Article:
Craske, Matthew W, Wilson, Jason S and Fogg, Paul C M orcid.org/0000-0001-5324-4293 (2024) Gene transfer agents:structural and functional properties of domesticated viruses. Trends in microbiology. pp. 1200-1211. ISSN 0966-842X

https://doi.org/10.1016/j.tim.2024.05.002

Trends in Microbiology

CellPress OPEN ACCESS

Review

Gene transfer agents: structural and functional properties of domesticated viruses

Matthew W. Craske [1,4], Jason S. Wilson [1,3,4], and Paul C.M. Fogg [1,2,*]

Horizontal exchange of DNA between bacteria and archaea is prevalent and has major potential implications for genome evolution, plasticity, and population fitness. Several transfer mechanisms have been identified, including gene transfer agents (GTAs). GTAs are intricately regulated domesticated viruses that package host DNA into virus-like capsids and transfer this DNA throughout the bacterial community. Several important advances have recently been made in our understanding of these unusual particles. In this review, we highlight some of these findings, primarily for the model GTA produced by *Rhodobacter capsulatus* but also for newly identified GTA producers. We provide key insights into these important genetic elements, including the differences between GTAs from their ancestral bacteriophages, their regulation and control, and their elusive evolutionary function.

Highlights

Gene transfer agents (GTAs) have recently been discovered and characterized in new species – including *Caulobacter*, *Phaeobacter*, *Dinoroseobacter*, *Wolbachia* – and the number of GTA candidates has been expanded massively by advanced bioinformatic analyses.

GTAs are derived from ancient bacteriophages, but they have developed characteristic properties that absolve them of the typical viral lifestyle.

Our understanding of GTA regulation has advanced rapidly in the past decade. Regulation is intricately controlled via quorum sensing, SOS response, stringent response, sigma factor 70, the pleiotropic regulator CtrA, two-component systems, phosphorelays, cyclic-di-GMP and GTA-specific activators/repressors.

The evolutionary function of GTAs has not been experimentally proven, but there is clear evidence of prolonged retention of GTA genes over millions of years and coadaptation with the host.

Introduction: what are GTAs?

Bacteriophages are selfish viruses of bacteria that infect and exploit the cellular machinery of the host cell to facilitate the integration of its genome into the host chromosome (lysogenic), rapid self-replication (lytic), or various intermediate states (e.g., chronic infection or pseudolysogeny) [1]. Lysogens or nonproductive life cycles are relatively stable until internal or external signals cause re-entry into the lytic cycle. During the lytic life cycle, replicated viral DNA is packaged into protective capsids and released into the environment, whereby the bacteriophage can infect and inject its genome into another host. Periodically, bacterial host DNA can be inadvertently incorporated into these capsid particles during this process and horizontally transferred to surrounding cells, referred to as generalized transduction [2]. GTAs are an unusual but widespread group of domesticated, selfless viruses that have been co-opted to serve bacterial hosts and are now intricately controlled by interlocking host regulatory circuits [3–5]. In contrast to bacteriophages, GTAs have no bias towards their own replication and instead exclusively package and transfer small 4–12 kb random DNA fragments of the host cell [6–9].

GTAs are descended from ancestral phages and have recently been classified as caudoviricete-derived **viriforms** (see Glossary) [10,11]. At least three distinct GTA clades have been proposed [10]: type I alphaproteobacterial GTAs (model host: *Rhodobacter* spp.), type II alphaproteobacterial GTAs (model host: *Bartonella* spp.), or spirochete GTAs (model host: *Brachyspira hyodysenteriae*). Other GTAs have been discovered, for example, in the deltaproteobacteria (*Desulfovibrio* spp.) and the archaeal species *Methanococcus voltae*, however insufficient information is available about the genes that encode them to make any phylogenetic inferences [12,13].

GTAs share many structural and life cycle properties with viruses but are distinct in classification and nomenclature. Importantly, GTAs differ from viruses in four main ways:

- GTAs encapsidate random pieces of host DNA and deliver these to compatible recipient cells.
- In all known cases, the GTA genome is fragmented across multiple loci in the host chromosome.

[1]Biology Department, University of York, York YO10 5DD, UK
[2]York Biomedical Research Institute (YBRI), University of York, York YO10 5NG, UK
[3]York Structural Biology Laboratory (YSBL), University of York, York YO10 5DD, UK
[4]These authors contributed equally to this work

*Correspondence:
paul.fogg@york.ac.uk (P.C.M. Fogg).

- GTAs are unable to fully package the genes that encode them.
- GTAs are replicated along with the host genome and inherited vertically.

The unusual nature of GTAs and their ancient and widespread occurrences in bacterial genomes has led to increased research in this area over the past decade. However, much is still unknown about the function and evolutionary benefit of GTA production – which is particularly vexing given that GTA production ultimately leads to the death of the producing cell and must, therefore, convey some benefit at the population level. Together with a recent GTA review by Banks *et al*. [3], which focused on genomic organization, structure, and regulation, we highlight here the latest research into the characteristic properties of GTAs, regulation on a molecular and transcriptional level, and the potential evolutionary functions of these fascinating viriforms. We also offer outstanding questions in these areas that will be of great interest to the community.

GTA characteristic properties

GTAs are morphologically indistinguishable from bacteriophages (Figure 1A) but differ in four characteristic ways. These include: (i) a reduction in the density of DNA packaged into the capsid compared with the near crystalline density observed for bacteriophage (Figure 1A); (ii) altered small and large **terminase** proteins that package host DNA into GTAs in a non-specific manner (Figure 1B); (iii) split GTA-encoding genetic loci that disrupt the ability of GTAs to package their own DNA (Figure 1C); and (iv) the DNA content of GTA particles contains biases to certain chromosomal locations, which is different from specific DNA packaging by bacteriophages but also varies between GTAs produced by different species.

DNA packaging capacity properties

The model GTA from *Rhodobacter capsulatus* (RcGTA) has several characteristics that reduce the amount of DNA that can be packaged into its capsid. RcGTA has a relatively small T=3 quasi-icosahedral head size, which is further reduced in size by an oblate capsid shape. This oblate shape is mediated by missing five hexameric faces around the circumference of the capsid relative to the portal [14] (Figure 1A). Further, the density of DNA within the packaged GTA head is reduced compared with tailed bacteriophage with similar head sizes (28–29 Å versus 21–25 Å spacing between DNA layers respectively, Figure 1A). It is intriguing that divergent GTAs appear to have a reduced packaging density, as this property has not been observed for any bacteriophages to date. Reduction in the quasi-crystalline, maximum efficiency packaging conserved in bacteriophage from which GTAs are derived points towards some evolutionary pressure for the uncoupling of GTA head size and amount of DNA packaged.

The combined properties of the capsid and packaging machinery means that RcGTAs can package only ~4 kb of DNA [6]. Similar packaging capacities have been observed for other GTAs containing oblate heads (Table 1), and fragments of 4–8 kb host DNA are characteristic of type I alphaproteobacterial GTA homologues [7], even those lacking structural characterization. Detection of these regular-sized encapsidated DNA fragments could represent a hallmark for experimental identification of GTAs. *Caulobacter crescentus* GTA (CcGTA) has an increased DNA capacity of ~8 kb, explained by electron micrographs which show that CcGTA has an icosahedral head [8]. DNA density within the head has yet to be determined. Still, it is expected to be reduced compared with bacteriophages with similar-sized capsids, based on the observed (8.3 kb) versus expected (14.2 kb) packaging length for a viral 40 nm capsid [15]. Overall, GTAs have a consistently low packaging capacity, and although detailed explanations for less well-characterized examples are lacking, it is expected that at least some of the features of RcGTA packaging are broadly applicable across GTA families.

Glossary

AHL: acyl-homoserine lactones are widespread autoinducer signaling molecules. Many bacteria use AHLs for quorum sensing to respond to changes in population density.
CtrA: cell-cycle transcriptional regulator A. A pleiotropic transcriptional regulator widespread across alphaproteobacteria required for the activation of early-stage RcGTA synthesis, late-stage maturation and release of RcGTAs, depending on its phosphorylation state.
Cyclic-dimeric-GMP (c-di-GMP): bis-(3′–5′)-cyclic dimeric GMP. Small, intracellular secondary molecules involved in signal transduction and the regulation of numerous bacterial processes including GTA production and biofilm formation.
GafA/GafYZ: GTA activation factors. GafA and GafY are autoregulatory transcription factors that directly bind to GTA loci and activate transcription. GafZ forms part of an antitermination complex that facilitates expression of the core CcGTA genes.
Guanosine tetraphosphate (ppGpp): an alarmone signaling molecule involved in the regulation of the stringent stress response of bacteria under amino acid/nutrient starvation.
Headful DNA packaging: a strategy used by certain bacteriophages to package their genome. The packaging machinery typically recognizes a sequence in the phage genome called a Pac site and cuts the DNA near to this site. DNA is translocated into the phage capsid until it is full, at which point the genome is cut a second time. The size of the capsid determines the amount of DNA that can be packaged.
RogA/Rc280: tight regulation of GTA activation is required to limit production to a small subpopulation of cells and to avoid population collapse. *Rhodobacter capsulatus* encodes an RTX-toxin domain protein, Rc280, that specifically represses GTA production by an unknown mechanism. Meanwhile, *Caulobacter crescentus* encodes an analogous repressor of GTA activators, RogA, that represses transcription of the GafYZ operon.
Run-off replication (ROR): a phenomenon where a region of the genome is amplified from a non-native origin of replication, for example, derived from a defective bacteriophage or an integrated plasmid.

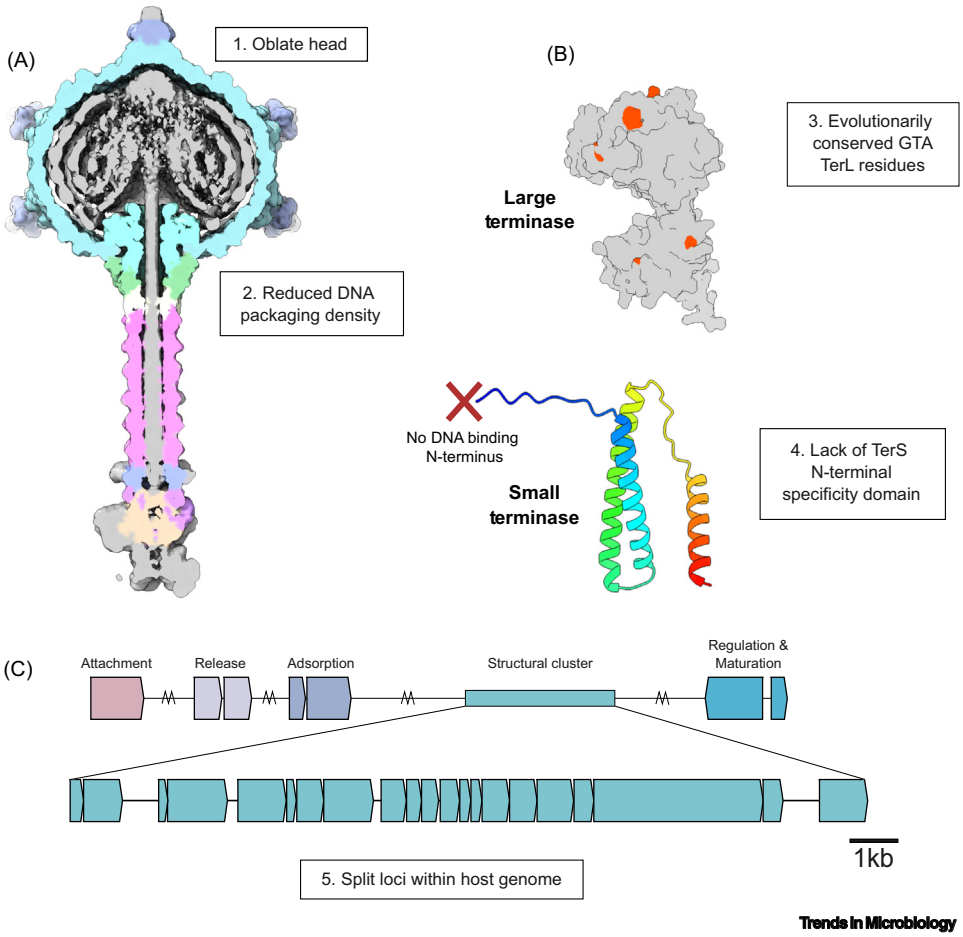

Figure 1. Schematic of the *Rhodobacter capsulatus gene transfer agent* (RcGTA) adaptations. (A) Cryo-electron microscopy (CryoEM) reconstruction of an intact RcGTA particle. RcGTA capsid (cyan) has oblate architecture (1), missing a central band of hexameric faces compared with icosahedral capsids. Internal DNA density (gray) is reduced compared with typical viral DNA packaging density (2). (B) The RcGTA large terminase protein contains several conserved residues compared with viral large terminases (illustrated in orange, 3). The predicted structure of the small terminase lacks the N-terminal globular DNA recognition domain (4). (C) The RcGTA genome is split into at least five distinct loci spread throughout the host genome, making it impossible for a single GTA to package all of the GTA-encoding genes.

Terminases: enzymes encoded by viriforms, many bacteriophages, and some eukaryotic viruses to package DNA through a portal in the capsid. Often encoded as two proteins, a large terminase (TerL) and a small terminase (TerS). TerL has nuclease and ATPase activity required to process DNA into the capsid, while TerS regulates these activities. Viral TerS also imparts specificity on the large terminase by recognizing specific DNA sequences (*cos*/*pac* sites).

Viriform: a highly diverse, polyphyletic collection of virus-derived particles that fulfil important roles for their host organism. Viriforms resemble viruses but do not preferentially package and transmit their own DNA.

Split loci of GTA genes

Unlike bacteriophages, GTA genes are generally encoded in multiple locations within the host genome. RcGTA is encoded by at least five loci dispersed across the genome [16] (Figure 1 and Table 1). The main cassette of RcGTA genes encodes most structural elements of the mature particle. Four other loci contain genes involved in GTA regulation, lytic release, and host cell attachment. As such, any increase in packaging capacity through mutation would still not allow a functional GTA genome to be packaged. A recent study identified 55 new gene families that coevolve with the RcGTA genes [17], suggesting that the number of loci involved in the GTA life cycle may not yet be fully understood. In *C. crescentus*, RNAseq [8] was used to show an increase in gene expression from ten loci associated with GTA production. The largest locus containing the structural genes with homology to RcGTA is ~18 kb, again too long to be packaged entirely within a single CcGTA particle.

Table 1. GTA properties

GTA	Species	Type strain	DNA capacity	Putative TerS	Known loci	Capsid morphology	Tail morphology	Refs
RcGTA	*Rhodobacter capsulatus*	SB1003	4.3 kb	11.5[a] kDa	5	Oblate (38nm)	Siphovirus[b]	[16]
CcGTA	*Caulobacter crescentus*	NA1000	8.3 kb	N.D[c]	2	Icosahedral (54nm)	Siphovirus	[8]
DsGTA	*Dinoroseobacter shibae*	DFL12	4.2 kb	12.9 kDa	≥4	Oblate (33 nm)	Siphovirus	[7]
BaGTA/ BgGTA	*Bartonella* spp	as4aup/ af165up	14 kb	11.7 kDa	2	Icosahedral (40 nm)	Unknown	[30,31]
DdGTA	*Desulfovibrio desulfuricans*	MO302	13.5 kb	13.5 kDa	N.D	Icosahedral (43 nm)	Podovirus	[13]
PpGTA	*Phaeobacter piscinae*	S26	N.D	12.5 kDa	≥4	Oblate (34 nm)	N.D	[53]
BhGTA	*Brachyspira hyodysenteriae*	B204	7.5 kb	N.D	2	Icosahedral (45 nm)	Tailed	[18,19]
MvGTA	*Methanococcus voltae*	PS-1	4.4 kb	14.6 kDa	N.D	Oblate (40 nm)	Tailed	[12,87]

[a]Experimentally verified.
[b]Siphovirus tail genes are present in the core CcGTA locus; however, tails have not been observed experimentally.
[c]N.D, not determined.

Some diversity is also observed in the organization of other GTA loci, especially in non-alpha proteobacterial GTAs. In the case of *B. hyodysenteriae* BhGTA (formally VSH-1), two gene clusters separated by 16.3 kb were identified from proteomic analysis of purified particles [18]. However, as these loci contain exclusively structural genes, lacking the portal, terminase, and other GTA-associated genes, more loci are expected to be associated with BhGTA. Candidate BhGTA genes at multiple locations have been predicted due to the presence of a putative regulatory hexanucleotide repeat that is also present upstream of the two confirmed loci; however, these will need to be confirmed experimentally [19].

In more distantly related alphaproteobacterial putative GTAs from *Holosporaceae* (including *Cytomitobacter primus, Cytomitobacter indipagum,* and *Nesciobacter abundans*), the large terminase gene is split from the main GTA locus [20]. In the case of the *Rickettsiales* endosymbiont *Sneabacter namystus,* the putative GTA loci are split between more than three regions. The large terminase gene is 258 bases downstream of the portal, separated by a small hypothetical protein, but the megatron tail gene is independent from the other structural genes [20]. These data emphasize the difficulty in identifying GTAs bioinformatically as the genes encoding GTAs can be ordered and split differently in different organisms, which in turn reduces the scoring obtained with bioinformatic tools designed for prophage detection. Crucially, the fragmented nature of GTA loci makes distinguishing them from remnant prophages challenging. GTA identification is confounded further by low spontaneous production frequencies and the difficulty of experimental confirmation of GTA expression in endosymbionts such as *Holosporaceae*.

GTA small and large terminase properties

GTAs, like tailed bacteriophages, employ terminase proteins to package DNA. The large terminase (TerL) proteins of viruses and GTAs contain an ATPase domain for generating the motive force to thread DNA through the portal, and a nuclease domain required to cut the DNA before and after packaging [21]. The small terminase (TerS) proteins regulate TerL activity and recognize the viral genome [22].

Due to a lack of any experimentally identified DNA sequence recognition sites, GTA packaging is predicted to use the **headful** mechanism. Headful packaging is also supported by the clustering of terminases based on sequence conservation [23]. Esterman *et al.* identified that large terminases of alphaproteobacteria GTAs form a distinct clade with phage headful packaging terminases from diverse viral genomes [23]. Further, the same study identified key amino acid

locations and properties that distinguish GTA terminases from viral terminases. However, none of these amino acids are within recognized active sites, and so their function is currently unknown.

The small terminase of RcGTA has recently been identified directly upstream of the large terminase and is essential for the packaging of *Rhodobacter* genomic DNA [24]. RcGTA TerS contains a C terminus involved in binding the ATPase domain of the large terminase, but lacks the N-terminal DNA binding domain (DBD) present in viral small terminases that is thought to recognize the packaging initiation site [22]. For some phage TerS, deletion of the DBD prevents DNA binding *in vitro* [25,26], and so the absence of the domain in GTA TerS may explain the observed lack of DNA specificity. Similar putative TerS genes with comparable domain architecture were identified upstream of other GTA large terminases, although no other TerS has been experimentally confirmed. Interestingly, for CcGTA, although a putative open reading frame (ORF) with small terminase features is present upstream of the large terminase gene, mutations and deletions in this region have little effect on DNA packaging [27], suggesting that CcGTA does not require a small terminase or that another protein is fulfilling its role.

Packaging bias

Although the exact function of RcGTA has not been confirmed, it has been termed a 'generalist' GTA, due to very little packaging bias across the genome [28]. The relatively closely related *Dinoroseobacter shibae* GTA (DsGTA) has a more distinct packaging bias, with some regions being highly over-represented or under-represented in packaged DNA [5,7]. CcGTA has under-represented regions of DNA located closer to the origin and terminus of the DNA, which are tethered at the poles of cells [5,8,29]. Steric hindrance of terminase binding to these regions may reduce DNA packaging of the tethered DNA.

In *Bartonellaceae* GTA (BaGTA), host adaptation factors are located close to an origin of replication derived from a defective prophage carried by the host. This prophage is unrelated to the BaGTA but affects the GTA packaging bias by increasing the copy number of genes in this region. The phage-derived origin and host adaptation factors are known as the **run-off replication (ROR)** gene cassette, and these genes are over-represented alongside prophage genes at the time of GTA production [28,30,31]. Further, disruption of the ROR abrogates gene transfer, suggesting functional coupling of the GTA and ROR loci possibly via a common regulator [31]. For BaGTA, the evolution of the more specialized GTA is thought to have derived from a more simplistic generalist GTA, with the bias imparting some advantage over the less biased generalist ancestor [32]. Evidence for this comes from the homology of the system to *Bartonella grahamii*, which lacks the coupling to the ROR, and so represents a more simplistic generalist GTA similar in function to RcGTA. However, in BaGTA, the addition of the ROR imparts a bias towards genes close to this region, therefore driving the transfer of these genes more strongly.

GTA regulation

GTAs are tightly and intricately regulated, with many interlocking controls to make sure that GTA production is only turned on at the appropriate time [4]. Our understanding of GTA regulation is predominantly based on an RcGTA-centric model; however, recent discoveries within other GTA-producing organisms have broadened our comprehension of how these systems are controlled. The timing and coordination of GTA production in most type I GTAs is controlled by the pleiotropic regulator **CtrA** and the direct activator GafA [8,33–36]. In *R. capsulatus*, CtrA is required to activate transcription of *gafA*, which then autostimulates its own expression and co-ordinates expression of the disparate RcGTA loci. Notably, *Caulobacter*-like GTAs (CcGTAs) are not thought to be controlled by CtrA and there are no canonical binding sites within CcGTA promoters [37], although lack of influence has not been confirmed experimentally. In *C. crescentus*, the specific GTA

activator is composed of two separate proteins: a transcription factor, GafY, and a transcription antiterminator, GafZ [8,27] that act together in a similar manner to the multidomain *R. capsulatus* GafA [4]. Transcription of *gafYZ* is autoregulated by GafY together with integration host factor (IHF) [27]. GafY is then responsible for the initiation of transcription of all structural and accessory genes required for CcGTA production [8,27]. The CcGTA core gene cluster contains multiple transcription termination sites. Transcription of the CcGTA genes is facilitated by an antitermination complex involving GafZ and NusA/G/E proteins [27].

A crucial part of GTA regulation is that expression of GTA genes must be strictly limited to avoid uncontrolled cell death. This process is likely to be mediated by **Rc280** in *R. capsulatus* and **RogA** in *C. crescentus*, which generally act to repress GTA production by inhibition of the explicit GTA activator **GafA/GafYZ** [8,38]. Stochastic relief of inhibition in a small subpopulation allows GTA production to occur (~0.15–3% in *R. capsulatus*) [6,8,36,38–40]. Analogous repressors in other GTA-producing species are yet to be discovered.

The intricate roles of phosphorylation and cyclic-dimeric-GMP

The activity of CtrA is modulated by **cyclic-dimeric-GMP (c-di-GMP)** levels and a phosphorelay signaling cascade including CckA-ChpT-CtrA [36,41–43]. In *R. capsulatus*, once GTA production is stimulated, high levels of intracellular c-di-GMP maintain CtrA in an unphosphorylated form and induction of *gafA* is enhanced by its own positive autoregulation [4,36,41,43]. GafA interacts with the RNA polymerase omega subunit to directly initiate transcription of early-stage RcGTA core structural and DNA packaging genes [4,44]. GafYZ does not contain an analogous RNA polymerase omega subunit protein–protein interaction domain, but instead directly interacts with the housekeeping sigma factor (σ70) [4,27]. Broader integration of GafYZ with the *Caulobacter* host regulatory circuits has yet to be demonstrated.

During late stationary phase, intracellular levels of c-di-GMP are reduced, by an unknown signal, and CckA phosphatase activity is switched to a kinase, stimulated by a PAS domain protein DivL, promoting phosphorylation of CtrA [34,36,41–43]. Although the phosphorelay cascade has a significant regulatory role in both the early and late stages of DsGTA and RcGTA production, this cascade functions as a cell cycle regulator within *C. crescentus* and does not regulate CcGTA production [41,42,45,46]. The switch to phosphorylated CtrA signals a change in regulation and, alongside GafA, induces transcription of late-stage RcGTA genes required for the release of mature RcGTA particles: head spikes (*ghsA/B*), tail fibers (*rcc00171*), maturation protein (*rcc001866*), and endolysin/holin (*rcc00555/6*) [36,47,48]. The protease ClpXP proteolytically regulates both phosphorylated states of CtrA, to control the temporal regulation of RcGTA production and is also required for the maturation of RcGTA functional particles [34].

A persulfide-responsive transcription factor, SqrR, indirectly regulates RcGTA expression through alterations of c-di-GMP levels, following induction by H_2O_2 [49]. In *D. shibae*, the majority of CtrA regulated genes contain LexA binding sites, including those involved in regulation of c-di-GMP levels, and LexA is essential for optimal RcGTA production [39,45,46]. Temporal control of RcGTA production, via the phosphorylation state of CtrA and intracellular c-di-GMP levels, is likely essential to ensure cell lysis occurs only when particles are mature and in the presence of compatible recipients [36,41,50,51].

Quorum sensing

Across all recognized type I alphaproteobacterial GTAs, high cell densities and stationary phase growth appear to be fundamental for GTA production and receipt [8,38,39,46,52]. A variety of environmental signals in *R. capsulatus* and *D. shibae* are coordinated by quorum sensing (GtaI/R,

LuxI/R) and a core transcriptional regulator, CtrA, to control the expression of the direct GTA activator GafA (*R. capsulatus/D. shibae*) [36,39]. As population size increases, the concentration of autoinducer N-acyl-homoserine lactone **(AHL)** signaling molecules accumulates and eventually relieves LuxR-type repression of *gafA* transcription and consequently stimulates GTA production [7,39,52]. In *Phaeobacter piscinae*, the production of the antibiotic secondary metabolite tropodithietic acid also acts as a quorum sensing signal and is proposed to restrict GTA expression after colonization of a novel niche [53,54].

Stringent response, ppGpp

Cytoplasmic concentration of the stringent response messenger signal guanosine-tetraphosphate (**ppGpp**) has conflicting implications for type I and type II GTA regulation. Synthesis of ppGpp under amino acid or carbon starvation conditions elicits type I RcGTA production [44] and the ppGpp receptor, RNA polymerase omega, is essential for RcGTA production [4]. Meanwhile, ppGpp alarmone signals are thought to prevent production of type II *Bartonella* GTAs (BaGTAs) in cells under starvation stress [31]. Here, actively replicating cells with low ppGpp levels are favored for BaGTA production and receipt [31]. In addition, amino acid transport and biosynthesis genes are required for optimal gene transfer, that is, nutrient stress likely dampens BaGTA production. However, it should be noted that a secondary lower peak of BaGTA expression also occurs in stationary phase, suggesting that BaGTA regulation is more nuanced and probably involves multiple competing factors [31].

Regulation of GTA receipt

Besides the indispensable role that CtrA has in RcGTA production, non-phosphorylated CtrA is activated within the majority of the remaining non-RcGTA-producing population and is required for optimal RcGTA receipt [50,51]. The biofilm formation associated gene, serine acetyltransferase (*cysE1*), is also required for maximal RcGTA receipt [55]. Initial, reversible binding of an RcGTA particle involves the display of a quorum sensing regulated capsular polysaccharide by recipient cells [14,48,56]. Transport of DsGTA/RcGTA-transduced DNA from the periplasm into the cytoplasm occurs via the Com protein family (ComEC, ComE, and ComM) and is regulated exclusively by CtrA in *D. shibae* and coregulated with quorum sensing in *R. capsulatus* [39,50,51]. Once in the cytoplasm, LexA regulates homologous recombination of translocated DNA onto the recipient genome by a RecA homologue and DprA in *D. shibae*, whereas these genes are controlled by non-phosphorylated CtrA and GtaI induction in *R. capsulatus* [39,50,51]. Both systems also appear to be the mechanism utilized for the uptake and recombination of BaGTA DNA, which suggests a conserved functional requirement of these pathways across both type I and type II alphaproteobacterial GTAs [31,39,57]. Receipt of RcGTAs can also be both positively and negatively affected by sub-minimum inhibitory concentrations (MICs) of antibiotics such as novobiocin [58]. The mechanism for this is unknown but could be related to stimulation of biofilm formation or part of a general response to DNA stress [59–61].

Evolutionary function of GTAs

The ability to horizontally transfer genetic material is a potentially valuable asset that facilitates rapid adaptation of bacteria to ever-changing environmental conditions, without the requirement of vertical inheritance [62]. It is necessary to appreciate how the gain of horizontally acquired genes, via conjugation or natural transformation, can overcome the associated metabolic costs of production [63,64]. Unlike these canonical mechanisms of horizontal gene transfer (HGT), GTA release requires lysis of the producing cells, up to 3% of the population for *R. capsulatus* or 6% for *Bartonella* spp., which complicates the evolutionary net-benefit of this process [28,31,40]. Despite the high cost of production, there is a growing collection of evidence that supports the maintenance of type I alphaproteobacterial GTAs over an estimated 700-million-year

period and provides insights into their possible ecological roles [65]. A genome-wide phenotyping study showed that deletion of RcGTA-like genes significantly reduced the fitness of three GTA-producing species, when grown with alternative carbon sources and other abiotic stressors [66,67]. If threatened by nutrient starvation, the sacrifice of a small subpopulation for GTA production may offer a last-resort attempt at survival for the greater good of the remaining population. An influx of beneficial allelic combinations may facilitate metabolic adaptations to such conditions and/or provide immediate relief to nearby cells through nutrient release following GTA production (cell lysis); akin to programmed cell death (Figure 2) [68,69].

Adaptations to reduce GTA production costs

The identification of various adaptations of type I GTAs reveals clues to how these genetic elements have evolved to reduce the cost of carriage for the host. GTA regions have an increased GC content relative to the rest of the host genome, which reduces the energy expenditure required to produce these proteins and is a hallmark feature that can be used to distinguish these elements from viral homologs – an otherwise challenging task due to significant sequence and organization similarities [65,66,68,70]. Using this classification, a high prevalence of RcGTA-like genes has been identified throughout the alphaproteobacteria (57.5% of 1423 genomes explored) [66]. To further optimize GTA protein production, a codon usage bias has been identified in type I GTAs, which promotes the incorporation of widely accessible tRNAs during translation [71]. Taken together, these adaptations are likely to facilitate high-efficiency, low-cost carriage of these GTAs, which is particularly important under nutrient-deficient conditions [44,68,71].

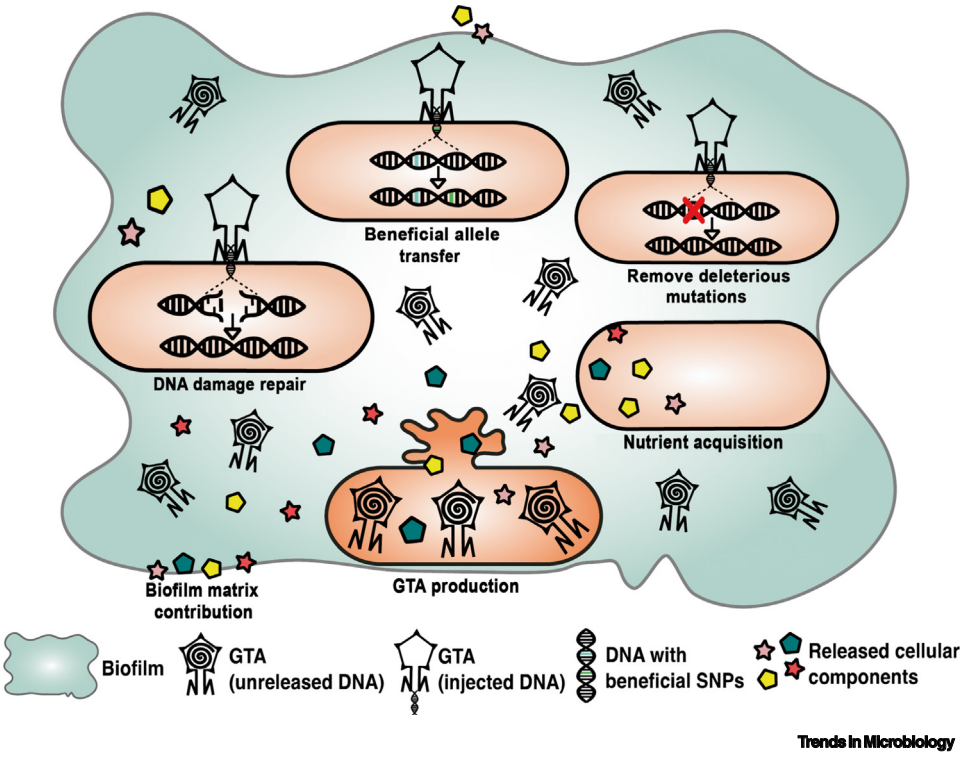

Figure 2. Diagram of the potential ecological functions of gene transfer agents (GTAs). The enclosed production of GTAs by a small subset of cells within a biofilm may provide a variety of ecological benefits to surrounding cells.

Potential ecological functions of GTA production

A study in *C. crescentus* provided evidence that the benefits provided by CcGTA production during stationary phase could be recombination-dependent, and not a result of nutrient release [8]. The authors reasoned that CcGTAs may function as a reservoir of template DNA, capable of rescuing neighboring cells damaged by double-stranded DNA breaks via homology-directed repair (Figure 2) [8] – whereas, in *P. piscinae* and *Bartonella henselae*, the advantages of GTA production are thought to be a consequence of increased rates of genetic exchange [28,31,53]. Intriguingly, a study of 32 genomes from geographically diverse *Phaeobacter* strains found that GTA genes were ubiquitous and detected an abundance of short DNA transfer events compatible with GTA activity [72].

In natural environments, GTA-mediated gene transfer events are reported to occur at a much greater frequency than those estimated for other recognized mechanisms of HGT [73]. These elevated rates of evolution are suggested to improve niche colonization of *P. piscinae* S26 and help avoid the accumulation of deleterious mutations (Muller's ratchet) of the intracellular parasite *B. henselae* [28,31,53,74]. Widespread identification of GTA gene homologs in other intracellular bacteria, which are also likely to be affected by Muller's ratchet, supports this suggested function [20,65,75]. However, Redfield and Soucy conclude that under environmentally relevant conditions, recombination alone was insufficient to compensate for the loss of cells by this process [76]. Additionally, the production of 'public goods' by a subset of sacrificial GTA producers inevitably results in population takeover by non-GTA producing 'cheater' cells, capable of receiving the benefits of GTA production without the requisite costs of cell lysis [76]. Although, growth-phase regulated GTA receptors (RcGTAs) and sequence similarity requirements should constrain the acquisition of these 'public goods' to closely related kin cells [51,77,78]. Recent revelations regarding the association between biofilms and GTAs may present an ideal ecological solution to overcome both confounding factors [17,55,76]. The enclosed biofilm structure may increase the chances of stable GTAs finding a compatible recipient cell before they are destabilized and limit loss of GTAs via diffusion, improving the overall efficiency of recombination (Figure 2) [17,55]. Furthermore, the biofilm matrix facilitates additional benefits of GTAs to supplement recombination. Released cellular components of GTA production may provide a source of nutrients for surrounding cells and contribute to the overall biofilm composition [17,79]. The authors propose that, although cheater cells could theoretically take over individual biofilms, the resultant loss of GTA production could increase their susceptibility to stressful conditions normally negated by GTA-mediated transfer [17,76]. Overall, GTAs are complex vehicles of genetic exchange that likely provide a diverse range of functions depending on the specific ecological requirements of their hosts, which collectively contribute to the global fitness of a population.

Concluding remarks

Over the last twenty-five years, great advances have been made in our knowledge of the structure of GTAs and how they are regulated, particularly for a few model species. However, there are many aspects of GTA biology, prevalence, and function that remain unanswered (see Outstanding questions). Current bioinformatics tools and GTA classifiers are heavily biased towards alphaproteobacterial GTAs [23,66] due to an over-representation of clear examples of this system for training. Better tools for identifying GTAs will be required to fully expand the GTA family and better methods to experimentally confirm candidates predicted by bioinformatics. Crucially, robust classifiers are required to distinguish GTAs from prophages in genome and metagenome data, and core physical characteristics to differentiate GTAs from other DNA encapsidating particles [80,81]. An urgent need for robust new methods of identifying and confirming GTAs is highlighted by the prophage WOStri from Wolbachia [82,83]. Recent studies have identified that this prophage may represent a GTA, but current methods are unable to confirm this assertion.

Outstanding questions

What is the evolutionary function of GTAs, and does the function of generalist versus specialist GTAs differ?

Are GTA packaging biases controlled by genomic architecture or adaptations made by GTA packaging enzymes?

What unique, universal features of GTAs can be exploited to identify new systems *in silico*?

How is a reduced DNA packaging density generated, and what is its importance?

What regulatory genes and pathways are responsible for the differences in regulation between different GTA systems?

What is the functional relevance of the association between biofilms and GTAs?

How do GTA-producing populations prevent population takeover by non-GTA producing cheater cells?

Recent methods of purifying GTAs from supernatants of *Rhodobacter* overproducer strains offer another means for expanding GTA families [84]. A simple two-step hydrophobic exchange chromatography followed by ion exchange can produce milligram quantities of active particles. Similar purification of putative GTAs would allow for rapid experimental validation of GTAs from new organisms and provide a means for proteomic confirmation of proteins involved in mature GTA structures. This approach would rely on there being sufficient levels of GTA production, which is often not the case. Detailed understanding of induction stimuli or genetic regulation could alleviate this problem.

Advances in our knowledge of GTA regulation have highlighted the importance of how small signaling molecules (AHLs, c-di-GMP, ppGpp) are involved in the control of different stages of GTA production [39,41,42,46,49,52,77,85,86]. The identification of a widespread direct GTA activator (GafA/GafYZ) and its repressor (Rc280/RogA) has revealed how these signals and host regulatory systems are integrated to directly activate the expression of RcGTA-like GTAs [4,8,27,36]. Future work should aim to identify regulatory genes involved in the regulation of other RcGTA-like GTAs (BaGTAs, CcGTAs, DsGTAs), how they differ from RcGTA regulation, and how we can use this to enhance GTA discovery/characterization.

Although the ecological function(s) responsible for outweighing the costs of GTA production remain unconfirmed, the observed evolutionary adaptations and long-term maintenance of these elements suggest that GTAs provide a selective advantage to their host [17,65,68,71]. GTAs are likely multifaceted elements which provide a range of biological functions (DNA repair, high-frequency gene transfer, a source of nutrients following GTA release) that collectively contribute to an overall fitness benefit, depending on the individual requirements of a population [8,28,53,55,71,73]. Further investigations are necessary to follow up on the recent observations between biofilms and GTAs and their proposed ecological relevance for GTA function [17,53,55]. Prospective research should also focus on experimentally demonstrating how GTA-producing populations prevent population takeover by non-GTA-producing cheater cells.

Acknowledgments

M.C. is funded by a Biotechnology and Biological Sciences Research Council (BBSRC) Doctoral Training Program Studentship, J.W. is funded by a BBSRC responsive mode grant (BB/V016288/1), and P.F. is jointly funded by a Wellcome Trust and Royal Society Sir Henry Dale Fellowship (109363/Z/15/A) and a BBSRC responsive mode grant (BB/V016288/1).

Declaration of interests

The authors declare no competing interests.

References

1. Mäntynen, S. *et al.* (2021) Black box of phage-bacterium interactions: exploring alternative phage infection strategies. *Open Biol.* 11, 210188
2. Chiang, Y.N. *et al.* (2019) Genetic transduction by phages and chromosomal islands: the new and noncanonical. *PLoS Pathog.* 15, e1007878
3. Banks, E.J. and Le, T.B.K. (2024) Co-opting bacterial viruses for DNA exchange: structure and regulation of gene transfer agents. *Curr. Opin. Microbiol.* 78, 102431
4. Sherlock, D. and Fogg, P.C.M. (2022) The archetypal gene transfer agent RcGTA is regulated via direct interaction with the enigmatic RNA polymerase omega subunit. *Cell Rep.* 40, 111183
5. Fogg, P.C.M. (2024) Gene transfer agents: the ambiguous role of selfless viruses in genetic exchange and bacterial evolution. *Mol. Microbiol.*, Published online March 21, 2024. https://doi.org/10.1111/mmi.15251
6. Hynes, A.P. *et al.* (2012) DNA packaging bias and differential expression of gene transfer agent genes within a population during production and release of the *Rhodobacter capsulatus* gene transfer agent, RcGTA. *Mol. Microbiol.* 85, 314–325
7. Tomasch, J. *et al.* (2018) Packaging of *Dinoroseobacter shibae* DNA into gene transfer agent particles is not random. *Genome Biol. Evol.* 10, 359–369
8. Gozzi, K. *et al.* (2022) Prophage-like gene transfer agents promote *Caulobacter crescentus* survival and DNA repair during stationary phase. *PLoS Biol.* 20, e3001790
9. Lang, A.S. *et al.* (2012) Gene transfer agents: phage-like elements of genetic exchange. *Nat. Rev. Microbiol.* 10, 472–482
10. Kogay, R. *et al.* (2022) Formal recognition and classification of gene transfer agents as viriforms. *Virus Evol.* 8, veac100
11. Kuhn, J.H. and Koonin, E.V. (2023) Viriforms – a new category of classifiable virus-derived genetic elements. *Biomolecules* 13, 289
12. Eiserling, F. *et al.* (1999) Bacteriophage-like particles associated with the gene transfer agent of methanococcus voltae PS. *J. Gen. Virol.* 80, 3305–3308
13. Rapp, B.J. and Wall, J.D. (1987) Genetic transfer in *Desulfovibrio desulfuricans*. *Proc. Natl. Acad. Sci. U. S. A.* 84, 9128–9130
14. Bárdy, P. *et al.* (2020) Structure and mechanism of DNA delivery of a gene transfer agent. *Nat. Commun.* 11, 3034

15. Petrovski, S. *et al.* (2012) Small but sufficient: the *Rhodococcus* phage RRH1 has the smallest known Siphoviridae genome at 14.2 kilobases. *J. Virol.* 86, 358–363
16. Hynes, A.P. *et al.* (2016) Functional and evolutionary characterization of a gene transfer agent's multilocus "genome". *Mol. Biol. Evol.* 33, 2530–2543
17. Kogay, R. and Zhaxybayeva, O. (2024) Co-evolution of gene transfer agents and their alphaproteobacterial hosts. *J. Bacteriol.* 206, e0039823
18. Matson, E.G. *et al.* (2005) Identification of genes of VSH-1, a prophage-like gene transfer agent of *Brachyspira hyodysenteriae*. *J. Bacteriol.* 187, 5885–5892
19. Stanton, T.B. *et al.* (2009) Identification of a divided genome for VSH-1, the prophage-like gene transfer agent of *Brachyspira hyodysenteriae*. *J. Bacteriol.* 191, 1719–1721
20. George, E.E. *et al.* (2022) Gene transfer agents in bacterial endosymbionts of microbial eukaryotes. *Genome Biol. Evol.* 14, evac099
21. Black, L.W. (2015) Old, new, and widely true: the bacteriophage T4 DNA packaging mechanism. *Virology* 479–480, 650–656
22. Lokareddy, R.K. *et al.* (2022) Viral small terminase: a divergent structural framework for a conserved biological function. *Viruses* 14, 2215
23. Esterman, E.S. *et al.* (2021) Evolution of DNA packaging in gene transfer agents. *Virus Evol.* 7, veab015
24. Sherlock, D. *et al.* (2019) Identification of the first gene transfer agent (GTA) small terminase in *Rhodobacter capsulatus*, its role in GTA production and packaging of DNA. *J. Virol.* 93, e01328–19
25. Gao, S. *et al.* (2016) Exclusion of small terminase mediated DNA threading models for genome packaging in bacteriophage T4. *Nucleic Acids Res.* 44, 4425–4439
26. Roy, A. *et al.* (2012) Small terminase couples viral DNA binding to genome-packaging ATPase activity. *Structure* 20, 1403–1413
27. Tran, N.T. and Le, T.B.K. (2023) Control of a gene transfer agent cluster in *Caulobacter crescentus* by transcriptional activation and anti-termination. *BioRxiv*, Published online November 12, 2023. https://doi.org/10.1101/2023.11.12.565669
28. Québatte, M. and Dehio, C. (2019) *Bartonella* gene transfer agent: evolution, function, and proposed role in host adaptation. *Cell. Microbiol.* 21, e13068
29. Tran, N.T. *et al.* (2017) SMC progressively aligns chromosomal arms in *Caulobacter crescentus* but is antagonized by convergent transcription. *Cell Rep.* 20, 2057–2071
30. Berglund, E.C. *et al.* (2009) Run-off replication of host-adaptability genes is associated with gene transfer agents in the genome of mouse-infecting *Bartonella grahamii*. *PLoS Genet.* 5, e1000546
31. Québatte, M. *et al.* (2017) Gene transfer agent promotes evolvability within the fittest subpopulation of a bacterial pathogen. *Cell Syst.* 4, 611–621.e6
32. Tamarit, D. *et al.* (2018) Origin and evolution of the bartonella gene transfer agent. *Mol. Biol. Evol.* 35, 451–464
33. Mercer, R.G. *et al.* (2010) Loss of the response regulator CtrA causes pleiotropic effects on gene expression but does not affect growth phase regulation in *Rhodobacter capsulatus*. *J. Bacteriol.* 192, 2701–2710
34. Westbye, A.B. *et al.* (2018) The protease ClpXP and the PAS domain protein DivL regulate CtrA and gene transfer agent production in *Rhodobacter capsulatus*. *Appl. Environ. Microbiol.* 84, e00275–18
35. Lang, A.S. and Beatty, J.T. (2000) Genetic analysis of a bacterial genetic exchange element: the gene transfer agent of *Rhodobacter capsulatus*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 859–864
36. Fogg, P.C.M. (2019) Identification and characterization of a direct activator of a gene transfer agent. *Nat. Commun.* 10, 595
37. Laub, M.T. *et al.* (2002) Genes directly controlled by CtrA, a master regulator of the *Caulobacter* cell cycle. *Proc. Natl. Acad. Sci. U. S. A.* 99, 4632–4637
38. Ding, H. *et al.* (2019) Induction of *Rhodobacter capsulatus* gene transfer agent (RcGTA) gene expression is a bistable stochastic process repressed by an extracellular calcium-binding RTX protein homologue. *J. Bacteriol.* 201, e00430–19
39. Koppenhöfer, S. *et al.* (2019) Integrated transcriptional regulatory network of quorum sensing, replication control, and SOS response in *Dinoroseobacter shibae*. *Front. Microbiol.* 10, 803
40. Fogg, P.C.M. *et al.* (2012) One for all or all for one: heterogeneous expression and host cell lysis are key to gene transfer agent activity in *Rhodobacter capsulatus*. *PLoS One* 7, e43772
41. Farrera-Calderon, R.G. *et al.* (2021) The CckA-ChpT-CtrA phosphorelay controlling *Rhodobacter capsulatus* gene transfer agent production is bidirectional and regulated by cyclic di-GMP. *J. Bacteriol.* 203, e00525–20
42. Pallegar, P. *et al.* (2020) Cyclic di-GMP-mediated regulation of gene transfer and motility in *Rhodobacter capsulatus*. *J. Bacteriol.* 202, e00554–19
43. Pallegar, P. *et al.* (2020) A two-component system acquired by horizontal gene transfer modulates gene transfer and motility via cyclic dimeric GMP. *J. Mol. Biol.* 432, 4840–4855
44. Westbye, A.B. *et al.* (2017) The *Rhodobacter capsulatus* gene transfer agent is induced by nutrient depletion and the RNAP omega subunit. *Microbiol. (Reading, Engl.)* 163, 1355–1363
45. Wang, H. *et al.* (2014) The CtrA phosphorelay integrates differentiation and communication in the marine alphaproteobacterium *Dinoroseobacter shibae*. *BMC Genomics* 15, 130
46. Koppenhöfer, S. and Lang, A.S. (2020) Interactions among redox regulators and the CtrA Phosphorelay in *Dinoroseobacter shibae* and *Rhodobacter capsulatus*. *Microorganisms* 8, 562
47. Westbye, A.B. *et al.* (2013) Phosphate concentration and the putative sensor kinase protein CckA modulate cell lysis and release of the *Rhodobacter capsulatus* gene transfer agent. *J. Bacteriol.* 195, 5025–5040
48. Westbye, A.B. *et al.* (2016) The gene transfer agent RcGTA contains head spikes needed for binding to the *Rhodobacter capsulatus* polysaccharide cell capsule. *J. Mol. Biol.* 428, 477–491
49. Shimizu, T. *et al.* (2022) Persulfide-responsive transcription factor SqrR regulates gene transfer and biofilm formation via the metabolic modulation of cyclic di-GMP in *Rhodobacter capsulatus*. *Microorganisms* 10, 908
50. Brimacombe, C.A. *et al.* (2014) *Rhodobacter capsulatus* DprA is essential for RecA-mediated gene transfer agent (RcGTA) recipient capability regulated by quorum-sensing and the CtrA response regulator. *Mol. Microbiol.* 92, 1260–1278
51. Brimacombe, C.A. *et al.* (2015) Homologues of genetic transformation DNA import genes are required for *Rhodobacter capsulatus* gene transfer agent recipient capability regulated by the response regulator CtrA. *J. Bacteriol.* 197, 2653–2663
52. Leung, M.M. *et al.* (2012) The GtaR protein negatively regulates transcription of the gtaRI operon and modulates gene transfer agent (RcGTA) expression in *Rhodobacter capsulatus*. *Mol. Microbiol.* 83, 759–774
53. Lindqvist, L.L. *et al.* (2023) Tropodithietic acid, a multifunctional antimicrobial, facilitates adaption and colonization of the producer, *Phaeobacter piscinae*. *mSphere* 8, e0051722
54. Henriksen, N.N.S.E. *et al.* (2022) Role is in the eye of the beholder-the multiple functions of the antibacterial compound tropodithietic acid produced by marine Rhodobacteraceae. *FEMS Microbiol. Rev.* 46, fuac007
55. Sherlock, D. and Fogg, P.C.M. (2022) Loss of the *Rhodobacter capsulatus* serine acetyl transferase gene, cysE1, impairs gene transfer by gene transfer agents and biofilm phenotypes. *Appl. Environ. Microbiol.* 88, e0094422
56. Alim, N.T.B. *et al.* (2023) Extracellular polysaccharide receptor and receptor-binding proteins of the *Rhodobacter capsulatus* bacteriophage-like gene transfer agent RcGTA. *Genes* 14, 1124
57. Westbye, A.B. *et al.* (2017) Guaranteeing a captive audience: coordinated regulation of gene transfer agent (GTA) production and recipient capability by cellular regulators. *Curr. Opin. Microbiol.* 38, 122–129
58. Bernelot-Moens, R. and Beatty, J.T. (2022) DNA gyrase inhibitors increase the frequency of bacteriophage-like RcGTA-mediated gene transfer in *Rhodobacter capsulatus*. *Genes* 13, 2071
59. Yaeger, L.N. *et al.* (2023) Central metabolism is a key player in *E. coli* biofilm stimulation by sub-MIC antibiotics. *PLoS Genet.* 19, e1011013
60. Whelan, M.V.X. *et al.* (2021) A novel high-content screening approach for the elucidation of *C. jejuni* biofilm composition and integrity. *BMC Microbiol.* 21, 2
61. Al-Gashgari, B. *et al.* (2023) Impact of chemicals and physical stressors on horizontal gene transfer via natural transformation. *Nat. Water* 1, 635–648

62. Soucy, S.M. *et al.* (2015) Horizontal gene transfer: building the web of life. *Nat. Rev. Genet.* 16, 472–482
63. Ilangovan, A. *et al.* (2015) Structural biology of the Gram-negative bacterial conjugation systems. *Trends Microbiol.* 23, 301–310
64. Ambur, O.H. *et al.* (2016) Steady at the wheel: conservative sex and the benefits of bacterial transformation. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 371, 20150528
65. Shakya, M. *et al.* (2017) Insights into origin and evolution of α-proteobacterial gene transfer agents. *Virus Evol.* 3, vex036
66. Kogay, R. *et al.* (2019) Machine-learning classification suggests that many alphaproteobacterial prophages may instead be gene transfer agents. *Genome Biol. Evol.* 11, 2941–2953
67. Price, M.N. *et al.* (2018) Mutant phenotypes for thousands of bacterial genes of unknown function. *Nature* 557, 503–509
68. Kogay, R. *et al.* (2020) Selection for reducing energy cost of protein production drives the GC content and amino acid composition bias in gene transfer agents. *MBio* 11, e01206–20
69. Peeters, S.H. and de Jonge, M.I. (2018) For the greater good: programmed cell death in bacterial communities. *Microbiol. Res.* 207, 161–169
70. Lang, A.S. *et al.* (2017) The distribution, evolution, and roles of gene transfer agents in prokaryotic genetic exchange. *Annu. Rev. Virol.* 4, 87–104
71. Kogay, R. and Zhaxybayeva, O. (2022) Selection for translational efficiency in genes associated with alphaproteobacterial gene transfer agents. *mSystems* 7, e0089222
72. Freese, H.M. *et al.* (2017) Trajectories and drivers of genome evolution in surface-associated marine *Phaeobacter*. *Genome Biol. Evol.* 9, 3297–3311
73. McDaniel, L.D. *et al.* (2010) High frequency of horizontal gene transfer in the oceans. *Science* 330, 50
74. Moran, N.A. (1996) Accelerated evolution and Muller's rachet in endosymbiotic bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 93, 2873–2878
75. Lang, A.S. and Beatty, J.T. (2007) Importance of widespread gene transfer agent genes in alpha-proteobacteria. *Trends Microbiol.* 15, 54–62
76. Redfield, R.J. and Soucy, S.M. (2018) Evolution of bacterial gene transfer agents. *Front. Microbiol.* 9, 2527
77. Brimacombe, C.A. *et al.* (2013) Quorum-sensing regulation of a capsular polysaccharide receptor for the *Rhodobacter capsulatus* gene transfer agent (RcGTA). *Mol. Microbiol.* 87, 802–817
78. Wall, J.D. *et al.* (1975) Gene transfer agents, bacteriophages, and bacteriocins of *Rhodopseudomonas capsulata*. *Arch. Microbiol.* 105, 217–224
79. Bayles, K.W. (2007) The biological role of death and lysis in biofilm development. *Nat. Rev. Microbiol.* 5, 721–726
80. Lücking, D. *et al.* (2023) Extracellular vesicles are the main contributor to the non-viral protected extracellular sequence space. *ISME Commun.* 3, 112
81. Grüll, M.P. *et al.* (2018) Small extracellular particles with big potential for horizontal gene transfer: membrane vesicles and gene transfer agents. *FEMS Microbiol. Lett.* 365
82. Fallon, A.M. (2022) Muramidase, nuclease, or hypothetical protein genes intervene between paired genes encoding DNA packaging terminase and portal proteins in *Wolbachia* phages and prophages. *Virus Genes* 58, 327–349
83. Fallon, A.M. and Carroll, E.M. (2023) Virus-like particles from *Wolbachia*-infected cells may include a gene transfer agent. *Insects* 14, 516
84. Langille, E. *et al.* (2022) Purification of functional gene transfer agents using two-step preparative monolithic chromatography. *Phage (New Rochelle)* 3, 194–203
85. Koppenhöfer, S. *et al.* (2022) Shared properties of gene transfer agent and core genes revealed by comparative genomics of Alphaproteobacteria. *Microb. Genom.* 8, mgen000890
86. Tomasch, J. *et al.* (2021) Connection between chromosomal location and function of ctra phosphorelay genes in alphaproteobacteria. *Front. Microbiol.* 12, 662907
87. Bertani, G. (1999) Transduction-like gene transfer in the methanogen *Methanococcus voltae*. *J. Bacteriol.* 181, 2992–3002