by knowing that the mutations can be found easily, how might experimental evolution help you address your biological questions? Are you interested in how your bug grows on a particular compound, resists a particular stress, or physiologically navigates the transition between environments? Select for them to do these things better! A recent example from my lab (Chou *et al*., 2009) shows just how much can be gained: (i) We got lucky and identified a mutation in an evolved isolate of *Methylobacterium* due to having noticed mRNA levels (via microarrays) for a hypothetical metal transporter had skyrocketed. (ii) Scanning across replicate populations, we found that 30 of 32 populations grown on methanol had fixed nearly identical mutations. (iii) We identified that the relevant selective pressure that made this mutation beneficial (by 18%) was limiting cobalt in our medium. (iv) Further analysis linked the benefit of overexpression of this novel cobalt transporter (*icuAB*) to a particular assimilatory pathway required during methanol growth that has two vitamin B_{12}-requiring enzymes. Interestingly, a traditional approach such as transposon mutagenesis to identify genes required for growth on low cobalt would have failed: deleting this transporter imparts a measurable but unremarkable 1.6% growth defect (due to a redundant copy).

In any genetic screen, mutants that are recovered are giving you the *answer* to what you were asking for, but you may not necessarily have known the *question*. As we freely admitted in our paper, we never intended to study cobalt metabolism (the recipe for our trace metals (Vishniac and Santer, 1957) had itself 'mutated' several times before I inherited it from my graduate advisor). How might one at least try to 'target' adaptation to processes you care about? To focus selection upon a single gene, Yousif Shamoo's group cleverly swapped a mesophilic enzyme

into a thermophile and cranked up the heat (Counago *et al*., 2006). Or to focus selection upon transport, the classic solution is to culture cells in chemostats, where growth rate is sub-maximal because of low levels of the stoichiometrically limiting nutrient (Novick and Horiuchi, 1961). My opinion, however, is that surprises such as our cobalt transport story can be among the most illuminating precisely because they uncover novel genes and physiological connections between them.

With experimental evolution and genome resequencing Microbiology is already well-poised to benefit from harnessing 'evolution in action' as a genetic approach to probe the function and optimization of biological systems, but I believe the future holds even more. As we strive for systems-level understanding of our model organisms, we will ultimately be able to generate testable, quantitative *predictions* of the probabilities of various evolutionary outcomes. This will require integrating frameworks for understanding what is possible from mutations (how they change functional properties of proteins and how these alterations propagate to physiological traits under selection) with how the interplay of selection and drift act in populations to shape the distribution of observed outcomes.

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Where reductionism meets complexity: a call for growth in the study of non-growth

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With the advent of metagenomics, we have unprecedented access to the genetic blueprint of the microbial world. Yet as metagenomic databases keep growing, our ability to interpret the information contained within them has not kept up. This conundrum arises from the fact that we cannot assign functions to the vast majority of their genes. As Jo Handelsman pointed out in a Crystal Ball piece two years ago, 'the glory of the last 50 years of microbiology is founded, in large part, on genetic analysis' (Handelsman, 2009). Amen. Yet as enticing as the prospect of environmental genetics or 'metagenetics' seems, how can we hope to interpret the unchartered world of environmental metagenomes when after more than a half-century of rigorous genetic and biochemical analyses, the functions of roughly a quarter of the genes in *Escherichia coli* – arguably the most well-studied organism on the planet – are still unknown (Karp *et al*., 2007)? Where have we gone wrong? Perhaps it is time to re-examine our assumptions about how to assign gene functions in light of lessons from the field.

Genetic analysis provides a powerful way to learn what genes are required for a phenotype of interest. Invented by physicists, it is steeped in reductionism, permitting clear insights into biological phenomena through the application of simple logical rules. If we want to know which genes are involved in a specific process for an organism that is genetically tractable, we make mutants and then design a screen or a selection that will permit us to assign a 'yes' or 'no' (or sometimes 'partial') level of involvement to any given one. Thus, a key question we must answer at the beginning is: what phenotype(s) do we care about? What conditions are most relevant for our favourite model organism or favourite uncultured microbial community in the environment? Clearly, there is no one answer. Even the concept of '*the* environment' is misleading, because organisms reside in a dynamic world, with changing physical, chemical and biological parameters. Given this complexity, is it even reasonable to think that reductionist approaches can be of value? Absolutely.

So where to begin? Recent work performed in Carol Gross' laboratory at UCSF provides an example. These investigators took a high-throughput approach to growing a collection of *E. coli* mutants under a battery of stressful conditions to assign roles to genes whose functions were unknown (Nichols *et al*., 2010). The idea was simple: if many conditions were tested, some of the unknown genes were bound to be involved in growth on some of them. The results bore this out, and, comfortingly, strains containing mutations in genes that had previously been shown to be involved in the response to particular stres-