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Catabolism of Host-Derived Compounds During Extracellular Bacterial Infections

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ABSTRACT

Efficient catabolism of host-derived compounds is essential for bacterial survival and virulence. While these links in intracellular bacteria are well studied, such studies in extracellular bacteria lag behind, mostly for technical reasons. The field has identified important metabolic pathways, but the mechanisms by which they impact infection and in particular, establishing the importance of a compound's catabolism versus alternate metabolic roles has been difficult. In this review we will examine evidence for catabolism during extracellular bacterial infections in animals and known or potential roles in virulence. In the process, we point out key gaps in the field that will require new or newly adapted techniques. J. Cell. Biochem. 115: 217–223, 2014. © 2013 Wiley Periodicals, Inc.

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To successfully cause infection, a bacterium must incorporate and metabolize molecules derived from the host. General maintenance and expression of virulence factors are minimal requirements, while efficient catabolism and resultant growth can lead to successful colonization and improved evasion of immune-mediated clearance. Here we will use the strict definition of catabolism: degradation of a compound yielding energy [Russell and Cook, 1995]. When we view bacterial catabolism in this way, the most straightforward analogy is to view the host as the growth medium [Brown et al., 2008]. This paradigm, while it is been around for 135 years and has seen intermittent resurgence [Pasteur, 1878; Garber, 1960], has seen many contributions within the last two decades. Armed with the powerful techniques of modern bacteriology, solving the problems of in vivo bacterial catabolism, growth, and resultant virulence should be easy, right?

In one sense the answer is, undoubtedly, yes. In the case of intracellular pathogens, the eukaryotic host cells generate the correct "growth medium" in the infected compartment. Experiments in tissue culture, presuming the correct cell type and growth condition, can very closely mimic the experience of the bacteria in a similar cell within the whole organism. These in vitro systems benefit tremendously from a few handy simplifications: (i) one or a few host cell types, (ii) high bacteria to host cell ratio, and (iii) facile monitoring and manipulation of both sides of the interaction. For many intracellular pathogens, their tractability has led to a reasonably good understanding of in vivo catabolism and its connection to virulence, particularly for *Legionella*, *Listeria*, *Salmonella*, and *Mycobacterium*, which have been well reviewed previously [Eisenreich et al., 2010] and will not be repeated here. Instead, this review focuses on extracellular bacterial pathogens; the little that is known, the vast areas that are not, and some thoughts about how to address the current unknowns. Our principle goal is to discuss what is known about the catabolic pathways governing in vivo growth and survival.

For extracellular bacteria, the primary technical issue can be boiled down to one thing: not knowing the growth medium. What looks to be straightforward turns out to be fraught with problems. We have certainly made important headway by using overly simplified models, but we are left with a wide gap between these models and reality. We lose the dynamic, homeostatic nature of the extracellular niche when we remove this niche from the organism or reconstitute it from parts [Smith, 2000]. Such in vitro characterizations of the extracellular milieu lack dynamics, while in vivo experiments are plagued with the problems readily overcome when studying intracellular pathogens: (i)

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numerous host cell types, tissues, and organs compared to a specific microenvironment within a specific cell type; (ii) overwhelming number of host cells to bacterial invaders; and (iii) poor spatial and temporal resolution of goings-on within the infection ([Smith, 2000] provides a thought provoking analysis of conceptual and technical problems associated with the study of catabolism in vivo). These issues do not make identifying important metabolic pathways overly difficult, rather they make dissecting the specific mechanism by which an implicated pathway alters in vivo growth, catabolism, and virulence incredibly hard. To bridge this gap in knowledge we need new techniques, greater incorporation of techniques from other fields, and a push towards mechanistic understanding of the importance of in vivo catabolism and specific catabolic pathways.

ASSESSING CATABOLISM VERSUS ALTERNATE METABOLIC USAGE

Catabolism has been extensively studied in vitro, where chemostats, microcalorimeters, and oxygen utilization measures have helped biochemists and microbiologists decipher the multiple roles of metabolized compounds during growth. Even within these in vitro systems, determining a compound's actual utilization requires both growth measures and flux analyses to extract information about the role of a compound in energy production versus growth yield [Russell and Cook, 1995]. Overlaid onto the challenges of studying catabolism in vivo is the issue that many important compounds have multiple roles within the cell. Amino acids can be catabolized as an energy source, but they also participate in protein synthesis, are interconverted to other amino acids, and can have other functions: proline is a useful osmoprotectant [Csonka and Hanson, 1991]; serine is used to synthesize phosphatidylserine and as part of the C1 acquisition cycle [Geiger et al., 2010; Anthony, 2011]; tryptophan feeds into formation of many secondary metabolites including virulence-related molecules like PQS produced by *Pseudomonas aeruginosa* [Farrow and Pesci, 2007], etc. Amino acids are not alone in having multiple functions: host-derived carbohydrates can often be used to decorate pathogen surfaces and incorporated into exopolysaccharides, while lipids can impact efflux mechanisms and envelope stress. In this review, we have tried to catalogue the strength of evidence for catabolism itself versus alternate usage of host-derived compounds. We will examine major classes of compounds in the following subsections, which are summarized in Table I.

CARBOHYDRATES

Colonization of mucosal surfaces is a key step in pathogenesis for many opportunistic pathogens. The mucosal surface is dominated by mucins, large glycoproteins that coat mucosal surfaces to trap particles and infectious agents [Thornton et al., 2008]. Mucins are enriched in sialic acid, an anionic nine-carbon carbohydrate [Vimr et al., 2004], thus it is not surprising that many mucosal pathogens have acquired pathways for sialic acid utilization. Some of the best evidence for catabolism of carbohydrates as a major source of energy is utilization of sialic acid by intestinal pathogens. Pathogenic species of *Vibrio* can be transmitted by the ingestion of contaminated food or water and subsequently colonize the host intestine. *V. cholerae* causes acute diarrheal disease and *V. vulnificus* results in gastroenteritis that can lead to septicemia in immunocompromised individuals. *V. vulnificus* carries the *nan* genes for sialic acid degradation on its core chromosome, whereas *V. cholerae* strains have the *nan* genes on the Vibrio Pathogenicity Island 2 (VPI-2) [Almagro-Moreno and Boyd, 2009; Jeong et al., 2009]. In vitro, deletion of the *nan* genes in both species results in the inability to utilize sialic acid as a sole carbon and energy source and in vivo studies demonstrate colonization and survival defects in the intestine of infected mice [Almagro-Moreno and Boyd, 2009; Jeong et al., 2009]. In vitro growth assays for both species reveal no toxic effects from sialic acid accumulation intracellularly or in the growth medium due to this defective catabolism, suggesting that the observed growth defects in vitro correlate with the colonization defect in the intestine. Jeong et al. postulate that sialic acid catabolism contributes to pathogenesis by contributing directly to multiplication (energy production and growth yield) and Almagro-Moreno et al. point out that it may serve as a carbon source in times of high competition when colonizing the intestine. Both groups concur that sialic acid catabolism as an energy source ensures survival during infection [Almagro-Moreno and Boyd, 2009; Jeong et al., 2009].

TABLE I. Summary of Studies on Extracellular Pathogens Implicating Catabolism of Host-Derived Compounds During Animal Infection

Bacteria	Disease	Catabolite	Reference
Gram positive			
Enterococcus faecalis	Endocarditis, UTI, bacteremia, meningitis	Lactate	Rana et al. (2013)
Staphylococcus aureus	Skin infections, endocarditis, pneumonia, meningitis, bacteremia	Proline	Schwan et al. (1998)
Steptococcus pneumonia	Pneumoina, meningitis, endocarditis, pericarditis, bacteremia	Sialic acid	Marion et al. (2011)
Gram negative			
Aggregatibacter actinomycetemcomitans	Perionditis, infective endocarditis	Lactate	Ramsey et al. (2011)
Borrelia burgdorferi	Arthritis, Lymes	Glycerol	Pappas et al. (2011), He et al. (2011)
Campylobacter jejuni	Gastroenteritis, Guillain–Barré syndrome	Serine	Velayudhan et al. (2004)
		Proline	Hofreuter et al. (2012)
		Aspertate	Guccione et al. (2008)
Escherichia coli	Gastroenteritis, UTI, neonatal meningitis	Serine	Anfora et al. (2007)
		Deoxyribose	Martinez-Jehanne et al. (2009)
		Fucose	Snider et al. (2009)
		Gluconate	Chang et al. (2004)
Haemophilus influenzae	Otitis media, pneumonia, meningitis, bacteremia	Sialic acid	Jurcisek et al. (2005)
Pseudomonas aeruginosa	Lung infection, keratitis, UTI, bacteremia, folliculitis, endocarditis	Alanine	Boulette et al. (2009)
Vibrio cholerae	Acute diarrhea	Methionine	Bogard et al. (2012)
		Sialic acid	Almagro-Moreno et al. (2009)
		Gluconate	Patra et al. (2012)
Vibrio vulnificus	Gastroenteritis, septicemia	Sialic acid	Jeong et al. (2009)

As with many potential catabolic substrates, sialic acid has other uses for some pathogens that can cloud the interpretation of in vivo studies. Sialic acid can be used to sialate lipids or exopolysaccharides as a means of immune evasion by bacterial pathogens [Vimr et al., 2004]. Deletions in genes encoding sialidases or sialic acid transporters have been shown to cause colonization and survival defects in nontypeable *Haemophilus influenzae*, the major causative agents of middle ear infections [Jurcisek et al., 2005], as well as respiratory tract infections by *Streptococcus pneumoniae* [Marion et al., 2011]. In these studies, while sialic acid utilization as a catabolic substrate is possible, alterations in sialylation are also a potential mechanism. In addition, it is critical to separate catabolism from incorporation. It is possible that while sialic acid is broken down as an energy source by some of these pathogens, its importance may be for supplying carbohydrate substrates for sugar-intensive biosynthetic processes like capsule synthesis or biofilm formation. Degradation of sialic acid requires an initial investment of ATP and produces acetate and ammonia during partial metabolism even prior to the catabolic steps that would lead to energy production [Vimr et al., 2004]. The resultant ammonia could function as a nitrogen source, while the acetate may be important in a more global sense because acetate, and its co-regulated acetyl-CoA and acetate phosphate pools, impact lysine acetylation [Verdin and Ott, 2013]. Lysine acetylation has been implicated in global regulation of central metabolism and multiple catabolic pathways [Wang et al., 2010]. Consequently, such metabolic byproducts can impact catabolism of other substrates indirectly.

The carbohydrates deoxyribose and fucose have been implicated as catabolic substrates for pathogenic strains of *Escherichia coli*. *E. coli* is a Gram negative bacterium that is a normal inhabitant of the human intestinal flora. Pathogenic strains can be transmitted through fecal–oral or fecal–ureter route where they can colonize the intestine or the urinary tract resulting in extreme diarrhea or urinary tract infections, respectively. For deoxyribose, many pathogenic *E. coli* contain the *deoK* operon encoding proteins for deoxyribose catabolism, allowing these organisms to use deoxyribose as a carbon source [Jonsen et al., 1959; Bernier-Febreau et al., 2004]. Strains incapable of deoxyribose catabolism have defects in intestinal colonization and are outcompeted by those strains capable of deoxyribose catabolism [Martinez-Jehanne et al., 2009]. It is known that deoxyribose is present in the intestinal mucus, the colonization defect of the *deoK* mutant is clear, and the presence of the *deoK* gene on one of the pathogenicity islands underlines its contribution to virulence, however, the evidence for direct catabolism is not strong.

It is well documented that the gut is populated with a variety of bacteria and that metabolite sharing is a common phenomenon. Freter′s nutrient theory states that in order for invading bacteria to compete and thrive in the intestine it must be able to more efficiently utilize a limiting nutrient better than resident bacteria [Freter et al., 1983]. Although *E. coli* can utilize fucose as a carbon source [Hacking and Lin, 1976], they do not contain genes encoding fucosidases [Hoskins et al., 1985], which cleave fucose from glycans. But by being in close proximity with the gut commensal *Bacteroidetes thetaiotamicron* and other strains, *E. coli* can capitalize on *B. thetaiotamicron*′s fucose cleavage by importing and catabolizing released fucose [Salyers et al., 1977; Xu et al., 2003]. In a bovine rectal colonization model, wild-type *E. coli* O157:H7 outcompeted a fucose catabolic mutant, *fucAO* (fucose aldolse and oxido-reductase, respectively) exhibiting the importance of fucose utilization for *E. coli* colonization and maintenance of the population [Snider et al., 2009].

E. coli in vitro gene expression studies showed that genes involved in catabolism of gluconate and other sugars were highly induced when cells were grown in intestine-like conditions, 50% mouse cecal mucus. Gluconate was shown to be the preferred nutrient in vitro through mutation in *edd*, which encodes a 6-phosphogluconate dehydratase, which catalyzes a key step in gluconate entry into the Entner–Doudoroff pathway. In a mouse cocolonization model, the *edd* mutant had a defect in initiation and maintenance of intestinal colonization [Chang et al., 2004]. The gluconate catabolism was also shown to be important for intestinal colonization of *V. cholerae*, as the *edd* mutant failed to colonize the intestine in an infant mouse model [Patra et al., 2012].

AMINO ACIDS

We previously remarked on the pleiotropic roles of amino acids within the cell. This integration with the whole metabolome often makes concrete conclusions about amino acid catabolism difficult. The best evidence for catabolic utilization comes from *Campylobacter jejuni*, for which serine is a preferred carbon and energy source [Leach et al., 1997]. *C. jejuni* is commensal to avian species but is spread to humans through the consumption of contaminated food and causes acute gastroenteritis. Mutations in L-serine deaminase (*sdaA*), which converts serine to pyruvate, lead to major *C. jejuni* colonization and survival defects in both the avian and mouse intestines, and the mouse liver [Velayudhan et al., 2004; Hofreuter et al., 2012]. Likewise, in uropathogenic *E. coli* (UPEC), L-serine can also be catabolized as a sole carbon and energy source [Su and Newman, 1991]. For UPEC, L-serine utilization is important for colonization of the bladder, as an *sdaAsdaB* double mutant was defective in colonization of the mouse bladder [Anfora et al., 2007]. The reaction catalyzed by serine deaminase directly produces pyruvate, strongly suggesting serine as a catabolic substrate. However, pyruvate is also a central player in anabolic reactions and while separable in vitro, in vivo attribution of mechanism is difficult. Also, given the additional product of the reaction, ammonia, the role of L-serine as a nitrogen source in these infection models cannot be wholly discounted.

As mentioned above, *C. jejuni* has a propensity to utilize amino acids within the intestines of mice and birds, rather than the dietary and mucin-derived carbohydrates that are often preferred by other intestinal pathogens [Velayudhan and Kelly, 2002]. Therefore, it is not surprising that *C. jejuni* genes responsible for amino acid catabolism, including aspartate and proline, have also been shown to be important in intestinal colonization [Guccione et al., 2008; Hofreuter et al., 2012]. Proline is also important during *Staphylococcus aureus* burn infections and abscess formation [Schwan et al., 1998]. In these studies, it is not easy to ascertain the role being played by proline, as the gene disruptions were made in the proline transporter. Proline can be transported into the cell and accumulated in the cytosol, where it can function as an osmoprotectant. Accumulation of various host-derived osmoprotectants is important for a variety of infectious agents and infection sites

[Sleator et al., 2001], therefore it is likely proline plays this role for *S. aureus*, though catabolism has not been ruled out.

Mekalanos′ group took a top down approach when it came to identifying factors that contribute to the virulence of *V. cholerae*. Using a suckling mouse intestine model, they paired individual LysR-type transcriptional regulator mutants with the parental strain and tested for in vivo fitness of the mutant compared to the wild type [Bogard et al., 2012]. Of the 38 LysR-family mutants tested, two mutants were impaired in colonizing the intestine, one of which was *metR*, the methionine biosynthesis regulator that also has a role in catabolism [Bogard et al., 2012]. Two MetR controlled genes that are particularly important for mouse intestine colonization are *metJ* which encodes a methionine repressor and *glyA1* which encodes serine hydroxymethyltransferase [Bogard et al., 2012]. GlyA1 is potentially interesting, as it controls the flux of glycine to pyruvate via serine, but also glycine biosynthesis via serine. Based on the evidence from *C. jejuni* infections mentioned previously, serine may be abundant in the intestine and could play a role in either the catabolic or anabolic reactions; particularly the later if glycine or other glycine precursors are limiting.

The concentrations of host-derived metabolites are substantially altered during bacterial infection, particularly near the site of infection [Beisel, 1975; Smith, 2000]. A dramatic case of such metabolic change occurs during chronic lung infections of people with the genetic disorder cystic fibrosis by the opportunistic pathogen *P. aeruginosa* [Burns et al., 1998]. During these chronic infections, tissue damage, immune response, and bacterial activities sculpt the mileu, resulting in thick mucus plugs in airways that can be expectorated as sputum [Boucher, 2004]. *P. aeruginosa* catabolizes many amino acids within sputum from cystic fibrosis patients in vitro, but displays a preference for alanine [Palmer et al., 2007]. The importance of alanine catabolism was demonstrated in a chronic rat lung infection model, showing that *P. aeruginosa* mutants incapable of converting alanine to pyruvate could not compete well against wild-type *P. aeruginosa* [Boulette et al., 2009]. Similar to the cases of serine catabolism in *C. jejuni* and UPEC, this is a deamination in *P. aeruginosa*, resulting in the formation of ammonia along with pyruvate. Therefore, while loss of alanine catabolism is among the likely mechanisms driving the mutant phenotype, the impact of pyruvate-dependent anabolic pathways and the role of the released ammonia need to be considered.

OTHER COMPOUNDS

Glycerol can be phosphorylated and incorporated into glycolysis and is also a critical contributor to phospholipid synthesis. In *Borrelia burgdorferi*, the causative agent of Lyme Disease, deletion of the glycerol catabolism gene *glpD*, which converts glycerol to dihdroxyacetone phosphate (DHAP), results in clear replication defects within the adult tick vector and nymph stage, while the deletion did not have any effect on growth or survival within the mouse model of infection [He et al., 2011; Pappas et al., 2011]. Deletion of *glpD* with the rest of the *glpDFK* operon resulted in a robust growth defect within the tick [He et al., 2011], likely because deletion of the entire operon limits glycerol incorporation into phospholipids in addition to elimination of a shunt of glycerol into glycolysis [Pappas et al., 2011].

L-Lactate can be the sole energy source and is the preferred carbon source for the opportunistic dental and endocardial pathogen *Aggregatibacter actinomycetemcomitans* (*Aa*), even in the presence of high-yield compounds such as glucose or fructose [Brown and Whiteley, 2009]. Like many bacteria, *Aa* exists in multispecies communities and is often found in conjunction with oral streptococci, which produce L-lactate as a catabolic product. *Streptococcus gordonii* and an *Aa lctD* (NAD independent L-lactate dehydrogenase) mutant co-inoculated into a mouse thigh abscess model was used to show that lactate catabolism in *Aa* is important in vivo for establishing polymicrobial infections [Ramsey et al., 2011].

The conversion of pyruvate to lactate by lactate dehydrogenase (*ldh*) is also an important part of NAD+ regeneration in fermentative systems, which allows the continued operation of glycolysis. An example can be found in *Enterococcus faecalis*, a pathogen that can cause endocarditis, bacteremia, urinary tract infections, and meningitis. *E. faecalis* has two *ldh* genes and when both are deleted there is a defect in colonization and persistence of the bacterium in an intravenous mouse model infection in the liver and kidney [Rana et al., 2013]. While NAD+ also functions in other metabolic processes, the flux required by glycolysis suggests that the *ldh* activity in *E. faecalis* plays its main role in enabling robust glycolysis.

THE IMPORTANCE OF CATABOLISM AND ITS LINK TO VIRULENCE

Catabolism of host-derived compounds is necessary for successful infection. These compounds may be derived directly from the host or by scavenging compounds released by other pathogenic or commensal bacteria [Freter et al., 1983]. The studies described above have determined metabolic pathways important for bacterial survival and growth within the host, but in most cases, direct evidence for catabolism contributing to the in vivo phenotype is limited (well described in [Vimr, 2013]). From the most human-centric standpoint, the exact mechanism is not critical to drive therapeutic development—efficacy and safety trumps mechanism. However, we think that understanding the direct mechanism may lead to better future therapies by suggesting synergistic targets to inhibit with the appropriate drug cocktail. For example, if serine was truly a major energy source for a pathogen, combining the serine pathway inhibitor with an inhibitor of the next most important energy source would likely boost efficacy. However, if the importance of serine is to provide a ready supply of glycine or contribution to C1 or C2 biosynthetic units, a better strategy would be to target alternate pathways that produce the limiting intermediate. Therefore, a more complete understanding of the nature of the metabolic deficiency during infection will benefit future antimicrobial therapeutic development.

Determining the link between catabolism and virulence can be difficult. It is obvious that eliminating catabolism of host-derived compounds decreases the pathogenicity of the bacterial population. On the other hand, microbiologists often look at virulence in terms of production of anti-host products termed virulence factors, while catabolic pathways important for survival would fall under the term virulence determinant. Under the heading of virulence determinant, we can certainly include all of the metabolic pathways discussed in this review. Each genetic mutation reduced the survival or growth of the bacteria and thus contributed to virulence in the model discussed.

While virulence determinants can work indirectly, all of the bacterial species mentioned here also produce secreted virulence factors. Most of these are transported through the general or type III secretion systems and target host cells or specific pathways to boost pathogenesis by increasing host damage and decreasing immune clearance. In the simplest sense, producing virulence factors requires energy and therefore are dependent on catabolic pathways. However, it is interesting to note two things. First, secreted proteins are generally less energy intensive than membrane or cytosolic proteins [Smith and Chapman, 2010] and consequently metabolically cheaper. Second, secreted virulence factors are a good investment during nutrient stress, as many described virulence factors, particularly extracellular enzymes transported by the general secretion system, can also be thought of as nutrient requisition systems [Rohmer et al., 2011], providing amino acids (proteases), sugars (glycosidases), nucleic acids (nucleases), and lipids, glycerol, and polar headgroups (lipases). The direct regulatory links between specific catabolism and virulence factor production are less readily apparent. However, it is very clear that, globally, the cell′s nutrient and energy status strongly impacts virulence factor production, reviewed in [Poncet et al., 2009].

METHODS: CURRENT AND A CALL FOR RENEWED AND NEWLY-ADAPTED TECHNIQUES

Most studies of bacterial catabolism have not started as such. With some exceptions described here, catabolic pathways have often been identified during medium or high-throughput screens to identify mutants with altered in vivo survival. Moderate throughput has generally relied on direct counts of surviving colonies, often in competition with wild type cells. High-throughput techniques that have been particularly successful are in vivo expression technology (IVET) [Slauch et al., 1994], signature-tagged mutagenesis (STM) [Lehoux and Levesque, 2000], and transposon insertion sequencing (TnSeq) [van Opijnen et al., 2009], all of which offer ways to assess large numbers of mutants simultaneously during infection. While generally useful, there are caveats associated with these methods of identification of catabolic pathways. One complication is that there are a variety of available carbon sources in a host—many at very high concentrations—so the contribution of any one carbon source may be relatively small, particularly for catabolically versatile bacteria.

Direct labeling. In the very simplest sense, we want to identify what compounds bacteria are eating in the host to provide them with energy, carbon, and nitrogen. There are direct and indirect ways to do this. A classic and still very useful method is to track the fate of radiolabeled substrates. While simple in vitro, many technical issues crop up in vivo. In general, a substrate is fed or injected into the animal and conversion of the substrate into radiolabeled CO_2, acetate, or some other catabolic product is monitored. By comparing to an uninfected animal one can determine the proportion of compound utilized during infection. However, practically this is made very difficult for two reasons. First, host metabolism changes during infection, alterations due to bacterial processes are difficult to separate from changes due to the way the host changes its metabolism to fight the pathogen [Beisel, 1975]. Second, there is the gut microbiota, which by sheer numbers, provide a huge catabolic reservoir to convert susbtrates of interest. This leaves direct labeling useful mainly in two cases: gnotobiotic mice and compartmentalized substrates. While the former eliminates or reduces issues with gut metabolism, the later takes advantage of the fact that many compartments and organs maintain stable pools of compounds that are not rapidly converted or moved from the compartment. Examples include particular proteoglycans in the joints, certain glycosphingolipids in the brain, and choline in the lung and brain. Other compounds that can be studied with this technique are those that are not catabolized by the host, such as L-carnitine, which while utilized in the carnitine/acylcarnitine shuttle system, is not catabolized by host cells [Peluso et al., 2000], although its anaerobic breakdown by the gut flora shows important links to cardiovascular health [Koeth et al., 2013].

Stable isotope probing. Stable isotope probing has been exceptionally useful for the study of catabolic processes in microbial ecology [Neufeld et al., 2007]. Briefly, an organism catabolizing a heavy-isotope substrate will incorporate the isotope into their DNA and the DNA of an otherwise "light" organism will shift towards "heavy." This shift is assessed with cesium gradient ultracentrifugation followed by fractionation. This technique falls victim to the same issues as direct radiolabeling, but the cesium gradient method allows discrimination of the source of the DNA by specific nucleic acid hybridization techniques. This technique, however, directly assesses the incorporation of label into DNA, an anabolic process. While these building blocks can be derived from catabolic end products and siphoned from catabolic intermediates, it is not a direct marker of catabolism.

Using transcriptional evidence to understand catabolism. One of the best ways to understand the range of possible food choices for the bacteria (but not their relative importance) is to look at the genes whose expression is induced in response to the host. As with the rest of bacterial catabolism in the host, the intracellular pathogens have yielded much more compelling transcriptional stories than their extracellular counterparts at the site of infection. For extracellular pathogens, the staggeringly abundant host RNA has generally made sensitive measurement of the bacterial transcriptome difficult. There are some exceptions, where bacterial abundance or localized infections have allowed ex vivo transcriptomics [Larocque et al., 2005; Bielecki et al., 2008; Chaffin et al., 2012], although this field is changing rapidly with many host RNA subtraction techniques and massively parallel sequencing. Therefore, many host–pathogen systems are now sufficiently sensitive to determine the transcriptome of the bacterial population during infection.

A particularly useful application of these techniques would be to couple transcriptomics and metabolomics during infection in order to determine what compounds the bacteria senses and simultaneously measure the fate of the related metabolites. In most infection sites, the bacteria may not be able to exceed host homeostasis for many metabolites, therefore simultaneous analysis of the local transcriptome of the host may help determine compounds that the host senses it must make more of, essentially responding to a small decrease by making or transporting more of a given compound.

While global transcriptomics has its place, often we are asking questions about specific pathways or genes. In these cases, sensitive and quantifiable reporters are critical tools for analysis. The current generation of reporter genes works well for microscopic evaluation (fluorescent proteins) and high levels of expression (*lux* and *lacZ*). While these are both quantifiable under optimal conditions, a better

proposition might be to use the power of reporter genes in combination with the sensitivity of radiodetection. One potential method we have been discussing is to use the SNAP and CLIP proteins (NEB) as reporters, with increasing the sensitivity and specificity by labeling with radioactive substrates instead of fluorescent or biotin-linked substrates. A strain carrying one of the reporters as a translational fusion to the gene of interest and the other to a control gene would allow relative expression values to be sensitively determined by SDS–PAGE of the labeled tissue homogenate followed by autoradiography.

CONCLUSIONS

Bacterial nutrition is a critical component of understanding infection and developing effective treatments. Part of this nutrition is utilization of compounds as energy sources, that is, catabolism. Here we have provided some glimpses of specific compounds used by pathogens, but we are confident that the current literature only scratches the surface of important host-derived compounds. The challenge for the field is to generate methods or new systems to determine the metabolic role of individual compounds for the infecting bacteria.

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