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MEETING REVIEW

Polysaccharide Utilization Loci: Fueling Microbial Communities

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ABSTRACT The complex carbohydrates of terrestrial and marine biomass represent a rich nutrient source for free-living and mutualistic microbes alike. The enzymatic saccharification of these diverse substrates is of critical importance for fueling a variety of complex microbial communities, including marine, soil, ruminant, and monogastric microbiota. Consequently, highly specific carbohydrate-active enzymes, recognition proteins, and transporters are enriched in the genomes of certain species and are of critical importance in competitive environments. In *Bacteroidetes* bacteria, these systems are organized as polysaccharide utilization loci (PULs), which are strictly regulated, colocalized gene clusters that encode enzyme and protein ensembles required for the saccharification of complex carbohydrates. This review provides historical perspectives and summarizes key findings in the study of these systems, highlighting a critical shift from sequence-based PUL discovery to systems-based analyses combining reverse genetics, biochemistry, enzymology, and structural biology to precisely illuminate the molecular mechanisms underpinning PUL function. The ecological implications of dynamic PUL deployment by key species in the human gastrointestinal tract are explored, as well as the wider distribution of these systems in other gut, terrestrial, and marine environments.

KEYWORDS *Bacteroidetes*, carbohydrate, carbohydrate-active enzymes (CAZymes), metabolism, microbiome, polysaccharide utilization loci (PULs), polysaccharides, symbiosis

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Complex carbohydrates, in the form of structural and storage polysaccharides, constitute the largest repository of metabolically accessible carbon in the biosphere (1, 2). The result of primary production, biomass carbohydrates thus present a ubiquitous energy source to fuel microbial life in both terrestrial and marine ecosystems (Fig. 1). Carbohydrate utilization is inextricably linked with the ability of microbes to persist in environments as diverse as freshwater, salt water, soil, and animal gastrointestinal tracts, particularly where competition for a common, and potentially temporally limited, pool of nutrients is fierce. The advent of improved culturing techniques and next-generation sequencing has granted us coveted access to a repository of genetic clues to the metabolic potential of key species across ecosystems (3–10). We are now in an era in which there is urgent need for post(meta)genomic functional analysis to elucidate the interplay between carbohydrate catabolism and microbial ecosystem dynamics.

THE UBIQUITY OF COMPLEX CARBOHYDRATES AND CAZymes

Complex carbohydrates—oligosaccharides and polysaccharides—are composed of a tremendous diversity of monosaccharide subunits and glycosidic linkages (11–13) (see Fig. 1 for examples). Therefore, a correspondingly large array of specific enzymes

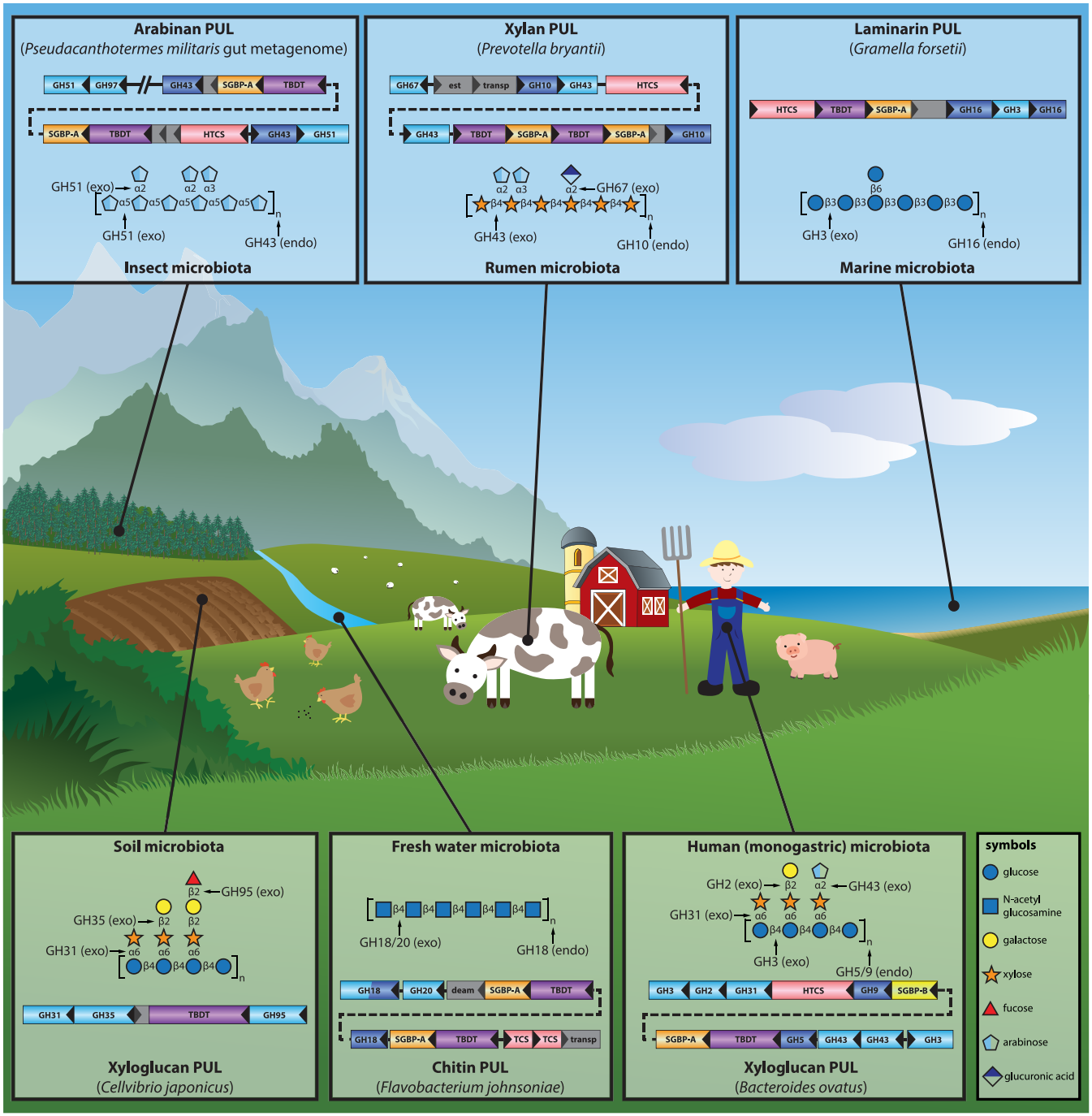

FIG 1 The ecological distribution of PULs in nature. PULs are found in a variety of microbial communities, highlighting the global role of this polysaccharide utilization strategy. Each semitransparent box contains a representative bacterial PUL from a distinct microbial ecosystem, as well as the schematic structure of the target glycan. Clockwise from the top left are the arabinan PUL from an unidentified bacterium from the gut of *Pseudocanthotermes militaris* (93), the xylan PUL from *Prevotella bryantii* (97), the laminarin PUL from *Gramella forsetii* (101), the xyloglucan PUL from *Bacteroides ovatus* (78), the chitin PUL from *Flavobacterium johnsoniae* (112), and the xyloglucan PUL from *Cellvibrio japonicus* (126). Genes are colored according to protein function as follows: blue, endo-GH; cyan, exo-GH; orange, SusD-homologous SGBP; yellow, other SGBP; purple, TBDT; pink, (hybrid) two-component sensor (HTCS/TCS); gray, unknown or other function (est, esterase; transp, transporter; deam, deaminase). Monosaccharides are represented by Consortium for Functional Glycomics symbols (154).

is required to effect complete saccharification and feed primary metabolism. Hence, there is demonstrable enrichment of genes encoding carbohydrate-active enzymes (CAZymes) in saprophytic and pathogenic microorganisms that attack plant and algal cell walls (14–17). Despite the complexity of dietary carbohydrates entering animal gastrointestinal tracts daily, most animal genomes are remarkably bereft of CAZyme-

encoding genes (14, 18, 19). Humans, for example, are intrinsically able to digest only a small group of relatively simple dietary carbohydrates, namely, starch, lactose, and sucrose (14). These observations have spurred considerable interest, dating back decades, in the contribution of complex carbohydrate degradation by intestinal microbiota to the nutrition of monogastric and ruminant animals (14, 20–24).

The CAZyme classification initiated by Bernard Henrissat is a key foundation for genomic, biochemical, and structural studies of the proteins and enzymes involved in complex carbohydrate degradation (25, 26). The CAZy database presently groups, on the basis of amino acid sequence, 145 families of glycoside hydrolases (GHs), 103 families of glycosyltransferases, 26 families of polysaccharide lyases (PLs), 16 families of carbohydrate esterases (CEs), 13 families of redox auxiliary activities, and 81 families of associated noncatalytic carbohydrate-binding modules (27–31; see also www.cazy.org and www.cazypedia.org). A key feature of the CAZy classification is the dissection of open reading frames to reveal discrete, and sometimes complex, CAZyme modular organization (30), which significantly increases the accuracy of bioinformatic analyses.

The predictive power of the CAZy classification has proven to be remarkably robust. Within a given family, key active-site residues, the catalytic mechanism, and the overall three-dimensional fold are strictly conserved (with very few exceptions [32]), while some families are further grouped into clans on the basis of a conserved catalytic mechanism and tertiary structure (25). Substrate specificity is, however, less easily divined because many large families (some with tens of thousands of members) are "polyspecific," i.e., encompassing members with distinct activity profiles, often on structurally related complex carbohydrates. Here, further division into subfamilies has been shown to be beneficial (28, 33–37). However, because of a comparative paucity of biochemically and structurally characterized members vis-à-vis the vast bulk of (meta)genomic sequence data (25-27, 38), bioinformatic predictions of CAZyme function in the context of microbial community ecology are still largely naive. Therefore, there is considerable scope to advance the field through a concerted, systems-based approach that incorporates microbial genetics, biochemistry, enzymology, and structural biology.

BACTEROIDETES AND THE PUL PARADIGM

Members of the Gram-negative phylum *Bacteroidetes* are widespread across diverse ecological niches, including marine, freshwater, and terrestrial habitats, and are notably abundant in microbiota of the alimentary canal. For example, *Bacteroidetes* bacteria, together with the members of the Gram-positive phylum *Firmicutes*, dominate the microbiota of the human colon (39, 40). *Bacteroidetes* bacteria are also profuse in the guts of plant biomass-consuming nonhuman animals (40–45). In animals, including humans, *Bacteroidetes* bacteria provide many symbiotic benefits, notably, the production of short-chain fatty acids by hydrolysis and fermentation of otherwise indigestible complex carbohydrates, which are absorbed and utilized by the epithelial cells of the gut (40, 46, 47).

A unique feature of *Bacteroidetes* genomes is the presence of polysaccharide utilization loci (PULs), a term first coined by Bjursell, Martens, and Gordon in 2006 (48) to describe clusters of colocalized, coregulated genes, the products of which orchestrate the detection, sequestration, enzymatic digestion, and transport of complex carbohydrates (49–51). PULs encode a complement of cell surface glycan-binding proteins (SGBPs), TonB-dependent transporters (TBDTs), CAZymes (most frequently GHs, but also PLs and CEs where substrate appropriate), and carbohydrate sensors/transcriptional regulators. The complexity of PULs often scales with that of their cognate substrates (Fig. 1) (52, 53) and may include ancillary enzymes such as proteases (54), sulfatases (55, 56), and phosphatases (57). These elegant systems constitute the major nutrient acquisition strategy deployed by *Bacteroidetes* bacteria and thus are intrinsically linked to the colonization of nutritional niches and the establishment of microbial ecosystems.

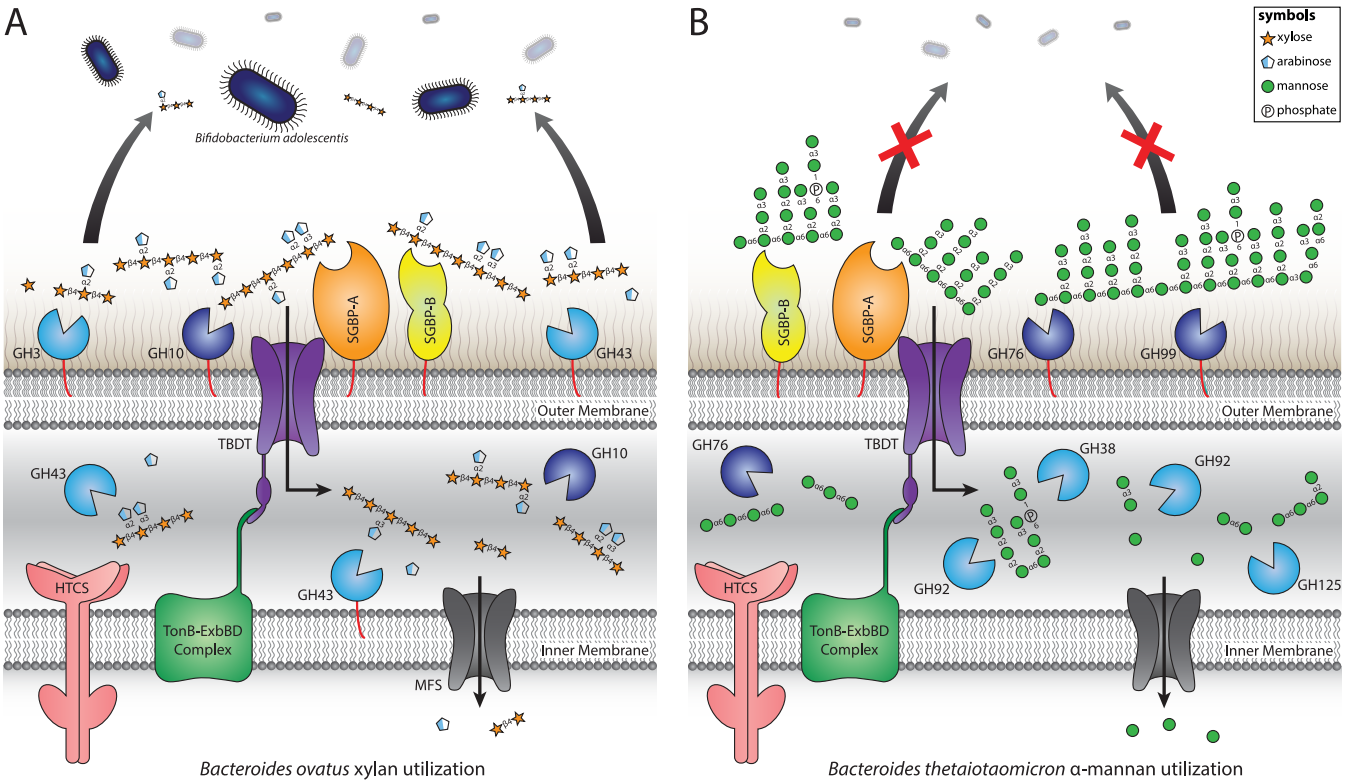

FIG 2 Nutritional foraging strategies encoded by PULs and their roles in microbial ecological interactions. (A) In a distributive mechanism, utilization of wheat arabinoxylan by *B. ovatus* releases partial breakdown products (PBPs) that diffuse into the extracellular environment and support the growth of *B. adolescentis* (79). (B) In contrast, the selfish mechanism employed by *B. thetaiotaomicron* in the digestion of yeast α-mannans results in the rapid import of extracellular products into the periplasm, where saccharification is culminated (80). The concerted actions of these two PUL models drive syntrophic and cooperative networks in the context of the complex microbial environment of the gut microbiota. Proteins are colored as in Fig. 1. Monosaccharides are represented by Consortium for Functional Glycomics symbols (154).

THE ARCHETYPAL PUL, THE Sus

The first evidence of a concerted molecular system for complex glycan degradation in *Bacteroidetes* bacteria was uncovered through pioneering studies of dietary starch utilization by the human gut symbiont *Bacteroides thetaiotaomicron*, which were initiated in the 1980s by Abigail Salyers and coworkers. Notably, initial cellular fractionation studies revealed that key enzymes and starch-binding proteins were individually localized to the cell surface, periplasmic space, and cytoplasm, thus suggesting the presence of a multiprotein carbohydrate-degrading system spanning both bacterial membranes (58). Indeed, subsequent studies precisely identified all of the components of this system, including proteins responsible for recognition and initial hydrolysis of starch at the outer membrane, translocation of glycans into the periplasm, further hydrolysis to monosaccharides, and transcriptional regulation (59–66). Together, eight genes were identified as part of a single gene cluster, collectively named the starch utilization system (Sus), which established a new paradigm of complex carbohydrate utilization (49, 51). Presently, the concerted operation of the Sus continues to be dissected through genetic, biochemical, and structural approaches (67–71) that, together with Salyers' seminal studies, outline a general cellular model for the study of other PULs (Fig. 2).

MOLECULAR ARCHITECTURES OF PUL SUBSYSTEMS

A hallmark of canonical PULs is the presence of at least one sequential pair of *susC* and *susD* homologs (49) that encode an outer membrane TBDT (Fig. 1 and 2, purple) and an N-terminally lipidated SGBP (Fig. 1 and 2, orange), respectively. In light of the considerable structural diversity among SGBPs (see below), SusD homologs are referred to as SGBP-A proteins. Genetic studies have revealed that the TBDT and SGBP-A

proteins are intimately associated; deletion of SGBP-A eliminates or reduces PUL function, yet growth can be rescued by complementation with SGBP-A variants in which substrate binding has been eliminated by site-directed mutagenesis (49, 67, 72). SGBP-A homologs show strong primary, secondary, and globular tertiary structural conservation, with topological variation in the extended substrate-binding surface accounting for carbohydrate specificity (72–76). Recent seminal crystal structures have revealed that SGBP-A homologs form flexible "lids" at the extracellular opening of their associated TBDTs and further highlight an integral role for SGBPs-A in selective nutrient transit (77).

Substrate binding is often assisted by one or more structurally distinct SGBPs (e.g., SGBP-B) (Fig. 1 and 2, yellow) that may have a specificity identical or complementary to that of SGBP-A. The lack of sequence conservation of the genes that encode these proteins often precludes definitive assignment as SusE (or SusF) homologs; hence, these auxiliary SGBPs are sometimes referred to as SusE positioned (or SusF positioned) with reference to their relative genetic organization. Despite this absence of sequence similarity, the crystal structures obtained to date have revealed that these N-terminal lipoproteins share extended multidomain tertiary structures that present substrate-binding faces in one or more distal C-terminal domains (70, 72).

Each PUL also contains a complement of CAZymes tasked with the dismantling of polysaccharides, beginning with the action of one or more cell surface-anchored endoglycanases (Fig. 1 and 2, blue) (78–80); in the Sus, this role is fulfilled by SusG, a GH13 endo-α1,4-glucanase (α-amylase) (65, 69). The resulting fragments are actively shuttled into the periplasmic space by the TBDT (e.g., SusC [61, 64]), where additional linkage-specific GHs (Fig. 1 and 2, cyan) act to release the component monosaccharides (or certain disaccharides) for metabolism in the cytosol. In the Sus, SusA (a GH13 α1,4-glucosidase) and SusB (a GH97 α1,6-glucosidase) are sufficient to hydrolyze all of the linkages in starch oligosaccharides (62, 71), while PULs directed toward more complex substrates typically have manifold exoglycosidases (reviewed in references 52 and 53; see Fig. 1 and 2 and below for specific examples).

PUL regulation is most commonly mediated by one of three mechanisms, the SusR sensor/regulator, extracytoplasmic function sigma (ECF-σ) factor–anti-σ-factor pairs, or hybrid two-component systems (HTCSs) (Fig. 1 and 2, pink). Other PUL-associated regulatory mechanisms include LacI, CRP, AraC (non-HTCS), SARP-OmpR, and classic TCSs (81). SusR is a predicted inner membrane-spanning receptor that binds starch-derived oligosaccharides (but not glucose) and triggers the upregulation of the remaining *sus* genes (63). Intriguingly, SusR appears to be the exception, rather than the rule, in the documented PUL catalogue (82). Rather, PUL regulation is most often orchestrated by ECF-σ–anti-σ pairs that are commonly associated with PULs targeting host-derived glycans (83) or HTCS proteins associated with PULs targeting a variety of plant cell wall carbohydrates (39, 84, 85). Regardless of the regulatory system utilized by a specific PUL, there generally appears to be a finely tuned interpretation of complex glycan signals that is necessary for a targeted, dynamic response; with some exceptions, monosaccharides are typically not inducers (39, 83, 86–89) and may, in fact, repress PUL expression (90).

PULOMICS

As introduced above, *susC-susD* pairs are hallmarks of PULs and have been used to enumerate PUL complements among the genomes of key human gut symbionts, including *B. thetaiotaomicron* (88 PULs), *Bacteroides ovatus* (126 PULs), and *Bacteroides cellulosilyticus* WH2 (113 PULs) (39, 48, 89). The abundance and diversity of PULs have been well documented as a result of these initiatives, which have enabled the comparative analysis of PULs from various gut *Bacteroidetes* bacteria and provided an essential foundation to understand nutrient niche colonization and community dynamics. For example, the genomes of *B. thetaiotaomicron* and *B. ovatus* both harbor ca. 100 PULs; however, strikingly few homologous PULs are shared between them, suggesting that these two symbionts have distinct glycan niches (39). Similarly, recent transcrip-

tomic analyses indicated that *B. xylanisolvens* dynamically responds to discrete structures of pectins and xylans through several differentially regulated PULs (91, 92). Such studies reflect the exquisite nutrient adaptation of individual *Bacteroidetes* bacteria.

Beyond the human gut, PULs have increasingly been identified in a variety of *Bacteroidetes* species (Fig. 1) through (meta)genomic, transcriptomic, and proteomic approaches (93–96). PULs have been identified in species outside the *Bacteroides* genus in diverse environments, including the ruminal *Prevotella bryantii* (97), the freshwater dweller *Flavobacterium johnsoniae* (98), and the gut microbiota of the termite *Pseudacanthotermes militaris* (99). Distinctly, PULs from the gliding soil bacterium *Cytophaga hutchinsonii* lack individual *susC-susD* pairs, but instead, two such pairs are encoded elsewhere in the genome (100).

The discovery of specialized CAZymes tasked with dismantling unique polysaccharides is particularly well illustrated by the PULs of several marine species that utilize highly sulfated algal polysaccharides. For example, PULs from *Zobellia galactanivorans* (55), *Formosa agariphila* (56), and *Gramella forsetii* (101) contain CAZyme portfolios tuned to the unique monosaccharide residues of algal polysaccharides and, not surprisingly, are also enriched in sulfatases (see also SulfAtlas, a new sulfatase classification database from the Marine Glycobiology and ABiMS teams at the Station Biologique de Roscoff [102; http://abims.sb-roscoff.fr/sulfatlas/]).

Recently, the development of automatic prediction tools by the CAZy team has led to the genesis of the PUL database (PULDB; http://www.cazy.org/PULDB/index.php). The PULDB presently catalogs ca. 4,000 predicted PULs from >70 *Bacteroidetes* bacteria, including *Alistipes*, *Bacteroides*, *Dysgonomonas*, *Odoribacter*, *Parabacteroides*, *Paraprevotella*, *Prevotella*, and *Tannerella* species. A key feature of the PULDB is that it, like the CAZy database, is anchored by experimentally characterized PULs (81).

INSIGHT FROM INTEGRATED FUNCTIONAL CHARACTERIZATION

Large-scale (meta)genomic approaches have clearly been instrumental in PUL discovery, as well as predicting the metabolic potential of diverse *Bacteroidetes* bacteria. However, refined functional characterization at the molecular and cellular levels remains critical for a full understanding of the roles of PULs in microbial communities. In a few cases, genomic and transcriptomic studies have been coupled with biochemical analyses of individual CAZymes, e.g., β-xylanases (103–105), arabinofuranosidases (93), β-glucanases (106, 107), and alginate lyases (108). Recently, a series of high-impact studies have combined genetic, enzymological, biophysical, and structural techniques to comprehensively characterize the molecular functions of individual PULs.

The pioneering study deploying this approach described the differential utilization of the fructans levan [β(2,6) linked] and inulin [β(2,1) linked] by several human-gut symbiotic *Bacteroides* species, revealing that each harbors a set of linkage-specific enzymes that are instrumental in defining nutritional preferences (86). Notably, the heterologous expression of these enzymes in species that lacked homologous activities resulted in increased fitness of the recipient on the target polysaccharide, showcasing that differences in gene content between species can translate to increased fitness on inaccessible substrates. This work also represents the first structural and functional information obtained for the HTCS, highlighting the importance of the periplasmic sensor domain in the binding of small oligosaccharides. Subsequent insightful structural biology revealed that this binding event was accompanied by a unique "scissor blade" closing mechanism thought to aid in the transduction of the signal across the membrane to trigger the upregulation of associated PUL genes (88).

Starch and fructans, discussed above, are comparatively simple storage polysaccharides that are composed of a single monosaccharide repeating unit. One of the first examples of the comprehensive characterization of a PUL directed toward a more complex plant cell wall polysaccharide was that of the xyloglucan utilization locus (XyGUL) from the human gut symbiont *B. ovatus* (Fig. 1) (78). The saccharification pathway of this ubiquitous, highly branched dietary glycan was determined through the biochemical and structural characterization of all eight GHs and two SGBPs from the

XyGUL, in harness with reverse genetics (72, 78, 109). These studies were instrumental in highlighting the adaptive evolution of GH cohorts among syntenic XyGULs. Furthermore, these syntenic XyGULs served as diagnostic markers of xyloglucan metabolic capacity among individual *Bacteroides* species and across human gut metagenomes (78).

Subsequently, comprehensive functional studies of PULs targeting other complex polysaccharides have been reported. A pair of xylan-targeting PULs from *B. ovatus*, PUL-XylS and PUL-XylL, was found to encode enzymes tailored for individual plant β-xylans that varied in their composition and branching (79). Recently, the detailed genetic, biochemical, and enzyme structural characterization of a galactomannan-specific PUL from *B. ovatus* revealed the interplay of two mannan-specific SGBPs, two GH26 endo-β-mannanases, and a GH36 exo-α-galactosidase in the deconstruction of this plant cell wall polysaccharide (110, 111). Among environmental bacteria, a complex chitin utilization locus from *Flavobacterium johnsoniae* has been extensively functionally characterized (112). Notable features of the system include two pairs of SusC/SusD homologs, a secreted chitinase composed of two GH18 modules separated by a chitin-binding module, and an intracellular glucosamine-6-phosphate deaminase. Taken together, these studies highlight the considerable insight systems-based analysis can bring to PUL structure-function studies in the context of the ecology of a variety of ecosystems.

In addition to common plant cell wall polysaccharides, specialized PULs devoted to the utilization of rare polysaccharides act to enhance the catabolic repertoire of selected gut species. The dynamic effects of the human diet on the adaptive evolution of the distal gut microbiota were recently highlighted in the seminal "sushi factor" study, which documented the presence of β-porphyranases (i.e., GH16 and GH86), algal polysaccharide-specific CAZymes, in the microbiota of Japanese populations (113). Notably, these CAZymes were found as part of PULs thought to be acquired by the gut bacterium *Bacteroides plebeius* via lateral gene transfer from porphyranolytic *Z. galactanivorans* associated with uncooked edible algae (i.e., nori) (114). Further evidence suggests that *B. plebeius* and other human gut *Bacteroides* spp. may have also acquired algal polysaccharide utilization genes from marine bacteria (108, 114).

The ability of gut bacteria to adapt to structurally complex dietary polysaccharides was highlighted by the discovery and detailed characterization of three PULs from *B. thetaiotaomicron* involved in the utilization of α-mannans from the yeast cell wall (80). Yeast residues in the intestine originate from either endogenous yeasts or the consumption of leavened foods and fermented beverages, products of technologies that have existed for only a few thousand years (57). Detailed biochemical and reverse genetic analyses of these *B. thetaiotaomicron* α-mannan and other PULs have been instrumental in enhancing our understanding of the roles of PUL acquisition in the evolving landscape of the gut. In this context, it is especially notable that one of the three *B. thetaiotaomicron* mannan PULs is located on a mobile element that is structurally similar and homologous to that harboring porphyran utilization genes in *B. plebeius* (53).

GIVE AND TAKE: THE ROLES OF GLYCAN UTILIZATION IN GUT ECOLOGY

The coordinated carbohydrate utilization systems of PULs represent an impressive evolutionary solution for capturing valuable carbon sources in competitive environments, which avoid the limitations of extracellular systems such as cellulosomes or freely diffusing enzymes employed by fungi and other bacteria. However, emerging research suggests that, in some cases, these apparently selfish PUL systems may be "leaky," with particular benefit to the community; partial breakdown products (PBPs) released by the action of certain PULs can be shared with neighboring bacteria and support the dynamic response of microbial communities (115, 116).

This distributive mechanism has been observed in xylan utilization by *B. ovatus*, where simple oligosaccharides produced at the cell surface diffuse into the extracellular environment and are utilized by *Bifidobacterium adolescentis*, a species lacking the

enzymatic machinery to catalyze this initial depolymerization step (Fig. 2A) (79). Interestingly, this form of syntrophy was observed only during the utilization of simple, linear xylans. These synergistic interactions therefore appear to be glycan and species specific and may reflect hierarchies in the selective metabolism of substrates. In this regard, the preferential degradation of some glycans over others is likely to play a central role in shaping the complex microbial relationships of the microbiota (117, 118).

The PUL-mediated liberation of PBPs contributes to the complex metabolic web of cross-feeding interactions that has been mapped between several *Bacteroidales* type strains (115), although in the context of the entire gut microbiota, these relationships are likely to be much more complex. For example, in some species, CAZymes are selectively packaged into outer membrane vesicles and released into the extracellular environment, where they are thought to mediate the production of free glycan fragments for use by the greater gut community (115, 119). Remarkably, certain species, such as *B. ovatus*, secrete enzymes that are not required for the utilization of glycans such as inulin by the bacterium itself or by its clonemates; rather, this effort appears to benefit other species in the gut community (116). This seemingly altruistic act results in significant fitness benefits for *B. ovatus* that are realized only in the context of a complex microbiota.

In contrast to the extensive and complex dynamic relationships that exist between cohorts of bacteria in the gut, certain species such as *B. thetaiotaomicron* exhibit relatively little collaboration during the digestion of complex glycans. This form of selfish metabolism is deployed by *B. thetaiotaomicron* during the utilization of yeast α-mannans, in which manno-oligosaccharides generated at the cell surface are rapidly imported into the periplasm for further breakdown, conferring no direct benefits on neighboring species (Fig. 2B) (80). To facilitate this process, surface mannanases appear to operate at a lower rate than homologous mannanases within the periplasm, ensuring that mass action effects do not impede transport.

As a result of the acquisition of rare carbohydrate-specific PULs and diverse glycan utilization strategies, glycan "generalists," or microorganisms capable of metabolizing a range of glycans, within the gut are endowed with multiple foraging strategies to ensure their survival in this highly competitive ecosystem.

EXTENDING THE PUL PARADIGM

As discussed above, the concept of the PUL was originally defined in the context of *Bacteroidetes* systems containing the hallmark tandem *susC-susD* homologs encoding TBDT-SGBP pairs (48, 49, 81). TBDTs are not specific to *Bacteroidetes* bacteria but are broadly distributed across Gram-negative bacteria, including alpha- and gammaproteobacteria living in association with biomass debris. Inspection of the genomes of such organisms reveals that TBDT- and CAZyme-encoding genes may be colocalized—analogous to canonical *Bacteroidetes* PULs—although *susD* homologs and sensor/regulator systems are notably absent (108, 120–122). Despite their limitations, these TBDT/CAZyme-encoding clusters thus arguably comprise a type of "polysaccharide utilization locus." Indeed, the coordinated action of such loci in the utilization of complex carbohydrates, including xylans and N-glycans, was first demonstrated in the plant pathogen *Xanthomonas campestris* pv. campestris (123–125). Arlat and coworkers thus advanced the term CUT (carbohydrate utilization locus-containing TBDT) to describe such systems (123).

Recently, an "abbreviated" XyGUL from the soil-dwelling, saprophytic gammaproteobacterium *Cellvibrio japonicus* (Fig. 1) has been the subject of reverse genetic, biochemical, and structural studies that highlight the concerted action of a TBDT and three periplasmic, side chain-specific exoglycosidases (126). Vis-à-vis the "complete" XyGUL of *B. ovatus* (Fig. 1) (78), the *C. japonicus* XyGUL lacks genes encoding a keystone extracellular endoxyloglucanase, which is provided elsewhere in the genome (127, 128). The lack of genes encoding a SusD (SGBP-A) homolog and a sensor/regulator system in the *C. japonicus* XyGUL, both of which are ubiquitous in *Bacteroidetes* PULs, mirrors observations in bacteria from other phyla (120). Furthermore, the TBDTs from *C.*

japonicus and *B. ovatus* XyGULs have distinct amino acid sequences, despite being functionally homologous (50, 126).

Although necessarily distinct in structure from their Gram-negative counterparts, Gram-positive *Firmicutes* bacteria also deploy elaborate cell surface-associated systems for the utilization of soluble and insoluble polysaccharides, especially cellulose and resistant starch (40, 129–133). Recently, inducible, substrate-specific gene clusters targeting a variety of plant- and host-based glycans were identified in the genomes of the human gut symbionts *Eubacterium rectale* and *Roseburia* species (134). These "Gram-positive PULs" (gpPULs) contain a minimum of one CAZyme, a carbohydrate transport system (most commonly ATP-binding cassette transporters), and a LacI- or AraC-like transcriptional regulator (134).

In the broader perspective, the identification of colocalized genes encoding CAZymes and transporters presents a valuable tool for bioinformatic analyses of complex carbohydrate utilization in bacteria. Continued comprehensive molecular characterization of all flavors of PUL systems will be crucial for understanding the evolution of nutrient acquisition across phyla and ecosystems.

CONCLUSION AND FUTURE PERSPECTIVES

Recent studies have clearly demonstrated the importance of combining genetic, biochemical, and structural tools to fully dissect the function of individual PULs and reveal their specific roles in mediating the dynamics of carbohydrate utilization in diverse environments. In turn, well-characterized PULs serve as genetic markers, enabling the prediction of complex carbohydrate metabolism with greater reliability. A picture that has emerged is that individual *Bacteroidetes* bacteria in complex environments, such as the human gut, contain partially overlapping sets of PULs, which indicate both the ability to respond dynamically to nutrient availability and niche specialization within a web of species. Further, comparison of syntenic PULs across species highlights the ongoing evolution of PUL specificity through stepwise changes in CAZyme cohorts (78, 79, 86).

In light of the limited number of biochemically characterized PUL CAZymes, SGBPs, TBDTs, and sensor-regulators, molecular structure-function studies are only in their infancy. The generally small number of characterized CAZymes versus available sequence data and the limits this places on functional prediction have been discussed above. With respect to substrate binding and import, the interplay among SGBPs, TBDTs, and endoglycanases remains to be fully elucidated at the molecular level (68).

Likewise, the mechanisms by which regulatory systems within *Bacteroidetes* PULs respond to carbohydrates and how signals are transduced into gene expression are not fully understood. In particular, a complete structural and functional portrait of these complex membrane-spanning systems, as well as a detailed understanding of the genetic signatures targeted by these proteins (135), will help fill a significant gap in our understanding of how PULs are regulated and may help usher in an era of designer communities and personalized intestinal medicine (136–138). Recently, a series of *cis*-encoded small RNAs were discovered in association with a subset of PULs in *Bacteroides fragilis* (139). These molecules are postulated to play a role in the suppression of host glycan-specific PUL systems in *Bacteroides* species, potentially adding a new layer of regulation to the strictly controlled hierarchical expression of these PULs. The role of monosaccharides in specific feedback inhibition of PUL expression is likewise an emerging area (90).

Functional genomic studies of PULs (broadly defined here to include all aspects of molecular characterization from transcriptomics though structural biochemistry) have provided critical insights into nutrient acquisition strategies and microbial ecological interactions. These insights will continue to deepen our understanding of microbial enzyme systems in human and animal nutrition and health, as well as their involvement in driving fundamental environmental processes such as global carbon cycling. Successively, these basic research initiatives have lasting impacts on a variety of industries,

serving to inform a wave of novel technologies with applications in industrial enzyme discovery (140, 141) and engineered microbial therapeutics (142–148).

Rapid advances toward next-generation solutions for animal agriculture that address losses in productivity, food safety, and/or food security that result from escalating restrictions on the use of antimicrobial growth promoters are paramount. In this regard, rigorous evaluation of prebiotic and probiotic outcomes (149) and establishment of realistic production benchmarks are mandatory before there will be further adoption by industry. Alternatively, the engineering of intestinal microorganisms, such as chimeric live vaccines (150, 151), CRISPR-based genome editing (152), and synthetic biology of secondary metabolism (153), holds vast potential and may ultimately transform how food is produced in the future.

Collectively, fundamental research on PUL function informs the development of a range of next-generation technologies aimed at the intentional manipulation of microbial communities, including bioengineered inducible synbiotic systems for metabolic selection and *in vivo* targeted delivery of therapeutics.

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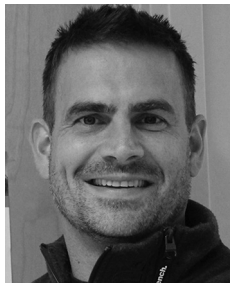

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