AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology and Molecular Biology Reviews®

REVIEW

RidA Proteins Protect against Metabolic Damage by Reactive Intermediates

Jessica L. Irons,[a] Kelsey Hodge-Hanson,[a*] Diana M. Downs[a]

[a]Department of Microbiology, University of Georgia, Athens, Georgia, USA

SUMMARY The Rid (YjgF/YER057c/UK114) protein superfamily was first defined by sequence homology with available protein sequences from bacteria, archaea, and eukaryotes (L. Parsons, N. Bonander, E. Eisenstein, M. Gilson, et al., Biochemistry 42:80–89, 2003, https://doi.org/10.1021/bi020541w). The archetypal subfamily, RidA (reactive intermediate deaminase A), is found in all domains of life, with the vast majority of free-living organisms carrying at least one RidA homolog. In over 2 decades, close to 100 reports have implicated Rid family members in cellular processes in prokaryotes, yeast, plants, and mammals. Functional roles have been proposed for Rid enzymes in amino acid biosynthesis, plant root development and nutrient acquisition, cellular respiration, and carcinogenesis. Despite the wealth of literature and over a dozen high-resolution structures of different RidA enzymes, their biochemical function

Citation Irons JL, Hodge-Hanson K, Downs DM. 2020. RidA proteins protect against metabolic damage by reactive intermediates. Microbiol Mol Biol Rev 84:e00024-20. https://doi.org/10.1128/MMBR.00024-20.

Address correspondence to Diana M. Downs, dmdowns@uga.edu.

* Present address: Kelsey Hodge-Hanson, AMICULUM USA, Chicago, Illinois, USA.

remained elusive for decades. The function of the RidA protein was elucidated in a bacterial model system despite (i) a minimal phenotype of *ridA* mutants, (ii) the enzyme catalyzing a reaction believed to occur spontaneously, and (iii) confusing literature on the pleiotropic effects of RidA homologs in prokaryotes and eukaryotes. Subsequent work provided the physiological framework to support the RidA paradigm in *Salmonella enterica* by linking the phenotypes of mutants lacking *ridA* to the accumulation of the reactive metabolite 2-aminoacrylate (2AA), which damaged metabolic enzymes. Conservation of enamine/imine deaminase activity of RidA enzymes from all domains raises the likelihood that, despite the diverse phenotypes, the consequences when RidA is absent are due to accumulated 2AA (or a similar reactive enamine) and the diversity of metabolic phenotypes can be attributed to differences in metabolic network architecture. The discovery of the RidA paradigm in *S. enterica* laid a foundation for assessing the role of Rid enzymes in diverse organisms and contributed fundamental lessons on metabolic network evolution and diversity in microbes. This review describes the studies that defined the conserved function of RidA, the paradigm of enamine stress in *S. enterica*, and emerging studies that explore how this paradigm differs in other organisms. We focus primarily on the RidA subfamily, while remarking on our current understanding of the other Rid subfamilies. Finally, we describe the current status of the field and pose questions that will drive future studies on this widely conserved protein family to provide fundamental new metabolic information.

KEYWORDS 2-aminoacrylate stress, RidA, YjgF/YER057c/UK114, enamine/imine metabolism

INTRODUCTION

For over 2 decades, RidA proteins from various organisms (including eukaryotes and prokaryotes) were assigned diverse functional roles based on experimental evidence, phylogenetic analyses, and genomic prediction. Despite differences in nomenclature spanning publications, only members of the RidA subfamily are found in all domains of life, and thus, proteins from eukaryotes discussed below are designated RidA herein. The earliest recognized reference to a RidA was a 1993 study of chromatin-associated proteins where RidA was isolated from rat liver based on its unusual solubility in perchloric acid (1). In this study, the protein was unnamed and simply classified as a small ~23-kDa acidic dimer with a pI of 4.67 under the conditions tested (*Salmonella enterica* RidA was similarly soluble in acid [J. L. Enos-Berlage and D. M. Downs, unpublished data]) (1). This early study referenced protein sequence alignments that were used to assign the protein (RidA) a heat shock or chaperone designation based on 18% to 27% identity to a motif in large heat shock proteins (82 to 90 kDa) from humans, mouse, fruit fly, and some single-celled organisms. This initial designation was not experimentally confirmed, yet it has since inundated the literature with predictions that other RidA proteins will demonstrate heat shock or chaperone activities, while physiological relevance remained undetermined (discussed below under Is Enamine Deaminase the Sole Activity of RidA Proteins?) (2–5).

Early Structural Analysis

Early structural analysis of RidA proteins hinted at potential substrate interactions and emphasized the structural conservation of the protein family. The first crystal structure of a Rid protein (RidA from *Escherichia coli*) was obtained in 1999 as part of a functional proteomic study (6). Presently, there are over 30 crystal structures of bacterial or eukaryotic Rid proteins in the Protein Data Bank (Table 1), all showing a homotrimeric complex with a chorismate mutase-like type (Fig. 1) (6–20). The structural conservation of RidA proteins was further emphasized in a study where the goat liver protein UK114 and the human monocyte protein p14.5 were purified and trimers were disassociated before mixing, which allowed both native and chimeric trimers to form, thus demonstrating the structural similarity between RidA

TABLE 1 Rid protein structures

Rid subfamily	Protein	Gene name or locus tag	Organism	PDB code	Reference
RidA	PSPTO-PSP	PSPTO_0072	*Pseudomonas syringae*	3K0T	17
	RidA/YjgF	*ridA/yjgF*	*Escherichia coli*	1QU9	6
	YabJ	*yabJ*	*Bacillus subtilis*	1QD9	8
	Hmf1	YER057c	*Saccharomyces cerevisiae*	1JD1	15
	L-PSP	Hrsp12	*Rattus norvegicus* (rat)	1QAH	To be published
	UK114	Hrsp12	*Capra hircus* (goat)	1NQ3	14
	Hp14.5	HRSP12	*Homo sapiens* (human)	1ONI	10
	TdcF	*tdcF*	*Escherichia coli*	2UYJ	13
	TdcF	*tdcF*	*Escherichia coli*	2UYP	13
	TdcF	*tdcF*	*Escherichia coli*	2UYK	13
	TdcF	*tdcF*	*Escherichia coli*	2UYN	13
	ST0811	STK_08110	*Sulfolobus tokodaii*	1X25	160
	HI0719	*HI_0719*	*Haemophilus influenzae*	1J7H	7
	RidA	RIDA	*Arabidopsis thaliana*	5HP7	20
	RidA	RIDA	*Arabidopsis thaliana*	5HP8	20
	SPy2060	Spy_2060	*Streptococcus pyogenes*	2EWC	To be published
	Cth_2968	Cthe_2798	*Clostridium thermocellum*	1XRG	To be published
	PA3499	PA3499	*Pseudomonas aeruginosa*	2IG8	To be published
	TTHA0137	TTHA0137	*Thermus thermophilus*	2CVL	To be published
	TTHA0137	TTHA0137	*Thermus thermophilus*	2CW4	To be published
	APE1501	APE1501	*Aeropyrum pernix*	2CWJ	To be published
	Mmf1	Mmf1	*Saccharomyces cerevisiae*	3QUW	18
	TM0215	TM0215	*Thermotoga maritima*	2B33	To be published
	PH0854	PH0854	*Pyrococcus horikoshii*	2DYY	To be published
	PAMC 21119	PAMC 21119	*Psychrobacter* sp.	6L8P	161
Rid2	YoaB	*yoaB*	*Salmonella enterica* serovar Typhimurium	3GTZ	To be published
	NMB1025	NMB1025	*Neisseria meningitidis*	3KJJ	To be published
	NMB1025	NMB1025	*Neisseria meningitidis*	3KJK	To be published
Rid6	Rv2704	MT2777.1	*Mycobacterium tuberculosis*	3I7T	16
Rid7	YjgH	*yjgH*	*Escherichia coli*	1PF5	To be published
RutC	RutC	*rutC*	*Escherichia coli*	3V4D	19
Aminomuconate deamininase	AmnE	*amnE*	*Pseudomonas* sp. AP-3	6IZH	162

proteins from different organisms and cell types. The numerous structural results became more valuable when the conserved biochemical activity of RidA was determined in the context of the metabolic system of *S. enterica* and confirmed in a purified system (21).

Functions Previously Ascribed to RidA

Before the definitive characterization of a biochemical activity for RidA enzymes from all domains of life in 2012 (22), publications involving studies of various organisms or cell types suggested a number of functions/activities for RidA (and other Rid family members). The functions reported for Rid proteins included RNase and/or general translation inhibitor (2, 3, 23, 24), aromatic deaminase (25–30), calpain activator (31), participant in pyrimidine utilization in *Escherichia coli* (32, 33), regulator of purine synthesis (34), fatty acid binding (35), and heat shock and/or chaperone protein (36). Additionally, RidA proteins were localized to plant chloroplasts, liver, kidney, intestine, and lung tissues of rat, and human monocytes (discussed below under Is Enamine Deaminase the Sole Activity of RidA Proteins?) (1–4, 37–40). To date, only the conserved deaminase activity (discussed below under 2AA Stress Response) has been rigorously demonstrated to be physiologically relevant. Thus, several key questions remain: do any or all of the functions reported for RidA rely on the conserved enamine/imine deaminase activity of the enzymes? Are there other activities that are physiologically relevant? Does the localization of RidA enzymes to specific organs, cell types, or organelles indicate novel physiological roles? And, beyond RidA, what functions do members of the Rid1 to -7 (Rid1–7)/RutC subfamilies perform in prokaryotes?

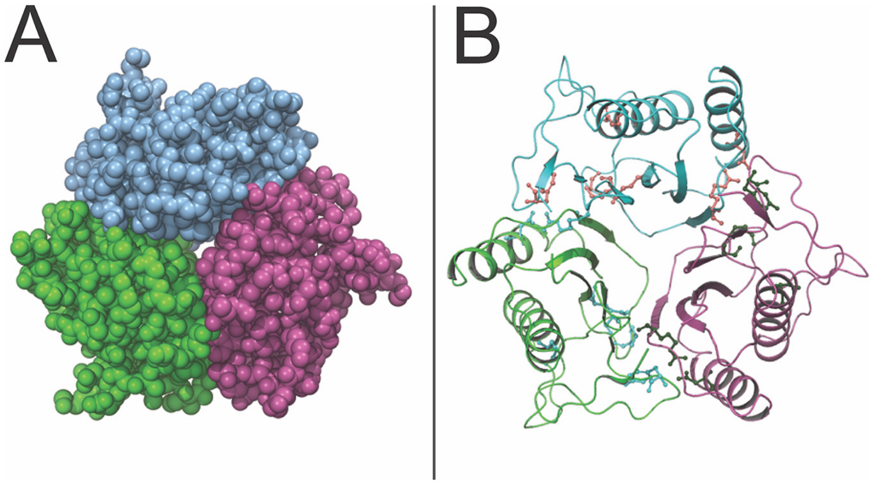

FIG 1 RidA structure. The secondary structure of RidA, generated by PyMOL and based on the solved crystal structure 1QU9 (Table 1) is shown. Each of the three colors (blue, green, and pink) represents one of the RidA monomers in the trimeric structure. Shown are the space-filling model of the RidA trimer (A) and the ribbon structure with each of the seven invariant residues highlighted on each monomer by ball-and-stick structures of different colors (B).

FUNCTIONAL INSIGHTS GENERATED SERENDIPITOUSLY

Early studies of the RidA family noted the striking sequence conservation among proteins from diverse species (*S. enterica* YjgF has 40% similarity to goat UK114) (41). The substantial conservation of RidA protein sequences throughout the domains of life suggested that the proteins likely share a biochemical activity. However, the diverse physiological contexts in which RidA enzymes had been described did not suggest a straightforward hypothesis for a conserved molecular mechanism. Rather, the critical insights into the conserved biochemical function and physiological role of RidA (formerly YjgF) were obtained by extensive and rigorous experimentation in *S. enterica* that was not initiated by a focused effort to define function. In a triumph of genetics, the conserved deaminase activity of RidA was ultimately uncovered through biochemical and genetic analyses of thiamine biosynthesis in *S. enterica*. Mutants of *S. enterica* lacking *purF* (phosphoribosylpyrophosphate amidotransferase, EC 2.4.2.14) and *gnd* (6-phosphogluconate dehydrogenase, EC 1.1.1.44) are unable to synthesize the pyrimidine moiety of thiamine (42). Null alleles of *ridA* were isolated as suppressor mutations that allowed the *purF gnd* strain to synthesize sufficient thiamine for growth (Fig. 2A) (41). These data ultimately led to the elucidation of RidA deaminase activity.

Prior knowledge of amino acid biosynthesis and the integration of the purine and thiamine biosynthetic pathways paved the way for understanding the role of RidA. In *S. enterica*, 5-phosphoribosylamine (PRA) is a precursor to purines and thiamine and is the product of the PurF-catalyzed reaction. Results from genetic analyses over several years found that PRA formation in the absence of RidA required an enzyme from branched-chain amino acid biosynthesis (IlvA, serine/threonine dehydratase, EC 4.3.1.19) and one involved in tryptophan biosynthesis (TrpD, anthranilate synthase, EC 4.1.3.27). In total, the data from these efforts led to a model in which the IlvA-generated enamine (aminocrotonate) was used by TrpD in the presence of phosphoribosylpyrophosphate (PRPP) to generate PRA (Fig. 2B). Significantly, these results were recapitulated *in vitro* (43, 44). The ability of RidA to prevent IlvA-dependent PRA formation *in vitro* and *in vivo* suggested that RidA could deaminate 2-aminocrotonate (2AC), making the metabolite unavailable for PRA synthesis. Subsequent experiments *in vitro* confirmed that RidA deaminated 2AC, defining the first physiological role of RidA (44).

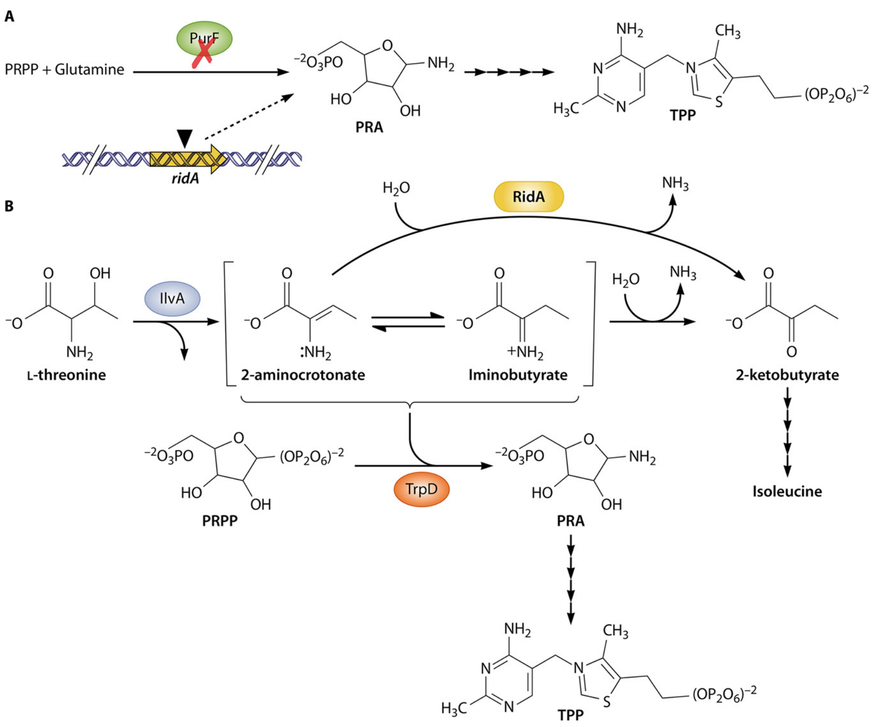

FIG 2 Lack of *ridA* uncovers a new pathway to synthesize phosphoribosyl amine (PRA). (A) PRA is the product of the PurF (phosphoribosylpyrophosphate amidotransferase, EC 2.4.2.14) enzyme (green) and an intermediate in the synthesis of the pyrimidine moiety of thiamine. Null alleles of *ridA* allowed *purF* mutants to grow in the absence of exogenous thiamine, indicating that a novel pathway to PRA had been activated (41). (B) The mechanism of PRA formation activated by the loss of RidA (yellow) required PRPP (phosphoribosylpyrophosphate), threonine, IlvA (blue, serine/threonine dehydratase, EC 4.3.1.19), TrpD (orange, anthranilate synthase, EC 4.1.3.27), and an uncharacterized intermediate in the formation of 2-ketobutyrate (41, 43, 44). If present, the RidA-catalyzed reaction removes 2-aminocrotonate and prevents its role in the formation of PRA.

2AA STRESS RESPONSE

A primary role for RidA in branched-chain amino acid or thiamine biosynthesis was not consistent with the conservation of the protein family through all domains of life, since many organisms lack these pathways but retain RidA. When an *S. enterica ridA* mutant was screened for a growth response to amino acids and/or vitamins central to metabolism, it was sensitive to exogenous serine. Furthermore, the specific activity of the pyridoxal 5′ phosphate-dependent isoleucine transaminase (IlvE, EC 2.6.1.42) isolated from a *ridA* mutant was ~40% of that in a wild-type strain (45). Both the sensitivity to serine and the reduced IlvE activity in the *ridA* mutant were abated by exogenous isoleucine, and genetic data showed that the role of isoleucine was as an allosteric inhibitor, not a nutrient suppressor (21, 41, 45). Importantly, the IlvA encoded by *S. enterica* (and several other organisms) is allosterically inhibited by isoleucine. Together, these data indicated that the deleterious effect of the *ridA* mutation was caused by a functional IlvA or, more likely, the product of IlvA. The promiscuous activity of IlvA with serine as a substrate, in combination with genetic and biochemical data, led to a working model for the role and impact of RidA in *S. enterica* (Fig. 3). The chemical mechanism of pyridoxal 5′ phosphate (PLP)-dependent dehydratases (like IlvA) involves the generation of an obligatory enamine intermediate. The serine-derived enamine 2-aminoacrylate (2AA), like other reactive enamines, is short-lived and known to form adducts with PLP in the active site of enzymes, thus inactivating the enzyme (46–57). The fact that serine dehydratases (SDHs) are broadly conserved suggested that the accumulation of 2AA was a physiologically relevant consequence of lacking RidA that could be extrapolated to other organisms.

A model implicating 2AA accumulation as the effect of a *ridA* mutation required that (i) 2AA was released from the active site of IlvA, (ii) 2AA could diffuse and react with a

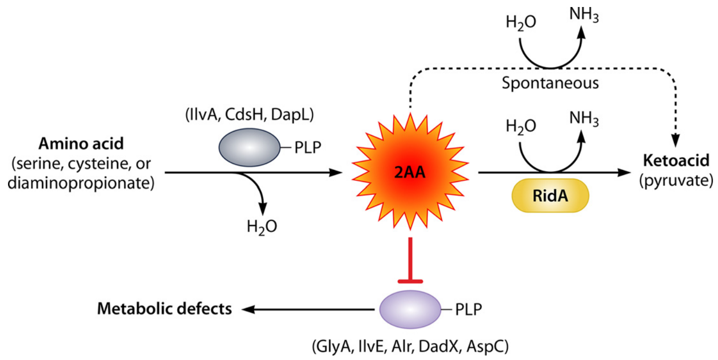

FIG 3 The RidA paradigm. The activities of some PLP-dependent enzymes (gray) generate 2-aminoacrylate (2AA, orange) as a catalytic intermediate. Some of this 2AA leaves that active site and can have one of three fates: (i) diffusion throughout the cell and inactivation of target PLP-dependent enzymes (blue), (ii) deamination by RidA (yellow), or (iii) nonenzymatic hydrolysis by solvent water. In the absence of RidA, the inactivation of PLP-dependent enzymes caused by accumulated 2AA can generate detectable metabolic phenotypes.

better nucleophile than water, and (iii) damage caused by the 2AA molecule resulted in the phenotype of the *ridA* mutant. The enamines generated by PLP-dependent dehydratases tautomerize to the respective imine before hydration in aqueous solution and formation of the respective ketoacid. Extensive mechanistic studies of PLP-dependent enzymes determined instability of imine/enamine intermediates, with the half-lives of the enamine/imine intermediates of threonine and serine estimated to be less than 3 min for threonine (58) and 1.5 s for serine (59). Due to the reactivity of enamines and the short half-lives of imine/enamine intermediates, final hydration *in vivo* was assumed to occur quickly, without requiring an enzyme. Both the phenotypic consequences of a *ridA* mutation and the *in vitro* activity of RidA showed that the latter assumption was incorrect (21, 22, 41, 45, 60).

RidA Proteins Are Enamine/Imine Deaminases

RidA enzymes increased the rate of hydrolysis of enamine/imine intermediates generated by IlvA *in vitro* (21). In this purified system at physiological pH, RidA increased the rate of 2-ketobutyrate formation from IlvA and threonine 2-fold, whereas at pH 9.5, the effect of RidA on the reaction rate was 4- to 5-fold (21). Importantly, at a pH of 9.5, the rate of pyruvate formation from serine increased 2.2-fold in the presence of RidA (21). These data supported the conclusion that RidA facilitated enamine or imine quenching and high pH contributed to the stability of the imine/enamine *in vitro* (21, 61, 62). It was posited that the effect of RidA could be more significant in the cellular environment. Subsequent data supported a model where 2AA could leave the IlvA active site and enter the active site of IlvE to cause damage, and these data explained the decreased IlvE activity observed in *S. enterica ridA* mutants (22). Significantly, these results could be recapitulated *in vitro*, where IlvE damage, as well as reduced specific activity, was prevented by the addition of purified RidA, validating the RidA paradigm *in vivo* and *in vitro* (22). Based primarily on *in vitro* activity assays, *Salmonella* YjgF was renamed RidA (reactive intermediate deaminase A) in 2012 (21). The Rid moniker was adopted for the protein superfamily and also describes the function shared by every RidA homolog tested *in vitro* to date, including *Pyrococcus furiosus* PF0668 (22), *Cucumis sativus* ChrD (22), *Homo sapiens* and *Capra hircus* (goat) UK114 (5, 22), and the RidA proteins of *Arabidopsis thaliana* (63), *Zea mays* (63), *Dermatophagoides farinae* (house dust mite) (64), *Bacillus subtilis* (22), *Escherichia coli* (65), *Pseudomonas aeruginosa* (66), *Campylobacter jejuni* (67), *Yersinia pestis* (68), and *Saccharomyces cerevisiae* (69) (Table 2).

RidA Protein Features

Early sequence alignments of RidA proteins, followed by multiple crystal structures, identified seven invariant residues presumed to comprise the active site (Fig. 1) (7). A

TABLE 2 Validated Rid deaminases

Organism	Protein	Subfamily	Reference(s)
Archaea			
Pyrococcus furiosus	PF0668	RidA	22
Bacteria			
Salmonella enterica	RidA	RidA	21
Salmonella enterica	YoaB	Rid2	107
Escherichia coli	RidA	RidA	65
Escherichia coli	TdcF	RidA	65
Escherichia coli	RutC	RutC	21
Bacillus subtilis	YabJ	RidA	21
Acinetobacter baylyi	ACIAD3089	Rid1	108
Campylobacter jejuni	Cj1388	RidA	67
Campylobacter jejuni	Cj0327	Rid2	67
Pseudomonas aeruginosa	PA5339	RidA	66
Pseudomonas aeruginosa	PA0814	Rid1	108
Pseudomonas aeruginosa	PA5083	Rid2	108
Pseudomonas syringae	PSPTO_0102	Rid2	108
Pseudomonas syringae	PSPTO_3006	Rid3	108
Pseudomonas fluorescens	PFL_1385	Rid3	108
Yersinia pestis	Y3551	RidA	68
Eukarya			
Homo sapiens	UK114	RidA	22
Cucumis sativus	ChrD	RidA	22
Arabidopsis thaliana	RidA	RidA	20, 63
Zea mays	RidA	RidA	63
Dermatophagoides farinae (house dust mite)	Derf34	RidA	64
Capra hircus (goat)	UK114	RidA	5
Saccharomyces cerevisiae	Mmf1	RidA	69
Saccharomyces cerevisiae	Hmf1	RidA	69

critical early report described structures obtained with keto acids or amino acids soaked into the active site and suggested key interactions. This work was published before elucidation of the conserved biochemical activity but was informed by a genetic link of RidA to branched-chain amino acid biosynthesis (41). This study found intermediates in isoleucine biosynthesis (2-ketobutyrate, 2-ketoisovalerate, tiglate, and methacrylate) and aromatic degradation (2-aminomuconate and 4-oxalocrotonate) bound to the RidA (7). The authors considered that the RidA could be involved in the conversion of an enamine/imine to a keto acid. However, since this reaction occurs spontaneously with free water, they concluded an enzyme should not be required (7). Structural studies found that the putative active site was nestled between subunits and functioned as a cleft where a water molecule was bound near a small-molecule substrate (7, 13). The water molecule was bound in the active site by an amino acid core rather than functional groups (7, 13). Although several residues that line the active site appeared to be conserved in multiple Rid sequences, a single arginine residue is necessary and sufficient to bind the substrate. Based on crystal structures, residue Arg105 (*S. enterica* numbering) forms hydrogen bonds with the substrate enamine/imine, and mutagenesis studies showed that this residue is essential for the 2AA deaminase activity of RidA (10, 13, 21). A later study using variant proteins indicated that conserved residues Cys107, Tyr17, and Glu120 were dispensable and supported the conclusion that Arg105 alone was critical to the conserved deaminase activity of RidA enzymes (21). The residue diversity in the active-site pocket of Rid subfamily members (Rid1–7 and RutC) could explain the broad substrate specificity (discussed below under Rid1–7 and RutC Subfamilies Provide a New Frontier for Metabolic Analyses).

Despite the many structural studies of Rid proteins, unanswered questions remain, including the substrate specificity (enamine or imine) of RidA, the role(s) of conserved residues in the active site, and the physiological functions and/or substrate specificities of other Rid subfamily members (Rid1–7 and RutC). Numerous residues are conserved in RidA homologs, yet only Arg105 has been found to be essential for biochemical activity (21). Perhaps addressing the effect of substitutions of other conserved residues will suggest a functional role that has yet to be determined. These approaches would also facilitate understanding the role of other Rid subfamily members (Rid1–7 and RutC) and could begin to shed light on the functions and substrates of these proteins.

INVOLVEMENT OF PLP-DEPENDENT ENZYMES

The RidA paradigm was described using *S. enterica*, which established the connection between RidA and 2AA generated by the isoleucine biosynthetic enzyme IlvA (Fig. 3) (41, 43–45, 60, 70). However, enamines/imines (like 2AA) are generated as a catalytic intermediate by various PLP-dependent enzymes (62, 71–79). In *S. enterica*, for instance, 2AA is generated and released by at least threonine/serine dehydratase (IlvA, EC 4.3.1.19), cysteine desulfhydrase (CdsH, EC 4.4.1.15), and diaminopropionate ammonia-lyase (DapL, EC 4.3.1.15) when serine, cysteine, or diaminopropionate, respectively, is used as a substrate (21, 80, 81, 87); all of these are PLP-dependent fold type II enzymes (83, 84). To date, the PLP-dependent enzymes known to be inactivated by 2AA are fold type I, III, or IV, including isoleucine transaminase (IlvE, EC 2.6.1.42) (22, 45, 85), alanine racemase (Alr/DadX, EC 5.1.1.1) (46), serine hydroxymethyltransferase (GlyA, EC 2.1.2.1) (86), and aspartate aminotransferase (AspC, EC 2.6.1.1) (65). The study of RidA in *S. enterica* and other organisms has led to a model where RidA functions as a 2AA stress modulator, preventing damage to PLP-dependent enzymes that leads to overall metabolic imbalance. In the cell, free 2AA meets one of three fates: (i) diffusion throughout the cell and reaction with various PLP-dependent enzymes, possibly inactivating them, (ii) tautomerization to the imine species followed by spontaneous deamination by water, or (iii) enzymatic deamination by RidA. As the study of RidA continues in *S. enterica* and other organisms, additional enzymes that either generate or are damaged by 2AA will likely be identified.

UNPREDICTED METABOLIC CONSEQUENCES

The circuitous path that led to the definition of a biochemical function and physiological role for RidA serves as an endorsement of using nontargeted, unbiased approaches to gain metabolic and functional insights that could not be generated *a priori*. Targeted approaches that rely on previous gene annotation, while sometimes successful, may occlude novel and/or unanticipated function, as illustrated by the case of RidA. Like many other proteins of unknown function, elimination of RidA failed to generate a dramatic phenotype in a wild-type strain of *S. enterica* unless the mutant was grown under specific nutrient conditions. Numerous genes of unknown function appear to have no significant function based on this single -pass criterium. However, in a novel genetic background and by chance, a *ridA* null allele resulted in a phenotype that allowed the locus to be characterized. Importantly, the 2AC deaminase activity and obscure role in thiamine synthesis could not explain the broad conservation of this enzyme family. Nonetheless, the obscure effect of a *ridA* mutation on thiamine synthesis informed testable models of the biochemical function, leading to the physiological role of RidA and the foundation of the RidA stress paradigm in *S. enterica* (and other organisms). This approach emphasizes the need for and the unpredictability of fundamental physiological genetic approaches where the experiments follow the outcomes, not *a priori* expectations, ultimately unraveling new connections.

The definition of the RidA deaminase activity contributed to a revised perspective on reactions thought to occur spontaneously with cellular water. Despite the short half-life of 2AA, the demonstration that 2AA was stable enough to be deleterious in the cell came from the initial growth defect of a *ridA* mutant. The elucidation of the RidA paradigm *in vivo*, and its reconstitution *in vitro*, showed for the first time that 2AA could leave the active site of the enzyme generator. Furthermore, 2AA could target the active site of other PLP-dependent enzymes where the expected damage, via covalent adducts, was produced. The kinetic parameters of RidA are difficult to measure due to the rapid turnover of 2AA in an aqueous environment, and this instability makes it impractical to measure 2AA accumulation *in vivo*. Instead, the accumulation of 2AA is

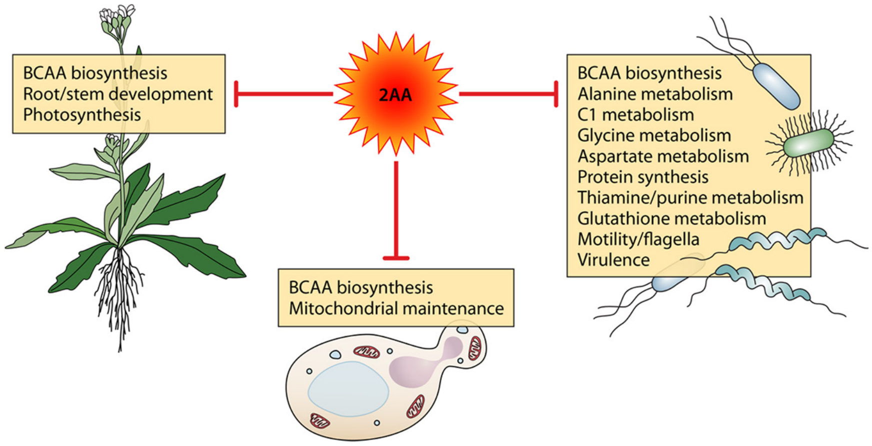

FIG 4 Lack of RidA has diverse consequences. Phenotypes have been reported for a variety of mutant organisms that lack RidA, including bacteria (*S. enterica* [41, 43, 45, 46, 60, 65, 70, 80, 86, 87, 154], *E. coli* [65], *P. aeruginosa* [66], and *C. jejuni* [67, 90]), plants (*A. thaliana* [107] and *Lycopersicon esculentum* [100]), and the yeast *S. cerevisiae* (69, 85, 91). The schematic assumes that 2-aminoacrylate (2AA), which accumulates in the absence of RidA, causes enzymatic damage that directly or indirectly perturbs the metabolic network affecting the relevant areas of metabolism.

detected by the scars it leaves, which include modification and decreased activity of target enzymes. The demonstration that a protein (RidA) catalyzes a reaction previously attributed to cellular water challenges us to think broadly about metabolic reactions and possible functions of uncharacterized proteins. Implications of the chemistry of the RidA paradigm for the metabolic network and the composition of the cellular milieu have been reviewed elsewhere (84).

PERTURBATION OF DIVERSE METABOLIC NETWORKS BY 2AA

Despite the conserved enamine/imine deaminase activity of RidA proteins, the consequences of lacking RidA defined so far are largely organism specific. In the absence of a RidA enzyme, phenotypes arise in bacteria, plants, and yeast; these include nutritional requirements, defects in mitochondrial maintenance, and perturbation of one-carbon (C1) metabolism (Fig. 4). Reports of the diverse impacts of removing RidA from a metabolic system beg the question, how does the lack of a conserved biochemical activity lead to diverse phenotypic outcomes? In mutant organisms that lack *ridA*, 2AA accumulates and damages PLP-dependent enzymes, which can affect multiple nodes of metabolism (65–67, 87). Thus, the lack of RidA generates numerous small perturbations that in combination result in broad phenotypic outcomes. The thresholds of different enzyme activities and metabolite pool sizes contribute to the breadth and severity of phenotypes associated with 2AA stress in different organisms.

Escherichia coli

The phenomenon discussed above is illustrated by considering the impact of 2AA accumulation in the closely related bacteria *S. enterica* and *E. coli*. Despite the conservation of all the components known to be relevant in the RidA paradigm, there are distinct phenotypic consequences of removing RidA in *E. coli* and *S. enterica* (65). In *E. coli*, neither a growth defect nor 2AA damage to IlvE was observed after the elimination of *ridA* alone or when the other encoded RidA, TdcF, was also absent. These data suggested a low-level accumulation of 2AA that could be explained by less IlvA activity, lower serine/threonine ratio, or other subtle metabolic differences between the two organisms. Importantly, only when 2AA was artificially increased in the *E. coli* mutant strain was a growth defect generated. Even then, unlike the *S. enterica ridA* mutant, where the growth defect was caused by inactivation of GlyA, the growth defect generated by 2AA in *E. coli* was not prevented by glycine. Instead, growth was restored by aspartate, indicating a distinct critical target of 2AA damage, potentially the PLP-dependent transaminase, AspC (65, 89). These results emphasized that new metabolic information could be obtained by exploring the RidA paradigm in different organisms.

Pseudomonas aeruginosa

P. aeruginosa encodes nine members of the Rid superfamily, two of which are annotated as RidA proteins (PA3123 and PA5339) (66). Of these loci, only a mutation in PA5339 (*ridA*) led to obvious metabolic defects. Analyses of these phenotypes highlighted both similarities and differences between the metabolic networks of *S. enterica* and *P. aeruginosa*. Similar to *S. enterica*, when *ridA* was absent in *P. aeruginosa*, there was a significant motility defect that was eliminated by exogenous isoleucine (66, 89). The growth defect of the *P. aeruginosa ridA* mutant with exogenous serine was more severe than those of *S. enterica* or *E. coli ridA* mutants (66). In contrast, the *P. aeruginosa ridA* mutant was only slightly sensitive to exogenous cysteine compared to the sensitivity of an *S. enterica ridA* mutant, and growth was not restored by glycine. *P. aeruginosa* encodes three known generators of 2AA, two serine/threonine dehydratases (IlvA) and one cysteine desulfhydrase (CdsH). The increased sensitivity of *P. aeruginosa ridA* mutants to serine suggested that 2AA accumulation proceeded largely through IlvA, since isoleucine eliminated all metabolic defects, presumably by preventing 2AA formation. The cause of the growth limitation in the *P. aeruginosa ridA* mutant was not identified. Despite encoding three glycine hydroxymethyltransferase (GlyA) homologs, alanine racemases (Alr and DadX), and a putative aspartate aminotransferase, no nutrient that would overcome the serine-induced growth defect was identified. The differences in the *S. enterica* and *P. aeruginosa ridA* mutant phenotypes further underscore the metabolic distinctions between organisms and highlight the potential for metabolic knowledge that can be gained from understanding the RidA system in *P. aeruginosa*. PA3123, an annotated RidA, is a less effective deaminase in an *S. enterica ridA* mutant and appears to be regulated in different cellular environments (see below under Regulation of Bacterial *ridA*s). These data suggest that *P. aeruginosa* may encode a housekeeping RidA (PA5339) and a secondary RidA (PA3123) or that PA3123 has an altogether different substrate *in vivo*. *P. aeruginosa* is an excellent model system to study the role of redundancy of RidA enzymes and the potential roles of other Rid subfamily members (Rid1–3 and RutC) (discussed below under Rid1–7 and RutC Subfamilies Provide a New Frontier for Metabolic Analyses).

Campylobacter jejuni

C. jejuni encodes two members of the Rid superfamily, a RidA and a Rid2 subfamily member (67). Similar to *ridA* mutants of *P. aeruginosa* and *S. enterica*, *C. jejuni ridA* (*ridA*/*cj1388*) mutants have phenotypes indicative of flagellar defects. In a complex medium, the *C. jejuni ridA* mutant has reduced motility and virulence, decreased autoagglutination, phage (NCTC 12673) sensitivity, and visible flagellar defects (67, 90). The *C. jejuni* phenotypes were striking in that they were the first phenotypes of a bacterial *ridA* mutant that were visible on complex medium. In *P. aeruginosa* and *S. enterica*, the motility defects of a *ridA* mutant occur on minimal medium and are eliminated when IlvA (and thus, 2AA generation) is inhibited by isoleucine. In contrast, in *C. jejuni*, genetic analysis showed that the biosynthetic serine/threonine dehydratase (IlvA) was not responsible for 2AA accumulation and motility defects. As such, *C. jejuni* provides a key system that should be probed to better understand the breadth of the cellular role(s) of RidA. In the *C. jejuni ridA* mutant, the 2AA generator and target enzymes, along with the role of the Rid2 (Cj0327), remain to be explored.

At present, the physiology of four bacterial species in the absence of *ridA* has been assessed. Each bacterium displayed unique properties of the RidA paradigm and presented questions that, when answered, will further our global understanding of metabolic architecture and 2AA stress. Each of the phenotypes that resulted in a nutritional requirement could be traced to 2AA damage of a metabolic enzyme, in agreement with the paradigm shown in Fig. 3. It was notable that three bacterial species lacking RidA had compromised motility, despite the lack of any flagellum/motility-specific 2AA enzyme target, except in *C. jejuni*. Pursuing the motility defects in a *ridA* mutant will either lead to a PLP-dependent enzyme that is indirectly involved and damaged by 2AA or a new class of 2AA targets. Either of these outcomes would contribute insights into the conserved connection of motility to RidA function.

RidA PREVENTS 2AA STRESS IN EUKARYOTIC ORGANISMS

While the RidA paradigm was defined in *S. enterica*, features of it are conserved in at least two eukaryotic organisms, *S. cerevisiae* and *A. thaliana* (63, 69, 91, 92). The function of RidA in *S. cerevisiae* highlights the essential role RidA proteins may play in eukaryotic systems. *S. cerevisiae* encodes two RidA enzymes, a cytoplasmic and a mitochondrion-targeted RidA. The loss of the mitochondrial RidA, Mmf1p (mitochondrial matrix factor 1p), leads to 2AA accumulation from serine dehydratases (Ilv1p or Cha1p, EC 4.3.1.19) in the mitochondria, causing metabolic stress and irreversible loss of mitochondrial DNA (69). *mmf1* mutants have reduced growth on dextrose, and the addition of isoleucine or threonine (isoleucine precursor) restores growth. In the absence of Mmf1p, respiration on glycerol was eliminated, due to the loss of the mitochondrial DNA and, ultimately, the mitochondria. The addition of an iron chelator to the growth medium of an *mmf1* mutant prevented loss of the mitochondria, suggesting that in the context of the yeast mitochondrion, RidA appeared to influence iron homeostasis (69). Further analysis of the mitochondrial system and exploration of a role for the cytoplasmic RidA will provide a glimpse into how this protein is involved in eukaryotic metabolism.

A. thaliana has a single RidA that is coexpressed with serine/threonine dehydratase (IlvA) and branched-chain aminotransferase-3 (BCAT3; IlvE in *S. enterica* nomenclature) during development (63). Coexpression data supported the hypothesis that RidA facilitated the deamination of an enamine generated by the dehydratase (IlvA), consistent with the RidA paradigm established in *S. enterica*. In defined growth medium without serine, the root growth of *A. thaliana ridA* mutants was significantly less than that of wild-type plants, and growth was further retarded by the addition of serine. The root growth defect in *A. thaliana* was partially restored by the addition of isoleucine. This result suggested that an isoleucine deficiency was caused by 2AA damage to BCAT3 or that exogenous isoleucine prevented the formation of 2AA and bypassed the need for RidA (45, 93, 94). The importance of RidA in *A. thaliana* root and plant development was further emphasized in a proteomic study in roots grown under exclusive ammonium or nitrate growth conditions where RidA (AT3G20390) was the most highly abundant protein under nitrate growth conditions. Although the authors of that study did not characterize the importance of RidA in plant roots, these studies underscore the significance of RidA enzymes in eukaryotic organisms and the potential for additional biochemical and genetic investigation (95).

IS ENAMINE DEAMINASE THE SOLE ACTIVITY OF RidA PROTEINS?

Over the past 2 decades, various functions in addition to enamine deaminase activity have been ascribed to RidA proteins. Significantly, every RidA homolog tested to date catalyzes 2AA deamination *in vivo* and *in vitro*, making this the only biochemical activity that unifies the proteins across domains. Therefore, the existence of numerous reports that fail to recognize the deaminase activity raises two key questions: (i) are the other observed effects and distinctive functions/activities attributable, directly or indirectly, to deaminase activity, or (ii) are there additional functions/activities inherent to RidA proteins, and if so, are these relevant *in vivo*?

Multiple Cellular Localizations

A number of the proposed roles for RidA homologs were suggested based on localization studies. Several studies highlighted localization of RidA (formerly perchloric acid-soluble protein [PSP]) to various eukaryotic tissues, including the lung (37), digestive tract (39), liver (2–4, 23, 40, 92, 96, 97), kidney (2, 3, 38, 98), brain (97), white blood cells (3, 24), and oocytes (99). Changes in the intracellular localization of RidA correlated with exposure to various compounds intended to elicit endoplasmic reticulum stress in rat kidney tissue (38). In plant cells, RidA was localized to the chloroplasts (similar to threonine dehydratase) (63, 100), and in yeast, RidA (Mmf1) was found in the mitochondria and the cytoplasm (Hmf1) (85, 91). In bacteria, RidA proteins lack secretory tags and are predicted to remain in the cytosol (101). The RidA homolog MCR_0348 appeared to be required for *Moraxella catarrhalis* attachment to human epithelial cells (102). The numerous reports of RidA localizing to specific cellular organelles suggest a role for RidA in these distinct cellular environments that is not yet clear. Perhaps the presence of RidA reflects the response to a metabolic state when 2AA is generated, the catabolism of serine, for instance. Homologs of the 2AA target, serine hydroxymethyltransferase (GlyA, EC 2.1.2.1), are found in all domains of life, and flux through this enzyme is key to C1 metabolism, as it catalyzes the interconversion of serine and glycine. As was discussed elsewhere, RidA may have a role in cancer biogenesis (88) where serine, glycine, and one-carbon units play a role in cancer cell proliferation (103). Under these growth conditions, RidA could be needed to counteract 2AA stress caused by the increased metabolism of serine.

Protein-Protein Interactions

It has been reported that RidA interacts with other components in the cell. Although any such interactions would be informative, the ability of RidA homologs across domains to function with bacterial components argues against an interaction impacting deaminase activity. A high-throughput yeast two-hybrid screen found that *Helicobacter pylori* RidA (HP0944) interacted with the chromosomal replication initiator DnaA/HP1529, the 50S large ribosomal subunit HP1318, and other proteins unrelated to DNA/RNA under the conditions tested (104). Disappointingly, the physiological relevance of these interactions was not confirmed, nor, to our knowledge, were any follow-up studies presented. In the same vein, immunoprecipitation studies suggested that human RidA interacted with a Y-box protein (YB-1) to regulate glucocorticoid receptor (GR)-mediated mRNA degradation (24, 105). The authors concluded that a GR, YB-1, and RidA (UK114) complex degraded monocyte chemoattractant protein 1 (MCP-1) mRNA. A later study showed that the arginine residue critical for deaminase activity (Arg105) was required for this mRNA degradation (105). These authors failed to incorporate knowledge of the deaminase activity in their discussion, a missed opportunity to connect a new observation with the characterized biochemical activity of RidA enzymes. Recently, the same authors demonstrated that the human RidA linked an RNase and a 3′-untranslated region (3′-UTR) protein, and this interaction did not require the Arg105 residue of the RidA (106). These most recent data could explain the RNase activity observed for RidA enzymes in older publications if the endoribonuclease-to-RidA linkage was strong enough to withstand fractional purification procedures. Data that describe interactions of RidA enzymes with other proteins are exciting and have the potential to provide insights into distinct roles for RidA. To make use of this potential, the interactions must be physiologically relevant rather than unique to specific experimental conditions. Without such a demonstration, it is difficult to evaluate the significance of reports of protein-protein interactions.

Chaperone Activity

The homology of RidA proteins to calpain-activating proteins (31) and/or heat shock proteins (1, 4) led investigators to analyze these proteins for chaperone activity. The sequence alignments for RidA and heat shock proteins suggest that human (p14.5) and mouse (Hrp12) RidA proteins have ∼24% sequence identity to a portion of human heat shock proteins Hsp90AA1 and Hsp90AB1. Some reports have shown chaperone activity for RidA proteins, though there are caveats with each study. For instance, fractions from the purification of human RidA (UK114) catalyzed calpain activation (31), but the fractions contained additional cellular components and the contribution of RidA was not rigorously determined. Furthermore, although DUK114 appeared to act as a chaperone during *in vitro* citrate synthase assays and overexpression seemed to protect *Drosophila* embryonic cells from heat shock, no chaperone activity was detected with purified *Drosophila melanogaster* RidA (DUK114) (36). In contrast to these studies, *C. hircus* (goat) RidA, UK114, was recently shown to be heat resistant and to hydrolyze imino acids produced by L- or D-amino acid oxidases (LOX or DOX, respectively) using L-/D-leucine, L-/D-alanine, L-methionine, or L-glutamine (5). These authors considered the conserved biochemical activity and purported properties of RidA proteins from the literature and confirmed a longstanding hypothesis that at least some RidA proteins are heat stable and biochemical activity remains after exposure to heat. These data coincide with the substrates and activities reported for *S. enterica* RidA (107, 108).

RNase Activity

Eukaryotic RidA proteins were originally designated perchloric acid-soluble proteins (PSPs) with endoribonuclease activity, an annotation that still permeates the literature and data sets. Crude extracts and fractions containing RidA (formerly PSP) proteins from rat liver (2, 23) and human monocytes (3) inhibited translation in cell-free assays. Heat treatment or precipitation with RidA-specific antibodies eliminated the effect on translation, leading the authors to conclude that RidA was responsible for the translation inhibition. Furthermore, purified human RidA protein (called p14.5) (2.5 to 20 μM) inhibited protein synthesis from tobacco mosaic virus (TMV) mRNA (109) and RidA from rat liver inhibited TMV mRNA translation in a dose-dependent manner, with 50% inhibition at 8 nM RidA (2). Evidence for the inhibition of translation by rat liver RidA was extended using mRNA encoding *E. coli* dihydrofolate reductase and endogenous globin mRNA (23). Separately, the RidA from the parasite *Entamoeba histolytica* (EhL-PSP) was associated with RNA degradation (110). As discussed above, recent reports suggest that the human RidA served as a molecular linker between a 3′-UTR protein and an RNase (RNase P) in HeLa cells and HEK293T cells (106).

Antiviral, Anticancer, and Allergen Roles

In addition to the examples of nondeaminase biochemical activity attributed to RidA proteins discussed above, several studies describe RidA-related processes without suggesting a molecular mechanism for the involvement of the protein. If validated, these reports provide exciting new directions to pursue while considering the role and functional diversity of the highly conserved RidA subfamily of proteins. In one provocative report, RidA (called Rhp-PSP) from *Rhodopseudomonas palustris* was shown to have antiviral properties against the tobacco mosaic virus (TMV) (109). When the purified protein was sprayed on TMV-infected tobacco plant leaves, the virus was inactivated in a RidA concentration-dependent manner. A mechanism by which purified RidA would act against TMV was not proposed or investigated further. More recently, RidA (called Lp16-PSP) from the edible mushroom *Lentinula edodes* was suggested as a potential anticancer treatment when studies showed that HL-60 cells treated with RidA exhibited morphological changes and induction of apoptosis and suffered chromosomal DNA cleavage and loss of mitochondrial membrane potential (111). A RidA protein (Der f 34) present in house dust mite (*Dermatophagoides farinae*) feces was found to be a major panallergen associated with human dust mite and fungal allergies (64). The occurrence of human antibodies to an allergen protein (RidA) in dust mite feces implies that RidA is secreted extracellularly or is exposed with other cytosolic contents after cell lysis. One study (discussed above) confirmed the heat stability of RidA from *C. hircus* (goat UK114), and examination of the heat stability of RidA enzymes from various organisms is needed before this designation can be assigned to all RidA proteins (5). The potential heat and acid stability of RidA could contribute to the antigenic properties of the protein isolated from *D. farinae* feces, as this protein would likely survive cell lysis. The stability of RidA proteins may be relevant in understanding the myriad of observations that have been made about this enzyme subfamily.

Modification of RidA

The results described above were generally in eukaryotic organisms where the direct link to metabolic status and the connection to 2AA is not trivial to assess. In contrast, observations made in *ridA* mutants of *E. coli* could easily be assessed in the context of the established enamine deaminase activity. It would benefit the field to know if such observations are integrated with 2AA stress *in vivo*. *E. coli ridA* mutants were reported to be more sensitive to peroxynitrite or hypochlorite (HOCl) than the wild type (112, 113). It is not clear whether these phenotypes were independent of the permeating effects of 2AA or whether the growth defect caused by peroxynitrite or HOCl ultimately stemmed from an inactivated PLP-dependent enzyme. *In vitro*, HOCl-modified RidA ($\text{RidA}_{\text{HOCl}}$) inhibited the rate of 2-ketobutyrate formation from threonine catalyzed by IlvA (113). The authors suggest that purified $\text{RidA}_{\text{HOCl}}$ and IlvA interact directly, but there are other potential explanations if one considers the components of the RidA paradigm that have been rigorously defined. For instance, residues required for RidA trimer formation or the critical Arg105 residue required for RidA deaminase activity could be targeted by HOCl and account for the decreased deaminase activity *in vitro*. Without additional work, it is unclear whether these observations expand our understanding of RidA function or simply expose global consequences of accumulating 2AA. The connection between the *in vitro* and *in vivo* results is essential to confirm physiological relevance, rather than simply expose an association. With observations made in *E. coli* and other genetically tractable organisms, there is a straightforward path to determining whether the phenotype(s) observed fits under the clearly defined paradigm of 2AA stress or provides new information that must be woven in to generate a more complete picture of the role of RidA.

Several reports suggest that a RidA enzyme is modified *in vivo*, though to our knowledge, no study has been conclusive or recapitulated. Early studies indicated that RidA proteins from rat liver were neither phosphorylated nor acetylated (1). In human liver cells, the 5′ coding region of RidA (p14.5) DNA was methylated and that methylation was associated with aberrant cell proliferation, reminiscent of the mechanism of silencing tumor-suppressing genes (92). *In vitro*, the mouse liver RidA homolog was phosphorylated by phosphate kinase (4). The purified rat heart RidA, PSP1, bound fatty acids in a 1:1 ratio (35), and RidA from *E. coli* might be capable of binding fatty acids (114). There is a formal possibility that if modified, RidA could perform functions other than the defined 2AA deaminase activity in the cell. At this point, no RidA modification has been shown to be reproducible or physiologically relevant.

A variety of roles were attributed to RidA in a number of organisms prior to the establishment of a biochemical activity of the protein and continue to be described without reference to the activity. These results raise the question of whether RidA has additional biochemical activities or whether each of the reports can be explained by a connection, direct or indirect, to the deamination of an enamine or other molecule. Such analyses will be key to tempering the conclusions and determining whether extrapolation is appropriate when considering RidA enzyme activities in specific organisms.

REGULATION OF BACTERIAL *ridA* GENES

A number of studies report that the expression of *ridA* transcript and/or accumulation of RidA protein in bacteria correlates with different cellular processes or metabolites. Mining these data sets has generated some provocative questions about the relevant effector or condition that could be tied to 2AA stress. For instance, RidA expression was associated with biofilm formation in *E. coli* (115) and *S. enterica* serovar Enteritidis (116). In *E. coli* strain ZK126 with an intact beta-glucoside operon (Bgl^{+}), *ridA* was positively regulated by the leucine-responsive regulatory protein (Lrp) and leucine antagonized its effect (117). Other correlations have been drawn between Rid protein expression and growth at higher temperatures in *Clostridium difficile* (118), amino acid metabolism in *P. aeruginosa* (119) and *Streptococcus pyogenes* (120), or D-/L-alanine metabolism in *P. aeruginosa* (121). A Rid protein in *S. pyogenes* was upregulated in strains with a defective two-component regulatory system (122). Data from these studies offer a starting point for efforts to define broader implications of 2AA stress or other roles for Rid proteins under changing conditions of the cell.

Pseudomonas aeruginosa ridA

Numerous global transcriptome studies have been performed in pathogenic organisms, including *P. aeruginosa*. Expression changes of genes encoding the designated RidA proteins in *P. aeruginosa*, PA5339 and PA3123, were identified in several global studies focused on adaptive stages of *P. aeruginosa*. Neither the direct regulators nor the significance of the RidA protein under these conditions was pursued. We determined that PA5339 is the *bona fide* RidA required to ameliorate 2AA stress-induced phenotypes in *P. aeruginosa*. PA3123, although designated a RidA, has a protein sequence that is divergent from the sequence of *S. enterica* RidA and had lower 2AA deaminase activity than PA5339 or the *S. enterica* RidA (66). Nevertheless, *P. aeruginosa* RidA (PA5339) accumulated more in cells during early attachment (i.e., early biofilm formation) than in unattached bacterial cells (123). PA3123 was regulated during various growth conditions; it was downregulated during swarming (two studies), under alginate-inducing conditions, and in the transition from planktonic growth to biofilm formation (124–127) and upregulated under conditions that mimic anaerobic urinary tract infection (128). The changes in Rid protein expression in *P. aeruginosa* observed in several global data sets suggest a role for these enzymes in various environments, potentially those that influence bacterial invasion and virulence. Similarly, the reports of differences in the expression levels of designated RidA proteins PA5339 and PA3123 raise the question of the potential role of the presumed housekeeping RidA (PA5339) compared to that of the secondary RidA, PA3123. Correct annotation of Rid proteins would have provided a context to address their role in these processes with a focus on the environmental conditions that were used to generate the regulatory data sets.

Campylobacter jejuni ridA

Unlike in *S. enterica*, *P. aeruginosa*, and *E. coli*, *C. jejuni* appears to have a direct transcriptional regulator of *ridA* (*cj1388*), HeuR. In total, the data suggest an inverse correlation between the expression of the *ridA* and iron availability, though the direct environmental signal for HeuR regulation has not been determined (90, 129). *C. jejuni* RidA (strain-specific gene designation 11168/*cj1388* or 81176/*cj1390*) has been identified in numerous global -omics studies due to its changing level of expression (130–136). In total, data from the studies of regulation of the RidA in *C. jejuni* in response to changing cellular conditions suggest this that protein (RidA) could play a direct or indirect role in virulence, antibiotic resistance, acid adaptation, growth with bile salts, and hydrogen peroxide and oxygen stress in this organism. When the data are taken together, these observations support the idea that Rid proteins affect diverse metabolic processes in all domains of life.

Yersinia pestis ridA

Yersinia pestis, the causative agent of the plague, encodes two Rid family members, including a protein that has been designated RidA (Y3551). *Y. pestis* has adapted to survive in the flea gut, where it forms a biofilm blockage that facilitates transmission of the bacteria into the bite site of the mammalian host (137, 138). *Y. pestis ridA* and several amino acid utilization genes were among the 150 genes most highly expressed during *Y. pestis* flea infection (139). Significantly, *Y. pestis* strains lacking the highly expressed locus (named the *nhaC* locus) that contains both Rid proteins were still able to form the biofilm flea gut blockage, suggesting that the role of the proteins is not specifically required for virulence (140). Rather, the high expression of the Rid genes along with genes that are critical in metabolism suggests these enzymes may play an important role in the *Y. pestis* adaptation to different environmental niches and nutrient conditions (68).

ACCUMULATION OF RidA IN EUKARYOTES

The accumulation patterns of RidA have been determined in some eukaryotic model systems. While we expect the physiology of these systems to differ from that of prokaryotes, some patterns of RidA accumulation in mammals are consistent with the RidA paradigm established in *S. enterica*. It is important to note that RidA is the only subfamily present in eukaryotes (107); thus, attempts should at least be made to correlate any observed effect with enamine deaminase activity before assigning a new role to the proteins. RidA (P23) expression was enhanced in the liver (and kidney) of glucose-starved rats, and the *ridA* mRNA expression decreased after glucose was added to the diet (141). Under starvation conditions, gluconeogenesis occurs in the liver, where glucogenic amino acids are used to synthesize glucose. Serine, a precursor to 2AA from the serine/threonine dehydratase (IlvA in *S. enterica*), is one of the best precursors to gluconeogenesis in the rat liver. PLP-dependent serine dehydratase (SDH) (EC 4.2.1.13) activity increases during glucose starvation, and its expression decreases during glucose feeding, following a pattern similar to that of *ridA* expression (142–145). Additionally, RidA (PSP1 from rat heart tissue) mRNA expression was upregulated on a diet supplemented with fatty acids, which are also known to stimulate gluconeogenesis (146, 147). The data that demonstrate the abundance of RidA in the liver and the regulation of RidA in response to nutrient supplementation could suggest that RidA is required to detoxify 2AA produced by the catabolism of serine during normal mammalian liver function (1, 2, 4).

Rid1–7 AND RutC SUBFAMILIES PROVIDE A NEW FRONTIER FOR METABOLIC ANALYSES

The Rid protein superfamily contains nine subfamilies (RidA, Rid1 to -7 [Rid1–7], and RutC) (107). While RidA is found throughout life, the Rid1–7 and RutC subfamilies are found in prokaryotes, primarily bacteria, where there are often multiple superfamily members in a single genome. Our understanding of the Rid1–7 and RutC subfamilies is in the early stages, and at present, the substrates and physiological roles of these proteins remain largely unknown. The PubSEED database provides a platform to differentiate between Rid subfamily members, though many organisms are not represented in this database (http://pubseed.theseed.org/), and it is difficult to determine a Rid subfamily designation based on protein sequence alone. A definitive method to identify the subfamilies, perhaps based on active-site residues or residues that define substrate specificity, would be beneficial. All Rid proteins characterized to date are trimeric and retain at least some of the 7 residues used to define the family (Table 1). Our data showed that Rid proteins (Rid1–3) that retain the Arg105 residue have imine deaminase activity on some substrates different than those of RidA *in vitro* (107, 108). Members of subfamilies Rid4–7 lack an active-site arginine residue (Arg105 in *S. enterica* RidA) that is required for deamination, which suggests that these subfamilies have a distinct function/activity(s) that has yet to be identified.

The number and diversity of subfamily members in an organism vary throughout bacterial species, further complicating efforts to predict the role or requirement for each enzyme (Table 3). For example, the *S. enterica* genome encodes a RidA, in addition to a YoaB and STM1549, which are assigned to subfamily Rid2 and Rid7, respectively. The genome of *E. coli* encodes five putative Rid proteins, including two RidA subfamily members, *C. jejuni* encodes one RidA and another subfamily member, *P. aeruginosa* encodes nine superfamily members, including two RidA proteins, *Burkholderia cepacia* encodes 17 putative Rid proteins, and *Bradyrhizobium japonicum* encodes 16 putative Rid proteins (65, 67, 90, 148).

A critical question moving forward is how the subfamilies Rid1–7 and RutC differ from RidA in their biochemical mechanisms, substrate specificities, and physiological role. One possibility is that while RidA is a stress response protein, other members of the Rid family are embedded in a specific metabolic pathway, needed for efficient metabolic flux and fitness of the organism under certain conditions. In this scenario, Rid1–3 subfamily proteins would accelerate reactions that occur nonenzymatically. The relevant reactions are assumed to be slow, possibly rate limiting, in the cellular milieu

TABLE 3 Rid proteins in representative organisms[a]

	No. of Rid proteins encoded								
Representative organism	**Total**	**RidA**	**Rid1**	**Rid2**	**Rid3**	**Rid4**	**Rid5**	**Rid6**	**Rid7**
Archaea									
Pyrococcus furiosus	1	1							
Sulfolobus tokodaii	1	1							
Bacteria									
Actinobacteria									
Mycobacterium smegmatis	9	4	4			1			
Mycobacterium tuberculosis	2		1					1	
Streptomyces coelicolor	12	9	1					2	
Streptomyces griseus	8	5	1			1		1	
Bacteroidetes									
Bacteroides fragilis	2	1					1		
Deinococcus-Thermus									
Thermus thermophilus	1	1							
Firmicutes									
Clostridium difficile	3	2		1					
Bacillus subtilis	1	1							
Streptococcus pyogenes	2	2							
Alphaproteobacteria									
Bradyrhizobium japonicum	16	9	1	5				1	
Caulobacter crescentus[b]	3	1	1		1				
Rhodopseudomonas palustris	6	3	1					1	1
Betaproteobacteria									
Neisseria meningitidis	2	1		1					
Bordetella pertussis CS	9	6	1	1					1
Burkholderia cepacia	17	8	3	2			1	1	2
Deltaproteobacteria									
Campylobacter jejuni	2	1		1					
Myxococcus xanthus	4	4							
Helicobacter pylori	1	1							
Gammaproteobacteria									
Acinetobacter baylyi[b]	7	4	1	2					
Erwinia amylovora	2			1				1	
Escherichia coli[b]	4	2		1					1
Haemophilus influenzae	3	2		1					
Pseudomonas aeruginosa	9	2	1	3					1
Pseudomonas putida	10	7		2			1		
Pseudomonas syringae[b]	8	5		1	1				1
Salmonella enterica	3	1		1					1
Yersinia pestis	3	1		1					
Eukarya									
Saccharomyces cerevisiae	2	2							
Arabidopsis thaliana	2	2							
Zea mays	1	1							
Homo sapiens	1	1							

[a] Genomes and annotations accessed from the PubSEED database (https://pubseed.theseed.org/).

[b] Organism additionally encodes RutC, a member of the Rid superfamily that has not been assigned a subfamily.

(21, 149, 150). We suggest that Rid family members ensure efficient flux through a pathway(s), thus increasing organism fitness under a relevant environmental condition. This overarching model is supported by new and emerging data, in addition to data mined from the literature that are described below.

Substrate Specificity

FAD- or NAD-dependent enzymes have been useful in expanding the substrate list of Rid enzymes *in vitro* (Fig. 5B) (107, 108, 151). For instance, the FAD-dependent

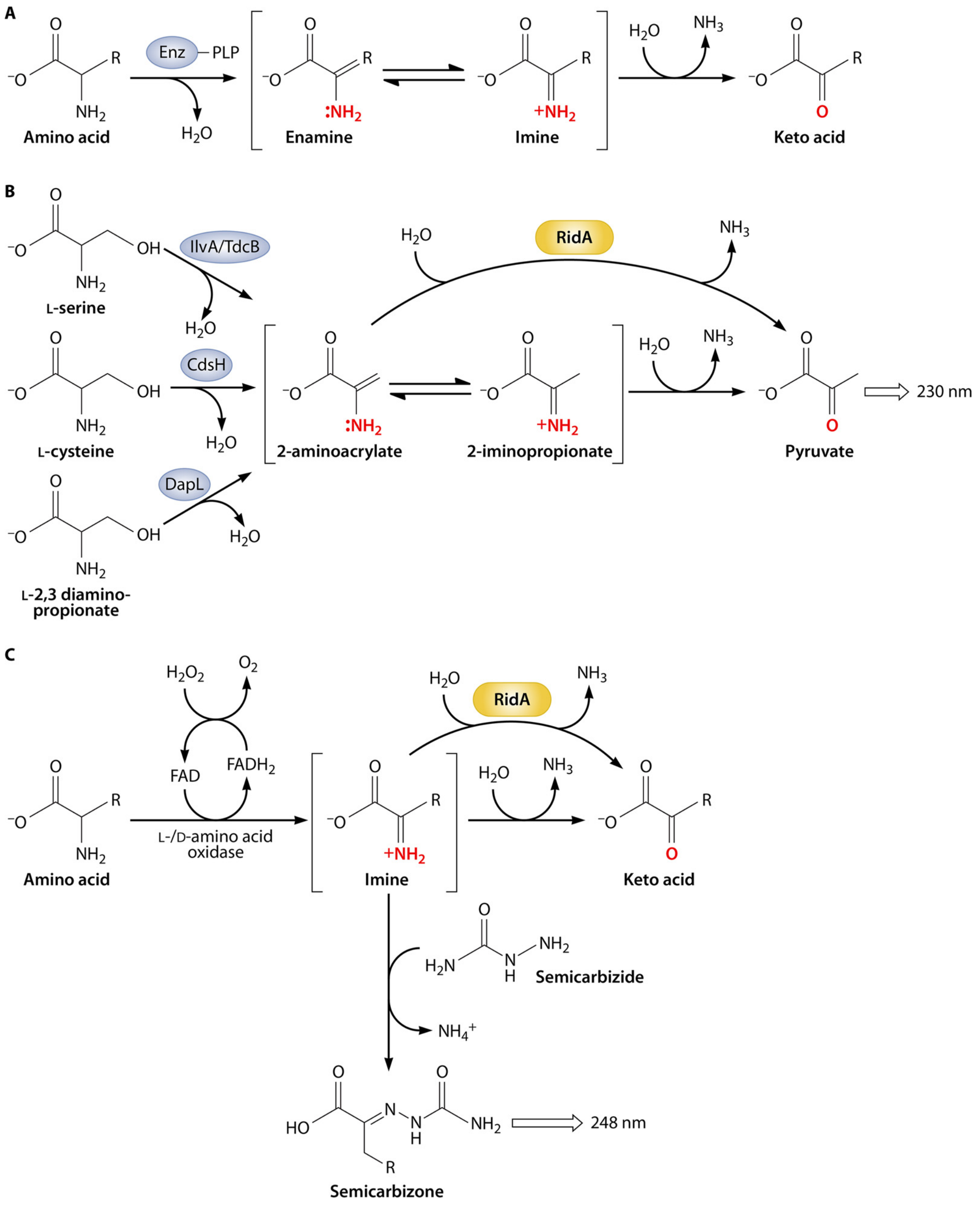

FIG 5 Multiple mechanisms of enamine and/or imine production. (A) The PLP-dependent generation of enamine/imine intermediates (shown in square brackets) from a generic amino acid is represented, where R is the side group of the amino acid. (B) The PLP-dependent generation of 2-aminoacrylate and iminopropionate from serine, cysteine, or 2,3-diaminopropionate is shown. These reactions are catalyzed by IlvA (serine/threonine dehydratase, EC 4.3.1.19), CdsH (cysteine desulfhydrase, EC 4.4.1.15), or DapL (diaminopropionate ammonia-lyase, EC 4.3.1.15), respectively. These mechanistic intermediates are deaminated by solvent water or a Rid protein to produce pyruvate. (C) The FAD-dependent imine production from an amino acid is shown as catalyzed by L/D amino acid oxidases. The resulting imines can be (i) deaminated by solvent water to produce a ketoacid, (ii) deaminated by a Rid protein, or (iii) derivatized by reaction with an added semicarbazide to produce a semicarbazone that is detected by absorbance at 248 nm. The functional group of generic amino acids is represented by R.

catalytic mechanism of D- or L-amino acid oxidases (DOX or LOX) generates an imine from various amino acids (Fig. 5C). The oxidase-dependent assays for imine deiminase have the advantage that they are relatively easy and can utilize commercially available enzymes to generate the imine *in situ* (107). In one study, in addition to RidA, six Rid enzymes with different subfamily designations were assayed for deaminase activity on eight imino acids generated *in situ* by LOX (108). The study

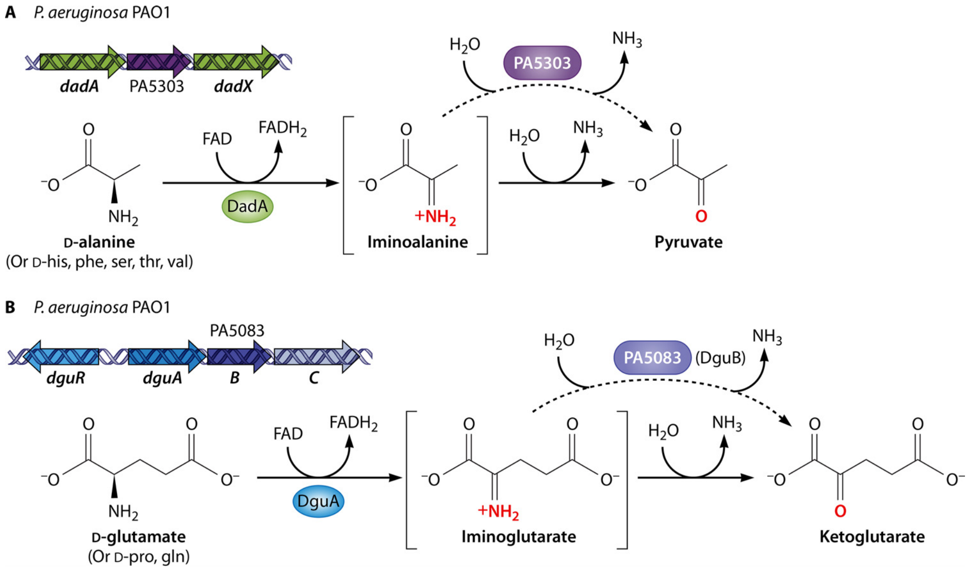

FIG 6 Gene synteny of Rid proteins in *P. aeruginosa*. The gene order of two operons in *P. aeruginosa* that include a *rid* homolog. Also shown is the enzyme which has the potential to generate an imine, to highlight the potential role for the Rid homolog. (A) *dadA* (DadX PLP dependent, alanine racemase, EC 5.1.1.1) and *dadX* (DadA FAD dependent, D-amino acid dehydrogenase, EC 1.4.5.1) are required for the utilization of L-alanine as a carbon source. DadA generates an imine intermediate that is the putative substrate of PA5303 (a Rid2) in a deamination reaction. (B) The *dguABC* locus is required for the catabolism of D-/L-glutamate. DguA generates an imine intermediate that is a potential substrate in a deamination reaction catalyzed by DguB (PA5083, a Rid2 protein).

provided the first indication that Rid proteins could be distinguished by substrate specificity. When two representatives each of Rid1, -2, and -3 proteins were compared to RidA, the trends of substrate specificities correlated with subfamily assignments and active-site residues were noted. Iminoarginine, iminophenylalanine, and iminohistidine were not significantly deaminated by RidA or Rid1 proteins but were efficiently deaminated by representatives of Rid2 and Rid3 subfamilies. In contrast, all Rid proteins tested, with the exception of RutC, have shown significant deaminase activity on 2AA (21, 108). Table 2 shows Rid1–3 deaminases that acted on 2AA generated by PLP-dependent dehydratases *in vitro*, as well as a broad range of imine intermediates generated by FAD- or NAD-dependent amino acid oxidases or NAD-dependent dehydratases *in vitro* (107, 108).

D-Amino Acid Metabolism

Gene clustering suggests that some Rid enzymes could function in pathways involved in D-amino acid utilization (119, 121, 152), antibiotic synthesis (153), and/or degradation of aromatic compounds (25–28, 30, 154). The model organism *P. aeruginosa* (strain PAO1) encodes nine Rid enzymes, some in loci that suggest potential substrates/activities (Fig. 6). For example, PA5303, a Rid2 subfamily member, is regulated by agmatine, putrescine, and D-/L-alanine and is encoded within the operon containing genes critical for alanine catabolism, *dadX* and *dadA* (119, 121). The PLP-dependent enzyme DadX (PLP dependent, alanine racemase, EC 5.1.1.1) and DadA (FAD-dependent, D-amino acid dehydrogenase, EC 1.4.5.1) are responsible for the conversion of L-alanine (and other L- or D-amino acids) to pyruvate and ammonia, and it is reasonable to suggest that PA5303 increases the rate of hydration of the imine intermediate from DadA if it is released from the enzyme active site (Fig. 6A) (119, 155, 156). Also in *P. aeruginosa* PAO1, PA5083 (Rid2), or DguB, is encoded in the *dguABC* operon, which is positively regulated by exogenous D-glutamate and D-glutamine (152). DguA is an FAD-dependent dehydrogenase that is required for growth on D-glutamate and likely goes through an imine intermediate in the formation of ketoglutarate (Fig. 6B). While the roles of PA5303 in D-/L-Ala and PA5083 in D-/L-Glu metabolism have not been explored, Rid2 proteins (PA0814 and PA5083) from PAO1 increased the rate of FAD-dependent reactions *in vitro* (107, 108). Each dehydrogenase in question (DadA or DguA) converts the nitrogen atom in the respective amino acid to an ammonium ion via oxidative deamination. Importantly, this reaction proceeds through an imine intermediate (Schiff base) that is converted to the free ammonium ion and a ketoacid. Thus, we expect an increased rate of ketoacid and ammonium ion formation *in vitro* when the respective Rid enzyme is added (5, 107, 108). Even if a role is demonstrated *in vitro*, a requirement for the Rid protein for pathway function *in vivo* may not be detectable. It is possible, and in fact likely, that lack of the Rid protein will generate a weak or undetectable phenotype under laboratory conditions. Cellular water is expected to catalyze the reaction at some rate, and the enamine/imine pathway intermediates are not likely toxic. Detection of a fitness defect when the relevant Rid protein is absent may require a high demand for the pathway that is not generated by laboratory conditions. Understanding the physiological importance of Rid1–3 subfamily proteins provides a challenging, yet exciting avenue of research for future investigators.

Aromatic Compound Degradation

The literature points to a role for Rid enzymes in the degradation of aromatic compounds in some bacterial species, based on their presence in operons with other components of the specific metabolic pathways. For example, upon closer examination, proteins from environmentally isolated bacterial species that participate in the breakdown of nitrobenzene, 2-nitrobenzoic acid, aminophenol, or tryptophan through a common reaction, the deamination of 2-aminomuconate to 4-oxalocrotonate, can be categorized as Rid proteins in *Pseudomonas* sp. AP3, *Pseudomonas pseudoalcaligenes* strain JS45, *Pseudomonas putida* strain HS12 (maintaining plasmid pNB1), *Pseudomonas fluorescens* strain KU-7, and *Burkholderia cepacia* strain J2315 (Fig. 7A) (25, 26, 28, 29). Similarly, a Rid homolog from *Bordetella* sp. strain 10d had 2-amino-5-carboxymuconic 6-semialdehyde deaminase activity, which aided in the metabolism of 4-amino-3-hydroxybenzoate (Fig. 7B) (30, 157). The unique 2-aminomuconate or 2-amino-5-carboxymuconic 6-semialdehyde deaminase activity of the Rid enzymes from these environmental isolates was hypothesized to promote cell proliferation in specific environmental niches and at a lower energetic cost than for other analogous pathways found in other bacteria (26). Readdressing these proteins and their activities in the light of current understanding of the Rid family and its general role in deamination is likely to generate new insights into these critical degradation pathways.

Uracil Degradation

In *E. coli*, a Rid family enzyme, RutC, is in the seven-gene *rut* operon. The Rut (py<u>r</u>imidine <u>ut</u>ilization) pathway is required to use uracil as a sole nitrogen source under specific growth conditions (32, 33). RutC was predicted to use peroxyaminoacrylate to produce 3-aminoacrylate (33), but it was notable that the proposed pathway included an aminoacrylate intermediate that was predicted to be spontaneously hydrated. Comparison of the catabolic pathway to the reaction that RidA catalyzes suggested that RutC would deaminate 3-aminoacrylate to malonic semialdehyde and ammonia (Fig. 8B). Recent studies have supported this hypothesis with the demonstration that when RutB generates 3-aminoacrylate *in situ*, RutC increases the rate of malonic semialdehyde formation (B. A. Buckner and D. M. Downs, unpublished data). Interestingly, in our hands, *rutC* mutants retain the ability to use uridine as a sole nitrogen source under the laboratory conditions tested. This result is not unexpected, since the reaction can be mediated by water. The assumption is that some condition(s) will generate the expected decrease in fitness of a *rutC* mutant when uridine is the nitrogen source.

In total, the reports described above highlight the possible diversity of substrates that Rid subfamily members act on and suggest physiologically relevant roles for these

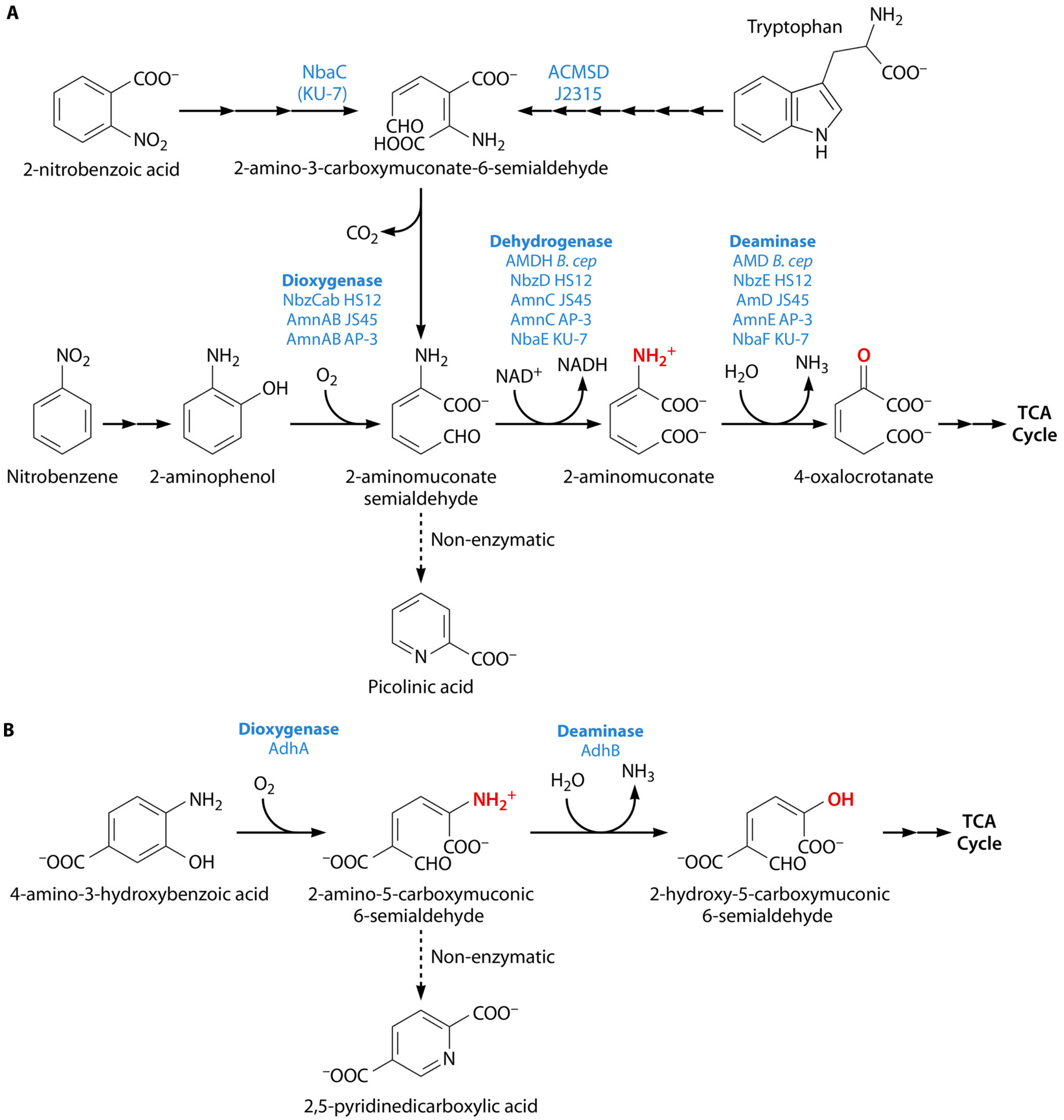

FIG 7 Rid subfamily members involved in degradation of aromatic compounds. (A) Pathways for the degradation of 2-nitrobenzoic acid, nitrobenzene, or tryptophan via a 2-aminomuconate semialdehyde intermediate have been identified in multiple organisms. Enzyme name(s) followed by organism designation are indicated at each step. The deamination of 2-aminomuconate to 4-oxalocrotonate is the step attributed to Rid proteins. Relevant organisms are *Pseudomonas* sp. AP-3, *Pseudomonas pseudoalcaligenes* strain JS45, *Pseudomonas putida* strain HS12, *Pseudomonas fluorescens* strain KU-7, and *Burkholderia cepacia* strain J23152. (B) The degradation of 4-amino-3-hydroxybenzoic by *Bordetella* sp. strain 10d (25, 26, 28–30, 157). This pathway involves 2-amino-5-carboxymuconic 6-semialdehyde, which is deaminated by AdhB, a Rid homolog. The sources of picolinic acid (A) and 2,5-pyridinecarboxylic acid (B) are indicated, with the dashed line representing the nonenzymatic formation of each.

enzymes. The Rut catabolic pathway provides a rigorously vetted example of the overall scheme proposed for the Rid protein superfamily members beyond the RidA subfamily. The less complete scenarios discussed above provide exciting opportunities to extend the understanding of the Rid family members beyond RidA.

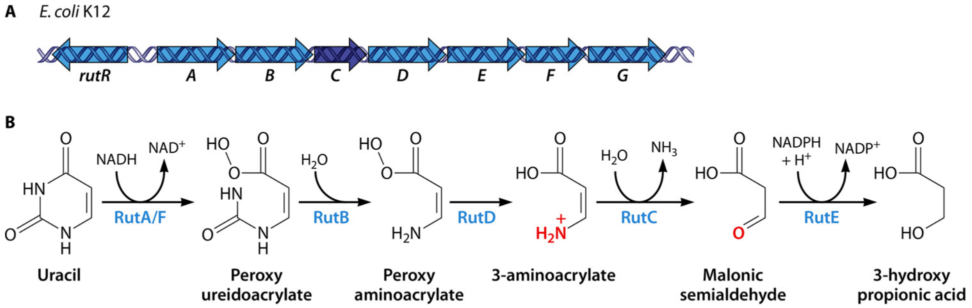

FIG 8 The Rut (pyrimidine utilization) pathway. The *rut* operon encodes enzymes involved in the Rut pathway for the utilization of uracil in *E. coli* (32, 33). The gene organization (A) and proposed pathway (B) are depicted. RutC is a Rid subfamily member and deaminates 3-aminoacrylate to malonic semialdehyde (Buckner and Downs, unpublished).

LESSONS LEARNED AND LINGERING QUESTIONS

A serendipitous mutation identified within the context of thiamine biosynthesis in an *S. enterica* model system led to the characterization of the 2-aminoacrylate stress paradigm. 2AA stress appears to be conserved across domains of life and is controlled by RidA proteins. The short half-life (~1.5 s) of 2AA partially explains why structure-function approaches made little progress in understanding the function of RidA prior to biochemical and genetic approaches. Similarly, the redundancy with a reaction catalyzed by water slowed understanding, since the reaction was presumed to be nonenzymatic *in vivo*. Though not initially considered, in hindsight, *in vivo* accumulation of reactive metabolites is a logical consequence of blocking a reaction. In total, the information presented herein highlights the different effects that metabolite accumulation can have. On one hand, the data are convincing that 2AA, which is normally managed by RidA, is a metabolite stressor and deleterious to the cell. On the other hand is the suggestion, based on emerging data and bioinformatic analyses, that metabolites that accumulate in the absence of other Rid members are simply pathway intermediates, not necessarily detrimental. It is the latter set of proteins that provide exciting questions for future studies. Exciting new questions in metabolism have been raised by studies of Rid proteins, and similarly, the history of the field provides some cautions. While they are critical components of efforts to define a protein function, depending too heavily on sequence analysis and annotation can conceal biochemical and mechanistic information. The Rid family is an example of the caveats that can be created using solely this approach. The rate of functional annotation lags behind the number of genes sequenced, such that genes of unknown function are likely to be misannotated or to lack annotation (63, 158, 159). In fact, in numerous studies referenced throughout this review, the Rid proteins had mis- or outdated annotations, preventing the investigators from contributing information to the Rid field.

Phenotypes are a coarse readout of the overall function of the metabolic network of an organism. Eliminating RidA results in multiple simultaneous perturbations, and thus, the impact depends on the distinct metabolic architecture of the particular organism. The diversity of phenotypes attributed to a lack of *ridA* does not imply there is a difference in biochemical activities of the relevant RidA enzymes, as suggested in the literature for over 20 years. Conserved deaminase activity of RidA enzymes has been demonstrated for enzymes from all domains *in vitro* and from *S. enterica in vivo*. In conclusion, the Rid system, along with the well characterized function of RidA, serves as another example (among many) where a bacterial system has been exploited for its simplicity and malleability to generate knowledge of a fundamental principle in biology.

ACKNOWLEDGMENTS

We thank Michael Paxhia for generating Fig. 1. Figures 2 through 8 were enhanced by Patrick Lane (ScEYEnce Studios).

This work is supported by National Institutes of Health (grant number GM095837 to D.M.D.). The authors state that they have no conflicts of interest in presenting this work.

REFERENCES

1. Levy-Favatier F, Cuisset L, Nedelec B, Tichonicky L, Kruh J, Delpech M. 1993. Characterization, purification, and cDNA cloning of a rat perchloric-acid-soluble 23-kDa protein present only in liver and kidney. Eur J Biochem 212:665–673. https://doi.org/10.1111/j.1432-1033.1993.tb17704.x.
2. Oka T, Tsuji H, Noda C, Sakai K, Hong YM, Suzuki I, Munoz S, Natori Y. 1995. Isolation and characterization of a novel perchloric acid-soluble protein inhibiting cellfree protein synthesis. J Biol Chem 270:30060–30067. https://doi.org/10.1074/jbc.270.50.30060.
3. Schmiedeknecht G, Kerkhoff C, Orso E, Stohr J, Aslanidis C, Nagy GM, Knuechel R, Schmitz G. 1996. Isolation and characterization of a 14.5-kDa trichloroacetic-acid soluble translational inhibitor protein from human monocytes that is upregulated upon cellular differentiation. Eur J Biochem 242:339–351. https://doi.org/10.1111/j.1432-1033.1996.0339r.x.
4. Samuel SJ, Tzung SP, Cohen SA. 1997. Hrp12, a novel heat-responsive, tissue-specific, phosphorylated protein isolated from mouse liver. Hepatology 25:1213–1222. https://doi.org/10.1002/hep.510250525.
5. Degani G, Barbiroli A, Regazzoni L, Popolo L, Vanoni MA. 2018. Imine deaminase activity and conformational stability of UK114, the mammalian member of the Rid protein family active in amino acid metabolism. Int J Mol Sci 19:945. https://doi.org/10.3390/ijms19040945.
6. Volz K. 2008. A test case for structure-based functional assignment: the 1.2 A crystal structure of the *yjgF* gene product from *Escherichia coli*. Prot Sci 8:2428–2437. https://doi.org/10.1110/ps.8.11.2428.
7. Parsons L, Bonander N, Eisenstein E, Gilson M, Kairys V, Orban J. 2003. Solution structure and functional ligand screening of HI0719, a highly conserved protein from bacteria to humans in the YjgF/YER057c/UK114 family. Biochemistry 42:80–89. https://doi.org/10.1021/bi020541w.
8. Sinha S, Rappu P, Lange SC, Mantsala P, Zalkin H, Smith JL. 1999. Crystal structure of *Bacillus subtilis* YabJ, a purine regulatory protein and member of the highly conserved YjgF family. Proc Natl Acad Sci U S A 96:13074–13079. https://doi.org/10.1073/pnas.96.23.13074.
9. Mistiniene E, Luksa V, Sereikaite J, Naktinis V. 2003. Oligomeric assembly and ligan binding of the members of the protein family YER057C/YIL051c/YJGF. Bioconjug Chem 14:1243–1252. https://doi.org/10.1021/bc0341066.
10. Manjasetty BA, Delbrück H, Pham D-T, Mueller U, Fieber-Erdmann M, Scheich C, Sievert V, Büssow K, Niesen FH, Weihofen W, Loll B, Saenger W, Heinemann U, Neisen FH. 2004. Crystal structure of *Homo sapiens* protein hp14.5. Proteins 54:797–800. https://doi.org/10.1002/prot.10619.
11. Mistiniene E, Pozdniakovaite N, Popendikyte V, Naktinis V. 2005. Structure-based ligand binding sites of protein p14.5, a member of protein family YER057c/YIL051c/YjgF. Int J Biol Macromol 37:61–68. https://doi.org/10.1016/j.ijbiomac.2005.08.008.
12. Burman JD, Stevenson CEM, Hauton KA, Sawers G, Lawson DM. 2003. Crystallization and preliminary X-ray analysis of the *E. coli* hypothetical protein TdcF. Acta Crystallogr D Biol Crystallogr 59:1076–1078. https://doi.org/10.1107/s0907444903007017.
13. Burman JD, Stevenson CE, Sawers RG, Lawson DM. 2007. The crystal structure of Escherichia coli TdcF, a member of the highly conserved YjgF/YER057c/UK114 family. BMC Struct Biol 7:30. https://doi.org/10.1186/1472-6807-7-30.
14. Deriu D, Briand C, Mistiniene E, Naktinis V, Grutter MG. 2003. Structure and oligomeric state of the mammalian tumour-associated antigen UK114. Acta Crystallogr D Biol Crystallogr 59:1676–1678. https://doi.org/10.1107/s0907444903014306.
15. Deaconescu AM, Roll-Mecak A, Bonanno JB, Gerchman SE, Kycia H, Studier FW, Burley SK. 2002. X-ray structure of *Saccharomyces cerevisiae* homologous mitochondrial matrix factor 1 (Hmf1). Proteins 48:431–436. https://doi.org/10.1002/prot.10151.
16. Thakur KG, Praveena T, Gopal B. 2010. Mycobacterium tuberculosis Rv2704 is a member of the YjgF/YER057c/UK114 family. Proteins 78:773–778. https://doi.org/10.1002/prot.22623.
17. Zhang H-M, Gao Y, Li M, Chang W-R. 2010. Crystal structure of the PSPTO-PSP protein from *Pseudomonas syringae pv. tomato str. DC3000* in complex with D-glucose. Biochem Biophys Res Commun 397:82–86. https://doi.org/10.1016/j.bbrc.2010.05.071.
18. Pu YG, Jiang YL, Ye XD, Ma XX, Guo PC, Lian FM, Teng YB, Chen Y, Zhou CZ. 2011. Crystal structures and putative interface of *Saccharomyces cerevisiae* mitochondrial matrix proteins Mmf1 and Mam33. J Struct Biol 175:469–474. https://doi.org/10.1016/j.jsb.2011.05.005.
19. Knapik AA, Petkowski JJ, Otwinowski Z, Cymborowski MT, Cooper DR, Chruszcz M, Krajewska WM, Minor W. 2012. Structure of *Escherichia coli* RutC, a member of the YjgF family and putative aminoacrylate peracid reductase of the rut operon. Acta Crystallogr Sect F Struct Biol Cryst Commun 68:1294–1299. https://doi.org/10.1107/S1744309112041796.
20. Liu X, Zeng J, Chen X, Xie W. 2016. Crystal structures of RidA, an important enzyme for the prevention of toxic side products. Sci Rep 6:30494. https://doi.org/10.1038/srep30494.
21. Lambrecht JA, Flynn JM, Downs DM. 2012. Conserved YjgF protein family deaminates reactive enamine/imine intermediates of pyridoxal 5′-phosphate (PLP)-dependent enzyme reactions. J Biol Chem 287:3454–3461. https://doi.org/10.1074/jbc.M111.304477.
22. Lambrecht JA, Schmitz GE, Downs DM. 2013. RidA proteins prevent metabolic damage inflicted by PLP-dependent dehydratases in all domains of life. mBio 4:e00033-13. https://doi.org/10.1128/mBio.00033-13.
23. Morishita R, Kawagoshi A, Sawasaki T, Madin K, Ogasawara T, Oka T, Endo Y. 1999. Ribonuclease activity of rat liver perchloric acid-soluble protein, a potent inhibitor of protein synthesis. J Biol Chem 274:20688–20692. https://doi.org/10.1074/jbc.274.29.20688.
24. Dhawan L, Liu B, Pytlak A, Kulshrestha S, Blaxall BC, Taubman MB. 2012. Y-box binding protein 1 and RNase UK114 mediate monocyte chemoattractant protein 1 mRNA stability in vascular smooth muscle cells. Mol Cell Biol 32:3768–3775. https://doi.org/10.1128/MCB.00846-12.
25. He Z, Spain JC. 1998. A novel 2-aminomuconate deaminase in the nitrobenzene degradation pathway of *Pseudomonas pseudoalcaligenes* JS45. J Bacteriol 180:2502–2506. https://doi.org/10.1128/JB.180.9.2502-2506.1998.
26. Davis JK, He Z, Somerville CC, Spain JC. 1999. Genetic and biochemical comparison of 2-aminophenol 1,6-dioxygenase of *Pseudomonas pseudoalcaligenes* JS45 to metacleavage dioxygenases: divergent evolution of 2-aminophenol meta-cleavage pathway. Arch Microbiol 172:330–339. https://doi.org/10.1007/s002030050787.
27. Takenaka S, Murakami S, Kim YJ, Aoki K. 2000. Complete nucleotide sequence and functional analysis of the genes for 2-aminophenol metabolism from Pseudomonas sp. AP-3. Arch Microbiol 174:265–272. https://doi.org/10.1007/s002030000203.
28. Muraki T, Taki M, Hasegawa Y, Iwaki H, Lau PC. 2003. Prokaryotic homologs of the eukaryotic 3-hydroxyanthranilate 3,4-dioxygenase and 2-amino-3-carboxymuconate-6 semialdehyde decarboxylase in the 2-nitrobenzoate degradation pathway of *Pseudomonas fluorescens* strain KU-7. Appl Environ Microbiol 69:1564–1572. https://doi.org/10.1128/aem.69.3.1564-1572.2003.
29. Park HS, Kim HS. 2001. Genetic and structural organization of the aminophenol catabolic operon and its implication for evolutionary process. J Bacteriol 183:5074–5081. https://doi.org/10.1128/jb.183.17.5074-5081.2001.
30. Orii C, Takenaka S, Murakami S, Aoki K. 2004. A novel coupled enzyme assay reveals an enzyme responsible for the deamination of a chemically unstable intermediate in the metabolic pathway of 4-amino-3-hydroxybenzoic acid in Bordetella sp. strain 10d. Eur J Biochem 271:3248–3254. https://doi.org/10.1111/j.1432-1033.2004.04258.x.
31. Melloni E, Michetti M, Salamino F, Pontremoli S. 1998. Molecular and functional properties of a calpain activator protein specific for *mu*-isoforms. J Biol Chem 273:12827–12831. https://doi.org/10.1074/jbc.273.21.12827.
32. Loh KD, Gyaneshwar P, Markenscoff Papadimitriou E, Fong R, Kim KS, Parales R, Zhou Z, Inwood W, Kustu S. 2006. A previously undescribed pathway for pyrimidine catabolism. Proc Natl Acad Sci U S A 103:5114–5119. https://doi.org/10.1073/pnas.0600521103.
33. Kim KS, Pelton JG, Inwood WB, Andersen U, Kustu S, Wemmer DE. 2010. The Rut pathway for pyrimidine degradation: novel chemistry and

toxicity problems. J Bacteriol 192:4089–4102. https://doi.org/10.1128/JB.00201-10.

34. Rappu P, Shin BS, Zalkin H, Mantsala P. 1999. A role for a highly conserved protein of unknown function in regulation of *Bacillus subtilis purA* by the purine repressor. J Bacteriol 181:3810–3815. https://doi.org/10.1128/JB.181.12.3810-3815.1999.
35. Sasagawa T, Oka T, Tokumura A, Nishimoto Y, Muñoz S, Kuwahata M, Okita M, Tsuji H, Natori Y. 1999. Analysis of the fatty acid components in a perchloric acid-soluble protein. Biochim Biophys Acta 1437: 317–324. https://doi.org/10.1016/S1388-1981(99)00025-6.
36. Farkas A, Nardai G, Csermely P, Tompa P, Friedrich P. 2004. DUK114, the *Drosophila* orthologue of bovine brain calpain activator protein, is a molecular chaperone. Biochem J 383:165–170. https://doi.org/10.1042/BJ20040668.
37. Matsumoto M, Kanouchi H, Suzuki K, Kaneki K, Kawasaki Y, Oka T. 2003. Purification and characterization of perchloric acid soluble protein from rat lung. Comp Biochem Physiol B Biochem Mol Biol 135:255–262. https://doi.org/10.1016/s1096-4959(03)00073-3.
38. Kanouchi H, Matsumoto M, Taga M, Yamada K, Oka T, Toné S, Minatogawa Y. 2005. Nuclear transfer of perchloric acid-soluble protein by endoplasmic reticulum stressors. Protein Sci 14:2344–2349. https://doi.org/10.1110/ps.051481105.
39. Kanouchi H, Miyamoto M, Oka T, Matsumoto M, Okamoto T, Tone S, Minatogawa Y. 2006. Perchloric acid-soluble protein is expressed in enterocytes and goblet cells in the intestine and upregulated by dietary lipid. Biochim Biophys Acta 1760:1380–1385. https://doi.org/10.1016/j.bbagen.2006.03.023.
40. Antonenkov VD, Ohlmeier S, Sormunen RT, Hiltunen JK. 2007. UK114, a YjgF/Yer057p/UK114 family protein highly conserved from bacteria to mammals, is localized in rat liver peroxisomes. Biochem Biophys Res Commun 357:252–257. https://doi.org/10.1016/j.bbrc.2007.03.136.
41. Enos-Berlage JL, Langendorf MJ, Downs DM. 1998. Complex metabolic phenotypes caused by a mutation in *yjgF* encoding a member of the highly conserved YER057c/YjgF family of proteins. J Bacteriol 180: 6519–6528. https://doi.org/10.1128/JB.180.24.6519-6528.1998.
42. Enos-Berlage JL, Downs DM. 1996. Involvement of the oxidative pentose phosphate pathway in thiamine biosynthesis in *Salmonella typhimurium*. J Bacteriol 178:1476–1479. https://doi.org/10.1128/jb.178.5.1476-1479.1996.
43. Browne BA, Ramos AI, Downs DM. 2006. PurF-independent phosphoribosyl amine formation in *yjgF* mutants of *Salmonella enterica* utilizes the tryptophan biosynthetic enzyme complex anthranilate synthase-phosphoribosyltransferase. J Bacteriol 188:6786–6792. https://doi.org/10.1128/JB.00745-06.
44. Lambrecht JA, Browne BA, Downs DM. 2010. Members of the YjgF/YER057c/UK114 family of proteins inhibit phosphoribosylamine synthesis in vitro. J Biol Chem 285:34401–34407. https://doi.org/10.1074/jbc.M110.160515.
45. Schmitz G, Downs DM. 2004. Reduced transaminase B (IlvE) activity caused by the lack of yjgF is dependent on the status of threonine deaminase (IlvA) in *Salmonella enterica* serovar Typhimurium. J Bacteriol 186:803–810. https://doi.org/10.1128/jb.186.3.803-810.2004.
46. Flynn JM, Downs DM. 2013. In the absence of RidA, endogenous 2-aminoacrylate inactivates alanine racemases by modifying the pyridoxal 5′-phosphate cofactor. J Bacteriol 195:3603–3609. https://doi.org/10.1128/JB.00463-13.
47. Arfin SM, Koziell DA. 1971. Inhibition of growth of *Salmonella typhimurium* and of threonine deaminase and transaminase B by beta-chloroalanine. J Bacteriol 105:519–522. https://doi.org/10.1128/JB.105.2.519-522.1971.
48. Roise D, Soda K, Yagi T, Walsh CT. 1984. Inactivation of the *Pseudomonas striata* broad specificity amino acid racemase by D and L isomers of beta-substituted alanines: kinetics, stoichiometry, active site peptide, and mechanistic studies. Biochemistry 23:5195–5201. https://doi.org/10.1021/bi00317a017.
49. Relyea NM, Tate SS, Meister A. 1974. Affinity labeling of the active center of L-aspartate-beta-decarboxylase with beta-chloro-L-alanine. J Biol Chem 249:1519–1524.
50. Kishore GM. 1984. Mechanism-based inactivation of bacterial kynureninase by beta-substituted amino acids. J Biol Chem 259:10669–10674.
51. Likos JJ, Ueno H, Feldhaus RW, Metzler DE. 1982. A novel reaction of the coenzyme of glutamate decarboxylase with L-serine *O*-sulfate. Biochemistry 21:4377–4386. https://doi.org/10.1021/bi00261a029.
52. Esaki N, Walsh CT. 1986. Biosynthetic alanine racemase of *Salmonella typhimurium*: purification and characterization of the enzyme encoded by the alr gene. Biochemistry 25:3261–3267. https://doi.org/10.1021/bi00359a027.
53. Bisswanger H. 1981. Substrate specificity of the pyruvate dehydrogenase complex from *Escherichia coli*. J Biol Chem 256:815–822.
54. Silverman RB, Abeles RH. 1976. Inactivation of pyridoxal phosphate dependent enzymes by mono- and polyhaloalanines. Biochemistry 15:4718–4723. https://doi.org/10.1021/bi00666a028.
55. Rando RR. 1974. Irreversible inhibition of aspartate aminotransferase by 2-amino-3-butenoic acid. Biochemistry 13:3859–3863. https://doi.org/10.1021/bi00716a006.
56. Walsh C. 1982. Suicide substrates: mechanism-based enzyme inactivators. Tetrahedron 38:871–909. https://doi.org/10.1016/0040-4020(82)85068-0.
57. Badet B, Roise D, Walsh CT. 1984. Inactivation of the *dadB Salmonella typhimurium* alanine racemase by D and L isomers of beta-substituted alanines: kinetics, stoichiometry, active site peptide sequencing, and reaction mechanism. Biochemistry 23:5188–5194. https://doi.org/10.1021/bi00317a016.
58. Flavin M, Slaughter C. 1964. An intermediate trapped by maleimides in a pyridoxal phosphate potentiated enzymatic elimination reaction. Biochemistry 3:885–893. https://doi.org/10.1021/bi00895a004.
59. Hillebrand GG, Dye JL, Suelter CH. 1979. Formation of an intermediate and its rate of conversion to pyruvate during the tryptophanase-catalyzed degradation of *S*-onitrophenyl-L-cysteine. Biochemistry 18: 1751–1755. https://doi.org/10.1021/bi00576a018.
60. Christopherson MR, Schmitz GE, Downs DM. 2008. YjgF is required for isoleucine biosynthesis when *Salmonella enterica* is grown on pyruvate medium. J Bacteriol 190:3057–3062. https://doi.org/10.1128/JB.01700-07.
61. Feldberg RS, Datta P. 1971. L-Threonine deaminase of *Rhodospirillum rubrum*. Purification and characterization. Eur J Biochem 21:438–446. https://doi.org/10.1111/j.1432-1033.1971.tb01490.x.
62. Datta P, Bhadra R. 1978. Biodegradative threonine dehydratase. Reduction of ferricyanide by an intermediate of the enzyme-catalyzed reaction. Eur J Biochem 91:527–532. https://doi.org/10.1111/j.1432-1033.1978.tb12706.x.
63. Niehaus TD, Nguyen TN, Gidda SK, ElBadawi-Sidhu M, Lambrecht JA, McCarty DR, Downs DM, Cooper AJ, Fiehn O, Mullen RT, Hanson AD. 2014. Arabidopsis and maize RidA proteins preempt reactive enamine/imine damage to branched-chain amino acid biosynthesis in plastids. Plant Cell 26:3010–3022. https://doi.org/10.1105/tpc.114.126854.
64. ElRamlawy KG, Fujimura T, Baba K, Kim JW, Kawamoto C, Isobe T, Abe T, Hodge-Hanson K, Downs DM, Refaat IH, Beshr Al-Azhary D, Aki T, Asaoku Y, Hayashi T, Katsutani T, Tsuboi S, Ono K, Kawamoto S. 2016. Der f 34, a novel major house dust mite allergen belonging to a highly conserved Rid/YjgF/YER057c/UK114 family of imine deaminases. J Biol Chem 291:21607–21615. https://doi.org/10.1074/jbc.M116.728006.
65. Borchert AJ, Downs DM. 2017. The response to 2-aminoacrylate differs in *Escherichia coli* and *Salmonella enterica*, despite shared metabolic components. J Bacteriol 199:e00140-17. https://doi.org/10.1128/JB.00140-17.
66. Irons J, Hodge-Hanson KM, Downs DM. 2018. PA5339, a RidA homolog, is required for full growth in *Pseudomonas aeruginosa*. J Bacteriol 200:e00434-18. https://doi.org/10.1128/JB.00434-18.
67. Irons J, Sacher JC, Szymanski CM, Downs DM. 2019. Cj1388 is a RidA homolog and is required for flagella biosynthesis and/or function in *Campylobacter jejuni*. Front Microbiol 10:2058. https://doi.org/10.3389/fmicb.2019.02058.
68. Martínez-Chavarría LC, Sagawa J, Irons J, Hinz A, Lemon A, Graca T, Downs D, Vadyvaloo V. 2020. Putative horizontally acquired genes, highly transcribed during Y*ersinia pestis* flea infection, are induced by hyperosmotic stress and function in aromatic amino acid metabolism. J Bacteriol 202:e00733-19. https://doi.org/10.1128/JB.00733-19.
69. Ernst DC, Downs DM. 2018. Mmf1p couples amino acid metabolism to mitochondrial DNA maintenance in *Saccharomyces cerevisiae*. mBio 9:e00084-18. https://doi.org/10.1128/mBio.00084-18.
70. Christopherson MR, Lambrecht JA, Downs D, Downs DM. 2012. Suppressor analyses identify threonine as a modulator of *ridA* mutant phenotypes in *Salmonella enterica*. PLoS One 7:e43082. https://doi.org/10.1371/journal.pone.0043082.
71. Eliot AC, Kirsch JF. 2004. Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annu Rev Biochem 73: 383–415. https://doi.org/10.1146/annurev.biochem.73.011303.074021.

72. Schnackerz KD, Ehrlich JH, Giesemann W, Reed TA. 1979. Mechanism of action of D-serine dehydratase. Identification of a transient intermediate. Biochemistry 18:3557–3563. https://doi.org/10.1021/bi00583a019.
73. Phillips AT, Wood WA. 1965. The mechanism of action of 5′-adenylic acid-activated threonine dehydratase. J Biol Chem 240:4703–4709.
74. Chargaff E, Sprinson DB. 1943. Studies on the mechanism of deamination of serine and threonine in biological systems. J Biol Chem 151:273–280.
75. Kredich NM, Foote LJ, Keenan BS. 1973. Stoichiometry and kinetics of inducible cysteine desulfhydrase from Salmonella typhimurium. J Biol Chem 248:6187–6196.
76. Collins JM, Monty KJ. 1973. The cysteine desulfhydrase of *Salmonella typhimurium*. Kinetic and catalytic properties. J Biol Chem 248:5943–5949.
77. Phillips RS, Demidkina TV, Faleev NG. 2003. Structure and mechanism of tryptophan indole-lyase and tyrosine phenol-lyase. Biochim Biophys Acta 1647:167–172. https://doi.org/10.1016/s1570-9639(03)00089-x.
78. Demidkina TV, Anston AA, Faleev NG, Phillips RS, Zakomyrdina LN. 2009. Spatial structure and mechanism of tyrosine phenol-lyase and tryptophan indole-lyase. Mol Biol (Mosk) 43:295–308. (In Russian.)
79. Barends TR, Domratcheva T, Kulik V, Blumenstein L, Niks D, Dunn MF, Schlichting I. 2008. Structure and mechanistic implications of a tryptophan synthase quinonoid intermediate. Chembiochem 9:1024–1028. https://doi.org/10.1002/cbic.200700703.
80. Ernst DC, Lambrecht JA, Schomer RA, Downs DM. 2014. Endogenous synthesis of 2-aminoacrylate contributes to cysteine sensitivity in *Salmonella enterica*. J Bacteriol 196:3335–3342. https://doi.org/10.1128/JB.01960-14.
81. Ernst DC, Anderson ME, Downs DM. 2016. L-2,3-Diaminopropionate generates diverse metabolic stresses in *Salmonella enterica*. Mol Microbiol 101:210–223. https://doi.org/10.1111/mmi.13384.
82. Reference deleted.
83. Liang J, Han Q, Tan Y, Ding H, Li J. 2019. Current advances on structure-function relationships of pyridoxal 5′-phosphate-dependent enzymes. Front Mol Biosci 6:4. https://doi.org/10.3389/fmolb.2019.00004.
84. Borchert AJ, Ernst DC, Downs DM. 2019. Reactive enamines and imines in vivo: lessons from the RidA paradigm. Trends Biochem Sci 44:849–860. https://doi.org/10.1016/j.tibs.2019.04.011.
85. Kim JM, Yoshikawa H, Shirahige K. 2001. A member of the YER057c/yjgf/Uk114 family links isoleucine biosynthesis and intact mitochondria maintenance in *Saccharomyces cerevisiae*. Genes Cells 6:507–517. https://doi.org/10.1046/j.1365-2443.2001.00443.x.
86. Flynn JM, Christopherson MR, Downs DM. 2013. Decreased coenzyme A levels in *ridA* mutant strains of *Salmonella enterica* result from inactivated serine hydroxymethyltransferase. Mol Microbiol 89:751–759. https://doi.org/10.1111/mmi.12313.
87. Ernst DC, Downs DM. 2016. 2-Aminoacrylate stress induces a context-dependent glycine requirement in *ridA* strains of *Salmonella enterica*. J Bacteriol 198:536–543. https://doi.org/10.1128/JB.00804-15.
88. Downs DM, Ernst DC. 2015. From microbiology to cancer biology: the Rid protein family prevents cellular damage caused by endogenously generated reactive nitrogen species. Mol Microbiol 96:211–219. https://doi.org/10.1111/mmi.12945.
89. Borchert AJ, Downs DM. 2017. Endogenously generated 2-aminoacrylate inhibits motility in *Salmonella enterica*. Sci Rep 7:12971. https://doi.org/10.1038/s41598-017-13030-x.
90. Reuter M, Periago PM, Mulholland F, Brown HL, van Vliet AH. 2015. A PAS domain-containing regulator controls flagella-flagella interactions in *Campylobacter jejuni*. Front Microbiol 6:770. https://doi.org/10.3389/fmicb.2015.00770.
91. Oxelmark E, Marchini A, Malanchi I, Magherini F, Jaquet L, Hajibagheri MA, Blight KJ, Jauniaux JC, Tommasino M. 2000. Mmf1p, a novel yeast mitochondrial protein conserved throughout evolution and involved in maintenance of the mitochondrial genome. Mol Cell Biol 20:7784–7797. https://doi.org/10.1128/mcb.20.20.7784-7797.2000.
92. Pozdniakovaite N, Popendikyte V. 2005. DNA methylation differences in human p14.5 gene promoter region in normal and proliferating cells. Dev Growth Differ 47:493–499. https://doi.org/10.1111/j.1440-169X.2005.00824.x.
93. Halgand F, Wessel PM, Laprévote O, Dumas R. 2002. Biochemical and mass spectrometric evidence for quaternary structure modifications of plant threonine deaminase induced by isoleucine. Biochemistry 41:13767–13773. https://doi.org/10.1021/bi0262348.
94. Wessel PM, Graciet E, Douce R, Dumas R. 2000. Evidence for two distinct effector-binding sites in threonine deaminase by site-directed mutagenesis, kinetic, and binding experiments. Biochemistry 39:15136–15143. https://doi.org/10.1021/bi001625c.
95. Coleto I, Vega-Mas I, Glauser G, González-Moro MB, Marino D, Ariz I. 2019. New insights on Arabidopsis thaliana root adaption to ammonium nutrition by the use of a quantitative proteomic approach. Int J Mol Sci 20:814. https://doi.org/10.3390/ijms20040814.
96. Ceciliani F, Faotto L, Negri A, Colombo I, Berra B, Bartorelli A, Ronchi S. 1996. The primary structure of UK114 tumor antigen. FEBS Lett 393:147–150. https://doi.org/10.1016/0014-5793(96)00850-2.
97. Nordin H, Matsumoto M, Suzuki K, Kaneki K, Natori Y, Kishi K, Oka T. 2001. Purification, characterization and developmental expression of chick (Gallus domesticus) liver PSP protein. Comp Biochem Physiol B Biochem Mol Biol 128:135–143. https://doi.org/10.1016/s1096-4959(00)00308-0.
98. Asagi K, Oka T, Arao K, Suzuki I, Thakur MK, Izumi K, Natori Y. 1998. Purification, characterization and differentiation-dependent expression of a perchloric acid soluble protein from rat kidney. Nephron 79:80–90. https://doi.org/10.1159/000044996.
99. D'Inca R, Marteil G, Bazile F, Pascal A, Guitton N, Lavigne R, Richard-Parpaillon L, Kubiak JZ. 2010. Proteomic screen for potential regulators of M-phase entry and quality of meiotic resumption in Xenopus laevis oocytes. J Proteomics 73:1542–1550. https://doi.org/10.1016/j.jprot.2010.03.017.
100. Leitner-Dagan Y, Ovadis M, Zuker A, Shklarman E, Ohad I, Tzfira T, Vainstein A. 2006. CHRD, a plant member of the evolutionarily conserved YjgF family, influences photosynthesis and chromoplastogenesis. Planta 225:89–102. https://doi.org/10.1007/s00425-006-0332-y.
101. Lopez-Campistrous A, Semchuk P, Burke L, Palmer-Stone T, Brokx SJ, Broderick G, Bottorff D, Bolch S, Weiner JH, Ellison MJ. 2005. Localization, annotation, and comparison of the *Escherichia coli* K-12 proteome under two states of growth. Mol Cell Proteomics 4:1205–1209. https://doi.org/10.1074/mcp.D500006-MCP200.
102. de Vries SP, Eleveld MJ, Hermans PW, Bootsma HJ. 2013. Characterization of the molecular interplay between *Moraxella catarrhalis* and human respiratory tract epithelial cells. PLoS One 8:e72193. https://doi.org/10.1371/journal.pone.0072193.
103. Locasale JW. 2013. Serine, glycine and one-carbon units: cancer metabolism in full circle. Nat Rev Cancer 13:572–583. https://doi.org/10.1038/nrc3557.
104. Rain JC, Selig L, De Reuse H, Battaglia V, Reverdy C, Simon S, Lenzen G, Petel F, Wojcik J, Schächter V, Chemama Y, Labigne A, Legrain P. 2001. The protein-protein interaction map of *Helicobacter pylori*. Nature 409:211–215. https://doi.org/10.1038/35051615.
105. Park OH, Park J, Yu M, An H-T, Ko J, Kim YK. 2016. Identification and molecular characterization of cellular factors required for glucocorticoid receptor-mediated mRNA decay. Genes Dev 30:2093–2105. https://doi.org/10.1101/gad.286484.116.
106. Park OH, Ha H, Lee Y, Boo SH, Kwon DH, Song HK, Kim YK. 2019. Endoribonucleolytic cleavage of M^6 A-containing RNAs by RNase P/MRP complex. Mol Cell 74:494–507.e8. https://doi.org/10.1016/j.molcel.2019.02.034.
107. Niehaus TD, Gerdes S, Hodge-Hanson K, Zhukov A, Cooper AJ, ElBadawi-Sidhu M, Fiehn O, Downs DM, Hanson AD. 2015. Genomic and experimental evidence for multiple metabolic functions in the RidA/YjgF/YER057c/UK114 (Rid) protein family. BMC Genomics 16:382. https://doi.org/10.1186/s12864-015-1584-3.
108. Hodge-Hanson KM, Downs DM. 2017. Members of the Rid protein family have broad imine deaminase activity and can accelerate the *Pseudomonas aeruginosa* D-arginine dehydrogenase (DauA) reaction in vitro. PLoS One 12:e0185544. https://doi.org/10.1371/journal.pone.0185544.
109. Su P, Feng T, Zhou X, Zhang S, Zhang Y, Cheng J, Luo Y, Peng J, Zhang Z, Lu X, Zhang D, Liu Y. 2015. Isolation of Rhp-PSP, a member of YER057c/YjgF/UK114 protein family with antiviral properties, from the photosynthetic bacterium *Rhodopseudomonas palustris* strain JSC-3b. Sci Rep 5:16121. https://doi.org/10.1038/srep16121.
110. Pena-Soler E, Fernandez FJ, Lopez-Estepa M, Garces F, Richardson AJ, Quintana JF, Rudd KE, Coll M, Vega MC. 2014. Structural analysis and mutant growth properties reveal distinctive enzymatic and cellular roles for the three major L-alanine transaminases of *Escherichia coli*. PLoS One 9:e102139. https://doi.org/10.1371/journal.pone.0102139.
111. Joseph TP, Chanda W, Mohammad AF, Kanwal S. 2017. Lp16-PSP, a member of YjgF/YER057c/UK114 protein family induces apoptosis and p21$^{WAF1/CIP1}$ mediated G_1 cell cycle arrest in human acute promyelo-

cytic leukemia (APL) HL-60 cells. Int J Mol Sci 18:2407. https://doi.org/10.3390/ijms18112407.

112. Lindemann C, Lupilova N, Müller A, Warscheid B, Meyer HE, Kuhlmann K, Eisenacher M, Leichert LI. 2013. Redox proteomics uncovers peroxynitrite-sensitive proteins that help *Escherichia coli* to overcome nitrosative stress. J Biol Chem 288:19698–19714. https://doi.org/10.1074/jbc.M113.457556.
113. Muller A, Langklotz S, Lupilova N, Kuhlmann K, Bandow JE, Leichert LI. 2014. Activation of RidA chaperone function by N-chlorination. Nat Commun 5:5804. https://doi.org/10.1038/ncomms6804.
114. Rangan KJ, Yang YY, Charron G, Hang HC. 2010. Rapid visualization and large-scale profiling of bacterial lipoproteins with chemical reporters. J Am Chem Soc 132:10628–10629. https://doi.org/10.1021/ja101387b.
115. Beloin C, Valle J, Latour-Lambert P, Faure P, Kzreminski M, Balestrino D, Haagensen JA, Molin S, Prensier G, Arbeille B, Ghigo JM. 2004. Global impact of mature biofilm lifestyle on *Escherichia coli* K-12 gene expression. Mol Microbiol 51:659–674. https://doi.org/10.1046/j.1365-2958.2003.03865.x.
116. Mangalappalli-Illathu AK, Vidović S, Korber DR. 2008. Differential adaptive response and survival of *Salmonella enterica* serovar enteritidis planktonic and biofilm cells exposed to benzalkonium chloride. Antimicrob Agents Chemother 52:3669–3680. https://doi.org/10.1128/AAC.00073-08.
117. Shukla S, Mahadevan S. 2019. The *ridA* gene of *E. coli* is indirectly regulated by BglG through the transcriptional regulator Lrp in stationary phase. Microbiology 165:683–696. https://doi.org/10.1099/mic.0.000806.
118. Ternan NG, Jain S, Srivastava M, McMullan G. 2012. Comparative transcriptional analysis of clinically relevant heat stress response in *Clostridium difficile* strain 630. PLoS One 7:e42410. https://doi.org/10.1371/journal.pone.0042410.
119. Boulette ML, Baynham PJ, Jorth PA, Kukavica-Ibrulj I, Longoria A, Barrera K, Levesque RC, Whiteley M. 2009. Characterization of alanine catabolism in *Pseudomonas aeruginosa* and its importance for proliferation in vivo. J Bacteriol 191:6329–6334. https://doi.org/10.1128/JB.00817-09.
120. LaSarre B, Federle MJ. 2011. Regulation and consequence of serine catabolism in *Streptococcus pyogenes*. J Bacteriol 193:2002–2012. https://doi.org/10.1128/JB.01516-10.
121. Chou HT, Kwon D-H, Hegazy M, Lu C-D. 2008. Transcriptome analysis of agmatine and putrescine catabolism in *Pseudomonas aeruginosa* PAO1. J Bacteriol 190:1966–1975. https://doi.org/10.1128/JB.01804-07.
122. Liu M, Hanks TS, Zhang J, McClure MJ, Siemsen DW, Elser JL, Quinn MT, Lei B. 2006. Defects in ex vivo and in vivo growth and sensitivity to osmotic stress of group A Streptococcus caused by interruption of response regulator gene vicR. Microbiology 152:967–978. https://doi.org/10.1099/mic.0.28706-0.
123. Crouzet M, Claverol S, Lomenech AM, Le Sénéchal C, Costaglioli P, Barthe C, Garbay B, Bonneu M, Vilain S. 2017. *Pseudomonas aeruginosa* cells attached to a surface display a typical proteome early as 20 minutes of incubation. PLoS One 12:e0180341. https://doi.org/10.1371/journal.pone.0180341.
124. Overhage J, Bains M, Brazas MD, Hancock RE. 2008. Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. J Bacteriol 190:2671–2679. https://doi.org/10.1128/JB.01659-07.
125. Damron FH, Barbier M, McKenney ES, Schurr MJ, Goldberg JB. 2013. Genes required for and effects of alginate overproduction induced by growth of *Pseudomonas aeruginosa* on Pseudomonas isolation agar supplemented with ammonium metavanadate. J Bacteriol 195:4020–4036. https://doi.org/10.1128/JB.00534-13.
126. Manos J, Arthur J, Rose B, Tingpej P, Fung C, Curtis M, Webb JS, Hu H, Kjelleberg S, Gorrell MD, Bye P, Harbour C. 2008. Transcriptome analyses and biofilm-forming characteristics of a clonal *Pseudomonas aeruginosa* from the cystic fibrosis lung. J Med Microbiol 57:1454–1465. https://doi.org/10.1099/jmm.0.2008/005009-0.
127. Tremblay J, Déziel E. 2010. Gene expression in *Pseudomonas aeruginosa* swarming motility. BMC Genomics 11:587. https://doi.org/10.1186/1471-2164-11-587.
128. Tielen P, Rosin N, Meyer AK, Dohnt K, Haddad I, Jänsch L, Klein J, Narten M, Pommerenke C, Scheer M, Schobert M, Schomburg D, Thielen B, Jahn D. 2013. Regulatory and metabolic networks for the adaptation of *Pseudomonas aeruginosa* biofilms to urinary tract-like conditions. PLoS One 8:e71845. https://doi.org/10.1371/journal.pone.0071845.
129. Johnson JG, Gaddy JA, DiRita VJ. 2016. The PAS domain-containing protein HeuR regulates heme uptake in *Campylobacter jejuni*. mBio 7:e01691-16. https://doi.org/10.1128/mBio.01691-16.
130. Taveirne ME, Theriot CM, Livny J, DiRita VJ. 2013. The complete *Campylobacter jejuni* transcriptome during colonization of a natural host determined by RNAseq. PLoS One 8:e73586. https://doi.org/10.1371/journal.pone.0073586.
131. Guccione EJ, Kendall JJ, Hitchcock A, Garg N, White MA, Mulholland F, Poole RK, Kelly DJ. 2017. Transcriptome and proteome dynamics in chemostat culture reveal how *Campylobacter jejuni* modulates metabolism, stress responses and virulence factors upon changes in oxygen availability. Environ Microbiol 19:4326–4348. https://doi.org/10.1111/1462-2920.13930.
132. Reid AN, Pandey R, Palyada K, Whitworth L, Doukhanine E, Stintzi A. 2008. Identification of *Campylobacter jejuni* genes contributing to acid adaptation by transcriptional profiling and genome-wide mutagenesis. Appl Environ Microbiol 74:1598–1612. https://doi.org/10.1128/AEM.01508-07.
133. Clark CG, Chong PM, McCorrister SJ, Simon P, Walker M, Lee DM, Nguy K, Cheng K, Gilmour MW, Westmacott GR. 2014. The CJIE1 prophage of *Campylobacter jejuni* affects protein expression in growth media with and without bile salts. BMC Microbiol 14:70. https://doi.org/10.1186/1471-2180-14-70.
134. Hao H, Li F, Han J, Foley SL, Dai M, Wang X, Wang Y, Huang L, Sun Y, Liu Z, Yuan Z. 2017. Cj1199 affect the development of erythromycin resistance in *Campylobacter jejuni* through regulation of leucine biosynthesis. Front Microbiol 8:16. https://doi.org/10.3389/fmicb.2017.00016.
135. Flint A, Sun YQ, Butcher J, Stahl M, Huang H, Stintzi A. 2014. Phenotypic screening of a targeted mutant library reveals *Campylobacter jejuni* defenses against oxidative stress. Infect Immun 82:2266–2275. https://doi.org/10.1128/IAI.01528-13.
136. Woodall CA, Jones MA, Barrow PA, Hinds J, Marsden GL, Kelly DJ, Dorrell N, Wren BW, Maskell DJ. 2005. *Campylobacter jejuni* gene expression in the chick cecum: evidence for adaptation to a low-oxygen environment. Infect Immun 73:5278–5285. https://doi.org/10.1128/IAI.73.8.5278-5285.2005.
137. Hinnebusch BJ, Erickson DL. 2008. *Yersinia pestis* biofilm in the flea vector and its role in the transmission of plague. Curr Top Microbiol Immunol 322:229–248. https://doi.org/10.1007/978-3-540-75418-3_11.
138. Hinnebusch BJ, Jarrett CO, Bland DM. 2017. "Fleaing" the plague: adaptations of Yersinia pestis to its insect vector that lead to transmission. Annu Rev Microbiol 71:215–232. https://doi.org/10.1146/annurev-micro-090816-093521.
139. Vadyvaloo V, Jarrett C, Sturdevant DE, Sebbane F, Hinnebusch BJ. 2010. Transit through the flea vector induces a pretransmission innate immunity resistance phenotype in Yersinia pestis. PLoS Pathog 6:e1000783. https://doi.org/10.1371/journal.ppat.1000783.
140. Chouikha I, Sturdevant DE, Jarrett C, Sun YC, Hinnebusch BJ. 2019. Differential gene expression patterns of Yersinia pestis and Yersinia pseudotuberculosis during infection and biofilm formation in the flea digestive tract. mSystems 4:e00217-18. https://doi.org/10.1128/mSystems.00217-18.
141. Levy-Favatier F, Leroux A, Antoine B, Nedelec B, Delpech M. 2004. Upregulation of rat P23 (a member of the YjgF protein family) by fasting, glucose diet and fatty acid feeding. Cell Mol Life Sci 61:2886–2892. https://doi.org/10.1007/s00018-004-4231-8.
142. Sandoval IV, Sols A. 1974. Gluconeogenesis from serine by the serine-dehydratase-dependent pathway in rat liver. Eur J Biochem 43:609–616. https://doi.org/10.1111/j.1432-1033.1974.tb03448.x.
143. Ishikawa E, Ninagawa T, Suda M. 1965. Hormonal and dietary control of serine dehydratase in rat liver. J Biochem 57:506–513. https://doi.org/10.1093/oxfordjournals.jbchem.a128109.
144. Ogawa H, Fujioka M, Su Y, Kanamoto R, Pitot HC. 1991. Nutritional regulation and tissue-specific expression of the serine dehydratase gene in rat. J Biol Chem 266:204127.
145. Zhong B, Sakai S, Saeki T, Kanamoto R. 2007. Excess leucine intake induces serine dehydratase in rat liver. Biosci Biotechnol Biochem 71:2614–2617. https://doi.org/10.1271/bbb.70512.
146. Williamson JR. 1966. Mechanism for the stimulation in vivo of hepatic gluconeogenesis by glucagon. Biochem J 101:11C–14C. https://doi.org/10.1042/bj1010011c.
147. González-Manchón C, Ayuso MS, Parrilla R. 1989. Control of hepatic

gluconeogenesis: role of fatty acid oxidation. Arch Biochem Biophys 271:1–9. https://doi.org/10.1016/0003-9861(89)90249-X.
148. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang H-Y, Cohoon M, de Crécy-Lagard V, Diaz N, Disz T, Edwards R, Fonstein M, Frank ED, Gerdes S, Glass EM, Goesmann A, Hanson A, Iwata-Reuyl D, Jensen R, Jamshidi N, Krause L, Kubal M, Larsen N, Linke B, McHardy AC, Meyer F, Neuweger H, Olsen G, Olson R, Osterman A, Portnoy V, Pusch GD, Rodionov DA, Rückert C, Steiner J, Stevens R, Thiele I, Vassieva O, Ye Y, Zagnitko O, Vonstein V. 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res 33:5691–5702. https://doi.org/10.1093/nar/gki866.
149. Kreuzer-Martin HW, Lott MJ, Ehleringer JR, Hegg EL. 2006. Metabolic processes account for the majority of the intracellular water in log-phase *Escherichia coli* cells as revealed by hydrogen isotopes. Biochemistry 45:13622–13630. https://doi.org/10.1021/bi0609164.
150. Zhou HX, Rivas G, Minton AP. 2008. Macromolecular crowding and confinement: biochemical, biophysical, and potential physiological consequences. Annu Rev Biophys 37:375–397. https://doi.org/10.1146/annurev.biophys.37.032807.125817.
151. Hafner EW, Wellner D. 1979. Reactivity of the imino acids formed in the amino acid oxidase reaction. Biochemistry 18:411–417. https://doi.org/10.1021/bi00570a004.
152. He W, Li G, Yang CK, Lu CD. 2014. Functional characterization of the *dguRABC* locus for D-Glu and D-Gln utilization in *Pseudomonas aeruginosa* PAO1. Microbiology 160:2331–2340. https://doi.org/10.1099/mic.0.081141-0.
153. Zelyas NJ, Cai H, Kwong T, Jensen SE. 2008. Alanylclavam biosynthetic genes are clustered together with one group of clavulanic acid biosynthetic genes in *Streptomyces clavuligerus*. J Bacteriol 190:7957–7965. https://doi.org/10.1128/JB.00698-08.
154. Zhou L, Wang J-Y, Wang J, Poplawsky A, Lin S, Zhu B, Chang C, Zhou T, Zhang L-H, He Y-W. 2013. The diffusible factor synthase XanB2 is a bifunctional chorismatase that links the shikimate pathway to ubiquinone and xanthomonadins biosynthetic pathways. Mol Microbiol 87:80–93. https://doi.org/10.1111/mmi.12084.
155. Franklin FC, Venables WA. 1976. Biochemical, genetic, and regulatory studies of alanine catabolism in *Escherichia coli* K12. Mol Gen Genet 149:229–237. https://doi.org/10.1007/BF00332894.
156. He W, Li C, Lu CD. 2011. Regulation and characterization of the *dadRAX* locus for D-amino acid catabolism in *Pseudomonas aeruginosa* PAO1. J Bacteriol 193:2107–2115. https://doi.org/10.1128/JB.00036-11.
157. Takenaka S, Sato T, Koshiya J, Murakami S, Aoki K. 2009. Gene cloning and characterization of a deaminase from the 4-amino-3-hydroxybenzoate-assimilating *Bordetella* sp. strain 10d. FEMS Microbiol Lett 298:93–98. https://doi.org/10.1111/j.1574-6968.2009.01699.x.
158. Babbitt PC. 2003. Definitions of enzyme function for the structural genomics era. Curr Opin Chem Biol 7:230–237. https://doi.org/10.1016/s1367-5931(03)00028-0.
159. Schnoes AM, Brown SD, Dodevski I, Babbitt PC. 2009. Annotation error in public databases: misannotation of molecular function in enzyme superfamilies. PLoS Comput Biol 5:e1000605. https://doi.org/10.1371/journal.pcbi.1000605.
160. Miyakawa T, Hatano KI, Lee WC, Kato Y, Sawano Y, Yumoto F, Nagata K, Tanokura M. 2005. Crystallization and preliminary X-ray analysis of the YjgF/YER057c/UK114family protein ST0811 from Sulfolobus tokodaii strain 7. Acta Crystallogr Sect F Struct Biol Cryst Commun 61:828–830. https://doi.org/10.1107/S1744309105023808.
161. Kwon S, Lee CW, Koh HY, Park H, Lee JH, Park HH. 2020. Crystal structure of the reactive intermediate/imine deaminase A homolog from the Antarctic bacterium Psychrobacter sp. PAMC 21119. Biochem Biophys Res Commun 522:585–591. https://doi.org/10.1016/j.bbrc.2019.11.139.
162. Chen Y, Chen Y, Jiang H, Lu D, Hu T, Bi G, Ran Y, Yu B, Dong H, Su D. 2019. A unique homo-hexameric structure of 2-aminomuconate deaminase in the bacterium Pseudomonas species AP-3. Front Microbiol 10:2079. https://doi.org/10.3389/fmicb.2019.02079.

Jessica L. Irons is a postdoctoral researcher at the University of Georgia, where she also completed her doctoral degree. As a graduate and postdoctoral researcher, she used the awesome power of genetics to study the integration of RidA in the metabolic systems of *Pseudomonas aeruginosa* and *Campylobacter jejuni*. As a graduate student at the University of Georgia, she was also an advocate for student mental health and championed initiatives to improve career exploration and development opportunities, peer support, student learning outcomes, and departmental communication for graduate students. As an undergraduate, she participated in two microbiology Research Experience for Undergraduates internships at the University of Wisconsin, studying the stringent response to nutrient starvation.

Kelsey Hodge-Hanson is a medical writer at AMICULUM, a global medical communications agency, who bolsters the Chicago STEM community through her work on the nonprofit board of directors for Chientist. As an undergraduate at the University of Wisconsin—Madison, she investigated vitamin biosynthesis, which ignited her interest in physiology. She earned her doctorate in microbiology at the University of Georgia, where she aligned her enthusiasm for genetics and biochemical assays with curiosity about the Rid family. As a postdoctoral researcher at Loyola University, she used genetics to study factors that govern biofilm dynamics. Her fascination with cell function continues to fuel her work communicating scientific information in a variety of therapeutic areas, including neurology, rare diseases, and oncology.

Continued next page

Diana M. Downs received her B.S. and Ph.D. from the University of Utah and postdoctoral training at the University of Wisconsin, Madison. She was a Professor in the Department of Bacteriology at the University of Wisconsin, Madison, until she moved to the University of Georgia in Athens, GA, to join the Department of Microbiology. Dr. Downs' group defined enamine deaminase as the biochemical activity of the RidA protein family that is conserved across domains. Her group's work integrated the biochemical activity with the physiological role of this protein to define the RidA paradigm of 2-amino acrylate stress. The Downs laboratory focuses on the function and integration of metabolic pathways, and her laboratory has been working in this area since 1993.