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Biosynthesis and function of 7-deazaguanine derivatives in bacteria and phages

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SUMMARY Deazaguanine modifications play multifaceted roles in the molecular biology of DNA and tRNA, shaping diverse yet essential biological processes, including the nuanced fine-tuning of translation efficiency and the intricate modulation of codon-anticodon interactions. Beyond their roles in translation, deazaguanine modifications contribute to cellular stress resistance, self-nonself discrimination mechanisms, and host evasion defenses, directly modulating the adaptability of living organisms. Deazaguanine moieties extend beyond nucleic acid modifications, manifesting in the structural diversity of biologically active natural products. Their roles in fundamental cellular processes and their presence in biologically active natural products underscore their versatility and pivotal contributions to the intricate web of molecular interactions within living organisms. Here, we discuss the current understanding of the biosynthesis and multifaceted functions of deazaguanines, shedding light on their diverse and dynamic roles in the molecular landscape of life.

KEYWORDS queuosine, archaeosine, toyocamycin, bacteriophage, restriction/modification, genetic code

INTRODUCTION

7-Deazapurines are analogs of nucleosides that feature pyrrolo[2,3-d]pyrimidine or pyrrolopyrimidine moieties. They can be found either as independent molecules or integrated into RNA or DNA polymers (1, 2). A distinctive structural characteristic of 7-deazapurines involves substitutions of the nitrogen atom typically located at the purine scaffold's position 7 (Fig. 1). Deazapurine-containing antibiotics such as tubercidin and sangivamycin (3) (Fig. 2) and deazapurine RNA modifications such as queuosine (Q) (4) and archaeosine (G^+) (5) were first described in the 1960s to 1980s. More recently, their large structural diversity has become apparent, propelled by the identification of synthesis pathway genes and the growing availability of diverse genome sequencing data.

Though the pyrrolopyrimidine core structure is strictly conserved, the deazapurines are a diverse class of molecules regarding their chemical structures, taxonomic distribution, and functional roles. While the diversity of 7-deazapurines has long been appreciated in the context of secondary metabolites, it was initially believed that only a few derivatives were present in nucleic acids, namely G^+, Q, and Q derivatives (1). However, recent reports of 7-deazapurines in DNA have altered this perspective, identifying eight distinct pyrrolopyrimidine modifications in bacteriophages so far (6) (Fig. 1).

Depending on the deazapurine, taxonomic distribution may be wide or narrow and the degree of enzymatic conservation of the biosynthetic pathway can vary. For example, Q is predicted to be present in the tRNAs of over 90% of sequenced bacterial and eukaryotic species (7, 8), while many pyrrolopyrimidine secondary metabolites are only found in select bacterial lineages (Table 1). The functions of deazapurines vary greatly depending on their final structures and/or location, as the same molecule can have different roles when located in RNA or DNA. G^+ in archaea plays a role in stabilizing tRNA tertiary structure (9), whereas the same modified base in DNA shields bacteriophages from restriction enzymes (10). Q is crucial for tRNA decoding efficiency or accuracy (11, 12) and has been adapted for regulatory functions in some individual species (13). Some deazapurines in bacterial DNA are components of restriction-modification islands (14). The natural functions of deazapurine secondary metabolites are not entirely understood but some have demonstrated anticancer, antiviral, or antibacterial activities (Table 1).

The exploration of various 7-deazapurine biosynthesis pathways has unveiled unprecedented enzyme chemistries and novel structural folds (1, 2). This has both practical applications and evolutionary significance, especially considering that many of these enzymes belong to the tunnel-fold (T-fold) family, which appears to have been recruited to execute different types of reactions on similar substrates (39).

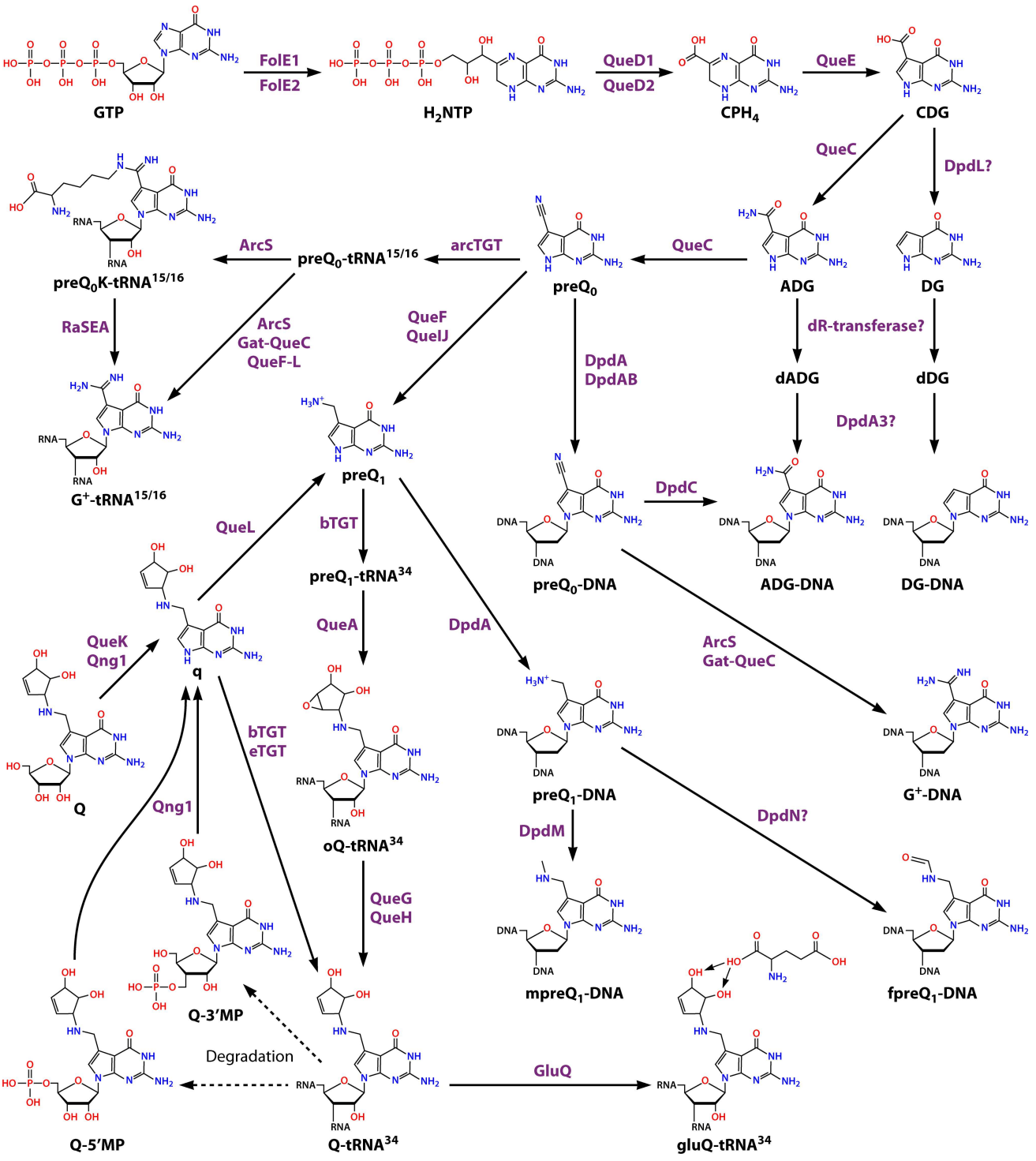

FIG 1 General pathway of 7-deazaguanine modifications in tRNA and DNA. The illustration includes representations of all final and distinctive molecules in the pathway, excluding redundant ones. Key proteins catalyzing reactions are highlighted in bold text adjacent to the corresponding arrows and as described in the text. When two proteins or more are listed, these are alternate enzymes catalyzing the same reactions. Unknown enzymes have question marks associated (?).

Certain precursors leading to the formation of the ultimate 7-deazapurine molecules can be reclaimed and viewed as micronutrients (40). Consequently, the notion that competition for these deazapurine precursors might influence the ecology of distinct niches, such as the mammalian microbiota or other host-associated environments, is just

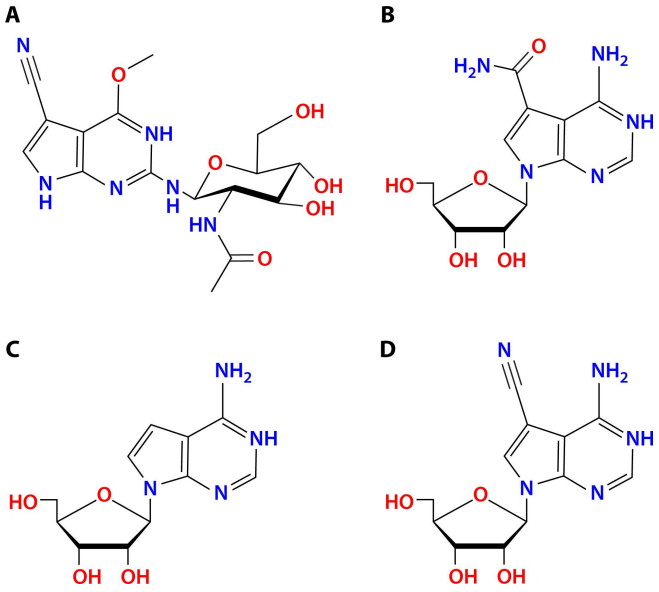

FIG 2 7-Deazapurine natural product representatives with entries in the MIBiG database. Huimycin (A), sangivamycin (B), tubercidin (C), and toyocamycin (D) are the only metabolites with known minimal annotations for the BGCs in the database. The rest of the compounds described in Table 1 remain orphaned. All those compounds have in common the $preQ_0$ scaffold in their chemical structures.

beginning to take root (41, 42). Thus, deazapurines are widespread metabolites with diverse biological functions, and the complete scope of their roles continues to reveal itself as our understanding advances.

$PREQ_0$ AND $PREQ_1$ ARE THE COMMON PRECURSORS FOR NUMEROUS DEAZAPURINE DERIVATIVES

$PreQ_0$, also known as 7-cyano-7-deazaguanine, serves as the precursor to most natural 7-deazaguanine-containing molecules (1, 2). Both $preQ_0$ and its derivative, 7-aminomethyl-7-deazaguanine ($preQ_1$), can not only be incorporated into DNA or RNA and undergo further modifications but also act as precursors to numerous secondary metabolites. The biosynthesis of $preQ_0$ occurs from guanosine-5'-triphosphate (GTP) in a four-step pathway (Fig. 1; Table 2) that remained enigmatic for over three decades. This pathway was eventually elucidated between 2004 and 2009: the gene candidates were identified by a combination of taxonomic distribution filters and gene neighborhood analyses and were validated by a combination of bioinformatic, genetic, and biochemical studies (43–46). These investigations also identified QueF as the enzyme responsible for the biosynthesis of $preQ_1$ from $preQ_0$ (47).

QueD: The first enzyme of $preQ_0$ biosynthesis is shared with the tetrahydrofolate and biopterin pathways

As predicted by the foundational work that identified GTP as the deazapurine precursor (48, 49), the $preQ_0$, tetrahydrofolate (THF), and biopterin (BH_4) synthesis pathways share a common first step with the formation of 7,8-dihydroneopterin triphosphate (H_2NTP) from GTP (Fig. 3) (46, 50). GTP cyclohydrolase I (EC 3.5.4.16), an enzyme class with various cofactors, mediates this reaction. The Zn^{2+}-dependent variant (FolE; COG0302) is used by most bacteria and mammals, which contrasts with the FolE2 type (COG1469) used by other bacteria and most archaea, which employs different metals like Mn^{2+} (51). Both FolE and FolE2 are members of the T-fold structural superfamily (39, 52). Shared pathway intermediates might enable shifts between THF and Q pools under conditions of GTP scarcity or elevated Q biosynthesis demand, as seen with riboflavin, another molecule

TABLE 1 7-Deazapurine derived natural products

Natural product	Bioactivity[a]	Organism	MIBiG BGC[b]	References
Tubercidin	AB, AP, AV, AT	*Streptomyces tubercidis*	BGC0001937	(1, 15)
Sangivamycin	AB, AT, AV	*Streptomyces rimosus* ATCC 14673	BGC0000879	(1, 16, 17)
Toyocamycin	AB, AT, AV	*Streptomyces rimosus* ATCC 14673	BGC0000879	(1, 17, 18)
Cadeguomycin	AB, AT	*Streptomyces hygroscopicus*	U	(19)
Kanagawamicin	AT	*Actinoplanes kanagawaensis*	U	(20, 21)
Echiguanine A	AT	*Streptomyces* M1698-50F1	U	(22)
Echiguanine B	AT	*Streptomyces* M1698-50F1	U	(22)
Ara-A	H	*Actinoplanes* sp. A9222	U	(23)
Dapiramicin A	AB, AF	*Micromonospora* sp. SF-1917	U	(24, 25)
Dapiramicin B	AB, AF	*Micromonospora* sp. SF-1917	U	(26)
5′-deoxyguanosine	H	*Thermoactinomycete* sp. A6019	U	(23)
Coaristeromycin	H	*Streptomyces* sp. A6308	U	(23)
Aristeromycin	H	*Streptomyces* sp. A6308	U	(23)
5′-deoxytoyocamycin	H	*Streptomyces* sp. A14345	U	(23)
Coformycin	H	Unclassified	U	(23)
5′-deoxy-5-iodotubercidin	AKI	*Hypnea valentiae*	U	(27)
4-amino-5-bromo-pyrrolo[2,3-d] pyrimidine	B	*Echinodictyum*	U	(27)
Huimycin	U	*Kutzneria albida* DSM 43870	BGC0002354	(28)
Tubercidin-5′-α-d-glucopyranose	CT, AF	*Plectonema radiosum*, *Tolypothrix tenuis*	U	(29)
Toyocamycin-5′-α-d-glucopyranose	CT, AF	*Plectonema radiosum, Tolypothrix tenuis*	U	(29)
Mycalisine A	AB	*Mycale* sp.	U	(30)
Mycalisine B	AM	*Mycale* sp.	U	(30, 31)
5′-deoxy-5-iodotubercidin	NB	*Hypnea valendiae*, *Didemnum voeltzkowi*	U	(32)
5-(methoxycarbonyl) tubercidin	CT	*Jaspis johnstoni*	U	(33)
Toyomycin	CT	*Jaspis johnstoni*	U	(34)
Rigidins B-D	CA	*Cystodytes* sp.	U	(35)
7-deazainosine	CT	*Aplidium pantherinum*	U	(36)
5′-deoxy-3-bromotubercidin	CT	*Didemnum voeltzkowi*	U	(37)
5′-deoxytubercidin	CT	*Didemnum voeltzkowi*	U	(37)
Unamycin B	AB	*Streptomyces . fungicidicus*	U	(38)
Vengicide	AB	*Streptomyces vendargensis*	U	(38)

[a]antibiotic (AB); antiparasitic (AP); antiviral (AV); antitumor (AT); antifungal (AF); herbicidal (H); adenosine kinase inhibitor (AKI); bronchodilator (B); unknown (U); antimitotic (AM); cytotoxic (CT); neuromuscular blocking (NB); calmodulin antagonistic (CA).
[b]BGCs reported in MIBiG with minimal annotation.

originating from GTP (53). The first committed step in preQ$_0$ synthesis, catalyzed by 6-carboxy-5,6,7,8-tetrahydropterin (6-CPH$_4$) synthase EC (EC4.1.2.50) or QueD (43, 44) (Fig. 1), is a textbook example of the difficulty in annotating paralogous families (54). The *Escherichia coli* QueD (or PTPS-I) protein was first annotated as 6-pyruvoyltetrahydropterin synthase (PTPS) because of its similarity with the mammalian homolog involved in biopterin synthesis, now named PTPS-II. QueD/PTPS-I and PTPS-II are both members of the T-fold derived COG0720 family (Pfam PF01242). Comparative genomic predictions (43) combined with genetic (43), biochemical (44), and structural studies (50, 55) revealed that slight variations in the active site can shift catalytic activity between PTPS-II and QueD/PTPS-I (Fig. 3). A third subgroup in this family, PTPS-III, catalyzes the conversion of H$_2$NTP into neopterin, bypassing the FolB and FolQ enzymes in the THF pathways of certain bacteria and parasites (Fig. 3) (56). Remarkably, some bacteria have bifunctional QueD/PTPS-III enzymes that must be used both for Q and THF biosynthesis (50). Another QueD variant, QueD2 was identified based on its signature motif and metal binding properties (Fig. 3) (50, 57). QueD is a Zn^{2+}-dependent (44, 55) lyase that catalyzes the elimination of triphosphate and acetaldehyde from H$_2$NTP. A coordinated zinc ion plays a key role, stabilizing oxyanion-containing intermediates leading to the product carboxylate. Comparative genomic analysis suggests QueD2 is regulated by the zinc uptake regulator (Zur) under metal-limiting conditions. The paralogs contain a second

TABLE 2 Known bacterial and archaeal Q and G^+ synthesis and salvage enzymes and transporters[b]

Protein name	Protein function	COG	KO	Cofactors
FolE/GCHI	GTP cyclohydrolase IA (EC 3.5.4.16)	COG0302	K01495	Zn
FolE2	GTP cyclohydrolase IB (EC 3.5.4.16)	COG1469	K09007	Mn
QueD	6-carboxy-5,6,7,8-tetrahydropterin synthase (EC 4.1.2.50)	COG0720[a]	K01737 (1)	Zn
QueD2	6-carboxy-5,6,7,8-tetrahydropterin synthase (EC 4.1.2.50)	COG0720[a]	K01737 (1)	Fe, Zn
QueE	7-carboxy-7-deazaguanine synthase (EC 4.3.99.3)	COG0602[a]	K10026	Fe
QueC	7-cyano-7-deazaguanine synthase (EC 6.3.4.20)	COG0603	K06920	ATP, Zn, NH4+
QueF type I	7-cyano-7-deazaguanine reductase (EC 1.7.1.13) Type II	COG0780, COG2904	K06879	NADPH
QueF type II	7-cyano-7-deazaguanine reductase (EC 1.7.1.13) Type I	COG0789	K09457	NADPH
bTgt	Queuine tRNA-ribosyltransferase (EC 2.4.2.29)	COG0343	K00773	Zn
QueA	S-adenosylmethionine:tRNA ribosyltransferase-isomerase (EC 2.4.99.17)	COG0809	K07568	SAM
QueG	Epoxyqueuosine reductase (EC 1.17.99.6)	COG1600	K18979	Fe, Cobalamin
QueH	epoxyqueuosine reductase (EC 1.17.99.6)	COG1636[a]	K09765	Fe
GluQ	Glutamyl-Q tRNA(Asp) synthetase (EC 6.1.1.B3)	COG0008[a]	K01894	Zn
aTGT	7-cyano-7-deazaguanine tRNA-ribosyltransferase (EC 2.4.2.48)	COG1370	K18779	Zn
ArcS	Archaeosine synthase alpha-subunit (EC 2.6.1.97; 2.6.1.-)	COG1549	K07557	Lys or Gln
RaSEA	Archaeosine synthase beta-subunit [EC 2.6.1.-]	COG1244[a]	K06936	
QueF-Like	QueF-like amidinotransferase (EC 2.6.1.-)		Pcal_0221 (2)	$NH4^+$
QPTR/YhhQ	Queuosine precursor transporter (TC 3.A.1.28)	COG1738	K09125	
QrtT/QueT	Energy-coupling factor transport system substrate-specific component (TC 3.A.1.28)	COG4708	K16923	
QueK	Queuosine hydrolase (EC 3.2.2.1)	COG1957	No KO, CD630_16820 (2)	
QueL	Queuine lyase (EC 4.3.99.M4)	COG1244[a]	CD630_16840 (2)	SAM
Qng1	Queuosine 5′-phosphate N-glycosylase/hydrolase (EC 3.2.2.-)	pfam10343 (3)	Sthe_2331 (2)	

[a]Not isofunctional COG.
[b]COG: Clusters of Orthologous Groups; KO: KEGG Orthology Number (1); KO not specific for QueD/PTPS-I (2); No KO number, posted a specific ID with experimental validation. (3); No COG number posted a pfam number instead.

zinc-binding site located in an inserted region not present in QueD, and based on its structure, a model has been proposed where the second metal site of QueD2 slows the dissociation of the catalytic metal (50, 57). The different COG0720 subgroups can be separated by strictly conserved signature motifs (50, 57, 58) but many annotation mistakes remain in most databases (59) and several discernable subgroups of this family remain to be functionally characterized [see Fig. S4 in (57)].

QueE: an atypical radical SAM enzyme

The next step in $preQ_0$ biosynthesis is the heterocyclic radical-mediated conversion of 6-carboxy-5,6,7,8-tetrahydropterin (CPH_4) to 7-carboxy-7-deazaguanine (CDG) catalyzed by 7-carboxy-7-deazaguanine synthase or QueE (EC 4.3.99.3) (60). First identified by *in silico* and genetics methods (43), QueE is a member of the radical SAM superfamily of enzymes [(61) and see https://radicalsam.org/explore.php?id=cluster-3-1&v=3.0] that perform a wide array of chemical reactions initiated by the highly reactive 5′-deoxyadenosyl radical (62) including C-H activation, atom/group transfer, isomerizations, bond cleavage, and rearrangements. QueE has been extensively studied using biophysical, structural, and biochemical approaches (60, 63–65). The overall mechanism involves nitrogen atom migration, resulting in ring contraction, followed by the elimination of ammonia. The role of QueE in catalysis is to stabilize and control the fate of high-energy radical intermediates. Among the larger family of radical SAM enzymes, QueE is atypical because of its dependence on an Mg^{+2} cation and overall turnover of the radical S-adenosylmethionine cofactor.

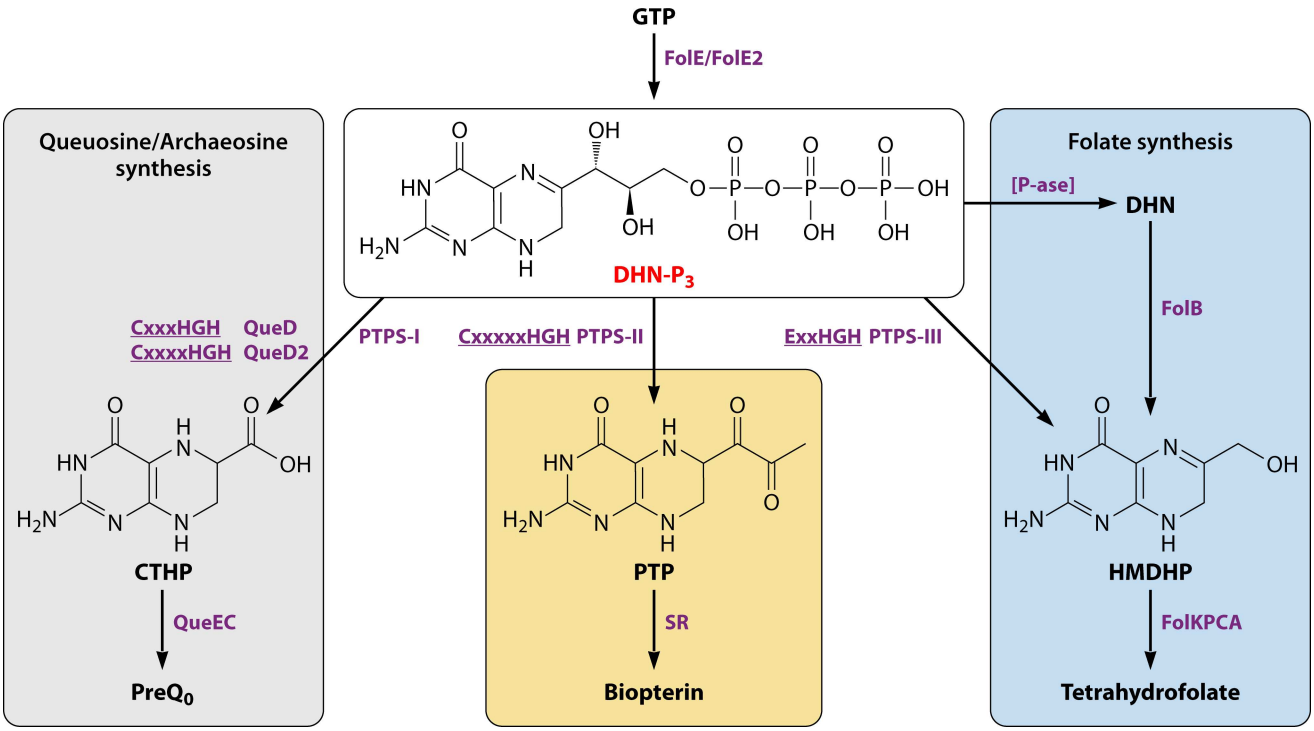

FIG 3 Functional roles of different PTPS subfamilies. Biosynthesis pathways in which PTPS-I, II, and III are involved. Specific reactions catalyzed by PTPS-I (QueD or QueD2), PTPS-II, and PTPS-III, and conserved motifs were identified. Abbreviations: GTP: guanosine triphosphate; FolE: GTP cyclohydrolase I; FolE2: GTP cyclohydrolase II; DHN-P3: dihydroneopterin-triphosphate; CTHP: 6-carboxytetrahydropterin; PTP: 6-pyruvoyl-tetrahydropterin; HMDHP: 6-hydroxymethyldihydropterin; preQ$_0$: 7-cyano-7-deazaguanosine; QueE: 7-carboxy-7-deazaguanine synthase; QueC: 7-cyano-7-deazaguanine synthase; SR: sepiapterin reductase; DHN: dihydroneopterin; [P-ase]: phosphatase; FolB: dihydroneopterin aldolase; FolK: 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase; FolP: dihydropteroate synthase; FolC: dihydrofolate:folylpolyglutamate synthase; FolA: dihydrofolate reductase.

QueC: an ATPase that catalyzes two reactions

The enzyme 7-cyano-7-deazaguanine synthase, also known as QueC (EC 6.3.4.20), plays a pivotal role in catalyzing the conversion of CDG into preQ$_0$ (45). This process involves two ATP molecules: one is consumed to generate a 7-amido-7-deazaguanine (ADG) intermediate while the other is used to process ADG into preQ$_0$ (66) (Fig. 1). Mutating two strictly conserved residues located within 7 Å of the phosphate ligand (K163A/R204A) in the *Bacillus subtilis* QueC protein, effectively halts the reaction at the ADG intermediate, showing the importance of these two residues in processing the second reaction from ADG to preQ$_0$ (67). Each monomeric subunit of the QueC homodimer consists of an N-terminal domain exhibiting a Rossman fold architecture, a characteristic feature shared with many nucleotide-binding proteins. In addition, it contains a helical zinc-binding C-terminal domain. Notably, the active site is predicted to reside at the interface between these two domains (68, 69). QueC-like encoding genes have been found in phage defense clusters, such as QatC in the QueC-like associated with ATPase and TatD DNase system (Qat) (70) or Cap9 in the type IV cyclic oligonucleotide-based anti-phage signaling system (CBASS) (71), yet their functions remain to be elucidated.

QueF: the four-electron reduction of preQ$_0$ to preQ$_1$ from nitrile to primary amine

In contrast to non-specific reductions by nitrogenases, the QueF-mediated reduction of preQ$_0$ to preQ$_1$ is the only known nitrile reduction found in a natural biosynthetic pathway (72). Although the catalytic activity was witnessed in the late 1970s (73), the NADPH-dependent 7-cyano-7-deazaguanine QueF (EC 1.7.1.13) enzyme was only characterized in 2004 (47). QueF was first identified by comparative genomic and genetic

studies and found to be involved in Q synthesis in *Acinetobacter baylyi* and *Bacillus subtilis* (43). The exact biochemical function was elucidated a few years later despite the initial misannotation that QueF was a GTP cyclohydrolase I based on its membership in the T-fold family (47). Sequence determinants specific to QueF's reductase activity have been identified and allow for the differentiation of QueF from GTP cyclohydrolases. As with other members of the T-fold family, the QueF active site pocket is located at the interface between subunits (72). Two types of QueF enzyme architectures have been characterized to date. In the QueF type I exemplified by *B. subtilis* YkvM (K09457), two independent subunits form the catalytic interface while in the QueF type II exemplified by *E. coli* YqcD (K06879), the interface is formed by two domains of the same subunit. Therefore, type II proteins are predicted to have arisen from a duplication of the type I domain with a catalytic inactive portion at the C-terminus (47). Mechanistic and structural studies identified Cys55 in *B. subtilis* QueF as a key catalytic residue, forming an α,β-unsaturated thioamide covalent intermediate with $preQ_0$, supporting a covalent catalysis reaction mechanism (72, 74–77). To prevent oxidation of the catalytic cysteine, QueF forms a large homodecameric complex with active sites at the inter-monomer interfaces, facilitated by an intermolecular disulfide bridge with another cysteine (78). Because of their unique reductase activity and their potential exploitation for biocatalysis (see in dedicated section), QueF enzymes have been extensively studied (76, 77). However, these characterized QueF enzymes are very specific for $preQ_0$, with only a limited number of other substrates reported (79).

An analysis of the distribution of the $preQ_1$ synthesis proteins (QueC, QueD, QueE, and QueF type I and Type II) and of the Q biosynthesis proteins [(Tgt), QueA see section below)] found 148 out of 7,267 bacterial genomes encoded all proteins but the QueF homologs (data extracted from https://www.kegg.jp/kegg-bin/view_ortholog_table?orthology=K01737+K10026+K06920+K06879+K09457+K00773+K07568 Release 108.0, October 1, 2023). This suggests a non-orthologous displacement for the yet unidentified enzyme catalyzing $preQ_0$ reductase activity, as enzymes with $preQ_1$ substrate specificity exist in those organisms, namely Tgt and QueA. Proposed candidates for this cryptic activity are members of the 2-hydroxyacyl-CoA dehydratase superfamily, but experimental validation remains to be performed (80).

Conservation and regulation of $preQ_0$/$preQ_1$ biosynthetic genes

The dedicated $preQ_0$/$preQ_1$ biosynthetic genes (*queD*, *queC*, *queE*, and *queF*) are generally physically clustered in different combinations (see https://www.kegg.jp/kegg-bin/view_ortholog_table?orthology=K01737+K10026+K06920+K06879+K09457+K18979+K09765+K00773+K07568). In some organisms, the *folE/folE2* genes can also be present in the clusters (Fig. 4). The regulation of $preQ_0$ and $preQ_1$ biosynthetic genes remains poorly understood in many organisms, including the model *E. coli* K12. The only known dedicated regulatory elements are $preQ_1$ responsive riboswitches, categorized into one of three classes (81). Members belonging to class-I were identified upstream of the *B. subtilis* $preQ_1$ biosynthesis operon (82) and are mainly found in Proteobacteria and Firmicutes. Class-II $preQ_1$ riboswitches are primarily present in Lactobacillales, while class-III is primarily found in Clostridiales (81). Not all $preQ_1$ riboswitches employ the same mechanisms: the *B. subtilis* class-I riboswitch uses ligand-triggered transcriptional termination, while the class-II *Streptococcus pneumoniae* R6 riboswitch utilizes metabolite-mediated sequestration of the Shine-Dalgarno sequence (83). A class-I $preQ_1$ riboswitch in *Carnobacterium antarcticus* was recently found to bind stacked effector molecules, a unique property in riboswitches studied to date (84). $preQ_1$ riboswitches have a characteristically short length, some as short as 25 nucleotides, making them suitable for structural and biophysical studies (85–87). They are used as inducible regulatory elements in synthetic biology (88, 89).

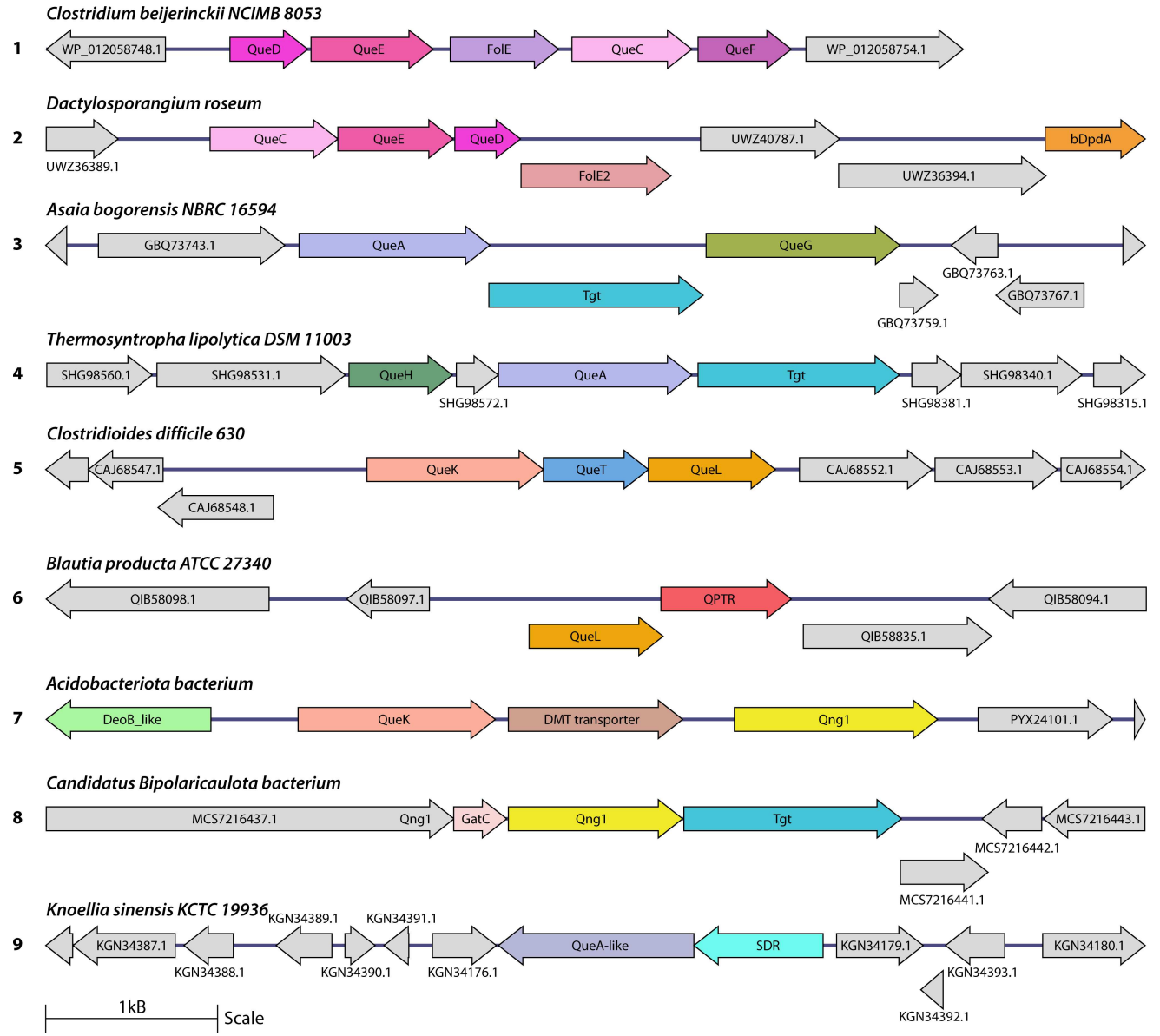

FIG 4 Physical clustering of genes encoding $preQ_0$/$preQ_1$ synthesis or Q synthesis/salvage enzymes. Different representatives of the gene neighborhood clusters discussed in the text are shown and were drawn using the GeneGraphics App (https://v2.genegraphics.net/) (90). Numeric protein identifiers are given for every example to retrieve specific information. All abbreviations of 7-deazapurine metabolism-related proteins are given in the text or Table 2 with the exception of short-chain dehydrogenase reductase (SDR). DeoB-like and QueA-like reflect that the functions might not be the same as the canonical DeoB (phosphopentomutase) or QueA enzymes.

DIVERSITY OF DEAZAGUANINE MODIFICATIONS IN NUCLEIC ACIDS

Transglycosylases insert free $preQ_0$, $preQ_1$, or Q bases in nucleic acids

$preQ_0$ and $preQ_1$ can be used directly as precursors of natural products (see dedicated section below). Their insertion as bases into nucleic acid molecules requires transglycosylase enzymes that exchange the deazapurine and guanine bases (Fig. 1 and 5). The founding members of this family of enzymes (Interpro ID: IPR002616) modify tRNAs and fall into three subgroups: (i) homodimeric bacterial tRNA-guanine transglycosylases (EC 2.4.2.29, bTGT) that exchange the wobble position guanine in tRNA with GUN anticodons with $preQ_1$; (ii) eukaryotic heterodimeric Queuine tRNA-ribosyltransferases [EC 2.4.2.64, eTGT composed of a catalytic subunit (QTRT1) and an accessory subunit

(QTRT2)] that introduce the Q base directly in the same target GUN-anticodon tRNAs; and (iii) archaeal tRNA-guanine transglycosylases (EC 2.4.2.48, arcTGT) that exchange the G at position 15, and sometimes at position 13 (91) with $preQ_0$ in many target tRNAs in most Archaea. The structures and catalytic mechanisms of these three groups of enzymes have been extensively studied and previously reviewed (92). The residues of the substrate binding pocket that allow discrimination between $preQ_0$, $preQ_1$, and Q substrates are well characterized (92, 93) as shown in Fig. 5.

In the last 5 years, the understanding of the functional diversity of those different subgroups has greatly expanded. A subgroup of bacterial enzymes was found to have shifted substrate specificity from $preQ_1$ to q in bacteria that live in queuine-rich environments (e.g., intracellular pathogens) (Fig. 5 and 6B) (7). Another subgroup, renamed DpdA for deazapurine in DNA, are homologs of archaeal TGT proteins encoded by bacteria or phages that have shifted their substrate specificities from RNA to DNA (14). In some bacteria, such as *Salmonella enterica* serovar Montevideo, DpdA incorporates $preQ_0$ into DNA only with the help of the DpdB protein (98, 99). The functional roles of DpdB are not yet clearly defined (and discussed below). Phage DpdA proteins do not require DpdB to insert 7-deazapurines into DNA (6, 10). Phage DpdA can be categorized into four groups that differ by their substrate specificities and potentially their sequence specificities. For example, the *E. coli* phages 9g and CAjan DpdA1 enzymes are specific for $preQ_0$ (10, 14) while the Haloarcula virus HVTV-1 DpdA4 is specific for $preQ_1$ (6). The DpdA2 enzymes seem to be more promiscuous. For example, the *Vibrio natriegens* phage nt-1 DpdA2 prefers $preQ_0$ while the *Vibrio* phage VH7D DpdA2 prefers $preQ_1$ but can insert $preQ_0$ and CDG into DNA, albeit at lower efficiencies, when $preQ_1$ is not available (10). The *in vivo* substrates of the DpdA3 enzymes are yet to be determined, but the

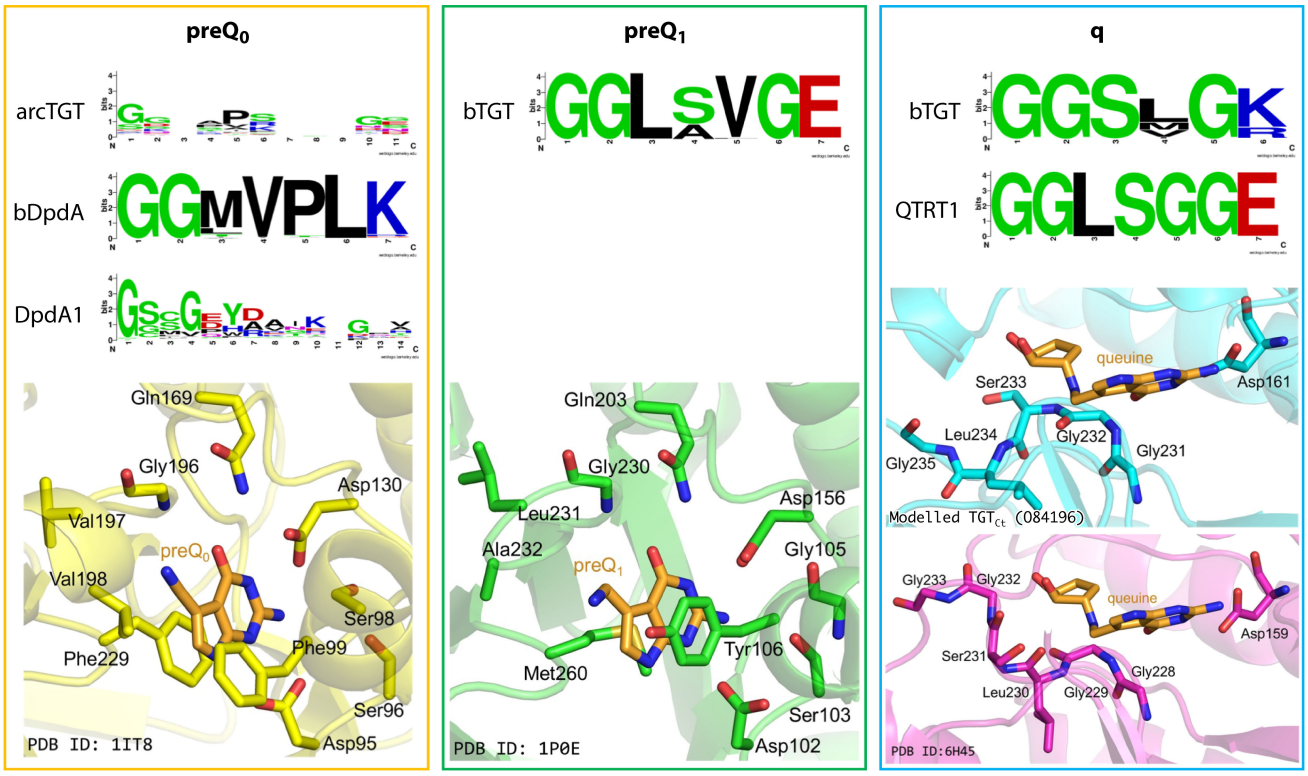

FIG 5 Substrate specificity of various deazaguanine transglycosylases. Logos on top represent the conserved region for 7-deazaguanine substrate binding and were generated from a relevant set of orthologous sequences of the protein depicted using MAFTT (94) and WebLogo2 (95). Representative structures for some orthologs are depicted on the bottom with their cognate substrate (colored in orange). Bacterial TGT from *Z. mobilis* complexed with $preQ_1$ (green, PDB ID: 1P0E), AlphaFold (96, 97) modeled *C. trachomatis* TGT with queuine (cyan, UniProt O84196), human TGT in complex with queuine (magenta, PDB ID: 6H45), and archaeal TGT from *P. horikoshii* complexed with $preQ_0$ (yellow, PDB ID: 1IT8). All structural illustrations were prepared using PyMOL (https://pymol.org/2/).

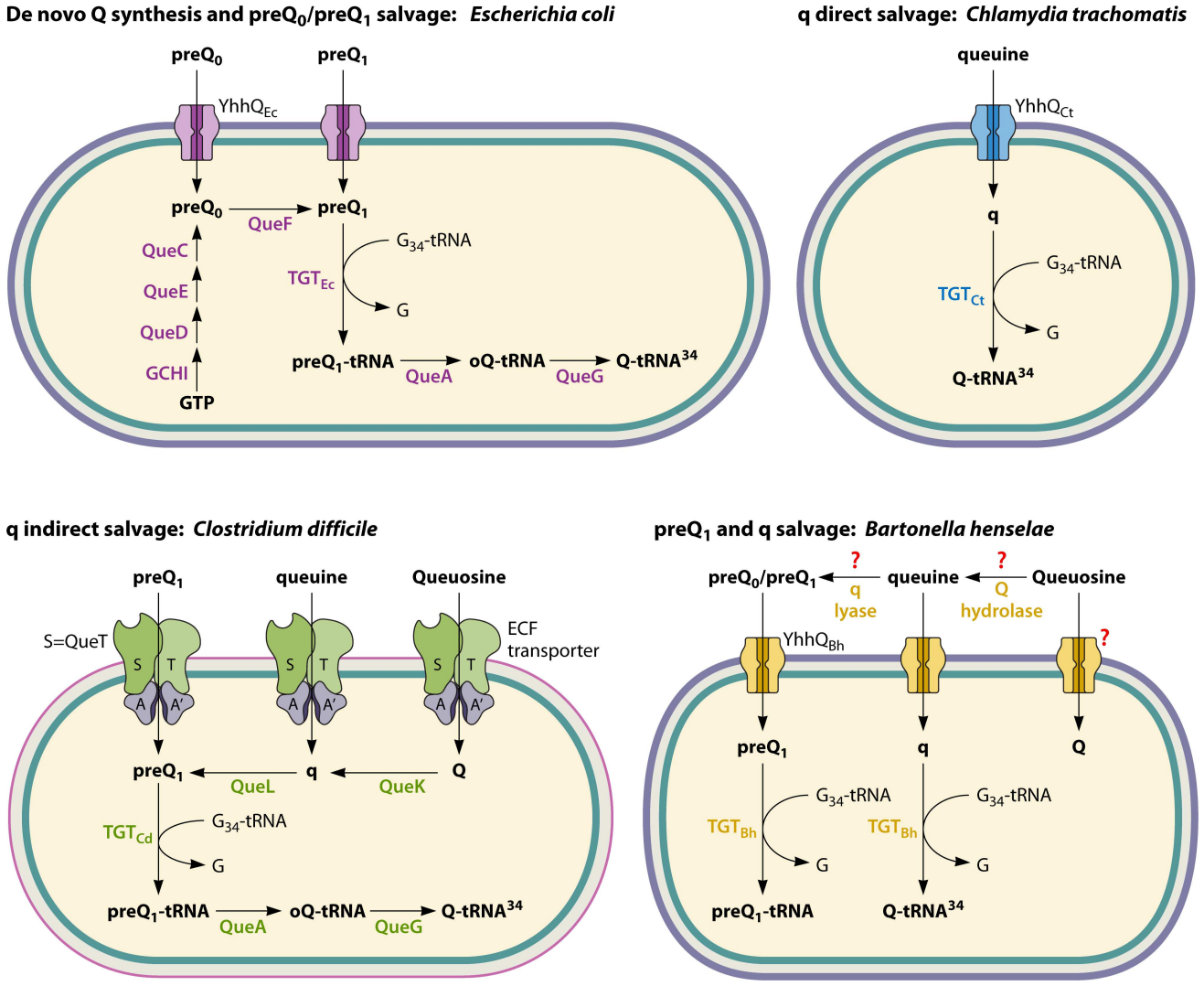

FIG 6 Known bacterial Q synthesis or salvage pathways. (Upper feft) *De novo* Q synthesis and $preQ_0$/$preQ_1$ salvage pathways in *E. coli*; (Upper right) q salvage pathway in *C. trachomatis*; (Lower left) $preQ_1$, q, and Q salvage pathways in *C. difficile*. The ECF transporters include four subunits: S, the substrate-specific transmembrane component (QueT); T, the energy-coupling module; A and A′, the pair of ABC ATPase. (Lower right) Possible q and Q salvage pathways in *B. henselae* Houston 1. All abbreviations of 7-deazapurine metabolism-related proteins are given in the text or Table 2.

phage genomes that encode them are modified with $preQ_0$ precursors such as CDG or ADG (6).

Target sequence specificity has been determined for two DpdA1 enzymes, both of which utilize $preQ_0$ as a substrate. The DpdA1 enzyme from Enterobacteria phage CAjan recognizes both "GA" and "GGC" sequences (100), while the DpdA enzyme from *Pseudomonas* phage Iggy specifically recognizes "GA" (101). Both enzymes replace the first guanine of the recognition sequence with $preQ_0$.

A predicted structural analysis of CAjan DpdA1 revealed striking similarities to TGTs (100). Notably, the binding pocket in CAjan DpdA1 resembles that of arcTGT, and both enzymes share two conserved catalytic aspartates. In addition, several residues (including Ser64, Phe67, Asp105, Gly153, Gly154, His132, and Phe189) are predicted to be involved in base binding activity. Asp206 is anticipated to catalyze the first step of the transglycosylation process, while Asp63 is likely responsible for the deprotonation of $preQ_0$ (100).

Diversity of deazapurine derivatives identified in phage DNA

To date, eight derivatives of 7-deazaguanine have been identified in phage genomes (Fig. 1). The first, known as dG^+, was initially discovered in Enterobacteria phage 9g (14), where it replaced ~25% of the guanine content. Subsequently, three additional modifications were observed in various phage genomes: 2′-deoxy-7-cyano-7-deazaguanosine ($dpreQ_0$), 2′-deoxy-7-amido-7-deazaguanosine (dADG), and 2′-deoxy-7-aminomethyl-7-deazaguanine ($dpreQ_1$) (10). $dpreQ_0$ was found in *Escherichia* phage CAjan, with 32% of the guanine undergoing modification, as well as in *Mycobacterium* phage Rosebush (28% guanine modification) and *Vibrio* phage nt-1 (0.1% guanine modification). dADG was found to modify 100% of the guanine in *Campylobacter* phage CP220 (102). It was hypothesized that QueC only performs the initial reaction resulting in ADG, which would then be loaded onto a 2′-deoxyribose and inserted into its genome by DpdA3 (Fig. 1), but this scenario needs to be further validated. The presence of dADG was also detected in small amounts within Halovirus HVTV-1, *Vibrio* phage nt-1, and *Mycobacterium* phage Rosebush, while it constituted the primary modification in *Salmonella* phage 7–11, affecting 0.02% of the guanine (10). In addition, $dpreQ_1$ was observed in Halovirus HVTV-1, where it modified ~30% of the guanine, and in *Streptococcus* phage Dp-1, affecting 1.7% of the guanine residues (10). In more recent discoveries, methylated and formylated forms of $preQ_1$ were identified, namely 2′-deoxy-7-(methylamino)methyl-7-deazaguanine ($mdpreQ_1$) and 2′-deoxy-7-(formylamino)methyl-7-deazaguanine ($fdpreQ_1$), alongside dCDG and its decarboxylated form, 2′-deoxy-7-deazaguanine (dDG) (6). mdpreQ1 was found in *Cellulophaga* phage phiSM, affecting 0.1% of the guanine residues, in combination with $dpreQ_1$, which modified 1.1% of the guanines. Similar findings were observed for *Cellulophaga* phage phi38:2 and phi 47:1. Meanwhile, $fdpreQ_1$ and dDG were both observed at a 100% replacement rate in *Flavobacterium* phage vB_FspM_immuto_2–6A and *Cellulophaga* phage phST, respectively. Lastly, dCDG was noted in Sulfolobus virus SVST-2, affecting 0.04% of the guanine content. The process through which dCDG is produced in the genome of *Sulfolobus* virus SVST-2 remains unclear but for the other seven modifications, the pathways have been nearly fully elucidated.

Phages that undergo modification with $dpreQ_0$ consistently carry the genes encoding the $preQ_0$ synthesis proteins FolE, QueE, QueD, and QueC, in addition to the signature enzyme DpdA (10, 14). Phages modified with dG^+ encode different non-orthologous enzymes like ArcS or Gat_QueC, which facilitate the final amidotransferase step akin to G^+ synthesis in Archaea (see below) (10). Phages encoding QueF are subject to modification by $dpreQ_1$, involving a change in the substrate of the cognate DpdA to $preQ_1$ (10). A $preQ_1$ methyltransferase, known as DpdM, has been identified and experimentally validated (6). DpdM is likely a metalloprotein with four cysteine residues capable of binding two metals. Furthermore, a proposed $preQ_1$ formyltransferase, DpdN, is a paralog of PurN involved in purine synthesis (103). A candidate CDG decarboxylase DpdL has also been suggested. DpdL, a member of the T-fold superfamily with a known affinity for pterins and purines (39), features an LxxxHRHxF signature motif binding a metal, indicative of an alkaline decarboxylation mechanism.

Synthesis of Q and Q derivatives in RNA

Once $preQ_1$ has been inserted in target tRNAs by the bTGT enzyme, two additional catalytic steps are required to finalize the synthesis of the Q molecule using quite unusual enzymes.

SAM is a ribose donor in the formation of epoxy-Q

The initial enzyme in the transformation of $preQ_1$-tRNA into Q-tRNA, known as S-adenosylmethionine:tRNA ribosyltransferase-isomerase or QueA (EC 2.4.99.17), transfers the ribose moiety from *S*-adenosylmethionine (SAM) with L-Met and adenine as byproducts. The identification of the *queA* gene occurred two decades ago in *E. coli* because it was

upstream of the *tgt* gene (104). This close syntenic association is prevalent, observed in ~50% of the 4,610 bacterial genomes in the KEGG database that encode both *tgt* and *queA* (data extracted from https://www.kegg.jp/kegg-bin/view_ortholog_table?orthology=K00773+K07568+K18979+K09765, Release 108.0, October 1, 2023).

Initial kinetic (105) and structural studies (106, 107) have revealed that this enzyme possesses a unique fold and operates through a fully ordered sequential bi-ter kinetic mechanism. In this mechanism, $preQ_1$-tRNA-Tyr binds first, followed by SAM, with product release occurring in the order of adenine, methionine, and epoxyQ-tRNA (or oQ-tRNA). The proposed mechanism involves a unique enzymatic pathway that includes sulfonium ylide and vinyl sulfonium intermediates (108). The five carbons from the ribose moiety of SAM are transformed into the cyclopropyl epoxide of the final product. As of now, no structures bound to substrates have been resolved, leaving the characterization of this unconventional use of SAM as a ribosyl donor incomplete (109).

The QueA protein family generally exhibits iso-functionality, except for members found in numerous Actinomycetes. In most bacteria within this clade, the Q modification is absent (110–112). QueA homologs in these Actinomycetes are encoded by genes that cluster with those encoding proteins of the short-chain dehydrogenase superfamily (Fig. 4) and should be renamed QueA-like.

Cobalamin-dependent or independent solutions for epoxy-Q reductase synthesis

In the late 1980s, it was discovered that the last step in Q synthesis, the reduction of epoxy-Q (oQ) to Q, is dependent on cobalamin (Vitamin B_{12}) (113). This insight originated from the observation that Q is present in *E. coli* tRNAs when grown anaerobically under fermentation conditions with limited iron but not in iron-abundant conditions. This pattern mirrored cobalamin biosynthesis, which is upregulated in anaerobic, iron-limited conditions but downregulated in aerobic or iron-abundant conditions. A genetic approach involving the deletion of *hemA,* a gene essential for cobalamin production, resulted in Q depletion in tRNA, accompanied by the accumulation of its direct precursor, oQ. This biochemical phenotype could be reversed by supplementing with 5-aminolevulinic acid. The gene responsible for the enzymatic reduction was identified 23 years later in 2011 through a biochemical screen of over 1,700 *E. coli* Keio collection deletion mutants, and the enzyme named epoxyqueuosine reductase (EC 1.17.99.6) or QueG (114). The QueG protein is homologous to cobalamin-dependent iron-sulfur proteins involved in halorespiration (114, 115). Recombinant QueG from *B. subtilis* exhibited activity on a synthetic substrate and hypomodified tRNAs from *queG*-deleted *E. coli*, requiring a reductant, a redox mediator, and stimulation by cobalamin. Structural studies of the *B. subtilis* QueG, including one with a bound tRNA-Tyr anticodon stem loop (indicating the positioning of the Q nucleoside in the enzyme's active site), have led to the proposal of a reaction mechanism involving the formation of a covalent cobalamin-tRNA intermediate (116).

A survey of bacterial genomes encoding the Q synthesis proteins revealed that QueG orthologs were absent in nearly half of these genomes (117), leading to the hypothesis that an alternative to QueG must exist in those genomes. A comparative genomics approach, focused on genomes lacking QueG, identified members of the DUF208 family as candidates for the missing epoxyQ-reductase enzyme and this hypothesis was validated using genetic approaches leading to renaming this family QueH (117). It was initially noted that purified recombinant QueH proteins did not bind cobalamin. Subsequent structural characterization of the *Thermotoga maritima* homolog confirmed that this protein adopted a novel fold, containing a [4Fe-4S] metallocluster with an intriguing adjacent, coordinated iron metal and an unprecedented mechanism for the reduction of epoxyqueuosine was proposed (118).

An updated analysis of the over 7,000 genomes present in the KEGG database shows that 65% of the genomes encoding both Tgt and QueA also encode the cobalamin-dependent QueG while 25% encode the cobalamin-independent

QueH (data extracted from https://www.kegg.jp/kegg-bin/view_ortholog_table?orthology=K07568+K18979+K09765+K00773). A small percentage of genomes (~9%) encoded both QueG and QueH (e.g., *Acinetobacter baylyi*) suggesting that in these organisms the availability of the cobalamin cofactor could drive the use of one enzyme over the other and that this could be a driving force behind the observed taxonomic distributions of *queG* and *queH*. Physical cluster data show that both *queG* and *queH* can sometimes be found adjacent to the *tgt* and *queA* genes (Fig. 4), reinforcing the strength of gene neighborhood information to link genes and functions (119). Finally, ~10% of the bacteria encoding Tgt and QueA lack homologs of both QueG and QueH, (e.g., in members of the *Polaribacter* clade). The pathway could stop at oQ in these organisms (e.g., as in *E. coli* MRE600) (120) or another reductase (specific or nonspecific) is yet to be discovered.

The only Q hypermodification found in bacteria is inserted by a paralog of glutamyl-tRNA synthase

Hypermodification of queuosine, by the addition of sugar or amino acid side chains, has been shown to occur sporadically and only for a subset of tRNAs. For example, galactosyl-Q and mannosyl-Q are only found in mammalian tRNAs (40) and the corresponding enzymes have only been recently identified (121). The only hypermodification identified in bacteria (mainly *Proteobacteria*) is glutamyl-Q (or GluQ) which is introduced specifically on the Q moiety present on tRNA-Asn by a paralog of glutamyl-tRNA synthase GluQ (YadB) (122–124).

Synthesis of archaeosine in DNA and RNA

The archaeosine base has been found in tRNA and DNA. In both molecules, its synthesis starts with the incorporation of $preQ_0$ into the target polymer by a member of the Tgt or DpdA family. $preQ_0$ moiety undergoes subsequent transformation into the archaeosine base through one or two catalytic steps, a process that varies depending on the organism (Fig. 1). The diverse enzymatic systems enabling the conversion of a nitrile to a formamidine moiety showcase instances of both convergent and divergent evolution (125). To date, three non-orthologous enzymatic systems catalyzing this reaction have been identified, all involving enzymes that are paralogs of those involved in queuosine biosynthesis.

Archaeosine synthase, or glutamine: $preQ_0$-tRNA amidinotransferase (ArcS, EC 2.6.1.97) was the first $preQ_0$ aminotransferase discovered nearly 15 years ago in *Haloferax volcanii* utilizing comparative genomics and genetics (126). ArcS, mainly found in *Euryarchaeota*, is a paralog of the aTgt enzyme, featuring an additional domain. It was first shown that the *Methanocaldococcus janaschii* ArcS could catalyze the amidinotransferase reaction *in vitro* (126). However, follow-up studies revealed a more complex pathway, requiring an additional radical-SAM enzyme, RaSEA (127). In *Methanosarcina acetivorans*, ArcS was observed to first link the ε-amino group of lysine to the cyano group of $preQ_0$. Subsequently, RaSEA activates the molecule for C-N bond cleavage, resulting in the formation of G^+ and 1-piperidine-6-carboxylic acid as a by-product (127). The vast majority (98%) of sequenced *Euryarchaeota* contain homologs of both ArcS and RaSEA, suggesting that the two-enzyme pathway is the primary route for G^+ synthesis in this clade (see data at https://www.kegg.jp/kegg-bin/view_ortholog_table?orthology=K06936+K07557). However, a few *Crenoarchaeota*, such as *Ignicoccus hospitalis* KIN4/I and *Thermofilum pendens*, only encode an ArcS homolog but no RaSEA homolog. In addition, ArcS homologs are found in dG^+ insertion clusters from phages lacking any neighboring radical-SAM encoding gene (10). This implies that a direct one-step route may be biologically possible and that further structural and biochemical studies are required for clarification (128).

In *Crenearchaeota*, the formation of G^+ can be catalyzed by distinct enzymes (129). For example, in *Pyrobaculum calidifontis,* this reaction is catalyzed by the QueF-like (QueF-L) ammonium: $preQ_0$-tRNA aminotransferase (EC 2.6.1.B18). This enzyme is a paralog of

QueF that lacks an NADPH-binding site but still makes a thioamide intermediate with the $preQ_0$-modified target tRNA and using NH_3 as a donor to make the G^+ product (129–131). In *Sulfolobus solfataricus*, the $preQ_0$ amidinotransferase reaction is likely catalyzed by a protein fusion between glutamine amidotransferase (Gat) and QueC (129) but the enzymatic details of this Gat-QueC remain unexplored. Both QueF-L and Gat-QueC homologs have been found in phages involved in the synthesis of the archaeosine base in DNA (10, 14), as discussed above. The molecular determinants that drive the substrate switch from RNA to DNA in these phage enzymes are still unknown.

Biosynthesis of dADG in bacteria: roles of DpdB and DpdC

The paradigm that deazapurine derivatives were found only in tRNA was broken by the detection of dADG and $dPreQ_0$ in bacterial genomic DNA, both originating from the $preQ_0$ precursor (14). In *Salmonella enterica* serovar Montevideo, a DpdA-DpdB complex integrates $preQ_0$ into DNA through a transglycosylation base exchange reaction, producing $dPreQ_0$, subsequently converted to dADG by DpdC (98, 99) (Fig. 1).

The *dpdB* gene is detected in 92% of genomes encoding DpdA proteins (132). It belongs to the DNA sulfur modification protein family DndB (IPR017642) which regulates the transcription of phosphorothioate (PT) DNA-modifying genes (133). The ATP hydrolysis function of DndB triggers the disassociation of the DndB-DNA complex, converting DndB-ATP into free DndB. This free DndB can then rebind to promoter DNA, thereby inhibiting transcription. DndB possesses a conserved DGQHR motif in its ATP-binding pocket, which corresponds to the DGQQR motif found in DpdB (14).

Although all DpdABC proteins can bind to DNA, DpdB shows the least DNA binding affinity (98), indicating that its ATPase activity, rather than DNA binding, is key for the base exchange reaction. A recent study on the DpdABC complex revealed that the DpdB ATP hydrolysis activity is essential for the *in vitro* base exchange reaction of DpdA (99). *dpdC* is found in 88% of the genomes harboring *dpdA* (132). DpdC possesses a domain resembling the peroxide stress protein YaaA (PF03883). The X-ray crystal structure analysis of *E. coli* YaaA revealed a positively charged cleft and a helix-hairpin-helix DNA-binding motif, characteristics shared by DNA repair enzymes (134). This aligns with the observation that DpdC has DNA-binding capabilities (98). *In vitro* incubation of $preQ_0$-modified DNA with DpdC resulted in the production of dADG-modified DNA, either with or independently of DpdA/B (99). This suggests that DpdC can convert $preQ_0$-modified DNA to ADG-modified DNA without relying on DpdA/B. However, it is important to note the possibility that DpdC first transforms free $preQ_0$ into ADG, which is then inserted into DNA by DpdA to create dADG. This hypothetical pathway has not yet been definitively excluded.

SALVAGE AND RECYCLING OF Q PRECURSORS

The biosynthesis of Q-tRNA imposes a significant demand on cellular energy and resources, involving the utilization of GTP, various metalloenzymes, and cofactors (Table 2). To mitigate this metabolic burden, all eukaryotes and many bacteria opt to salvage precursors rather than synthesize Q *de novo*. Eukaryotes acquire the queuine base (q), derived from Q-tRNA, through food or microbiota (40). While most bacteria appear to salvage $preQ_0$ and $preQ_1$, some also employ Q salvage routes, particularly among pathogens (7, 135) (Fig. 6). Dedicated transporters facilitating these salvage routes are only beginning to be characterized. Furthermore, given that Q is the only known tRNA modification that can be recycled, it is evident that cellular mechanisms must exist for reusing/recycling Q degradation products such as Q nucleosides and their phosphate derivatives. Their identity and role in Q metabolism are slowly emerging.

Diversity of transporters involved in salvaging Q

Although several strong transporter candidates had been predicted *in silico* (136, 137), the first experimental evidence for Q precursor salvage was reported in 2017 for the

COG1738 aka YhhQ family classified as the vitamin uptake transporter (VUT) family (TC 2.A.88). It has since been reclassified as Queuosine Precursor Transporter or QPTR (135). Genes encoding members of this family are associated strongly with Q pathway genes when analyzed by comparative genomic approaches (Fig. 4). QPTR (UniProt: P37619) from *E. coli* was shown to transport both $preQ_0$ and $preQ_1$ (Fig. 6A) with a slight preference for the latter (135).

The substrate specificity of Q precursor transporters can be predicted from the presence and absence of Q pathway genes (7, 135): QPTR homologs found in bacteria that harbor *queF*, *tgt*, *queA,* and *queG/H* are predicted to transport both $preQ_0$ and $preQ_1$, while those in organisms that lack *queF* but harbor downstream enzymes are predicted to only transport $preQ_1$. Noteworthy, some bacteria only encode TGT and QPTR homologs. The implication, supported by experimental validation of the corresponding *Chlamydia trachomatis* D/UW-3/CX genes, is that in these organisms, the TGT enzymes and QPTR transporters have switched their substrate specificity for queuine as observed in eukaryotes (7, 135) (Fig. 6B). There are some exceptions, such as *Bartonella henselae* that encode only TGT and QPTR enzymes that have retained the capacity to use $preQ_1$ as a substrate (138) (Fig. 6D).

The size of QPTR family members ranges from ~19 to 32 kDa and exhibit a predicted six transmembrane helices, and like other transporters, must be located at the inner membrane with a C-terminal inside appendage in the cytosol (135, 139, 140). However, as there are no known structural homologs present in the Protein Data Bank (PDB) for the QPTR family, it has not been possible to determine the residues involved in substrate recognition that could explain the observed shifts in substrate specificity (7, 135).

Energy-coupling factor (ECF)-type transporters are a subfamily of ATP-binding cassette (ABC) transporters (136, 141). While being exclusive to prokaryotes, they consist of two identical copies of cytoplasmic ATPases (A and A′) and two transmembrane units, namely the transmembrane component (T) and substrate-binding component (S). Group I ECF transporters use a dedicated energy coupling module [TAA′; e.g., *Rhodobacter capsulatus bioMNY* (A, T, S) transports biotin] while group II ECF transporters share the energy coupling module with other S components that transport different molecules (e.g., *Bacillus subtilis thiT* (S) transports thiamine) (141). Comparative genomic analyses predicted that members of the ECF family transported $preQ_1$: the group I QrtTUVW and the group II ECF-QueT (137). These predictions have been experimentally validated in only one organism, *Clostridioides difficile,* that encodes three ECF-QueT homologs (7). The heterologous expression of a reconstituted ECF complex in *E. coli* shows that one of the S components (CD630_16830) can transport $preQ_1$ and Q while another (CD630_2097) could only transport $preQ_1$ (7) (Fig. 6C). The distribution of QPTR/YhhQ and QueT/QrtT homologs in bacteria predicted to be transporting a Q precursor is sporadic (see data at https://www.kegg.jp/kegg-bin/view_ortholog_table?orthology=K00773+K09125+K16787+K16786+K16785+K16923+K01737+K10026+K06920+K06879+K09457+K07566, Release 108.0, October 1, 2023), suggesting that many more bacterial Q precursor transporters are yet to be identified. Candidates identified from gene fusion and physical clustering studies are currently under investigation (de Crécy-Lagard laboratory, unpublished).

Salvage enzymes can regenerate $preQ_1$ and Q from Q/QMP derived from tRNA degradation

Queuosine hydrolases are responsible for catalyzing the hydrolysis of the queuosine ribonucleoside to produce the queuine base and ribose. Two families with Q hydrolase activity have been characterized so far, QueK and Qng1 (7, 142, 143). Initially predicted through the analysis of genes regulated by $preQ_1$ riboswitches and named IunH (82), the first experimentally validated queuosine hydrolase is encoded by a gene under the predicted control of a $preQ_1$ riboswitch in *C. difficile* (7). Renamed QueK, this enzyme belongs to the Ca^{++}-dependent nucleoside hydrolase family (Fig. 6C). Further analyses, including sequence and structural assessments, identified signature motifs for

specifically annotating the QueK subgroup. The *queK* gene is frequently found in physical clusters, both with ECF-*queT* genes and *yhhQ* genes (Fig. 4).

Another family with Q hydrolase activity has recently been biochemically and structurally characterized. Initially designated DUF2419, this family was observed to co-distribute with the eukaryotic TGT enzyme subunit QTRT1 (8). Genetic studies demonstrated the involvement of members from this family in Q salvage in *S. pombe* and plants, although the precise reaction remained undetermined, despite structure modeling hinting at a potential nucleoside hydrolase role. Recent biochemical and structural characterizations confirm that the homolog from *S. thermophilus, S. pombe*, and humans, named Qng1, indeed hydrolyzes Q *in vitro* (142, 143). However, it preferentially targets the Q-5′MP and Q-3′MP substrates (143). The widespread presence of Qng1 homologs in many bacteria, along with their clustering with *tgt* genes or potential Q hydrolase genes (Fig. 4), suggests potential involvement in Q salvage, recycling, and/or degradation in these organisms. This hypothesis is yet to be experimentally validated and would require a TGT dedicated to queuine incorporation [similar to *C. trachomatis* (7)] or another enzyme for further breakdown of queuine into $preQ_1$ that could be used as substrate by canonical bacterial TGTs.

While numerous bacteria, including intracellular pathogens like *C. trachomatis*, directly salvage Q with a TGT with altered specificity from $preQ_1$ to Q (Fig. 6B), a subset of pathogenic bacteria has developed an indirect queuine salvage pathway (7) (Fig. 6C). In those organisms, $preQ_1$ is regenerated from queuine through the action of a recently identified enzyme, queuine lyase or QueL, which belongs to the radical-SAM family (see https://radicalsam.org/explore.php?id=cluster-2-7&v=3.0). The uncommon chemical mechanism involves a radical-mediated cleavage of a C-N bond along with the generation of cyclopentenone compounds.

QueL encoding genes are generally located in an operon with q*ueT* and *queK* but also with *yhhQ* genes (Fig. 4). These physical clustering associations suggest that queuine is imported from an environment where it is available (e.g., in mammalian blood) and recycled to form $preQ_1$ that, in turn, can be salvaged by most bacterial *tgt* without necessitating any changes in their sequence for substrate specificity adaptation.

We recently performed a phylogenomic prediction of intracellular organisms that encode the direct q pathway by encoding only a full-length TGT [**Fig, 7** of (138)]. We found it was prevalent and predicted in nearly all members of the *Dietziaceae*, *Gordioniaceae*, and *Anasplamataceae* families and half of the species in the *Borreliaceae* and *Corynabacteriaceae* families that all include major human pathogens. We also did a prediction of organisms that rely on the q indirect pathway (Fig. 7). These are sparse, spread all around the bacterial tree, and are mainly members of the *Fusobacteriia*, *Clostridia*, *Spirochaetia,* and *Erysipelotrichia* classes. Of note, other yet unidentified Q lyases might exist and current analyses are likely to underestimate the prevalence of the indirect q salvage capabilities.

PHYLOGENETIC DISTRIBUTION OF THE Q PATHWAY IN BACTERIA AND ARCHAEA

bTGT is the signature enzyme of the Q pathway, as it is the enzyme responsible for the base exchange. Hence, when a given genome harbors a *tgt* gene, it can be inferred with confidence that the corresponding organism salvages or synthesizes Q. However, one should ensure that *tgt* annotations are correct, as otherwise biological inferences will be erroneous. Common annotation issues arise due to the presence of *tgt* gene fragments and the miscalling of *dpdA* genes as *tgt* genes. The fragmentation of the *tgt* gene is commonly observed in organisms such as *Bartonella quintana* that have lost the Q pathway (138). Analysis of 4,245 complete representative genomes in the BV-BRC database (version 3.31.12) revealed they encoded 3,714 proteins annotated as queuine tRNA-ribosyltransferase (EC 2.4.2.29). In all, 50 (~12%) of those were shorter than 260 amino acids in length and further analyses showed these were *tgt* gene fragments, like in *B. quintana* (138). bDpdA proteins are currently annotated as "archaeosine

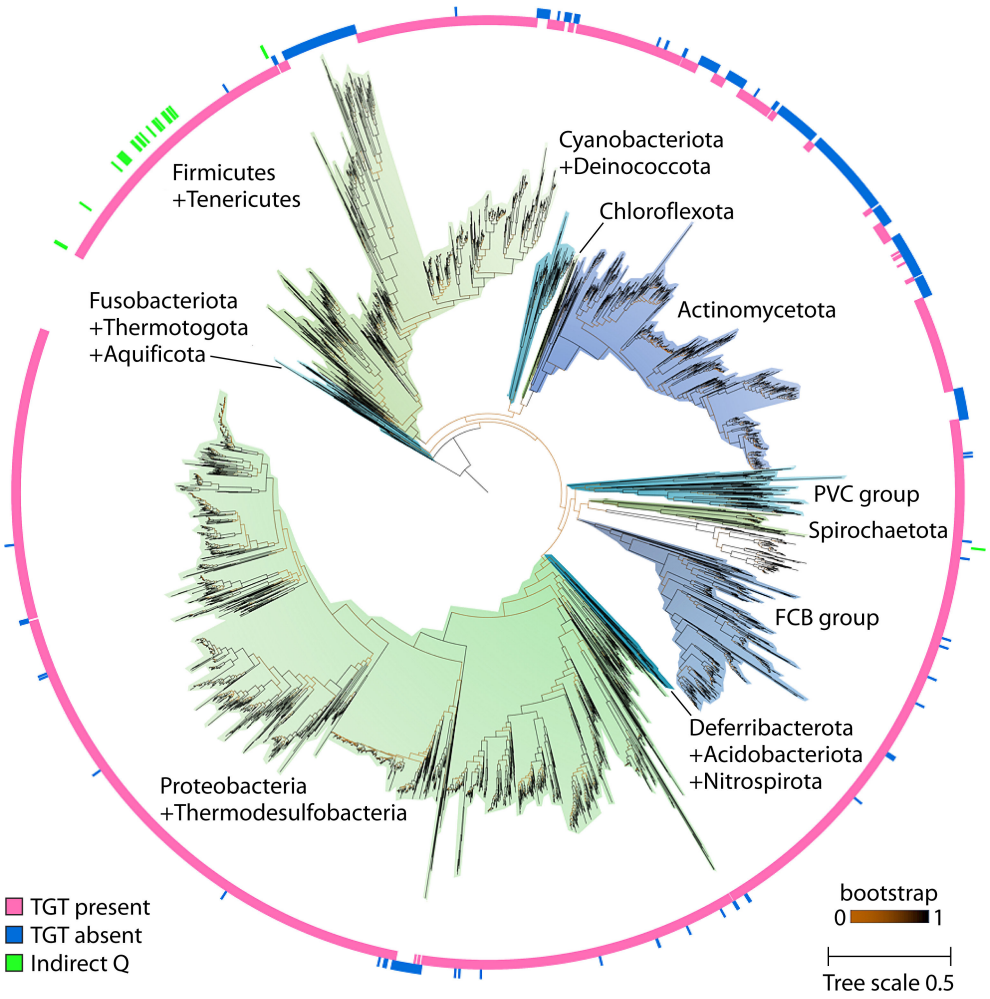

FIG 7 Presence and absence of encoded signature Q pathways protein TGT and of indirect q salvage pathways in representative bacterial genomes. A maximum likelihood tree of 10 concatenated ribosomal proteins was created for the species 4,231 complete representative genomes in the BV-BRC database (https://www.bv-brc.org/, version 3.31.12) (144) and the presence (red) or absence (blue) of a full length (>200 aa) TGT encoded protein annotated as Queuine tRNA-ribosyltransferase (EC 2.4.2.29) were noted in the outside circles. Genomes that encode the indirect Q salvage pathway (QueA, QueG/H, QueK, and QueL) are noted in green (outer circle). For better visualization, the branches are grouped and colored by phyla or clade. The branches were colored by bootstrap support value. The tree was visualized using the iTOL platform https://itol.embl.de/ (version 6.8.1) (145). The branch length scale bar indicates the evolutionary distance of 0.5 amino acid substitutions per site.

tRNA-ribosyltransferase (EC 2.4.2.-) type 5" in the BV-BRC database so they cannot be mistaken for bTGT. In other databases such as Uniprot, DpdA proteins are sometimes incorrectly annotated as queuine tRNA-ribosyltransferase (see Verru16b_03187, Uniprot ID A0A1D8AZ010) though the two families can be readily differentiated based on sequence similarity and gene neighborhoods (14).

The analysis of the *tgt* gene distribution across bacterial kingdoms shows it is uniformly spread, with independent losses in various clades (Fig. 7). This supports the hypothesis that Q was present in the common ancestor of bacteria. The clades that have lost *tgt* are the *Actinomycetiae* class, the *Tenericute* phylum, and the *Lactobacillaceae* family. For a few individual organisms in these groups, the absence of Q in tRNA has been experimentally validated (112, 146). Across the broader phylogeny, the loss of *tgt* is sporadic. It occurs mainly in symbionts or intracellular pathogens with minimal genomes even if the Q synthesis pathways are retained in many of such organisms, as seen in several *Buchera* or *Rickettsia* species (147, 148).

It was previously thought that the Q modification was only found in bacteria and not in archaea. However, it was recently reported that the entire Q pathway is encoded in some archaea, notably in *Woesearcheaota* genomes (149). A current limitation of these observations is the lack of experimental validation. To date, Q has not been observed in any archaeal tRNA.

DIVERSITY OF DEAZAPURINE-DERIVED NATURAL PRODUCTS

Microorganisms are prolific producers of a diverse array of natural products (NPs) (150) also referred to as secondary or specialized metabolites, which often confer fitness advantages in their environments (151). Exploring the biosynthetic capacities of the microbial world has revealed numerous anticancer (152), anti-inflammatory, photoprotectant (153), and antibiotic (154) NPs, including those harboring 7-deazapurine moieties (155). Deazapurine-derived NPs have recently attracted increasing scientific interest due to their diverse chemical structures and biological activities (156–158). The distinctive chemistry and biology of these nucleosides and nucleoside-like compounds offer an intriguing path to investigate their range of structures, biosynthetic pathways, and evolutionary histories.

Pyrrolopyrimidine-derived NPs exhibit a remarkable diversity, still far from being fully described. They are exemplified by the nucleosides toyocamycin from *Streptomyces toyocaensis* and *S. rimosus*, as well as sangivamycin from *S. rimosus*, that exhibit diverse bioactive properties including antibiotic, antitumor, and antiviral activities (16, 18). Tubercidin, discovered in *S. tubercidis*, also demonstrates versatile characteristics, including antimicrobial, antiparasitic, antiviral, and antitumor properties (15). Beyond these exemplars, the broader array of deazapurine-derived compounds reveals a myriad of functionalities, underscoring their potential as promising sources for novel therapeutic agents (Table 1).

The central precursor, $preQ_0$, serves a dual role, being not only a critical participant in DNA and tRNA modifications but also a precursor for putative NPs. In fact, $preQ_0$ itself has shown anticancer properties (159), potentially contributing to the bioactivity seen in its downstream NPs. Although sangivamycin, toyocamycin, tubercidin, huimycin, kanagawamicin, echiguanine, cadeguomycin, and dapiramicin share a common pyrrolopirimidine core, deazapurine-derived NPs have notable chemical diversity with examples of distinct structural modifications. Commonly, deazapurine-derived metabolites attach a ribose moiety to their core (17). The biosynthesis of pyrrolopyrimidines is carried out by a series of reactions, which is initiated with the conversion of GTP to $preQ_0$, orchestrated by pivotal genes including *folE*, *queD*, *queE*, and *queC* as previously described. For example, the toyocamycin biosynthetic gene cluster harbors dedicated homologs from $preQ_0$ biosynthesis. ToyD catalyzes the reaction of GTP to H_2NTP (as in GTP cyclohydrolase I; FolE), ToyB catalyzes H_2NTP to CPH_4 (as in QueD), ToyC catalyzes CPH_4 to CDG (as in QueE), and ToyM catalyzes CDG to $preQ_0$ (as in QueC). From here, further tailoring occurs depending on the gene content of the biosynthetic gene cluster (1, 28). For example, the presence and/or regulation of other biosynthetic genes can drive the conversion of one NP to another, as in *Streptomyces rimosus* where both toyocamycin and sangivamycin are produced through a common biosynthetic pathway (17). Sangivamycin emerges as a downstream product of toyocamycin under certain regulatory conditions that, when met, modify toyocamycin by a nitrile hydratase (TNHase), introducing a non-heme iron or non-corrin cobalt ion to amide nitrogen and cysteine sulfurs (1). In another example, huimycin is produced by $preQ_0$ methylation by HuiC (a SAM-dependent methyltransferase) before attaching N-acetylglucosamine through the glycosyltransferase HuiG (28). These tailoring variations represent only a subset of what has been described and what is yet to be discovered. As with other NPs, their unique chemistries can influence their interactions with enzymatic, biological, and ecological processes.

While many deazapurine-derived natural compounds have been identified (Table 1), their biosynthetic pathways remain elusive. This renders these compounds "orphans" in

a sense, as the genes responsible for their biosynthesis have not yet been characterized. In the MIBiG (**M**inimum **I**nformation about **Bi**osynthetic **G**ene Cluster) database (160), only tubercidin, toyocamycin, sangivamycin, and huimycin have their biosynthetic gene clusters (BGCs) partially annotated. In part, this is due to the lack of their biosynthetic rules being incorporated in conventional genome mining algorithms, such as antiSMASH (161). This gap underscores the need for orthogonal approaches to unveil the genetic diversity of these intriguing deazapurine-derived NPs, de-orphan known NPs by linking them to their cognate BGCs, and mine genomes for BGCs predicted to be novel examples of the class. As more putative deazapurine BGCs are identified, biosynthetic genes must be carefully annotated to properly predict their enzymatic functions. Comparative genomic analyses have uncovered genetic signatures associated with DNA (*dpdAs* genes) (14) and tRNA modifications (bacterial *tgt* genes and their homologs in archaea *arcTGT*/*arcS*) (91). If we exclude regions that encode these characteristic DNA and tRNA-modifying enzymes, the remainder may be BGCs responsible for generating deazapurine-derived NPs.

FUNCTION OF DEAZAPURINES

Functions of Q and G^+ in RNA

Complex roles of Q in decoding accuracy and efficiency

Q is exclusively found at position 34 of the anticodon stem-loop of the four tRNAs with GUN anticodons that decode the NAC/U codons encoding His/Tyr/Asn/Asp, all located in split codon boxes (Fig. 8). Over the last 20 years, a combination of studies in different organisms using +1 or −1 frameshifts (162, 163), amino acid misincorporations (11, 12, 164), stop-codon readthrough assays (13, 165), and sense codon reassignment analyses (166) have been employed to better understand Q function. Decoding of reporter genes with enrichments of C or U ending Q-dependent codons (13, 167–169), structures focusing on codon/anticodon interactions in the ribosome decoding sites (170, 171), evolutionary analyses (172), and ribosome profiling studies (12, 173) can be combined in a model where the presence of Q can stabilize or destabilize the interactions of the $Q_{34}U_{35}N_{36}$ anticodon with N_1A_2U/C_3 codons in the ribosome A site. This, in turn, homogenizes the translation rates of C or U ending codons and modulates the efficiency of second codon mismatch in both directions near cognate recognition (for Cys and Gly) (12, 164). The specific role of the Q modification in translation speed and accuracy does, however, vary greatly between tRNA isoacceptors and organisms (Fig. 8). For example, RiboSeq provides a genome-wide measurement of translation speed at every codon (174). Applied to mammals, it suggests that Q increases the speed or efficiency of decoding at all NAC/U codons but with a marked difference in ratios: the NAU codons are more dependent on the Q modification than are the NAC codons (173) as predicted from the pioneering studies of Grosjean and Nishimura that found that Q-containing tRNAs bind better to U than to C codons (4, 175). In *Schizosaccharomyces pombe* however, Q increases the translation speed of the codons G/CAC but not of the G/CAU Asp and His codons, whereas it decreases the translation speed of the A/UAU but not of the A/UAC Asn and Tyr codons (12). These results are consistent with the theory of Grosjean and Westhof (170) where codon-anticodon strength is equilibrated across the genetic code and Q plays different roles for intermediate strength codons (e.g., Asp, His) compared to weak codons (e.g., Asn, Tyr). The only bacterial RiboSeq study of Q-deficient mutants was recently performed in *Vibrio cholerae* where the absence of Q led to a more efficient translation of UAU (Tyr) and GAU (Asp) (13). These results contrast with a recent analysis of EGFP reporter genes recoded with only C or U ending Q-codons. Here, in an *E. coli queF* mutant, the U-ending codon reporter gene is translated less efficiently (20%) (168). Of note, the distinct types of Q-dependent U ending codons were not differentiated in this study. Some of the differences observed between codons could be caused by the presence of the hypermodification of Glu-Q on tRNA-Asp (166). It has recently been observed that *Shewanella glacialimarina* phage 1/4 influences the level of Q in

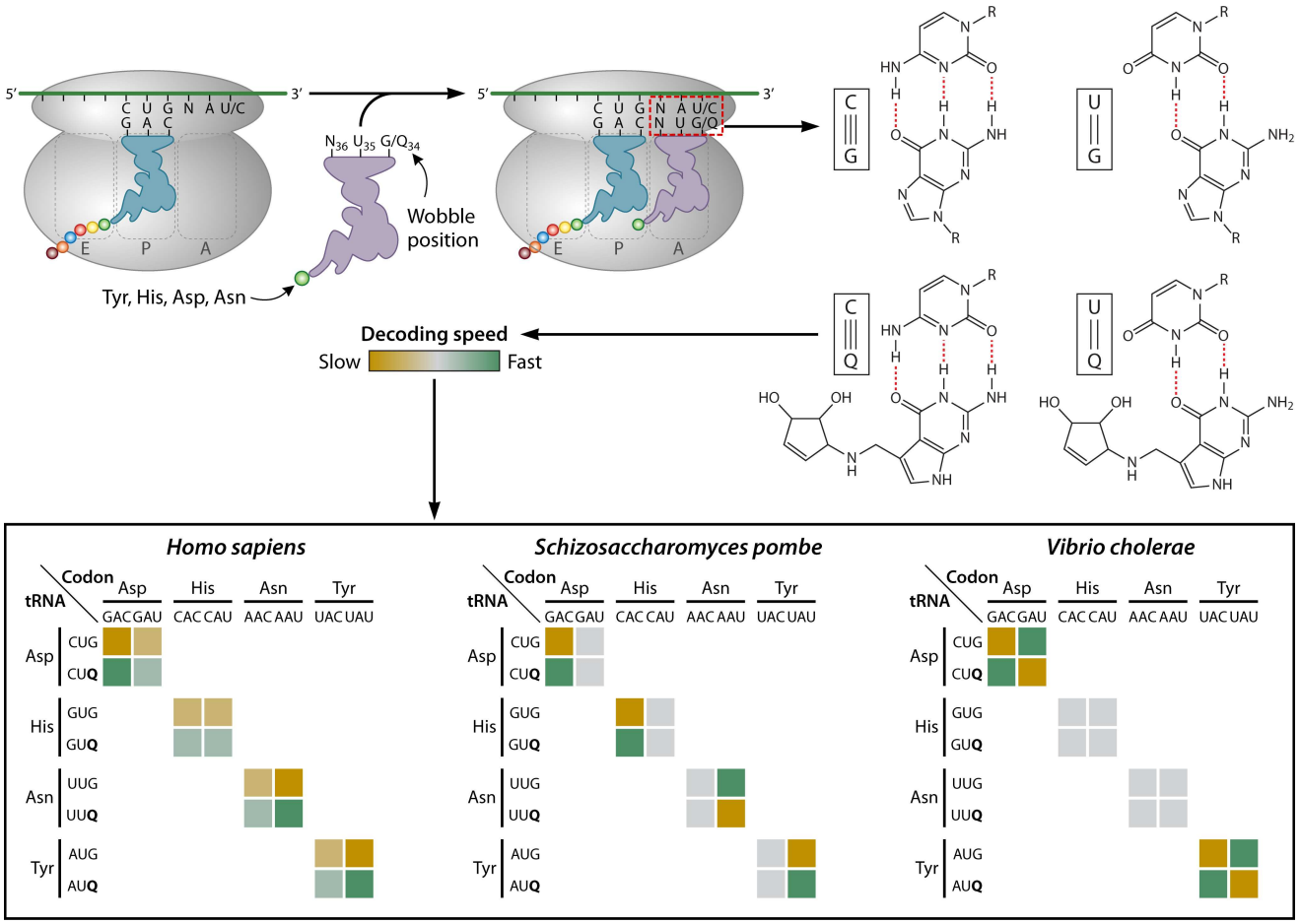

FIG 8 Effects of Q on decoding speed in different organisms. Decoding of GUN codons by Q-modified tRNAs in ribosomal A sites. The hydrogen pairing pattern of the wobble base is affected by the presence/absence of Q and decoding speed has been measured by RiboSeq in three organisms to date: *H. sapiens* (173), *S. pombe* (12), and *V. cholerae* (13).

tRNA throughout the phage infection cycle, lowering it in the early stage and gradually increasing it (176). It is proposed to help in the translation of the genes expressed late in the cycle to have a preferred GUA codon for tyrosine decoding.

Although Q at position 34 clearly fine-tunes the efficiency and accuracy of translation, its role varies with each of the four Q-modified tRNAs and one must be cautious not to generalize findings from one organism to another without further experimental validations. Indeed, the role of Q is a consequence of an evolutionary adaptation to all other components of the translation machinery, including the presence of other tRNA modifications or the codon usage that is specific to every species. Q is predicted to have been present at the origin of bacteria (177), yet organisms can adapt to life without this modification, as it has been repeatedly lost along the tree of life (Fig. 7). More studies are required to understand the role of Q in translation in different bacteria and in the few Archaea where it is present. This could include RiboSeq analyses of Q^+ and Q^- in *E. coli* and *B. subtilis*, to better link the pleiotropic phenotypes caused by Q deficiency (discussed below) and the underlying molecular mechanisms.

Q is rarely a determinant for other enzymes interacting with tRNAs

Q functions as a determinant for Dnmt2, the enzyme responsible for inserting the m^5C38 modification in tRNAs in Eukaryotes (178). However, it is not known to act as a determinant for any modification enzyme in bacteria. In *E. coli*, tRNA extracted from a Q^- strain

does not show any variations in the levels of other tRNA modifications (unpublished data from Dedon and de Crécy-Lagard). Although it has been reported that tRNA-Tyr in *E. coli* is less efficiently charged when Q34 is replaced by C34 (179), the absence of significant growth defects in an *E. coli tgt* mutant suggests that this defect may not be relevant *in vivo* (180, 181). Q has been implicated in protecting against ribonuclease cleavage in mammals (182), but this protective role has not been observed in bacteria to date. Ribotoxin E5 specifically cleaves Q-modified tRNAs *in vivo*, yet Q is not a determinant for recognition (183).

Pleiotropic phenotypes linked to Q or GluQ deficiency vary across organisms

As listed in Table 3, the absence of Q leads to a wide variety of phenotypes across bacteria. Until recently, it was thought that Q was dispensable as several important model organisms such as *Saccharomyces cerevisiae*, *Arabidopsis thaliana,* or *Mycoplasma genitalium* have lost the enzymatic capacity for the modification (184) and the *tgt* mutant of *E. coli* does not show any growth defects in most conditions (180). The only notable phenotype was the virulence deficiency of a *Shigella flexneri* Q^- strain caused by a reduced expression of the *virF* regulator (185). Several studies in the last 3 years have now changed this view with roles in oxidative stress resistance, biofilm formation, and metal homeostasis emerging as common themes.

TABLE 3 Phenotypes linked to Q genes deficiency or overexpression in bacteria

Organism	Phenotype	References
Oxidative stress		
Escherichia coli	*tgt* mutant is slightly more sensitive to oxidative stress.	(181)
Streptococcus thermophilus	*tgt* mutant is more sensitive to oxidative stress	(186)
Vibrio cholerae	Translation of regulator of oxidative stress *rtxA* is increased in *tgt* mutant	(13)
Metal homeostasis		
Escherichia coli	*tgt* mutant is more resistant to cobalt and nickel and more sensitive to cadmium	(181)
Acinetobacter baumanii	*queD* and *tgt* expression induced by metal sequestration enzyme and metal limitation reduces Q levels in tRNA	(57, 187)
Arthrobacter viscosus	Overexpression of the *queC* gene in *E. coli* confers aluminum resistance	(188)
Erwinia amylovira	*yhhQ* and *queF* overexpressed in high copper	(189)
Neisseria meningitidis	*queC* and *queF* induced by zinc limitation	(190)
Pseudomonas putida	*queF/cinQ* expression is induced by copper but mutant does not give any copper sensitivity	(191)
Agrobacterium tumefaciens	QueF is highly induced by manganese limitation	(192)
Virulence		
Shigella flexneri	Reduced virulence in *tgt* mutant because of decreased levels of VirF	(193)
Rhizobium meliloti	Mutants in *queC*, *queF,* and *tgt* are deficient in triggering cytoskeleton modification in uninvaded Hela cells	(194)
Escherichia coli	Biofilm and cell aggregates diminish in a *ΔqueF* and iboth with the addition of LPS l levels increase when some of the Q synthesis genes are overexpressed	(168)
Miscellaneous		
Escherichia coli	*tgt* mutant has fitness defect in the stationary phase	(180)
	Growth defect with streptomycin but not ampicillin or spectinomycin	(181)
Streptococcus gordonii	*queA* mutant has fitness cost in the stationary phase	(195)
Vibrio cholerae	TnSeq data show that mutations in *tgt* and *queADEF* genes confer sensitivity to sub-MIC tobramycin	(196)
Bacillus subtilis	Overexpression of *queCDEF* genes in VBNC cells and *queG* mutant more sensitive to Kan in those cells	(197)
Pseudomonas simiae	TnSeq data show that *queA* mutants are deficient in deoxyribose catabolism	(198)
Staphylococcus epidermidis	*queF*, *queH,* and *tgt* induced by pH	(199)
Bacillus subtilis	Sporulation and biofilm reduced in *ΔqueF* mutant	(168)
Pseudomonas putida	Growth inhibition of *E. coli* by *P. putida* increased when *queF* is overexpressed	(168)

Two types of oxidative stress phenotypes have been linked to Q deficiency in bacteria to date. The first is a mild sensitivity to oxidative stress as seen in *S. thermophilus* and *E. coli* (Table 3) that is not yet understood at the molecular level, but it is possible that this sensitivity may be due to a general response to protein aggregation triggered by conditions that affect translation speed, as previously discussed (181). This mechanism tying Q and oxidative stress could be conserved between kingdoms (200, 201). The second is a Modification Tunable Transcript (or MoTT) regulatory mechanism (202, 203), as seen in *V. cholerae* where the translation of the *rtxA* gene is decreased under Q excess because it is enriched in tyrosine encoding TAT codons (13). In *V. cholerae*, RtxA inactivates the main oxidative stress activator SoxR. High Q levels would lead to an increased oxidative stress response than would low Q levels. This response is required to resist aminoglycosides and would explain why the *V. cholerae* Q^- mutants are more sensitive to tobramycin (196). It was also shown in *V. cholerae* that the transcription of the *tgt* gene is regulated by the central regulator CRP and by the stringent response. This leads to an intricate regulatory model where stress increases the levels of Q, leading to decreased levels of TrxA and the induction of oxidative stress response through the activation of SoxR. This second mechanism appears species specific as the regulation observed in *V. cholerae* is absent in the fellow *Enterobacteriaceae E. coli*.

The role of metals in Q synthesis was first observed by one of the pioneers in the study of Q synthesis, Helga Kersten, who found that the presence of iron and B_{12} in the media affected the ratio of oQ/Q in *S. typhimurium* and *E. coli* (113) because the last enzyme in the Q pathway in this organism is the B_{12}-dependent iron-sulfur cluster enzyme QueG (114). Additional sporadic observations have linked metal and Q synthesis genes over the years (Table 3). Most of the enzymes in Q synthesis are metal dependent (57, 204) (Table 2). FolE, QueD, QueC, and Tgt are zinc-dependent enzymes and QueE is an iron-dependent radical-SAM enzyme. Comparative genomic analyses of genes in the Zur regulon predicted that Q might be required under zinc limitation in certain organisms (50, 205). In addition, metal limitation was shown to lower Q levels in *Acinetobacter baumanii* and induce the expression of Q biosynthesis genes (57, 187), but it is yet to be shown that these phenotypes are part of a regulatory circuit. The *E. coli tgt* mutant is more resistant to nickel and cobalt and more sensitive to cadmium (181). The sensitivity to nickel is possibly caused by a lower expression of the nickel transporter encoding operon *nikABCDE* when Q is absent, but the underlying mechanism has not been elucidated. One hypothesis that is yet to be experimentally validated is that NikR the repressor is enriched in TAT codon and could be efficiently translated in the absence of Q (13).

A recent study combining proteomic, codon-usage analyses, and phenotypic validations in several model bacteria reported reduced biofilm formation in *queF* mutants of both *E. coli* and *B. subtilis* (168). The study also linked several other virulence-related traits/proteins to Q deficiency and/or to an enrichment in Q-dependent U ending codons. The authors proposed that Q could have a general role in regulating bacterial virulence by modulating the translation of virulence genes. This hypothesis is yet to be validated with RiboSeq data, recoding of target genes, and identification of the signal(s) that would modulate Q levels in conditions where virulence genes would be differentially expressed.

Q biosynthesis is complex, requires several metals (as discussed above), draws on many building blocks from central metabolism (GTP, SAM, ATP, cobalamin) (Table 2), and shares intermediates with essential cofactors such as tetrahydrofolate (Fig. 3). Thus, Q could be used to monitor many aspects of cellular physiology (206). We anticipate that the next few years will reveal more examples of regulatory roles of the Q modification in bacteria as more phenotypes get reported and their molecular mechanisms get fully dissected (as done to date only in the case of the *V. cholerae* RtxA). This will likely be accelerated as new methods for Q detection become more accessible (207–212) and more RiboSeq data sets of Q-deficient cells are generated.

We emphasize, however, that based on the current known cases, MoTT-dependent regulations when identified will be very species-specific. In many organisms, the absence of Q might just lead to a mild increase in amino acid misincorporations and/or aggregation phenotypes (12) that could become problematic under additional proteotoxic stresses, as with many other tRNA modifications deficient cells (213).

The precise role of the hypermodified Glu-Q tRNA-Asp is not fully understood, but it has been associated with stress resistance, observed through its co-transcription with the stringent response-regulated gene *dksA* in many *gammaproteobacteria*, including a *Shigella flexneri* mutant that lacks the Glu-Q modification that demonstrates increased sensitivity to osmotic stress (214).

Q and its precursors are micronutrients

All eukaryotes salvage the queuine (q) base derived from bacterial Q directly from the microbiota or indirectly through the diet (40). The last 10 years have seen a reemergence of Q as a micronutrient important for human health (215, 216), particularly for optimal brain function (217, 218). Even if it is yet to be explored, queuine should also be an important micronutrient for the health of most plants but crucifers that have lost Q biosynthesis genes (8). Another unexplored area is how bacteria compete for Q precursors particularly in specific niches. Different bacteria of the microbiota can make Q *de novo*, be $preQ_1$/$preQ_0$/Q scavengers, or have lost all genes of the pathway (7, 168). This may encourage competition between sympatric organisms for Q as is observed for B vitamins (219, 220). Indeed, Q supplementation leads to an increased level of α-diversity among intestinal microbiota (42). The amount of Q produced and utilized by the gut microbiome will have health consequences on the host that are just starting to be appreciated. For example, the gut microbiome is enriched in Q-producing bacteria in obese mice (41) or chickens raised outdoors compared to indoors (221).

Structural role of G^+ in tRNA stability

Since its discovery in the early 1990s (5), the proposition that G^+, primarily located at position 15 and occasionally at 13 in archaeal tRNAs (91), plays a structural role has been substantiated. This hypothesis has been confirmed both *in vivo* and *in vitro* across various archaeal models. Experiments involving random and targeted deletion of G^+ synthesis genes in thermophilic and mesophilic archaea (9, 91, 222, 223) demonstrated that the absence of G^+ in tRNAs resulted in a thermosensitivity (Ts) phenotype in the hyperthermophile *Thermococcus kodakarensis* but not in the mesophiles *Haloferax volcanii* and *Methanosarcina mazei*. A comparison of thermal denaturation profiles between fully modified *T. kodakarensis* tRNAs and naked transcripts with or without G^+ revealed that the presence of G^+ protected from melting, particularly in the transcripts. These findings, obtained by two independent laboratories, collectively underscore the role of this modification in adapting to high growth temperatures. Nevertheless, the conservation of this modification in most sequenced Archaea (i.e., not only limited to thermophiles), the absence of a Ts phenotype in G^+-deficient *M. mazei,* and the cold-sensitive phenotype of G^+-deficient *H. volcanii* suggest that the roles of G^+ might extend beyond thermotolerance.

Function of deazapurines in DNA

dADG is used by bacteria to discriminate self from non-self

The bacterial *dpdABC* genes that modify genomic DNA with dADG are located in genomic islands called *dpd* islands. These islands contain a consistent set of nine genes (DpdABC-DpdEGIJKD) with minor variations, that are sporadically distributed around the bacterial phylogenetic tree and most certainly spread through horizontal gene transfer (14). Classical transformation efficiency experiments revealed that plasmids extracted from cells expressing *dpdABC* and subsequently modified with dADG were more efficiently transformed in host cells harboring *dpdEFGHIJ* than plasmids extracted

from cells expressing *dpdAB* and hence modified with $preQ_0$ or unmodified plasmids. These differences in transformation efficiencies disappeared after the disruption of any of the *dpdEGIJKD* genes (98).

These features bear a resemblance to the well-characterized "self-nonself discrimination" mechanism of methylation-based Restriction-Modification (R-M) systems. R-M systems, typically composed of a methyltransferase (MTase) and a restriction endonuclease (REase), are considered primitive immune systems in bacteria, protecting against bacteriophages or other invading DNA. A similar defensive feature suggests that DpdABC-DpdEGIJKD constitutes a novel dADG-based R-M system, recognizing the dADG status of invading foreign DNA, such as plasmids (14, 98). DpdABC modifies DNA with dADG, while DpdEGIJKD acts as the cognate restriction enzymes that recognize foreign DNA lacking ADG modification and may initiate its cleavage.

Deazapurines are used as anti-restriction strategies by phages

The suggestion that 7-deazaguanine modifications might impede digestion by restriction enzymes was proposed upon their discovery in Enterobacteria phage 9g (14), given the observed resistance of the phage's DNA to initial digestion (224). Subsequent comprehensive testing by New England Biolabs on this phage DNA revealed that only restriction enzymes interacting with guanines would be inhibited (225). Notably, EcoRV, known to have guanine in its recognition site, was entirely inhibited when the guanine was replaced by a 7-deazaguanine (226). Further exploration involving various bacteriophages with 7-deazaguanine derivatives confirmed that all natural 7-deazaguanines protect against digestion (6, 10). A connection between the recognition sequence of *Enterobacteria* phage CAjan ("GA" and "GGC") and the range of inhibited restriction enzymes was established, indicating that only enzymes with recognition sites containing "GA" are affected (100). In addition, $dPreQ_0$ in *Pseudomonas* phage iggy was found to protect against Cas9 digestion and potentially other DNA-degrading defense systems (101).

Function of deazapurine small molecules

In therapeutic contexts, toyocamycin, while potent against tumors, also demonstrates substantial host toxicity (3, 17). Tubercidin and its analogs are potent antimicrobials, particularly against *Candida* species and *Mycobacterium tuberculosis* (227). Sangivamycin and echiguanines A-B exhibit high cytotoxicity and inhibit protein kinase C. The mechanism of action for echiguanines could be involved in phosphatidylinositol turnover and with cell surface tyrosine kinase receptors [members of the platelet-derived growth factor (PDGF) family]. On the other hand, sangivamycin might have two possible mechanisms of action: cell death by apoptosis [i.e., protein kinase C (PKC) and c-Jun NH_2-terminal kinase (JNK) activation] and by growth arrest [i.e., cyclin-dependent kinase (CDK) inhibition, DNA damage, and p21 induction) associated with multidrug-resistant breast cancer lines (16, 22). Collectively, these compounds intervene in cellular processes associated with adenine nucleosides, exhibiting diverse effects rather than targeting a singular cellular entity or process (1). As such, these compounds showcase various modes of action, leading to hypotheses that their impacts on cellular metabolism can occur at multiple levels. Despite the paucity of identified self-resistance mechanisms for 7-deazaguanines in the producing organisms, the extracellular release of these molecules in the culture might suggest that it safeguards the producing strains (1). However, there is no empirical evidence for this assumption and further analyses are required to validate this hypothesis.

DETECTION AND BIOTECHNOLOGICAL USES OF DEAZAPURINES

7-deazapurine detection methods

Gel-based assays coupled with Northern blotting that separate and detect Q-modified tRNA through the addition of acryloylaminophenyl boronic (APB) acid have made Q detection accessible to numerous laboratories since the 1980s (228). However, the utility of APB gels is confined to Q detection alone. Traditionally, the detection and quantification of other deazapurines relied on liquid chromatography-mass spectrometry (LC-MS) (229). Although lacking single nucleotide resolution, the heightened sensitivity of these methods now allows the detection of modifications with minimal starting material, making them indispensable when combined with synthetic chemistry for the discovery of new modifications (230, 231).

Nanopore technology was successfully used to detect $preQ_0$ in phage DNA (100, 101) but next-generation methods have also recently been developed to detect Q, $preQ_1$, and $preQ_0$ in RNA. To be detected at the single nucleoside level, chemical treatment (211) or labeling of the tRNA by a non-natural $preQ_1$ derivative (232) must be performed before sequencing, even though some polymerases have been found to make more mistakes in the presence of Q and hence can be used to detect the modification by mapping the errors (208, 212). Direct sequencing by nanopore was recently used to detect Q and $preQ_1$ in tRNA (208, 212). This method does not require prior treatments or labeling but does require comparing modified to unmodified samples, reminiscent of bisulfide sequencing strategies to measure DNA methylation patterns. The toolbox for deazapurine detection is expanding with single base resolution methods and although many are not yet cost-effective for tRNA modifications, they may be critical to survey the locations of deazapurine modifications in DNA.

Biotechnological uses of deazapurines and their biosynthetic enzymes

At the frontiers of unique biochemistry and microbial defense systems, deazapurines and their biosynthetic enzymes have emerged as versatile molecular tools in green chemistry, genetic engineering, and pharmaceutical development. Harnessing the enzymatic reduction of nitrile to primary amine would be of great interest for green chemistry applications (233). This reaction is crucial in synthetic chemistry, traditionally involving harmful reducing agents and complex blocking/deblocking processes (234–237). Exploring enzymatic nitrile reduction offers an alternative to synthetic methods. Despite QueF enzymes typically favoring $preQ_0$ as a substrate, advancements in enzyme design raise the possibility of using QueF as a template for creating versatile nitrile reductases that accept a broader range of substrates. A systematic screening of QueF type I, II, and QueF-like enzymes could identify homologs that could accept non-canonical substrates and serve as the basis for directed evolution approaches that could greatly expand our chemical/enzymatic toolkit (238).

Wild-type bacteria encode multiple defense systems against mobile genetic elements (MGEs), such as plasmids, transposons, and bacteriophages (239). In many cases in both bacteria and archaea, the first line of defense is provided by restriction/modification systems. Several of these MGEs are employed in genetic engineering applications, including plasmids in complementation assays, transposons in mutagenesis, and strategies against pathogens (e.g., phage therapy). Thus, it has been proposed to use 7-deazaguanine DNA modifications as a shield to protect against a wide variety of restriction enzymes (225) [and potentially other defense mechanisms (101)] during the initial entry of the MGE (International Patent Application No. PCT/US20/21886). Consequently, the 7-deazaguanine-modified MGE would withstand the first defensive barrier of the target bacteria, avoiding degradation by an organism's restriction-modification systems.

As we discussed before, pyridopyrimidines derivatives exhibit utility as anticancer, antiviral, and antibiotic agents, which can lead to the discovery of deazapurine-like NPs or serve as a scaffold for synthetic or mimics NPs (240, 241). For instance,

some FDA-approved drugs such as ribociclib (242) fododesine, and ruxolitinib contain 7-deazapurine moieties, which are being used to treat breast cancer, leukemia, and pleural mesothelioma, respectively (243). In another study, using 7-deazapurine, as an alternative to conventional purine structures, researchers were able to synthesize compounds that exhibited significantly enhance characteristics. These compounds not only displayed greater potency but also showcased an increased level of selectivity. Moreover, the resultant compounds exhibited favorable pharmacokinetic properties, making them highly promising candidates for applications in the context of cardiac troponin I-interacting kinase (TNNI3K) (244).

7-Deazapurines act as important analogs of biogenic purine nucleosides. Upon replacing the N7 atom with carbon, these compounds gain increased electron density, enabling diverse substituents at the C7 position. This increased electron density makes 7-deazapurines particularly versatile in terms of their chemical functionalization. These modifications may be crucial in the development of compounds with enhanced biological activity, especially in the context of interactions with nucleic acids like DNA and RNA (157).

CONCLUSIONS

The recent surge in 7-deazapurine-related research prompted this review. Following the initial exploration of Q and G^+ in the 1980s, the field experienced a downturn by the end of the century, with only a few papers annually. However, the advent of whole-genome sequences, coupled with the rising interest in epigenetics and epitranscriptomics, has sparked a renaissance, resulting in over 20 papers per year. This body of knowledge remains however confined to specialists, as pathway databases inadequately capture it. Comparison among Gene Ontology (245), KEGG (246), and MetaCyc (247) (Table 4) reveals that while Q synthesis is well documented, archaeosine synthesis is incomplete, and DNA modification pathways, even for those like dG^+ published seven years ago, are nonexistent. Consequently, recent papers may overlook that $preQ_0$-related clusters in certain phage genomes pertain to DNA modification genes, not tRNA modification genes (248).

TABLE 4 Comparison of deazapurine-related pathway annotations in knowledge databases

DatabAse objects	Pathway names	Notes
Metacyc[a]		Good coverage of RNA modifications with a few missing enzymes or intermediates, No DNA modifications, one natural product.
PWY-6700	Pathway: queuosine biosynthesis I (*de novo*)	Q and GluQ synthesis complete
PWY-8105	Pathway: queuosine biosynthesis II (queuine salvage)	Direct q salvage, no transporter, no Qng1
PWY-8106	Pathway: queuosine biosynthesis III (queuosine salvage)	Indirect pathway from *C. difficile*, complete with transporter (RXN-21036)
PWY-6703	Pathway: $preQ_0$ biosynthesis	Missing ADG intermediate
PWY-6720	Pathway: toyocamycin biosynthesis	Complete
PWY-6711	Pathway: archaeosine biosynthesis I	ArcS missing raSEA
PWY-7923	Pathway: archaeosine biosynthesis II	QueF-like but missing Gat-QueC
KEGG[b]		All pathways nonexistent except for $preQ_0$
map00790	Folate biosynthesis	Only $preQ_0$ synthesis as part of the folate pathway map, no ADG intermediate
Geneontology.org[c]		Queuosine synthesis is well described; the other pathways are inexistent or fragmentary
GO:0046116	Queuosine metabolic process	From GTP to Q in Bacteria, missing FolE2, and QueH
GO:0002927	Archaeosine-tRNA biosynthetic process	Very fragmentary in terms of enzyme captures
GO:1990397	Queuosine salvage	Just QPTR no other transporter gene
CHEBI:134606	Toyocamycin	Not linked to genes
CHEBI:45075	$preQ_0$	Not linked to genes

[a]https://metacyc.org.
[b]https://www.kegg.jp/kegg/.
[c]https://amigo.geneontology.org/amigo/landing.

This review addresses lingering questions that will drive future research. Although pathways for $preQ_0$-derived molecules are better understood than ever, validation or discovery of some pathway enzymes is still required (Fig. 1). Many transporters for Q precursors are unidentified, and comprehension of how Q precursors circulate within microbial communities or between communities and hosts remains inadequate. The newfound associations between Q, considered a quasi-vitamin source (215), and various human diseases (249, 250) have spurred investigations into Q synthesis in the human microbiota (251), but more studies are needed to fully understand the interplay of the gut microbiome and the diet in supplying the Q precursors to the human host. Q degradation is unexplored, and like other modified ribonucleosides (252, 253), Q may serve as a carbon or nitrogen source.

Furthermore, knowledge about the regulation of 7-deazapurine synthesis genes in bacteria without riboswitches is nonexistent. Mechanistically, the observed differences in how Q affects the decoding speed of U or C-ending GUN codons between species (Fig. 8) lack understanding. In addition, the prevalence of regulatory circuits using Q, as described in *V. cholerae* (13), and whether $preQ_0$ and $preQ_1$ have roles as signaling molecules remain unknown.

Comparative genomics of the enzymatic machinery involved in the production of deazapurine NPs can unveil valuable insights into the evolutionary forces that shape these pathways, including patterns of conservation, duplication, and adaptation (151, 254). Importantly, 7-deazapurines are not confined solely to bacterial sources. Their occurrence in sponges and algae underscores their broader distribution (1), perhaps suggesting an ancient ancestral origin and potential biological interactions mediated by this privileged structural motif that has perpetuated it across diverse evolutionary lineages. The structural diversity seen in these compounds might reflect an evolutionary divergence that has occurred to adapt to distinct niches.

The potential use of 7-deazaguanine derivatives in biotechnology is a promising avenue for exploration. These DNA modifications, protecting against restriction enzymes and possibly other defense systems, offer opportunities for research involving naturally isolated bacteria that are challenging to genetically manipulate. Alternatively, bacteriophages modified with 7-deazaguanine emerge as strong candidates for phage therapy, given their increased likelihood of surviving the initial infection round (International Patent Application No. PCT/US20/21886).

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AUTHOR BIOS

Valérie de Crécy-Lagard, after obtaining a bachelor's degree at Ecole Polytechnique in 1987 and a Ph.D. in microbial genetics at the Pasteur Institute (Paris) in 1991, worked in diverse academic and industrial settings using the power of bacterial genetics to study primary and secondary metabolism as well as mechanisms of regulation by proteolysis. In the past 25 years, her work has focused on combining comparative genomic analysis with experimental methods to discover the function of the many "unknowns" found in sequenced genomes, first at the Scripps Research Institute and then, since 2004, in the Microbiology and Cell Science Department at the University of Florida where she is now a Distinguished Professor. This led to solving many long-standing mysteries, particularly in the fields of coenzyme metabolism and transfer RNA (tRNA) modification. In parallel, she collaborates with biotech groups on using long-term cultures to evolve microorganisms with specific traits.

Geoffrey Hutinet holds a bachelor's degree from the University of Paris XI in France, earned in 2011, and completed his Ph.D. at INRAE of Jouy-en-Josas, France, in 2014. During his Ph.D., he delved into bacteriophages and protein-DNA interactions. In early 2015, Geoffrey joined the University of Florida as a post-doctoral fellow, rising to the position of Biology Scientist III in 2019. His work initially focused on a newly discovered system inserting deazaguanine into bacterial DNA and shifted to a similar system in bacteriophages that he identified. Geoffrey Hutinet played a pivotal role in uncovering various types of deazaguanine DNA modifications and deciphering the pathways leading to them. Currently serving as a Visiting Assistant Professor at Haverford College, he continues his research on deazaguanine modification systems and expands to explore other modification systems, aiming to comprehend their roles in virus-host interactions.

José D. D. Cediel-Becerra earned his Summa Cum Laude bachelor's degree in biology from the Industrial University of Santander in Colombia in 2021. His honors research thesis focused on uncovering natural products (NP) with photoprotective potential in bacterial wild-type strains. As an undergraduate, José published his thesis findings as the first author, leading him to receive the laureate thesis award. Fueled by his interests in the NP field, bacterial genomics, and computational biology, José joined the Microbiology and Cell Science Ph.D. Program at the University of Florida in 2022. Currently, in his second year, he is dedicated to developing computational approaches that describe the yet-to-be-discovered biosynthetic gene cluster (BGC) diversity in the microbial world. José is particularly passionate about unraveling the biosynthetic machinery that escapes conventional genome miner algorithms. Throughout his Ph.D. dissertation, José aims to develop a tool for targeted genome mining and to elucidate the potential diversity of 7-deazapurine BGCs.

Yifeng Yuan obtained a bachelor's degree in biotechnology at Jilin University in China in 2009, a Master's Degree at California State University in 2012 and a Ph.D. in microbiology at University of Florida, in 2019. Yifeng Yuan worked at the department of biological engineering at MIT as a postdoctoral associate. He worked in diverse academic fields from cancer biology and signaling transduction to bioinformatics and microbiome metagenomics. He discovered gene families involved in deazaguanine and phosphorothioate modification and developed sequencing technology to map phosphorothioate modifications in bacterial genomes. Since 2023, he worked at the department of microbiology and cell science at University of Florida as a biological scientist. He continues interest in combining comparative genomic analysis, machine-learning bioinformatics and multi-omics approaches to connect genes and phenotypes, with an emphasis on bacterial epigenetics and epitranscriptomics and microbiome-host interactions.

Rémi Zallot received his Doctorate from Université Bordeaux 2 as a plant biochemist. During his work at the University of Florida, while exploring queuosine and B vitamin pathways from plants and microbes, he learned how comparative genomics is leveraged to guide and define the characterization of unknown genes. Subsequently, at the University of Illinois at Urbana-Champaign, he characterized enzymes from the human gut microbiome and contributed to the development and promotion of the EFI web tools, integral to the genomic enzymology approach. With a European-funded MSCA fellowship at Swansea University, he investigated uncharacterized CYPs in Mycobacterium species. At the Manchester Institute of Biotechnology, he characterized specialized metabolism enzymes. Since June 2023, Dr. Zallot is a Lecturer at the Department of Life Sciences of Manchester Metropolitan University. He is focused on characterizing relevant genes from microbial human pathogens and continues to develop and promote bioinformatics approaches.

Marc G. Chevrette received a B.Sc. in Molecular Biology and Bioinformatics from Rensselaer Polytechnic Institute, master's degrees in Bioengineering and Genetics from Harvard University Extension and the University of Wisconsin-Madison, respectively, a Ph.D. in Genetics from the University of Wisconsin-Madison, and postdoctoral training at the Wisconsin Institute of Discovery. Marc was the Head of Experimental Genomics at Warp Drive Bio and an Associate at the Broad Institute of MIT & Harvard. He is currently an Assistant Professor in the Department of Microbiology and Cell Science at the University of Florida.

R. M. Madhushi N. Ratnayake earned her B.Sc. (Special) degree in Chemical Biology from the University of Colombo, Sri Lanka in 2016 along with a Diploma in Information Technology in 2013. She worked as a Chemist at the Food and Environment Laboratory, Bureau Veritas, Sri Lanka before commencing her doctoral studies at the University of Florida. Under the mentorship of Professor Steven D. Bruner, her research focused on elucidating the transcriptional regulation of genotoxin colibactin biosynthesis. She explored microviridins as inhibitors to the human transmembrane protease and studied the structure and function of bacterial transporters and nucleoside hydrolases involved in queuosine salvage allowing her to gain expertise in Biochemistry, X-ray crystallography, and Molecular Biology. Madhushi earned her Ph.D. in Chemistry from the University of Florida in 2023. Currently, she continues pursuing research as a postdoctoral fellow at the School of Medicine Basic Sciences, Vanderbilt University with Prof. John Kuriyan.

Marshall Jaroch completed a bachelor's degree in Microbiology from the University of South Florida in Tampa, Florida, in 2016 and began his career at Brammer Bio in Alachua, Florida, where he gained a strong foundation in modern analytical techniques while working in the Assay Development and Analytics department. He returned to academia in 2019, joined the Microbiology and Cell Sciences Department at the University of Florida in Gainesville, Florida, and completed his Ph.D. in 2023. After graduating, he began postdoctoral training at the Oral Biology Department at the University of Florida in 2023 and is currently investigating the mechanisms of metal homeostasis in oral pathogens. He has contributed to the understanding of tRNA modifications in Gram-positive organisms, particularly during his Ph.D., by utilizing bioinformatic-guided experimental investigations.

Samia Quaiyum holds a bachelor's and master's degree in microbiology from Stamford University Bangladesh. Additionally, she pursued a second master's degree in Biodiversity and Molecular Biology at Hokkaido University, Japan. This was supported by a Japanese government scholarship (MEXT). Samia also completed an internship at Algarve University, Portugal, focusing on Marine Biodiversity and Climate Change. In 2020, Samia earned her Ph.D. in Environmental Molecular Microbiology from the Faculty of Agriculture, Hokkaido University. She specializes in bacterial cell degradation in aerobic and anaerobic environments. She served as a Research Assistant at the National Institute of Advanced Industrial Science and Technology Hokkaido Center, Japan, from 2017 to 2020. Currently, Samia is part of the Microbial Genomics and Genetics lab at the University of Florida, acquiring expertise in Molecular Biology, Microbiology, and Bioinformatics, she is set to complete her 3-year postdoctoral training in January 2024.

Steven Bruner received a bachelor's degree in Chemistry from Boston College and a Ph.D. in Chemistry from Harvard University in Gregory Verdine's group. Postdoctoral training was in Christopher Walsh's group at Harvard Medical School. Steven was an Assistant Professor at Boston College before moving to the University of Florida where he is currently a Professor in the Chemistry Department. The Bruner group uses organic chemistry, enzymology. and structural biology to probe enzyme mechanisms.