Bacterial transformation: distribution, shared mechanisms and divergent control

Calum Johnston[1,2], Bernard Martin[1,2], Gwennaele Fichant[1,2], Patrice Polard[1,2] and Jean-Pierre Claverys[1,2]

Abstract | Natural bacterial transformation involves the internalization and chromosomal integration of DNA and has now been documented in ~80 species. Recent advances have established that phylogenetically distant species share conserved uptake and processing proteins but differ in the inducing cues and regulatory mechanisms that are involved. In this Review, we highlight divergent and common principles that govern the transformation process in different bacteria. We discuss how this cumulative knowledge enables the prediction of new transformable species and supports the idea that the main role of internalized DNA is in the generation of genetic diversity or in chromosome repair rather than in nutrition.

Parasexual
A term used to describe a form of reproduction in which genetic recombination occurs in the absence of meiosis, wherein one 'partner' is DNA.

Homologous recombination
The exchange of DNA sequences between identical or similar molecules. In the case of transformation, this involves the host chromosome and internalized single-stranded DNA (ssDNA).

[1]*Centre National de la Recherche Scientifique, LMGM-UMR5100, F-31000 Toulouse, France.*
[2]*Université de Toulouse, UPS, Laboratoire de Microbiologie et Génétique Moléculaires, F-31000 Toulouse, France.*
Correspondence to J.-P.C.
e-mail: claverys@biotoul.fr
doi:10.1038/nrmicro3199
Published online
10 February 2014

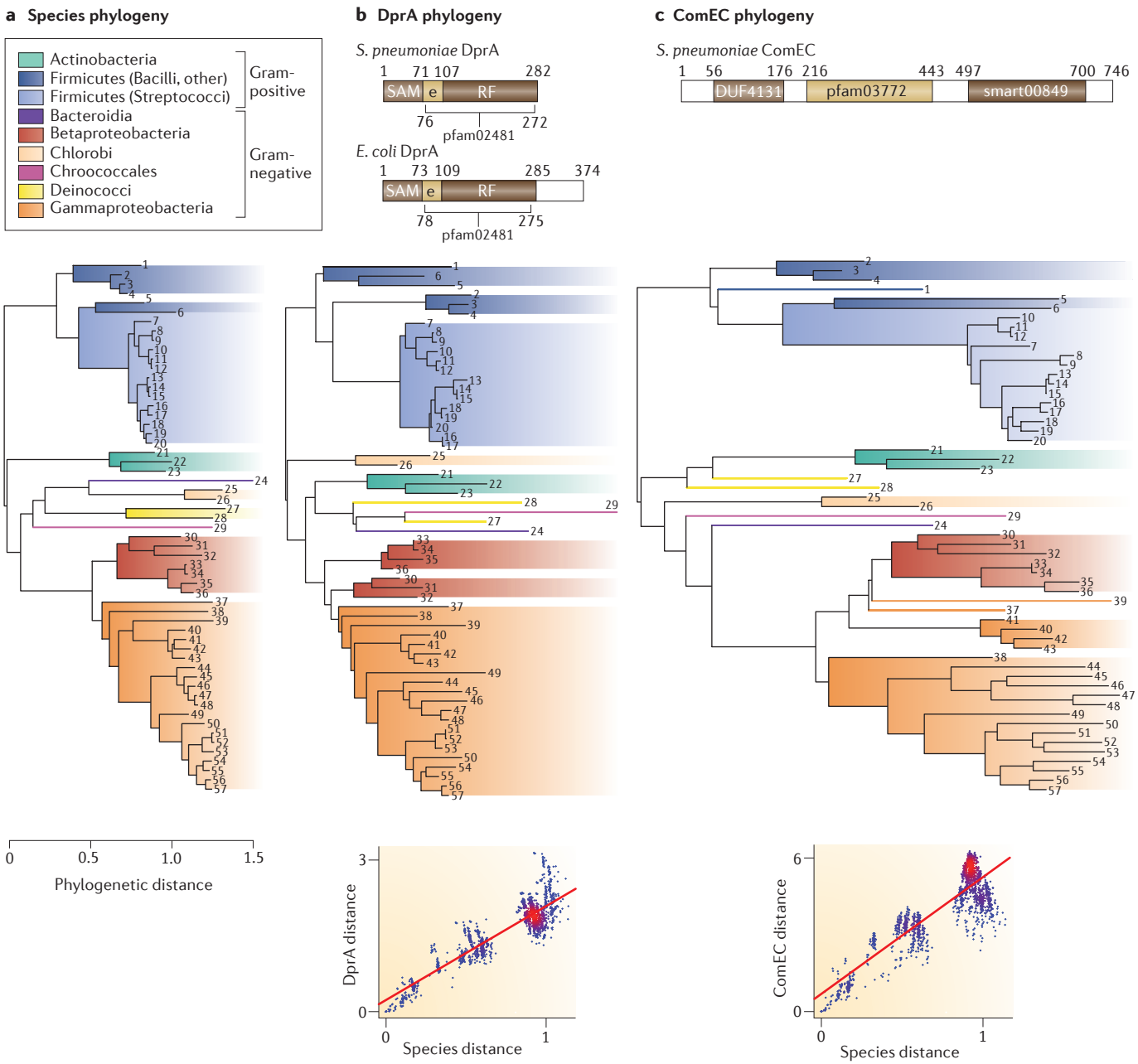
Natural bacterial transformation, which was first discovered in the Gram-positive bacterium *Streptococcus pneumoniae* (also known as pneumococcus)[1], is regarded as a parasexual process that involves two partners: exogenous DNA and a recipient cell. Internalization of exogenous DNA and integration into the recipient genome by homologous recombination enables bacteria to acquire new genetic traits and to adapt to changing environmental conditions, promoting — for example — resistance to antibiotics and evasion of vaccines[2]. Unlike other mechanisms of horizontal gene transfer, such as transduction and conjugation, transformation is entirely directed by the recipient cell and all required proteins are encoded in the core genome. Most transformable bacteria do not permanently express the proteins that are involved but instead require specific conditions to develop competence for genetic transformation. Competence is thus a transient 'window of opportunity' for DNA internalization and thereby enables subsequent transformation.

The number of species that are known to be naturally transformable has almost doubled in 20 years[3,4]. A total of 82 species have now been shown to be naturally transformable (Supplementary information S1 (table)). This number could be an overestimate as, in several cases, only a single report documents transformation and molecular proof of natural transformation (such as inactivation of a key DNA-uptake or processing gene — for example, *comEC* or *dprA*; see below) is lacking. Nevertheless, an analysis of the phylogenetic distribution of 57 of these 82 species shows that transformability is spread throughout the main taxa, with Gram-positive and Gram-negative bacteria equally represented (FIG. 1a).

Over the past 10 years, several reviews have addressed various aspects of transformation, including its prevalence and the mechanisms of DNA uptake and processing[5–9]. Other reviews have focused on competence regulation in Gram-positive[10] and Gram-negative bacteria[11]. In this Review, we aim to provide an update of recent studies that describe the molecular mechanisms that control competence, with an emphasis on Gram-positive organisms, including *S. pneumoniae* and species that were not previously known to be naturally transformable, such as *Streptococcus thermophilus* and *Staphylococcus aureus*. We also discuss recent insights that have been obtained for the Gram-negative species *Vibrio cholerae*, *Helicobacter pylori* and *Haemophilus influenzae*. We compare how competence is regulated in these phylogenetically distinct bacteria, focusing on common principles and species-specific mechanisms. Finally, we revisit the question of the role of imported DNA and discuss the prediction of new transformable species.

Transformation: a brief overview

With the exception of *H. pylori* (BOX 1), all transformable bacteria are thought to share common mechanisms of



Transduction
A mechanism of horizontal gene transfer, in which DNA is accidentally transferred to a new host by a bacteriophage vector.

Conjugation
A mechanism of horizontal gene transfer by cell-to-cell contact that is primarily used for plasmid transfer but occasionally leads to chromosomal transfer.

Regulons
Sets of genes that are under coordinated control by dedicated regulatory circuits.

DNA uptake and processing (FIG. 2). These mechanisms rely on conserved proteins, which are mainly encoded by a set of genes that are simultaneously expressed at the onset of competence. In this Review, we will refer to these gene arrays as *com* regulons. Exogenous double-stranded DNA (dsDNA) is the substrate for transformation: one strand is degraded and the other is internalized in single-stranded form through the ComEC transmembrane channel[12] in a 3′–5′ orientation. This model is mainly based on observations from *Bacillus subtilis* and *S. pneumoniae*, which are the two species for which key aspects of transformation have been established. Internalized single-stranded DNA (ssDNA) is bound by the transformation-dedicated DprA (DNA processing A) protein, which loads the recombinase RecA onto ssDNA[13]. RecA polymerizes on the ssDNA and promotes a homology search in the host chromosome. When a homologous sequence is found, RecA-driven homologous recombination occurs, which involves pairing of the ssDNA with a homologous strand of chromosomal DNA to form a transformation heteroduplex. These duplexes can be fully homologous or can contain heterologous DNA (such as pathogenicity islands), which remain in single-stranded form and are flanked by the homologous regions (FIG. 2). Thus, ComEC, DprA and the ubiquitous recombinase RecA have key roles in natural transformation.

Conserved DNA uptake. Gram-positive and Gram-negative bacteria rely on highly similar DNA-uptake systems, with the only major difference being the requirement for transport across the outer membrane in Gram-negative

◀ Figure 1 | **Phylogenetic distribution of naturally transformable species, the DNA-uptake protein ComEC and the DNA-processing protein DprA.** **a** | Phylogenetic tree of naturally transformable species (which was generated as described in REF. 85), on the basis of a collection of 137 conserved protein sequences. The numbers refer to species that are detailed in Supplementary information S1 (table) . The parametric bootstrap[167] values were greater than 96% for all branches except those at the origin of clades 16–20 (66%) and 41–42 (50%). **b** | Phylogenetic analysis of DprA in naturally transformable species. The upper panel shows a schematic of *Streptococcus pneumoniae* and *Escherichia coli* DprA proteins, with the known domains indicated. The centre panel shows a phylogenetic tree that was constructed using subsequences from the pfam02481 domain. The parametric bootstrap[167] values were greater than 90% for all branches except those at the origin of clades 14–15 (87%), 13–17 (75%) and 30–31 (70%). In the lower panel, a comparison of the rate of evolution of DprA sequences and species evolution is shown. The evolutionary distances were computed as described in REF. 85 (each blue dot corresponds to the distance computed for a given species pair and the blue colour gradually changes to red when spot density increases). The data suggest that DprA evolves at a rate that is threefold higher than the rate of species evolution. **c** | Phylogenetic analysis of ComEC in naturally transformable species. The upper panel shows a schematic of *S. pneumoniae* ComEC protein, with the known domains indicated. The centre panel shows a phylogenetic tree constructed using subsequences from the pfam03772 domain. The parametric bootstrap[167] values were greater than 92% for all branches except those at the origin of clades 5–20 (89%), 13–20 (74%), 31–32 (71%), 42–43 (77%), 49–57 (86%), and 54–57 (80%). The lower panel shows a comparison of the rate of evolution of ComEC sequences and species evolution, which suggests that ComEC evolves at a rate that is six-fold higher than the rate of species evolution. The DprA and ComEC trees were computed using PhyML from a multiple alignment obtained using the MUSCLE program and trimmed with the trimAl program, as described in REF. 86; 242 and 168 informative sites, respectively, were used for tree computation. The Le and Gascuel (LG) model of sequence evolution with a Γ-correction (which incorporates four categories of evolutionary rates) was used and the shape parameter and the proportion of invariant sites that were estimated from the data were selected by the ProtTest3 program[86]. The branch supports were estimated using 100 replicates of non-parametric bootstrap. e, extension of Rossman fold; RF, Rossman fold; SAM, sterile alpha motif.

organisms, which involves the PilQ secretin channel[6] (FIG. 2). Although protein nomenclature varies between species, we mainly use the *B. subtilis* nomenclature as the DNA-uptake system was first characterized in detail in this bacterium. For DNA transport across the outer layer of the envelope (which consists of the outer membrane and the peptidoglycan layer), transformable Gram-negative species use proteins that are related to those involved in the assembly of type II secretion systems and type IV pili (T4P)[14,15]. It has been proposed that a similar structure to T4P, known as a competence pseudopilus, participates in DNA transport not only in Gram-negative bacteria but also in Gram-positive bacteria. However, despite a correlation between the presence of T4P and competence in Gram-negative species, whether pili themselves have a direct role in transformation has remained unclear[6]. In Gram-positive bacteria, competence pseudopili are encoded in the *comG* operon and ComGC is the major pilin[6,16]. Protrusion of competence pseudopili beyond the cell surface was considered to be limited. This assumption originated from the observation that a *B. subtilis* cell typically contains 40–100 ComGC monomers and, assuming that each monomer is ~1 nm in length, this would result in a pseudopilus with an overall length of 40–100 nm (compared with the ~55 nm thickness of the periplasm and the cell wall)[17]. However, a bona fide T4P of 2–3 μm in length was recently identified in *S. pneumoniae* cells[18]. This appendage binds directly to exogenous dsDNA and is required for transformation; it has therefore been named the transformation pilus (Tfp) (FIG. 2). As the pneumococcal Tfp remained elusive despite many years of investigation, and given the presence of T4P-related structures in both Gram-negative and Gram-positive species, it seems probable that both groups use a Tfp to convey exogenous DNA to the dsDNA receptor ComEA[19]. Pilus retraction[20] might participate in DNA transport but experimental evidence is currently lacking.

ComEA presumably delivers dsDNA to a protein that generates ssDNA for internalization (FIG. 2). In *S. pneumoniae*, the nuclease EndA degrades one DNA strand, thereby enabling import of the complementary strand through the ComEC pore[21,22] (FIG. 2). A protein with a similar function to EndA has not yet been identified in other species[6,23]. Recruitment of EndA to midcell was recently documented and was shown to require the presence of ComEA but not ComEC or Tfp[24]. Midcell-bound dsDNA was found to colocalize with ComEA and EndA, which suggests that uptake occurs at this position in *S. pneumoniae*[24], whereas polar DNA uptake is observed in *B. subtilis*[25]. These data suggest that there is no general rule that governs the subcellular location of the DNA-transport machinery. In Firmicutes, the associated ComFA protein is thought to function as an ATP-dependent translocase that drives internalization of ssDNA[6,26].

Thus far, only *H. pylori* is an exception to this general model (BOX 1), as it does not rely on Tfp for transfer of exogenous DNA to the periplasm, although a distant homologue of the ComEC channel (Supplementary information S1 (table)) is required for the uptake of transforming DNA[27,28]. Although this finding suggests that all naturally transformable bacteria encode a ComEC homologue, this hypothesis has not previously been rigorously tested. Therefore, a phylogenetic tree of ComEC homologues from transformable species was constructed and compared with species and DprA phylogenetic trees (see below). The data reveal that the species, DprA and ComEC trees show good congruence (FIG. 1), which suggests that ComEC is stably maintained as part of the core genome of transformable species.

Conserved DNA processing. Homologous recombination is initiated in all organisms by ssDNA; thus, internalized transforming ssDNA is an ideal substrate for recombination. However, homologous recombinases (such as RecA in prokaryotes and Rad51 in eukaryotes) require cofactors called recombination mediator proteins (RMPs)[29] to load onto ssDNA and assist the recombination process. DprA of *S. pneumoniae* has been proposed to be a transformation-dedicated RMP that facilitates RecA loading onto internalized ssDNA[8,13]. Further structure–function studies of DprA support this view and have identified residues that are important for interactions with RecA, as well as revealing the importance of DprA dimerization for recombination[30]. An investigation of the conservation of DprA in prokaryotes has revealed that its pfam02481 domain (FIG. 1b) is present in 84% of 317 completely sequenced bacterial genomes[13]. Moreover, an

Transformation heteroduplex
A DNA duplex that is comprised of one strand of host chromosomal DNA and a complementary strand of internalized DNA.

Pili
Filamentous extracellular appendages that are present in some bacteria and that participate in different processes. Transformation pili promote the capture of exogenous DNA for uptake.

Box 1 | ***Helicobacter pylori*: the black sheep**

It is often said that there is an exception to every rule. In the case of natural bacterial transformation, this exception seems to be the Gram-negative gastric pathogen *Helicobacter pylori*.

An atypical DNA uptake machinery

Rather than relying on a transformation pilus (Tfp) for DNA uptake, *H. pylori* uses a type IV secretion system[122]. This secretion system, which is known as competence protein B (ComB), was shown to be essential for transformation[122,123]. However, a homologue of the ComEC transmembrane pore is also present and is required for transformation[27,28]. Notably, this ComEC protein is only distantly related to other members of the family; it is much shorter (437 amino acids compared with 746 amino acids for pneumococcal ComEC) and lacks the carboxy-terminal domain (smart00849; FIG. 1c). It has been suggested that DNA uptake involves a two-step process, in which ComB transports DNA into the periplasm, followed by subsequent import into the cytoplasm through ComEC[28]. This differs substantially from the mechanism involving Tfp that is used by all other bacteria that have been characterized so far, including both Gram-positive and Gram-negative species (FIG. 2).

Constitutive competence?

Unlike bacteria that require environmental and cellular signals to induce competence, *H. pylori* is often described as being constitutively competent[124]. However, this conclusion was made on the basis of a study in which peaks of transformation were observed at different growth phases, but periods of no transformation were also observed[125], which suggests that regulation of competence must occur at some level in this species. Clearly, further work is required before concluding that *H. pylori* competence is really constitutive.

Intriguingly, it has also been reported that fluoroquinolone-mediated DNA damage in *H. pylori* triggers genetic exchange[124]; however, although a number of competence genes are induced, the induction of *comEC* and *dprA* was not observed. This is surprising considering that the encoded proteins have crucial roles in natural transformation in other species. At the regulatory level, the recombinase RecA was found to be required for the induction of competence, which led to the suggestion that RecA is involved in sensing and transmitting the DNA damage signal[124]. However, considering that LexA, which is the master regulator of the SOS response, is absent in *H. pylori*, the mechanism of RecA-mediated induction remains elusive. Finally, as this study was carried out using the *H. pylori* strain for which distinct peaks of competence are observed during growth[125], caution is needed when interpreting variations in *com* gene expression, as these variations could be the consequence of growth perturbations.

Despite these potential limitations, it is clear that *H. pylori* is an exception to several of the general rules that govern natural transformation in other bacteria. Transcriptomic studies of gene expression are currently lacking and are needed to determine the genes that are highly expressed when transformation rates are maximal and to establish a model for this unusual transformation system.

analysis of the genome sequences of transformable species shows that DprA is conserved in all of these species, which strongly suggests that a functional DprA protein is a conserved characteristic of naturally transformable species (Supplementary information S1 (table)). There is also high phylogenetic congruence between the DprA and species phylogenetic trees (FIG. 1a,b), which indicates that DprA is stably maintained as part of the core genome of transformable species. Although many species that encode DprA have not been shown to be transformable, a role for DprA in processes other than transformation has not been found. Thus, DprA may be a remnant of ancestral transformability or, alternatively, some of these species may be transformable but this has not yet been detected (see below).

Despite the observation of longer phylogenetic distances between ComEC genes than between DprA genes (FIG. 1b,c), which suggests a faster evolution rate for ComEC, the congruent phylogenies of two key transformation proteins and transformable species suggests that transformation is an ancient process that was inherited from a common ancestor.

Divergent central competence regulators

Competence is a regulated property and regulation occurs at specific growth phases in different bacteria. It is induced during early exponential phase in *S. pneumoniae*, but restricted to stationary phase in *B. subtilis*. *H. pylori* and *Neisseria* spp. are exceptions, and are generally described as being 'constitutively competent' (although this is questionable for *H. pylori*; BOX 1). Despite conserved DNA-uptake and processing mechanisms, competence regulation varies considerably among transformable species. Central competence regulators, which are defined as proteins that directly control the *com* regulon, include dedicated alternative sigma factors (alternative σ factors), transcription activators and transcription co-regulators. Interestingly, all known central competence regulators function as activators. By contrast, even though competence is regarded as an SOS substitute in some species[10,31,32], the master regulator (LexA) of the SOS response functions as a repressor until the SOS response is triggered (reviewed in REF. 33). Below, we focus on the best characterized examples of each type of central competence regulator to illustrate the stark regulatory differences that exist.

Alternative σ factors. A number of transformable species rely on an alternative σ factor to regulate competence. The best characterized alternative σ factor, σ^X of *S. pneumoniae*, is encoded by two identical *comX* genes[34] and is widely distributed among the Firmicutes. σ^X forms a complex with RNA polymerase that recognizes an 8 bp sequence in the promoter of all *com* genes that are required for transformation[35] (with the exception of *endA*) resulting in the induction of competence (FIG. 3a). It is likely to be the central competence regulator in all streptococci[36] as a similar role for σ^X has been identified in several species[37–39]. Reliance on an alternative σ factor for competence regulation is unlikely to be exclusive to streptococci as σ^X is also present in Lactobacillales[36], and a σ factor that is phylogenetically related to σ^X (known as σ^H) has recently been shown to control competence in the distantly related Firmicute *Staphylococcus aureus*[40].

Transcription activators. The best characterized example of a transcription activator that controls competence is the *B. subtilis* ComK protein[41]. In this bacterium, competence is induced in stationary phase when ComK binds to its own promoter, which creates an autostimulatory cycle that is central to the development of competence (reviewed in REF. 10). Unusually, only 15% of the population becomes competent, which is attributed to stochastic fluctuations in the cellular levels of ComK, resulting in so-called bistability[42–47]. ComK drives the expression of more than 100 genes, including all the genes required for transformation (FIG. 3a), and most of these genes are directly regulated by the binding of ComK to recognition sites within their promoters[48–50].

Alternative sigma factors (alternative σ factors). Alternative RNA polymerase cofactors that direct the RNA polymerase to specific promoters.

Transcription co-regulators Proteins that lack DNA-binding activity but can interact with and stimulate the activity of a transcription activator, thus indirectly promoting transcription.

SOS response A global response to DNA damage that enables DNA repair in bacteria.

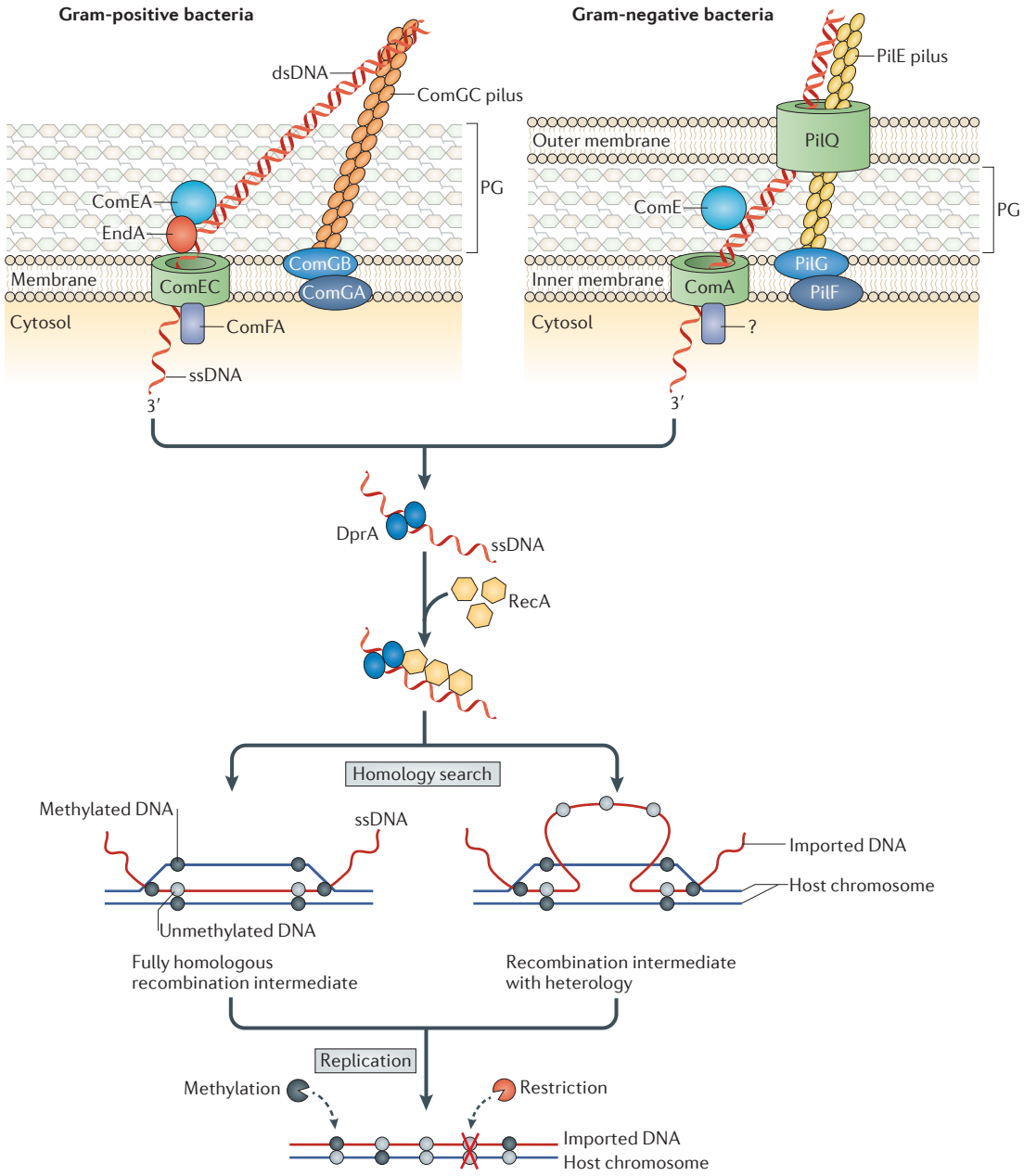


Figure 2 | **An overview of the transformation process.** The key steps of transformation in Gram-positive and Gram-negative species are shown. The DNA-uptake machinery generally comprises a transformation pilus (Tfp), which consists mainly of ComGC subunits in Gram-positive bacteria and captures exogenous double-stranded DNA (dsDNA), the DNA receptor ComEA and the transmembrane pore ComEC. In *Streptococcus pneumoniae*, the EndA nuclease receives DNA from the DNA receptor ComEA and degrades one DNA strand , whereas unidentified nucleases (or strand-separating proteins) generate single-stranded DNA (ssDNA) for uptake in other species. In Firmicutes, ssDNA internalization through ComEC is presumably driven by the ATP-dependent translocase ComFA. In Gram-negative bacteria, such as *Neiserria gonorrhoeae*, the PilQ secretin channel enables the pilus (which is mainly composed of PilE subunits) to cross the outer membrane and dsDNA is transported across the outer membrane through PilQ. In both Gram-positive and Gram-negative cells, additional proteins are required for DNA uptake (for example, the ComGA and ComGB proteins of Firmicutes). A homologue of the ComFA translocase might be present in Gram-negative bacteria, but this is currently unclear. Internalized ssDNA is presumably bound by DprA (DNA processing protein A), which recruits the recombinase RecA. RecA polymerizes on ssDNA and promotes a homology search along chromosomal DNA, followed by strand exchange. The transformation heteroduplex that forms can be a fully homologous double-stranded recombination intermediate, or if the imported DNA contains heterologous sequences (such as a pathogenicity island) flanked by homology, a recombination intermediate with a single-stranded loop is formed. If heterologous donor DNA is unmethylated (light grey circles), this DNA remains fully unmethylated in the recipient chromosome after replication. The methylation and restriction activities of the restriction–modification (R–M) system compete (dashed arrows) for access to this sensitive DNA, and restriction can kill transformants and limit heterologous transformation. PG, peptidoglycan.

Transcription co-regulators. The competence activator Sxy in *H. influenzae* and its orthologue[51], TfoX, in *V. cholerae* are transcription co-regulators. Interactions between cyclic AMP (cAMP)[52,53] and its receptor protein CRP[54] (cAMP receptor protein) are required but not sufficient to induce competence in *H. influenzae*. The *sxy* gene was subsequently shown to be essential for competence[55] and the encoded protein is thought to function as a positive regulator, despite lacking a DNA-binding motif[56]. Microarray studies identified 25 genes in the *com* regulon that depend on both CRP and Sxy for expression[57]. Sites of 22 bp were found in the promoters of these genes (known as CRP-S sites) and the binding of CRP was shown to be dependent on Sxy. Thus, Sxy has been suggested to function as an activating cofactor for CRP, preferentially directing it to competence-specific CRP-S sites rather than canonical CRP sites[56,57].

In *V. cholerae*, TfoX is essential for competence[58] and, as it is an orthologue of Sxy, it presumably functions as a CRP-activating cofactor[59]. Surprisingly, TfoX does not directly regulate expression of the DNA-uptake genes *comEA* and *comEC*, which were recently shown to be controlled by the transcription activator QstR[60]. Although *qstR* itself is positively regulated by TfoX, this is the first example of bipartite regulatory control of a *com* regulon (FIG. 3a).

Figure 3 | **Divergent competence regulatory cascades. a** | Competence genes can be regulated by alternative σ factors (for example, σ^X in streptococci such as *Streptococcus pneumoniae*), transcription factors (such as ComK in bacilli such as *Bacillus subtilis*) and transcription co-regulators (such as the cAMP receptor protein (CRP) cofactor TfoX in *Vibrio cholerae*). Although TfoX directly regulates most of the *com* genes in *V. cholerae*, it indirectly activates *comEA* and *comEC* by regulating the expression of *qstR*[60]. **b** | Distribution of the two distinct two-component systems that regulate competence in streptococci: ComDE and ComRS. The ComDE system is present in *S. pneumoniae* and the *Streptococcus mitis* and *Streptococcus anginosus* groups, and ComRS is present in the *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus bovis* and *Streptococcus pyogenes* groups. Both of these systems regulate expression of the central competence regulator σ^X. *Lactococcus lactis* was used as an outgroup in the distribution analysis. Filled circles with white digits indicate species that have been shown to be naturally transformable. Scale bar represents phylogenetic distance. For information on the genomes and methods used to create the tree, see REF. 85. Two additional transformable species included here are *Streptococcus infantarius* (species NC_016826, genome CJ18) and *Streptococcus macedonicus* (species NC_016749, genome ACA-DC 198)[39]. Part **b** is modified, with permission, from REF. 85 © (2013) Proceedings of the National Academy of Sciences, USA.

Regulating central regulators

In addition to important differences between the central regulators of competence in transformable species, the regulatory cascades that govern the activity of these regulators are also diverse.

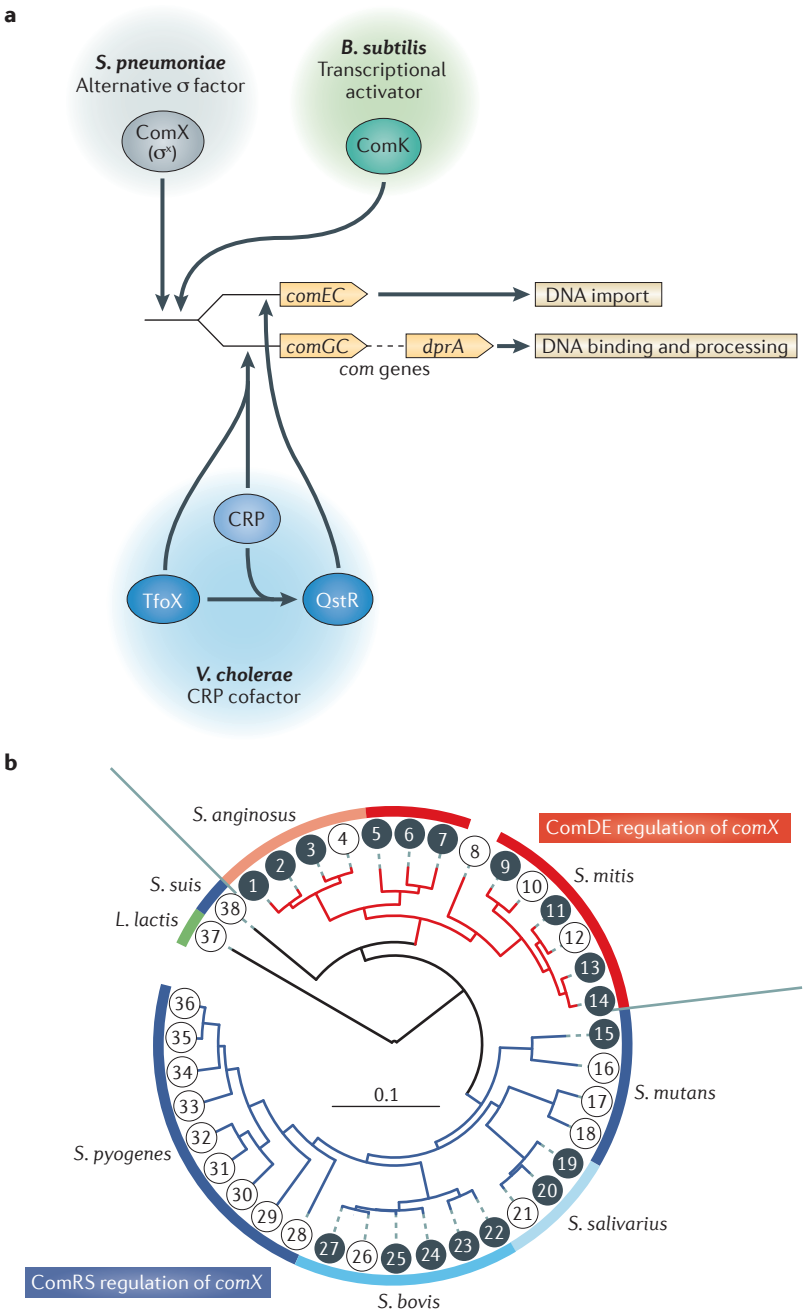
Two-pronged regulation of ComK. Expression of the central regulator of competence in *B. subtilis* is controlled at the transcriptional and post-translational levels[61]. Briefly, five proteins directly control *comK* expression: ComK itself[62] and the response regulator DegU[63] activate transcription, whereas transition state regulatory protein AbrB[64], GTP-sensing transcriptional pleiotropic repressor CodY[65] and Rok (repressor of *comK*)[66] inhibit transcription. The post-translational control of ComK stability involves ComS, ClpCP and the adaptor protein MecA. After formation of a ternary complex comprised of MecA, ComK and ClpC, MecA targets ComK to the ClpP protease for degradation[67,68]. ComS, which is the product of a small ORF that is embedded in the *sfrA* operon[69], has a key role in promoting the stability of ComK by displacing it from this ternary complex[67,68]. Expression of the *srfA* operon, and thus ComS production, also depends on a complex regulatory system involving the response regulator ComA[70] (BOX 2), which is interconnected with other adaptive responses (for example, sporulation), as well as the transcriptional regulators CodY[65] and peroxide-responsive repressor PerR[71] (reviewed in REF. 10).

Species-specific regulation of σ^X expression. The competence regulator σ^X is controlled by two distinct regulatory cascades in different streptococcal species. In *S. pneumoniae*, competence is initiated by a 17-residue competence-stimulating peptide (CSP), which is encoded by the *comC* gene and is exported and matured by the dedicated ComAB exporter[72–74]. At a critical concentration, extracellular CSP activates the two-component signal-transduction system (TCS) ComDE[73]. Interactions between CSP and the transmembrane histidine kinase ComD presumably lead to autophosphorylation of ComD, which then phosphorylates and thereby activates the response regulator ComE[75]. Phosphorylated ComE (ComE-P) directly activates expression of *comAB* and *comCDE* by binding to direct repeats in the promoters of these operons, thereby creating an autocatalytic feedback loop that amplifies the signal and coordinates competence in the population. ComE-P uses the same mechanism to directly activate the *comX* genes[75], which results in the production of σ^X (BOX 2) and thus promotes expression of the proteins that are required for transformation.

Although pneumococcal σ^X is regulated by ComDE, phylogenomic analysis has shown that many streptococci lack this TCS, which suggests that a different regulatory circuit is present in other species[36]. This alternative circuit, which was recently characterized in *S. thermophilus*[76,37] and *Streptococcus salivarius*[37], involves the transcriptional activation system ComRS. The system is induced by expression of the ComS peptide, which is processed (probably by the Eep membrane protease) to produce mature XIP (*comX*-inducing peptide). XIP initially accumulates extracellularly[77], but at a critical concentration, XIP is imported back into the cytoplasm by the oligopeptide permease Opp (also known as Ami)[76] and directly interacts with ComR[37,78] (which belongs to the Rgg[79] transcriptional regulator family[80]). The ComR–XIP

Orthologue
A homologous gene that is derived by a speciation event from a single ancestral sequence. Orthologues typically carry out equivalent functions in closely related species.

Two-component signal-transduction system (TCS). A system that comprises a histidine kinase and a response regulator; TCSs enable bacteria to sense signals (including those in the extracellular environment) and to regulate genes accordingly.



1. *Streptococcus intermedius*
2. *Streptococcus constellatus*
3. *Streptococcus anginosus* (strain CCUG_39159)
4. *Streptococcus anginosus* (strain 1_2_62CV)
5. *Streptococcus gordonii* (strain Challis)
6. *Streptococcus sanguinis* (strain SK36)
7. *Streptococcus cristatus*
8. *Streptococcus australis*
9. *Streptococcus infantis*
10. *Streptococcus peroris*
11. *Strepotococcus oralis*
12. *Streptococcus sanguinis* (strain ATCC_49296)
13. *Streptococcus mitis* (strain B6)
14. *Streptococcus pneumoniae* (strain R6)
15. *Streptococcus mutans* (strain UA159)
16. *Streptococcus macacae*
17. *Streptococcus criceti*
18. *Streptococcus downei*
19. *Streptococcus salivarius*
20. *Streptococcus thermophilus*
21. *Streptococcus vestibularis*
22. *Streptococcus equinus*
23. *Streptococcus infantarius*
24. *Streptococcus macedonicus*
25. *Streptococcus gallolyticus*
26. *Streptococcus pasteurianus*
27. *Streptococcus bovis*
28. *Streptococcus agalactiae*
29. *Streptococcus urinalis*
30. *Streptococcus parauberis*
31. *Streptococcus uberis*
32. *Streptococcus porcinus*
33. *Streptococcus ictaluri*
34. *Streptococcus equi*
35. *Streptococcus dysgalactiae*
36. *Streptococcus pyogenes*
37. *Lactococcus lactis*
38. *Streptococcus suis*

complex then activates transcription of *comX*, which promotes the development of competence (BOX 2).

Streptococcus mutans deserves a specific mention. Whereas *S. pneumoniae* possesses two paralogous[51] TCSs (ComDE and BlpRH), phylogenomic analysis has shown that *S. mutans* possesses only the orthologue of BlpRH, which was unfortunately annotated ComDE[36]. As its name suggests, in *S. pneumoniae* BlpRH controls the expression of the *blp* regulon, which encodes a group of bacteriocins[81]. The phylogenetic differences between ComDE and BlpRH have mostly been ignored, and even the most recent literature[82] still refers to the *S. mutans* TCS as ComDE. However, the master regulatory system of competence in *S. mutans* has since been shown to be ComRS[38]. This suggests that the operon controlled by the mistakenly annotated ComDE TCS in *S. mutans* actually regulates bacteriocin production and should be renamed accordingly. This would involve changing the incorrectly annotated *S. mutans* CSP to BIP (for bacteriocin- or *blp*-inducing peptide). However, the BlpRH locus has been shown to activate competence in *S. mutans*. How is this possible? Activation is clearly indirect and involves delayed stimulation of ComRS[83,84]. Consistent with the observation that competence is induced under stressful conditions in some species (see below), we propose that bacteriocin production by a fraction of the population puts a stress on the non-producing population (those cells that lack immunity to the bacteriocin), which eventually induces competence via the ComRS system.

Although both the ComDE and ComRS systems rely on an exported peptide (XIP and CSP, respectively), XIP interacts with membrane-bound ComD extracellularly in *S. pneumoniae*, whereas CSP is actively imported back into the cell to directly interact with ComR in *S. thermophilus* and *S. mutans*. A phylogenetic tree of streptococci updated on the basis of concatenation of a set of orthologous core proteins[85] enables visualization of the distribution of ComDE and ComRS regulatory networks within the streptococci (FIG. 3b). This analysis shows that ComDE-dependent regulation occurs in the *Streptococcus mitis* and *Streptococcus anginosus* groups, whereas ComRS-dependent regulation occurs in the *Streptococcus bovis*, *S. salivarius*, *S. mutans* and *Streptococcus pyogenes* groups. Thus, two divergent competence regulatory cascades have evolved, both culminating in tight control of streptococcal σ^X production and competence.

Varied regulatory mechanisms. The regulatory cascades that are described above document transcriptional activation of a central competence regulator. However, a large variety of other regulatory mechanisms exist in different transformable species.

Several diverse mechanisms that control the translation of competence regulators have been documented in different species. For example, in *V. cholerae*, the sugar polymer chitin is required for transcription and translation of the competence activator TfoX[58,86]. It was recently shown that a 102 nucleotide non-coding small RNA (sRNA) — TfoR — the expression of which is stimulated by the transmembrane regulator TfoS in the presence of chitin[87], activates translation of TfoX by interacting directly with

Box 2 | **Autoinducers, peptides and competence**

The signalling molecules that induce competence are either peptides or autoinducers. Peptides are synthesized by the ribosome, whereas autoinducers are produced by dedicated synthases (see below).

Synthesis of signalling molecules

Two autoinducers are involved in the induction of competence in *Vibrio cholerae*: These are CAI-1 (cholerae autoinducer 1) and AI-2 (autoinducer 2), which are produced by the synthases CqsA[126] and LuxS[127], respectively (see the figure, part **a**). In *Bacillus subtilis*, two peptide pathways control competence. The main peptide involved is ComX (a small peptide consisting of ten amino acids), which is initially synthesized as a 55-residue precursor and is post-translationally modified by ComQ before export to the extracellular environment[10] (see the figure, part **b**). The signalling pentapeptide CSF (competence and sporulation factor) is also involved; its precursor (PhrC) is exported by Sec and then processed into CSF by one of three redundant proteases[128–130] (see the figure, part **b**). Similarly, the 17-residue competence-stimulating peptide (CSP) of *Streptococcus pneumoniae* is processed and exported by the ComAB transporter from a 45-residue pre-CSP protein that is encoded by *comC*[10] (see the figure, part **c**). Interestingly, there are only two CSP pherotypes in *S. pneumoniae*, whereas the closely related commensal bacterium *Streptococcus mitis* possesses multiple different pherotypes[10]. Unlike in *S. pneumoniae*, the ComX-inducing peptide (XIP) precursor ComS (which consists of 24 residues) of *Streptococcus thermophilus* lacks a dedicated exporter. In this species, ComS is possibly exported by the Sec translocon and undergoes one or more unknown maturation steps to produce the heptapeptide XIP[37,77] (see the figure, part **d**).

Sensing and responding to autoinducers

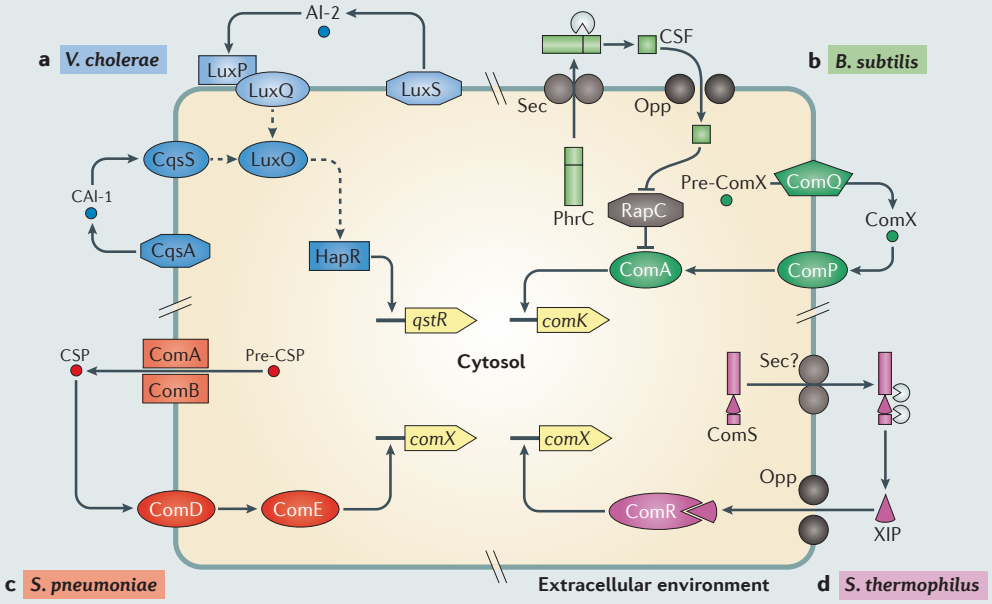
Extracellular CAI-1 is sensed by the sensor kinase CqsS[131], whereas AI-2 is sensed by the LuxPQ receptor complex[132] (see the figure, part **a**). In the absence of these autoinducers, the response regulator LuxO is phosphorylated and the downstream regulatory cascade inhibits production of the HapR regulator (for full details, see REF. 133), whereas at high cell densities both signals promote dephosphorylation of LuxO, thereby promoting HapR production[134] (see the figure). HapR drives the production of QstR, which is the transcriptional activator of *comEA* and *comEC*, resulting in the induction of competence[60].

Sensing and responding to peptides

Competence peptides are either sensed extracellularly or are actively imported back into the cytosol. In *B. subtilis*, ComX is sensed extracellularly by the histidine kinase ComP, which autophosphorylates and passes a phosphoryl group to its cognate response regulator ComA[10] (see the figure, part **b**). Phosphorylated ComA (ComA-P) activates the transcription of ComS, which results in ComK accumulation and competence development. Similarly to ComX, CSP stimulates the ComD histidine kinase, which phosphorylates the ComE response regulator in *S. pneumoniae*. Phosphorylated ComE (ComE-P) actively stimulates expression of σ^X, which leads to the induction of competence[75] (see the figure, part **c**). Conversely, XIP is imported by the oligopeptide permease Opp[76] and activates ComR intracellularly to drive σ^X expression in *S. thermophilus*[37] (see the figure, part **d**). In *B. subtilis*, CSF is also imported through Opp and indirectly activates competence by inhibiting the ComA-antagonizing protein RapC[10] (see the figure, part **b**).

Modulating CSP production

CSP is not a true quorum-sensing device as its accumulation does not depend on pneumococcal cultures reaching a critical cell density. By contrast, its production is induced in response to external[31] or cellular cues. This is illustrated by direct and indirect control of *comCDE* expression by the CiaRH two-component system (not shown)[135]. The response regulator CiaR regulates the expression of small RNAs that inhibit competence by masking the ribosome binding site of the *comC* mRNA, thus repressing the production of CSP[136]. CiaR also regulates the membrane-associated HtrA protease, which is proposed to sense errors in translation and repress competence by digesting extracellular CSP when error rates are low[137,138].



Bacteriocins
Small peptides that are produced by bacteria to inhibit the growth of other species (sometimes closely related species) to which the producer possesses an immunity mechanism.

Non-coding small RNA
A functional RNA molecule that is not translated into a protein.

CAI-1
(Cholerae autoinducer 1).The main quorum-sensing signalling molecule of the human pathogen *Vibrio cholerae*; it has been identified as (S)-3-hydroxytridecan-4-one.

AI-2
(Autoinducer 2). An inter-genera signalling molecule that is involved in quorum-sensing; it has been identified as the furanyl borate diester (2S,4S)-2-methyl-2,3,3,4-tetrahydroxy-tetrahydrofuran borate.

CSF
(Competence and sporulation factor). A signalling molecule that contributes to quorum-sensing in *Bacillus subtilis*. CSF is a pentapeptide that is produced from Phr precursor peptide, which is re-imported by the oligopeptide permease Opp.

Pherotypes
Streptococci that produce the same competence-stimulating peptide (CSP) belong to the same pherotype.

the *tfoX* mRNA[88]. This interaction enables the ribosome to access the ribosome binding site (RBS) on the *tfoX* mRNA, which is otherwise sequestered by a secondary structure in the 5′ end of the mRNA. Nutrient starvation induces natural competence in *H. influenzae*[89], *Actinobacillus pleuropneumoniae*[90] and *Actinobacillus suis*[91], and a recent study showed that depletion of purine nucleotides activates translation of the *sxy* mRNA, which encodes the central competence regulator Sxy[91]. Conversely, high levels of purines inhibit translation of *sxy*; thus, competence in *H. influenzae* is modulated in response to nucleotide pools. Although the exact mechanism remains unclear, regulation is dependent on a stem structure in the *sxy* mRNA; this is consistent with the finding that RNA secondary structure regulates the translation of *sxy* and the induction of competence[92]. Another example of translational regulation occurs in *S. aureus*, in which an inverted repeat that is immediately upstream of the *sigH* translational start site sequesters the RBS within a stem-loop secondary structure, thus inhibiting the production of σ^H (REF. 40).

Post-translational regulation of central competence regulators is not limited to *B. subtilis* ComK (see above). In *S. thermophilus*, the adapter protein MecA was recently shown to function as a negative regulator of competence, and it interacts with both ClpC and σ^X (REF. 93), which is consistent with a role for ClpC in σ^X degradation[94]. The Clp proteases are also involved in σ^X regulation in *S. pneumoniae*, as ClpE targets σ^X to ClpP for degradation[95]. In this species, a competence-dependent protein ComW contributes to the stability and activation of σ^X, although the underlying mechanisms remain unclear[95].

The regulatory systems that are described above illustrate the wide variety of molecular mechanisms that control competence. These regulatory cascades are induced by a range of environmental or endogenous triggers, which are summarized below.

Recurrent themes and inducing cues

An examination of the environmental and cellular cues that trigger competence reveals that common themes exist. However, the signals that are involved are diverse and sometimes have opposing effects in different bacterial species.

Autoinducers, peptides and competence. Induction of competence in *S. pneumoniae* has frequently been described as one of the first examples of quorum-sensing in bacteria. However, if quorum-sensing is defined in the strictest sense as a mechanism that relies on a critical cell density for induction, then pneumococcal competence is not consistent with this definition, as it can develop at widely different cell densities[10] and is specifically induced by external stimuli[31] (for example, by antibiotics such as streptomycin or the fluoroquinolones; BOX 2). Instead, the pneumococcal competence model can be considered to be an example of cell–cell signalling, in which cells coordinate the response to environmental and cellular triggers, irrespective of cell density. Coordination involves the synthesis and export of CSP, which promotes competence in neighbouring pneumococci; thus, CSP functions as an alarmone or a timing device[10]. The use of peptides or autoinducers as an induction cue is a recurring theme in transformable species; however, the diversity of the peptides that are involved (in terms of their synthesis, structure and mode of sensing; BOX 2) supports the conclusion that competence regulatory cascades evolved independently in different species[10,36].

Nutritional status. A frequently encountered signal for the induction of competence is the availability of nutrients in the extracellular environment. Exhaustion of nutrients coincides with maximal transformability in *H. influenzae*, which becomes competent on entry into stationary phase[55] or following transfer from rich to defined non-growth medium[89]. Similarly, competence spontaneously develops in *B. subtilis* in stationary phase, which suggests that nutritional exhaustion is an induction cue[10]. However, nutrient starvation is not a universal trigger of competence; in *Acinetobacter baylyi*, competence is induced by high nutrient levels[96], and in *S. thermophilus*, competence is induced by casein-derived peptides[78].

The situation is more complex in *V. cholerae*, as competence is induced by the absence of glucose, which shows that there is a link between competence and carbon catabolite repression (CCR)[97]. However, it is difficult to conclude that *V. cholerae* competence is a response to nutrient starvation as it is also induced by the biopolymer chitin[58], which is a source of both carbon and nitrogen and is abundant in the aquatic habitat of the bacterium[98]. The divergence of inducing cues is further illustrated by *S. pneumoniae* and *H. influenzae*, which are two species that inhabit the human nasopharynx, where nutrients are in limited supply[99]. Nutrient starvation induces competence in *H. influenzae*, whereas no nutritional signal has been reported for *S. pneumoniae*. By contrast, *S. pneumoniae* becomes competent during early exponential growth in the laboratory (when nutrients are not limiting).

Stressful conditions. Competence can also be induced in response to environmental stresses that threaten cell survival. Sublethal concentrations of aminoglycoside and fluoroquinolone antibiotics were shown to induce competence in *S. pneumoniae*[31] and *Legionella pneumophila*[100]. Competence in both species is also induced by exposure to DNA-damaging agents, such as ultraviolet (UV) radiation and mitomycin C. However, neither fluoroquinolones nor mitomycin C induce competence in *S. thermophilus*; these compounds actually reduce transformation[101] (see below). Hydroxyurea generates stalled replication forks and was shown to induce competence in *S. pneumoniae*[32] and *L. pneumophila*[100], which suggests that there is a link between replication stress and the induction of competence.

As described above, competence is induced in *H. influenzae*[91] by depletion of purine pools. There is also a link between nucleotide pools and competence in other species; for example, the central competence regulator CodY (which represses competence in *B. subtilis*[65]) senses and responds to GTP levels[10,102]; and in *V. cholerae*,

Quorum-sensing
The regulation of gene expression in response to cell density; secreted inducing molecules are sensed and induction occurs only when a critical cell density is reached.

Cell–cell signalling
A mechanism of cell–cell communication in which an inducing molecule is produced and can be sensed by neighbouring cells, resulting in coordinated gene expression. This mechanism is not necessarily dependent on cell density owing to the fact that inducer expression can be regulated by external signals.

Alarmone
A signalling molecule that is produced by bacteria in response to stress, which stimulates the expression of proteins involved in cellular processes that counteract the stress.

Autoinducers
Non-proteinaceous chemical signalling molecules that are involved in cell–cell signalling and quorum-sensing.

Carbon catabolite repression
(CCR). A regulatory mechanism in which the regulation of phosphotransferase systems enables the sequential utilization of carbon sources.

Mitomycin C
A DNA-damaging agent that crosslinks target DNA and is toxic to bacterial cells.

Hydroxyurea
A synthetic compound that promotes the stalling of bacterial replication forks by depleting nucleotide pools.

Box 3 | **The pros and cons of the DNA-for-food hypothesis**

Importance of nutritional signals?
The recently documented positive correlation between the depletion of purine pools and induction of competence in *Haemophilus influenzae* has been used to support a nutritional role for competence[91]. Alternatively, changes in nucleotide pools could function as markers of replication stress, which induces competence in *Streptococcus pneumoniae* and *Legionella pneumophila*[32]. This illustrates the difficulty of drawing conclusions on the basis of a single observation. More generally, it no longer seems justifiable to use inducing cues and conditions to infer the present-day role of competence and the selective pressure for its maintenance[10]. For example, nutrient depletion induces the SOS response in *Escherichia coli*[139], but it has not been suggested that this response has any nutritional function.

Recycling internalized DNA
The inclusion of *comEB* — which encodes deoxy-CMP deaminase (an enzyme that is required for the salvage of dCMP) — in a DNA-uptake operon of *Bacillus subtilis*[140] was used to support a nutritional role for the imported DNA[107]. However, *comEB* orthologues are not induced in competent *S. pneumoniae* or *H. influenzae*, arguing against a universal role[10]. Similarly, although digestion of internalized DNA into nucleotides has been documented in *H. influenzae*[141] and *S. pneumoniae*[142], the selective internalization of self-species DNA in *H. influenzae*[143] and the induction of DNA release only from other pneumococci and closely related streptococci via fratricide in *S. pneumoniae*[144,145,146] are inconsistent with the import of DNA for catabolism. More generally, if DNA is used as food, why is uptake limited by tight control of competence in *S. pneumoniae*[75,85], by modulation of DNA binding and uptake in *Neisseria gonorrhoeae*[147] or by the use of species-specific DNA-uptake sequences (DUS) in the Pasteurellaceae[148] and Neisseriacae[149]?

Nucleotide import versus DNA import
The *Vibrio cholerae* extracellular nuclease Dns is argued to have an important role in nutrition at low cell density when competence is switched off[150]; however, Dns is specifically repressed at high cell density[150] by the same regulatory circuit that induces competence (FIG. 3a). Although it is unclear whether uptake of long single-stranded DNA (ssDNA) is more efficient for nutrient provision than the import of nucleotides by dedicated transporters, preventing degradation of exogenous DNA is clearly crucial to enable transformation. Furthermore, Dns[150] activity constitutes an example of the previously suggested efficient route for the consumption of environmental DNA[15]; thus, it seems counterintuitive to assemble a complex transformation machinery to achieve the same goal.

DNA as a nutrient source?
It was previously reported that *E. coli* uses extracellular DNA as the sole source of carbon and energy, and that some *com* genes are essential for this use[151,152]. However, the importance of the *comEC* orthologue *ycal* was not investigated. Investigation of *ycal* is needed to establish if DNA internalization occurs through the transforming DNA uptake machinery. In addition, the conditions for induction of the *com* regulon in *E. coli* remain unknown (BOX 4). Furthermore, recent attempts to confirm these observations in three independent laboratories did not succeed and suggested that there were possible inconsistencies in the previous data. For example, the amount of DNA that was used as the sole carbon source could not account for the increase in biomass that was observed, which would imply other nutrient sources were present[153].

the presence of cytidine impedes *comEA* expression and prevents transformation[103]. Whether these observations indicate that nucleotide pools function as a sensor of replication stress or, alternatively, represent nutritional cues, is discussed in the next section.

Thus, despite similarities among competence regulatory cascades (such as the use of peptides as inducing cues), it is clear that they have almost certainly evolved independently in different species (which is exemplified by the use of different peptides for induction; BOX 2). Furthermore, although common competence-inducing cues also exist, none of these are universal inducers of competence and, in fact, some have opposing effects in different bacteria. Finally, there are probably other unidentified competence-inducing signals, which could provide clues to the conditions under which competence is favourable for a given species.

Fratricide
The ability of competent pneumococcal cells to promote lysis of non-competent neighbouring pneumococci and closely related streptococci, liberating DNA for transformation and virulence factors.

Roles of imported DNA

The evolutionary raison d'être of importing DNA for nutrition, genome maintenance or genome diversification remains controversial[11,104,105]. Although competence undoubtedly facilitates genetic exchange, it has been proposed that the nutritional benefits of internalizing DNA provide a sufficient selective advantage to account for its evolutionary maintenance[106]. However, the arguments surrounding this hypothesis are inconclusive (BOX 3). By contrast, an earlier report concluded that an example of a competence gene selected to cause genetic exchange did not exist[107]. Since then, at least three competence-induced genes (*dprA*, *recA* and *ssbB*) have been shown to favour genetic exchange via the stabilization and/or processing of internalized ssDNA into recombinants, and a fourth gene (*dpnA*) has been shown to favour genetic exchange via the specific protection of heterologous sequences that are integrated into the chrosomome.

Conservation and roles of DNA-processing proteins. Both DprA and RecA are required for protection of incoming ssDNA in *S. pneumoniae*, and their absence results in the immediate degradation of internalized DNA[108]. However, this protective role might not be universal, as plasmid establishment in *B. subtilis*[109] is independent of RecA, which suggests that internalized plasmid DNA is still protected when this protein is absent. In *S. pneumoniae*, further protection from endogenous nucleases is afforded by SsbB, which is the transformation-dedicated paralogue of the essential house-keeping ssDNA-binding protein SSB[110] and SsbB has been shown to promote chromosomal transformation[111]. Although SsbB is also found in *B. subtilis*[8], it is not ubiquitous among transformable species[8]. For example, *H. influenzae* lacks a transformation-dedicated SSB paralogue, but SSB is induced at competence[112], which suggests that it might be a substitute for SsbB in this species and in other transformable species.

Importantly, DprA and RecA are crucial for homologous recombination. Although *recA* is ubiquitous in bacteria, its induction during competence has thus far only been documented in *B. subtilis* and *S. pneumoniae*[10]. In *S. pneumoniae*, *recA* expression was found to be responsible for generating 95% of transformants[113], which suggests that *recA* recruitment to the *com* regulon facilitates chromosomal integration of internalized DNA. By contrast, DprA is specific for transformation and loads RecA onto ssDNA[13,30]. DprA is highly conserved in transformable species (FIG. 1b); this universality among transformable species and its demonstrated role in homologous recombination provide the strongest argument that DNA is imported for chromosomal integration.

DNA for genome repair or genome diversification? Although the active protection of internalized ssDNA from endogenous nucleases is incompatible with the

hypothesis that imported DNA is primarily used as a source of nutrition, the generation of recombinants does not distinguish between whether the imported DNA is used for repair or genome diversification. Direct support for the DNA-for-diversity hypothesis is provided by the competence-induced DpnA protein of *S. pneumoniae*[114], which was recently shown to be crucial for the acquisition of heterologous DNA, such as pathogenicity islands[115]. DpnA belongs to the DpnII restriction–modification system (R–M system), which also includes a classic restriction enzyme and a dsDNA methylase[116]. In general, R–M systems possess only these two activities; however, DpnA is an unusual methylase that modifies ssDNA. Restriction enzymes potentially antagonize transformation by a mechanism that is proposed to involve the post-replication degradation of newly integrated unmethylated heterologous sequences, thereby killing potential transformants[117] (FIG. 2c). However, methylation of internalized ssDNA by DpnA has been shown to protect the transformed chromosomes from attack by the DpnII restriction enzyme. Thus, methylation of ssDNA by DpnA promotes the acquisition of heterologous sequences[115]. As heterologous sequences are of no use for genome maintenance, this study provides the first direct evidence that imported DNA in *S. pneumoniae* is primarily used to generate genome diversity[115,118]. The recruitment of *dpnA* to the *com* regulon of *S. pneumoniae* thus suggests that competence has evolved owing to an evolutionary pressure to increase genome diversification.

Limiting DNA import. The existence of various mechanisms that limit competence (see below) or the uptake of exogenous DNA in different species (BOX 3) are also difficult to reconcile with the DNA-as-food hypothesis. The limitation of DNA uptake might arise from the inherent risk of altering the order of genes and the overall structure of the recipient chromosome, which can occur when exogenous DNA is integrated. Mechanisms that trigger the lysis of close relatives that share the same environment (BOX 3) — leading to the release of DNA — also minimize this risk, although even transformation with self-DNA was recently shown to trigger the formation of large tandem duplications, ranging in size from ~100 kb to ~900 kb at several chromosomal locations in *S. pneumoniae*[119]. Considering that duplicated genes provide opportunities for the evolution of new traits, these events illustrate not only the genetic plasticity that is potentially afforded by transformation but also the inherent risk of disrupting chromosome organization.

Roles of competence

Although competence is generally defined as the ability to internalize exogenous DNA, some competence-dependent phenomena might not rely on DNA uptake. Evidence that competence per se enhances pneumococcal survival during stress was recently obtained[32]. This is reminiscent of an earlier observation that competent pneumococci show a transient increase in resistance to UV radiation[32], possibly owing to the temporary increase in the cellular levels of recombination proteins, such as RecA, which are needed for repair. In another striking example, a relationship between competence and virulence in *Listeria monocytogenes* has also been documented[120]. Although this species is not known to be naturally transformable, it possesses a *comK* gene that is interrupted by a temperate prophage. Prophage excision is specifically induced during growth of the bacterium in macrophages, leading to restoration of the *comK* coding sequence and induction of the *com* regulon, which promotes bacterial escape from phagosomes[120]. The precise role of competence remains unclear, as only the ComEC channel was required, whereas Tfp and ComEA were dispensable, which strongly suggests that DNA uptake does not contribute to virulence.

More generally, the number of genes that comprise the *com* regulon varies widely between different species, which raises questions regarding the roles of the additional genes. In *H. influenzae*, the Sxy regulator controls a very limited set of genes (26 in total), 17 of which are absolutely required for transformation[112]. By contrast, ComK drives the expression of more than 100 genes in *B. subtilis*, whereas only a quarter of these genes are required for transformation[10]. Expression of the *B. subtilis com* regulon was thus suggested to result in a differentiated state known as the K-state, one feature of which renders cells naturally transformable[48]. Similarly, although *S. pneumoniae* σ^X controls the expression of all of the key transformation genes, at least 70 genes of the regulon are not required for transformation[35]. As a result, it was proposed that transient induction of the σ^X regulon should be referred to as the X-state (rather than competence)[10]. However, notably, in both *B. subtilis* and *S. pneumoniae*, the most highly expressed genes in the *com* regulons are those that are required for transformation.

In view of the induced gene set, the K-state in *B. subtilis* was proposed to be "…a global adaptation to stress, distinct from sporulation, which enables the cell to repair DNA damage, to acquire new fitness-enhancing genes by transformation, to use novel substrates and to detoxify environmental poisons" (REF. 48). Induction of the X-state by mitomycin C, together with the absence of an SOS system in *S. pneumoniae,* led to the proposal that the X-state is an SOS substitute, in which CSP functions as an alarmone[10,31]. Similarly, *L. pneumophila* lacks a prototypical SOS response and this organism also becomes competent in response to DNA damage, which strengthens the hypothesis that competence evolved to be a global stress response in SOS-deficient bacteria[32,100].

However, competence has not evolved to be an SOS substitute in all transformable species. Several species, such as *B. subtilis*, *V. cholerae* and *S. thermophilus*, have maintained both a functional SOS system and a *com* regulon. In *S. thermophilus*, the two systems even seem to be antagonistic: the fluoroquinolones and mitomycin C both induce the SOS system and simultaneously reduce transformability[101]. This observation further illustrates the diverse behaviour of transformable species, particularly with respect to the mechanism of induction and the roles of competence.

Paralogue
One of a pair of homologous genes that are derived by a duplication event from a single sequence. Paralogous relationships occur both within and between genomes, and paralogues can evolve to have novel functions.

Restriction–modification system
(R–M system). A bacterial immune system that protects cells from invading foreign DNA, such as that injected by bacteriophages. Most of these systems encode a restriction enzyme that cleaves specific sequences in unmethylated DNA and a methylase that methylates the host genome, thereby protecting it from restriction.

K-state
An alternative for competence in ComK-possessing bacteria (Bacilli), representing induction of the ComK regulon.

X-state
An alternative for competence in σ^X-possessing bacteria (Streptococci), representing induction of the σ^X regulon.

Box 4 | **The long road from prediction to the demonstration of transformation**

Although the identification of a full set of transformation genes or the presence of an orthologue of a central competence regulator might suggest that a new species is transformable, the examples below show that it is a long road from prediction to the actual demonstration of transformation.

From 'synthetic' transformation to natural transformation

Streptococcus thermophilus is the most encouraging example for those seeking new transformable species. This streptococcal species was initially shown to transform only after 'synthetic' expression of sigma factor X (σ^X) (REF. 154) and was therefore listed as a transformable species in a previous review[4]. Subsequent studies have confirmed that natural transformation occurs in this species [37,76], with the accompanying discovery of a new regulatory cascade controlling σ^X expression in streptococci, which is distinct from the archetypal cascade that is used in *Streptococcus pneumoniae* (FIG. 3b). Similarly, synthetic overexpression of the σ^X-related σ^H factor in *Staphylococcus aureus* resulted in the induction of *com* operons[155]. However, it took almost 10 years to show that two distinct mechanisms potentially lead to the activation of endogenous σ^H and the subsequent transformation of *S. aureus*[40].

From prediction to 'synthetic' expression of a *com* regulon

Compared with *S. thermophilus* and *S. aureus*, demonstrating transformation in other species has turned out to be more of a challenge. Overexpression of a gene encoding σ^H in *Lactobacillus sakei* leads to *com* regulon induction[156]; however, this did not lead to detectable transformation. Similarly, although *Streptococcus pyogenes* encodes all the genes that are required for natural transformation as well as the alternative sigma factor σ^X (REF. 157), it has not been possible to transform this species under laboratory conditions, despite attempts to synthetically activate the *com* regulon[158] and the demonstration of natural activation of the *com* regulon by the competence-inducing peptide XIP via σ^X (REF. 159). It has been suggested that transformation is blocked at the stage of DNA uptake[159], but this requires further study.

Transformation among the Bacilli

Natural transformation has been documented only in *Bacillus subtilis*, *Bacillus licheniformis*[160] and *Bacillus amyloliquefaciens*[161], even though homologues for most genes that are required for natural transformation are present in almost all Bacilli in addition to a *comK* gene orthologue[162], which suggests that transformation is widespread within this genus. In support of this view, synthetic expression of *B. subtilis comK* in *Bacillus cereus* resulted in chromosomal transformation[163].

Transformation among Gammaproteobacteria, including *Escherichia coli*

Homologues of the competence activators Sxy and TfoX have been identified in many Gammaproteobacteria, which suggests that there is a common competence regulatory mechanism in this taxon[164]. In *E. coli*, initial studies showed that the regulator cAMP receptor protein (CRP) binds to non-canonical CRP-binding sites (CRP-S) and is dependent on Sxy, which is the master regulator of competence in Gammaproteobacteria[164]. Microarray analyses then showed that the CRP-S regulon included all of the *E. coli com* gene homologues[165]. A study recently showed DNA uptake in *E. coli* by overexpression of native *sxy*, which confirms that this bacterium does encode a functional uptake machinery[166]. However, bona fide transformation could not be shown, and the environmental conditions that render *E. coli* naturally competent remain elusive. As exemplified by *S. thermophilus* and *S. aureus*, it is hoped that it is only a matter of time (and perseverance on the part of researchers in the field) before transformation-permissible conditions are found for some of these species.

Switching off competence

Compared with the abundance of information that has been gained on the mechanisms of competence induction in various species, the mechanisms by which competence is turned off have received little attention. However, the shut-down of pneumococcal competence was recently shown to involve both ComE[75] and DprA[85]. Shut-down relies on ComE-mediated repression of its cognate promoters, particularly P_{comC} — the promoter of the *comCDE* operon[75]. In addition, DprA directly interacts with ComE-P to block ComE-P-driven transcription[85]. Owing to a differential transcriptional activation and repression of P_{comC} and P_{comX} by ComE and ComE-P respectively, DprA predominantly accelerates the shut-down of the promoters of the *comX* genes, which, together with the instability of σ^X (REF. 95), ensures the rapid cessation of σ^X-driven expression[85]. Notably, phylogenetic analyses showed that the acquisition of this new regulatory function by DprA had affected the evolution of its amino-terminal domain (which interacts with ComE) in all streptococci that rely on ComE for the direct regulation of *comX*. This suggests that DprA has a similar role in competence shut-down in these other streptococcal species[85]. However, the potential advantage of using this key DNA-recombination protein for the regulation of competence remains elusive. As DprA directly interacts with ssDNA[13], it has been proposed that DprA regulates pneumococcal and streptococcal competence as a function of DNA availability; however, this hypothesis remains to be experimentally verified[85].

Predicting new transformable species

Whole-genome sequences have revealed that several bacterial species that were not previously known to be naturally transformable contain complete sets of genes required for the uptake of exogenous DNA. This observation raises the question of whether such gene sets are the signature of active transformation or whether they are only remnants of an ancestral ability to transform[121]. The prediction of new transformable species is now aided by the identification of genes encoding proteins that are dedicated to transformation (for example, DprA). However, although suggestive, the mere presence of all transformation genes is insufficient to obtain

spontaneous transformants (BOX 4), as expression of these genes is typically only transient and requires specific environmental conditions. Knowledge of competence regulatory cascades in model species can be helpful for predicting central regulators that are used by related species, as exemplified by σ^X in streptococci.

In attempts to predict a new transformable species, it is important to bear in mind that the identification of a putative central competence regulator might not be sufficient to define the conditions that are most favourable for the spontaneous transformation of a species (BOX 4). At least two potential caveats must be considered. First, it is possible that there is an alternative mechanism of regulation, as illustrated by the division of streptococci into two subgroups that rely on completely different regulatory circuits (the ComDE and BlpRH TCSs) to control σ^X expression (FIG. 3b). It is therefore important to carry out robust phylogenetic analyses to avoid drawing misleading parallels between different transformation models, as exemplified by *S. mutans* (see above). Second, the possible existence of species-specific competence-inducing signals (such as chitin in the case of *V. cholerae*[58]) should also be considered. Owing to these potential caveats, it can take several years to unravel the regulatory circuits and/or natural conditions that control the spontaneous induction of natural competence, after the demonstration of 'synthetic' competence or 'synthetic' transformation (which involves the use of strains that are engineered to express the genes required for transformation), as illustrated for *S. thermophilus* (BOX 4).

Stumbling blocks for demonstrating transformation. To determine whether or not a particular species is transformable, a large number of isolates should be studied, as transformability within a single species is often sporadic. Particular attention should be devoted to the choice of chromosomal markers for the selection of transformants. The use of plasmids should be avoided owing to the poor efficiency of plasmid transformation, which has been observed in both *B. subtilis* and *S. pneumoniae*. Reconstitution of a plasmid replicon following uptake of ssDNA fragments is very inefficient compared with insertion of DNA into the chromosome, in part because SsbB antagonizes plasmid transformation[111]. Chromosomally inserted cassettes should only be used if donor DNA is available from a strain with a genetic background that is similar to the recipient, to guarantee methylation of donor DNA and thereby avoid antagonization of transformation by R–M systems[117].

Concluding remarks

In contrast to the overall conservation of the DNA-uptake and processing machinery in naturally transformable species, there is an impressive variety of competence regulatory cascades. Important challenges for future research include determining not only how and when competence is turned on but also the cues and mechanisms that are involved in switching competence off. This should provide new insights into the biology of natural genetic transformation and might shed a different light on the debated role of imported DNA. Further investigations are also needed to elucidate the mechanistic aspects of DNA uptake and processing, such as the mechanisms that enable insertion of the DNA-transport machinery into the cell wall of competent bacteria. In addition, future work needs to address how the Tfp facilitates access of exogenous DNA to its receptor, whether this involves pilus retraction, and how dsDNA is converted into ssDNA before delivery to the transmembrane channel. Whether transformation-dedicated proteins other than DprA are required for integration of internalized DNA into the chromosome, including for the final resolution of recombination intermediates, also remains unknown. Solving these questions will improve our understanding of a central process that is a major contributor to the genetic plasticity of bacteria and that is shared by an increasing number of phylogenetically diverse species.

1. Griffith, F. The significance of pneumococcal types. *J. Hyg.* **27**, 113–159 (1928).
2. Croucher, N. J. *et al.* Rapid pneumococcal evolution in response to clinical interventions. *Science* **331**, 430–434 (2011).
3. Lorenz, M. G. & Wackernagel, W. Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev.* **58**, 563–602 (1994).
4. Johnsborg, O., Eldholm, V. & Håvarstein, L. S. Natural genetic transformation: prevalence, mechanisms and function. *Res. Microbiol.* **158**, 767–778 (2007).
5. Averhoff, B. & Friedrich, A. Type IV pili-related natural transformation systems: DNA transport in mesophilic and thermophilic bacteria. *Arch. Microbiol.* **180**, 385–393 (2003).
6. Chen, I. & Dubnau, D. DNA uptake during bacterial transformation. *Nature Rev. Microbiol.* **2**, 241–249 (2004).
7. Allemand, J. F. & Maier, B. Bacterial translocation motors investigated by single molecule techniques. *FEMS Microbiol. Rev.* **33**, 593–610 (2009).
8. Claverys, J. P., Martin, B. & Polard, P. The genetic transformation machinery: composition, localization and mechanism. *FEMS Microbiol. Rev.* **33**, 643–656 (2009).
9. Allemand, J. F., Maier, B. & Smith, D. E. Molecular motors for DNA translocation in prokaryotes. *Curr. Opin. Biotechnol.* **23**, 503–509 (2012).
10. Claverys, J. P., Prudhomme, M. & Martin, B. Induction of competence regulons as general stress responses in Gram-positive bacteria. *Annu. Rev. Microbiol.* **60**, 451–475 (2006).
11. Seitz, P. & Blokesch, M. Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. *FEMS Microbiol. Rev.* **37**, 336–363 (2012).
12. Draskovic, I. & Dubnau, D. Biogenesis of a putative channel protein, ComEC, required for DNA uptake: membrane topology, oligomerization and formation of disulphide bonds. *Mol. Microbiol.* **55**, 881–896 (2005).
13. Mortier-Barrière, I. *et al.* A key presynaptic role in transformation for a widespread bacterial protein: DprA conveys incoming ssDNA to RecA. *Cell* **130**, 824–836 (2007).
This study identifies DprA as a transformation-dedicated loader of RecA onto transforming ssDNA, which is a step that is crucial for the formation of transformation recombinants.
14. Hobbs, M. & Mattick, J. S. Common components in the assembly of type 4 fimbriae, DNA transfer systems, filamentous phage and protein-secretion apparatus: a general system for the formation of surface-associated protein complexes. *Mol. Microbiol.* **10**, 233–243 (1993).
15. Dubnau, D. DNA uptake in bacteria. *Annu. Rev. Microbiol.* **53**, 217–244 (1999).
16. Mann, J. M., Carabetta, V. J., Cristea, I. M. & Dubnau, D. Complex formation and processing of the minor transformation pilins of *Bacillus subtilis*. *Mol. Microbiol.* **90**, 1201–1215 (2013).
17. Chen, I., Provvedi, R. & Dubnau, D. A macromolecular complex formed by a pilin-like protein in competent *Bacillus subtilis*. *J. Biol. Chem.* **281**, 21720–21727 (2006).
This paper documents the presence of a short pilus-like structure in *B. subtilis*. This is the first identification of a competence-specific pseudopilus in Gram-positive bacteria.
18. Laurenceau, R. *et al.* A Type IV pilus mediates DNA binding during natural transformation in *Streptococcus pneumoniae*. *PLoS Pathog.* **9**, e1003473 (2013).
By identifying a long pilus that is essential for transformation in *S. pneumoniae*, this study shows that transformation pili can protrude far beyond the cell wall of Gram-positive bacteria to capture exogenous DNA.
19. Provvedi, R. & Dubnau, D. ComEA is a DNA receptor for transformation of competent *Bacillus subtilis*. *Mol. Microbiol.* **31**, 271–280 (1999).
20. Burrows, L. L. Weapons of mass retraction. *Mol. Microbiol.* **57**, 878–888 (2005).
21. Puyet, A., Greenberg, B. & Lacks, S. A. Genetic and structural characterization of EndA. A membrane-bound nuclease required for transformation of *Streptoccus pneumoniae*. *J. Mol. Biol.* **213**, 727–738 (1990).
22. Bergé, M. *et al.* Uptake of transforming DNA in Gram-positive bacteria: a view from *Streptococcus pneumoniae*. *Mol. Microbiol.* **45**, 411–421 (2002).

23. Seitz, P. & Blokesch, M. DNA-uptake machinery of naturally competent *Vibrio cholerae*. *Proc. Natl Acad. Sci. USA* **110**, 17987–17992 (2013).
24. Bergé, M. J. *et al.* Midcell recruitment of the DNA uptake and virulence nuclease, EndA, for pneumococcal transformation. *PLoS Pathog.* **9**, e1003596 (2013).
This study shows that the EndA nuclease is recruited to midcell during pneumococcal competence, which indicates that DNA uptake is likely to occur at this site.
25. Hahn, J. *et al.* Transformation proteins and DNA uptake localize to the cell poles in *Bacillus subtilis*. *Cell* **122**, 59–71 (2005).
This study shows that DNA uptake occurs at the cell poles in competent *B. subtilis*.
26. Londoño-Vallejo, J. A. & Dubnau, D. Mutation of the putative nucleotide binding site of the *Bacillus subtilis* membrane protein ComFA abolishes the uptake of DNA during transformation. *J. Bacteriol.* **176**, 4642–4645 (1994).
27. Yeh, Y. C., Lin, T. L., Chang, K. C. & Wang, J. T. Characterization of a ComE3 homologue essential for DNA transformation in *Helicobacter pylori*. *Infect. Immun.* **71**, 5427–5431 (2003).
28. Stingl, K. *et al.* Composite system mediates two-step DNA uptake into *Helicobacter pylori*. *Proc. Natl Acad. Sci. USA* **107**, 1184–1189 (2010).
29. Beernink, H. T. & Morrical, S. W. RMPs: recombination/replication mediator proteins. *Trends Biochem. Sci.* **24**, 385–389 (1999).
30. Quevillon-Cheruel, S. *et al.* Structure–function analysis of pneumococcal DprA protein reveals that dimerization is crucial for loading RecA recombinase onto DNA during transformation. *Proc. Natl Acad. Sci. USA* **109**, E2466–E2475 (2012).
31. Prudhomme, M. *et al.* Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. *Science* **313**, 89–92 (2006).
This paper reports the discovery that pneumococcal competence is induced in response to environmental stresses such as antibiotics and DNA-damaging agents.
32. Charpentier, X., Polard, P. & Claverys, J. P. Induction of competence for genetic transformation by antibiotics: convergent evolution of stress responses in distant bacterial species lacking SOS? *Curr. Opin. Microbiol.* **15**, 1–7 (2012).
33. Butala, M., Zgur-Bertok, D. & Busby, S. J. The bacterial LexA transcriptional repressor. *Cell. Mol. Life Sci.* **66**, 82–93 (2009).
34. Lee, M. S. & Morrison, D. A. Identification of a new regulator in *Streptococcus pneumoniae* linking quorum sensing to competence for genetic transformation. *J. Bacteriol.* **181**, 5004–5016 (1999).
35. Peterson, S. *et al.* Identification of competence pheromone responsive genes in *Streptococcus pneumoniae*. *Mol. Microbiol.* **51**, 1051–1070 (2004).
36. Martin, B., Quentin, Y., Fichant, G. & Claverys, J. P. Independent evolution of competence regulatory cascades in streptococci? *Trends Microbiol.* **14**, 339–345 (2006).
This paper reports the phylogenetic analysis of the streptococcal ComDE TCS and establishes that *S. mutans* and several other streptococci lack this master competence regulator, which suggests that there is an alternative competence regulatory circuit in these species.
37. Fontaine, L. *et al.* A novel pheromone quorum-sensing system controls the development of natural competence in *Streptococcus thermophilus* and *Streptococcus salivarius*. *J. Bacteriol.* **192**, 1444–1454 (2010).
38. Mashburn-Warren, L., Morrison, D. A. & Federle, M. J. A novel double-tryptophan peptide pheromone controls competence in *Streptococcus* spp. via an Rgg regulator. *Mol. Microbiol.* **78**, 589–606 (2010).
39. Morrison, D. A., Guedon, E. & Renault, P. Competence for natural genetic transformation in the *Streptococcus bovis* Group Streptococci *S. infantarius* and *S. macedonicus*. *J. Bacteriol.* **195**, 2612–2620 (2013).
40. Morikawa, K. *et al.* Expression of a cryptic secondary sigma factor gene unveils natural competence for DNA transformation in *Staphylococcus aureus*. *PLoS Pathog.* **8**, e1003003 (2012).
41. Mohan, S. & Dubnau, D. Transcriptional regulation of *comC*: evidence for a competence-specific transcription factor in *Bacillus subtilis*. *J. Bacteriol.* **172**, 4064–4071 (1990).
42. Maamar, H. & Dubnau, D. Bistability in the *Bacillus subtilis* K-state (competence) system requires a positive feedback loop. *Mol. Microbiol.* **56**, 615–624 (2005).
This paper shows that stochastic variation in *comK* expression in *B. subtilis* populations determines which cells become competent.
43. Smits, W. K. *et al.* Stripping *Bacillus*: ComK auto-stimulation is responsible for the bistable response in competence development. *Mol. Microbiol.* **56**, 604–614 (2005).
44. Dubnau, D. & Losick, R. Bistability in bacteria. *Mol. Microbiol.* **61**, 564–572 (2006).
45. Smits, W. K., Kuipers, O. P. & Veening, J. W. Phenotypic variation in bacteria: the role of feedback regulation. *Nature Rev. Microbiol.* **4**, 259–271 (2006).
46. Maamar, H., Raj, A. & Dubnau, D. Noise in gene expression determines cell fate in *Bacillus subtilis*. *Science* **317**, 526–529 (2007).
47. Leisner, M., Stingl, K., Frey, E. & Maier, B. Stochastic switching to competence. *Curr. Opin. Microbiol.* **11**, 553–559 (2008).
48. Berka, R. M. *et al.* Microarray analysis of the *Bacillus subtilis* K-state: genome-wide expression changes dependent on ComK. *Mol. Microbiol.* **43**, 1331–1345 (2002).
49. Hamoen, L. W. *et al.* Improving the predictive value of the competence transcription factor (ComK) binding site in *Bacillus subtilis* using a genomic approach. *Nucl. Acids Res.* **202**, 5517–5528 (2002).
50. Ogura, M. *et al.* Whole-genome analysis of genes regulated by the *Bacillus subtilis* competence transcription factor ComK. *J. Bacteriol.* **184**, 2344–2351 (2002).
51. Kuzniar, A., van Ham, R. C., Pongor, S. & Leunissen, J. A. The quest for orthologs: finding the corresponding gene across genomes. *Trends Genet.* **24**, 539–551 (2008).
52. Wise, E. M. Jr., Alexander, S. P. & Powers, M. Adenosine 3′:5′-cyclic monophosphate as a regulator of bacterial transformation. *Proc. Natl Acad. Sci. USA* **70**, 471–474 (1973).
53. Dorocicz, I. E., Williams, P. M. & Redfield, R. J. The *Haemophilus influenzae* adenylate cyclase gene: cloning, sequence, and essential role in competence. *J. Bacteriol.* **175**, 7142–7149 (1993).
54. Chandler, M. S. The gene encoding cAMP receptor protein is required for competence development in *Haemophilus influenzae* Rd. *Proc. Natl Acad. Sci. USA* **89**, 1626–1630 (1992).
55. Redfield, R. J. *sxy-1*, a *Haemophilus influenzae* mutation causing greatly enhanced spontaneous competence. *J. Bacteriol.* **173**, 5612–5618 (1991).
56. Macfadyen, L. P. Regulation of competence development in *Haemophilus influenzae*. *J. Theor. Biol.* **207**, 349–359 (2000).
57. Redfield, R. J. *et al.* A novel CRP-dependent regulon controls expression of competence genes in *Haemophilus influenzae*. *J. Mol. Biol.* **347**, 735–747 (2005).
58. Meibom, K. L. *et al.* Chitin induces natural competence in *Vibrio cholerae*. *Science* **310**, 1824–1827 (2005).
This paper reports the discovery that competence in *V. cholerae* is induced by chitin, which is a sugar polymer that is abundant in the aquatic habitat of this bacterium.
59. Blokesch, M. A quorum sensing-mediated switch contributes to natural transformation of *Vibrio cholerae*. *Mob. Genet. Elements* **2**, 224–227 (2012).
60. Lo Scrudato, M. & Blokesch, M. A transcriptional regulator linking quorum sensing and chitin induction to render *Vibrio cholerae* naturally transformable. *Nucleic Acids Res.* **41**, 3644–3658 (2013).
This paper provides the first documentation of bipartite regulation of competence genes, in which they are directly controlled by two distinct regulators.
61. Hamoen, L. W., Venema, G. & Kuipers, O. P. Controlling competence in *Bacillus subtilis*: shared use of regulators. *Microbiology* **149**, 9–17 (2003).
62. Hamoen, L. W., Van Werkhoven, A. F., Dubnau, D. & Venema, G. The competence transcription factor of *Bacillus subtilis* recognizes short A/T-rich sequences arranged in a unique, flexible pattern along the DNA helix. *Genes Dev.* **12**, 1539–1550 (1998).
63. Hamoen, L. W., Van Werkhoven, A. F., Venema, G. & Dubnau, D. The pleiotropic response regulator DegU functions as a priming protein in competence development in *Bacillus subtilis*. *Proc. Natl Acad. Sci. USA* **97**, 9246–9251 (2000).
64. Hamoen, L. W. *et al.* The *Bacillus subtilis* transition state regulator AbrB binds to the -35 promoter region of comK. *FEMS Microbiol. Lett.* **218**, 299–304 (2003).
65. Serror, P. & Sonenshein, A. L. CodY is required for nutritional repression of *Bacillus subtilis* genetic competence. *J. Bacteriol.* **178**, 5910–5915 (1996).
66. Hoa, T. T., Tortosa, P., Albano, M. & Dubnau, D. Rok (YkuW) regulates genetic competence in *Bacillus subtilis* by directly repressing *comK*. *Mol. Microbiol.* **43**, 15–26 (2002).
67. Turgay, K., Hamoen, L. W., Venema, G. & Dubnau, D. Biochemical characterization of a molecular switch involving the heat shock protein ClpC, which controls the activity of ComK, the competence transcription factor of *Bacillus subtilis*. *Genes Dev.* **11**, 119–128 (1997).
This paper provides *in vitro* characterization of the interplay between ComK, ClpCP, MecA and ComS and demonstrated that accumulation of *B. subtilis* ComK is regulated by proteolysis.
68. Turgay, K., Hahn, J., Burghoorn, J. & Dubnau, D. Competence in *Bacillus subtilis* is controlled by regulated proteolysis of a transcription factor. *EMBO J.* **17**, 6730–6738 (1998).
69. Nakano, M. M. *et al. srfA* is an operon required for surfactin production, competence development, and efficient sporulation in *Bacillus subtilis*. *J. Bacteriol.* **173**, 1770–1778 (1991).
70. Roggiani, M. & Dubnau, D. ComA, a phosphorylated response regulator protein of *Bacillus subtilis*, binds to the promoter region of *srfA*. *J. Bacteriol.* **175**, 3182–3187 (1993).
71. Hayashi, K. *et al.* The H_2O_2 stress-responsive regulator PerR positively regulates *srfA* expression in *Bacillus subtilis*. *J. Bacteriol.* **187**, 6659–6667 (2005).
72. Hui, F. M. & Morrison, D. A. Genetic transformation in *Streptococcus pneumoniae*: nucleotide sequence analysis shows *comA*, a gene required for competence induction, to be a member of the bacterial ATP-dependent transport protein family. *J. Bacteriol.* **173**, 372–381 (1991).
73. Pestova, E. V., Håvarstein, L. S. & Morrison, D. A. Regulation of competence for genetic transformation in *Streptococcus pneumoniae* by an auto-induced peptide pheromone and a two-component regulatory system. *Mol. Microbiol.* **21**, 853–864 (1996).
74. Håvarstein, L. S., Coomaraswamy, G. & Morrison, D. A. An unmodified heptadecapeptide pheromone induces competence for genetic transformation in *Streptococcus pneumoniae*. *Proc. Natl Acad. Sci. USA* **92**, 11140–11144 (1995).
75. Martin, B. *et al.* ComE/ComE ~ P interplay dictates activation or extinction status of pneumococcal X-state (competence). *Mol. Microbiol.* **87**, 394–411 (2012).
76. Gardan, R. *et al.* The oligopeptide transport system is essential for the development of natural competence in *Streptococcus thermophilus* strain LMD-9. *J. Bacteriol.* **191**, 4647–4655 (2009).
This paper reports the seminal discovery of the involvement of an oligopeptide transporter in a competence regulatory cascade, which paved the way for the discovery of ComRS in *S. thermophilus*.
77. Gardan, R. *et al.* Extracellular life cycle of ComS, the competence-stimulating peptide of *Streptococcus thermophilus*. *J. Bacteriol.* **195**, 1845–1855 (2013).
78. Fontaine, L. *et al.* Mechanism of competence activation by the ComRS signalling system in streptococci. *Mol. Microbiol.* **87**, 1113–1132 (2013).
79. Sulavik, M. C., Tardif, G. & Clewell, D. B. Identification of a gene, *rgg*, which regulates expression of glucosyltransferase and influences the Spp phenotype of *Streptococcus gordonii* Challis. *J. Bacteriol.* **174**, 3577–3586 (1992).
80. Fleuchot, B. *et al.* Rgg proteins associated with internalized small hydrophobic peptides: a new quorum-sensing mechanism in streptococci. *Mol. Microbiol.* **80**, 1102–1119 (2011).
81. de Saizieu, A. *et al.* Microarray-based identification of a novel *Streptococcus pneumoniae* regulon controlled by an autoinduced peptide. *J. Bacteriol.* **182**, 4696–4703 (2000).
82. Federle, M. J. & Morrison, D. A. One if by land, two if by sea: signalling to the ranks with CSP and XIP. *Mol. Microbiol.* **86**, 241–245 (2012).
83. Lemme, A. *et al.* Subpopulation-specific transcriptome analysis of competence-stimulating-peptide-induced *Streptococcus mutans*. *J. Bacteriol.* **193**, 1863–1877 (2011).
84. Son, M. *et al.* Microfluidic study of competence regulation in *Streptococcus mutans*: environmental inputs modulate bimodal and unimodal expression of *comX*. *Mol. Microbiol.* **86**, 258–272 (2012).

85. Mirouze, N. *et al.* Direct involvement of DprA, the transformation-dedicated RecA loader, in the shut-off of pneumococcal competence. *Proc. Natl Acad. Sci. USA* **110**, E1035–E1044 (2013).
86. Yamamoto, S., Morita, M., Izumiya, H. & Watanabe, H. Chitin disaccharide (GlcNAc)2 induces natural competence in *Vibrio cholerae* through transcriptional and translational activation of a positive regulatory gene *tfoXVC*. *Gene* **457**, 42–49 (2010).
87. Yamamoto, S. *et al.* Regulation of natural competence by the orphan two-component system sensor kinase ChiS involves a non-canonical transmembrane regulator in *Vibrio cholerae*. *Mol. Microbiol.* **91**, 326–247 (2013).
88. Yamamoto, S. *et al.* Identification of a chitin-induced small RNA that regulates translation of the *tfoX* gene, encoding a positive regulator of natural competence in *Vibrio cholerae*. *J. Bacteriol.* **193**, 1953–1965 (2011).
89. Herriott, R. M., Meyer, E. M. & Vogt, M. Defined nongrowth media for stage II development of competence in *Haemophilus influenzae*. *J. Bacteriol.* **101**, 517–524 (1970).
90. Bosse, J. T. *et al.* Natural competence in strains of *Actinobacillus pleuropneumoniae*. *FEMS Microbiol. Lett.* **298**, 124–130 (2009).
91. Sinha, S., Mell, J. & Redfield, R. The availability of purine nucleotides regulates natural competence by controlling translation of the competence activator Sxy. *Mol. Microbiol.* **6**, 1106–1119 (2013).
92. Cameron, A. D., Volar, M., Bannister, L. A. & Redfield, R. J. RNA secondary structure regulates the translation of *sxy* and competence development in *Haemophilus influenzae*. *Nucleic Acids Res.* **36**, 10–20 (2008).
93. Boutry, C. *et al.* Adpaptor protein MecA is a negative regulator of the expression of late competence genes in *Streptococcus thermophilus*. *J. Bacteriol.* **194**, 1777–1788 (2012).
94. Biornstad, T. J. & Havarstein, L. S. ClpC acts as a negative regulator of competence in *Streptococcus thermophilus*. *Microbiology* **157**, 1676–1684 (2011).
95. Sung, C. K. & Morrison, D. A. Two distinct functions of ComW in stabilization and activation of the alternative sigma factor ComX in *Streptococcus pneumoniae*. *J. Bacteriol.* **187**, 3052–3061 (2005).
96. Nielsen, K. M., Bones, A. M. & van Elsas, J. D. Induced natural transformation of *Acinetobacter calcoaceticus* in soil microcosms. *Appl. Environ. Microbiol.* **63**, 3972–3977 (1997).
97. Lo Scrudato, M. & Blokesch, M. The regulatory network of natural competence and transformation of *Vibrio cholerae*. *PLoS Genet.* **8**, e1002778 (2012).
98. Keyhani, N. O. & Roseman, S. Physiological aspects of chitin catabolism in marine bacteria. *Biochim. Biophys. Acta* **1473**, 108–122 (1999).
99. Murphy, T. F. & Brauer, A. L. Expression of urease by *Haemophilus influenzae* during human respiratory tract infection and role in survival in an acid environment. *BMC. Microbiol.* **11**, 183 (2011).
100. Charpentier, X., Kay, E., Schneider, D. & Shuman, H. A. Antibiotics and UV radiation induce competence for natural transformation in *Legionella pneumophila*. *J. Bacteriol.* **193**, 1114–1121 (2011).
101. Boutry, C. *et al.* SOS response activation and competence development are antagonistic mechanisms in *Streptococcus thermophilus*. *J. Bacteriol.* **195**, 696–707 (2013).
102. Ratnayake-Lecamwasam, M., Serror, P., Wong, K. W. & Sonenshein, A. L. *Bacillus subtilis* CodY represses early-stationary-phase genes by sensing GTP levels. *Genes Dev.* **15**, 1093–1103 (2001).
103. Antonova, E. S., Bernardy, E. E. & Hammer, B. K. Natural competence in *Vibrio cholerae* is controlled by a nucleoside scavenging response that requires CytR-dependent anti-activation. *Mol. Microbiol.* **86**, 1215–1231 (2012).
104. Michod, R. E., Bernstein, H. & Nedelcu, A. M. Adaptive value of sex in microbial pathogens. *Infect. Genet. Evol.* **8**, 267–285 (2008).
105. Vos, M. Why do bacteria engage in homologous recombination? *Trends Microbiol.* **17**, 226–232 (2009).
106. Redfield, R. J. Genes for breakfast: the have-your-cake-and-eat-it-too of bacterial transformation. *J. Hered.* **84**, 400–404 (1993).
107. Redfield, R. J. Do bacteria have sex? *Nature Rev. Genet.* **2**, 634–639 (2001).
108. Bergé, M., Mortier-Barrière, I., Martin, B. & Claverys, J. P. Transformation of *Streptococcus pneumoniae* relies on DprA- and RecA-dependent protection of incoming single strands. *Mol. Microbiol.* **50**, 527–536 (2003).
109. Kidane, D. *et al.* Evidence for different pathways during horizontal gene transfer in competent *Bacillus subtilis* cells. *PLoS. Genet.* **5**, e1000630 (2009).
110. Morrison, D. A., Mortier-Barrière, I., Attaiech, L. & Claverys, J. P. Identification of the major protein component of the pneumococcal eclipse complex. *J. Bacteriol.* **189**, 6497–6500 (2007).
111. Attaiech, L. *et al.* Role of the single-stranded DNA binding protein SsbB in pneumococcal transformation: maintenance of a reservoir for genetic plasticity. *PLoS Genet.* **7**, e1002156 (2011).
112. Sinha, S., Mell, J. C. & Redfield, R. J. Seventeen Sxy-dependent cyclic AMP receptor protein site-regulated genes are needed for natural transformation in *Haemophilus influenzae*. *J. Bacteriol.* **194**, 5245–5254 (2012).
113. Mortier-Barrière, I., de Saizieu, A., Claverys, J. P. & Martin, B. Competence-specific induction of *recA* is required for full recombination proficiency during transformation in *Streptococcus pneumoniae*. *Mol. Microbiol.* **27**, 159–170 (1998).
114. Lacks, S. A., Ayalew, S., de la Campa, A. G. & Greenberg, B. Regulation of competence for genetic transformation in *Streptococcus pneumoniae*: expression of *dpnA*, a late competence gene encoding a DNA methyltransferase of the *Dpn*II restriction system. *Mol. Microbiol.* **35**, 1089–1098 (2000).
115. Johnston, C. *et al.* Programmed protection of foreign DNA from restriction allows pathogenicity island exchange during pneumococcal transformation. *PLoS Pathog.* **9**, e1003178 (2013).
This study shows that a competence-induced pneumococcal methylase is crucial for protecting transformant chromosomes from restriction.
116. Cerritelli, S., Springhorn, S. S. & Lacks, S. A. DpnA, a methylase for single-strand DNA in the *Dpn* II restriction system, and its biological function. *Proc. Natl Acad. Sci. USA* **86**, 9223–9227 (1989).
117. Johnston, C., Martin, B., Polard, P. & Claverys, J. P. Post-replication targeting of transformants by bacterial immune systems? *Trends Microbiol.* **21**, 516–521 (2013).
118. Johnston, C., Polard, P. & Claverys, J. P. The DpnI/DpnII pneumococcal system, defence against foreign attack without compromising genetic exchange. *Mob. Genet. Elements* **3**, e25582 (2013).
119. Johnston, C. *et al.* Natural genetic transformation generates a population of merodiploids in *Streptococcus pneumoniae*. *PLoS Genet.* **9**, e1003819 (2013).
120. Rabinovich, L. *et al.* Prophage excision activates *Listeria* competence genes that promote phagosomal escape and virulence. *Cell* **150**, 792–802 (2012).
121. Claverys, J. P. & Martin, B. Bacterial "competence" genes: signatures of active transformation, or only remnants? *Trends Microbiol.* **11**, 161–165 (2003).
122. Hofreuter, D., Odenbreit, S. & Haas, R. Natural transformation competence in *Helicobacter pylori* is mediated by the basic components of a type IV secretion system. *Mol. Microbiol.* **41**, 379–391 (2001).
123. Karnholz, A. *et al.* Functional and topological characterization of novel components of the comB DNA transformation competence system in *Helicobacter pylori*. *J. Bacteriol.* **188**, 882–893 (2006).
124. Dorer, M. S., Fero, J. & Salama, N. R. DNA damage triggers genetic exchange in *Helicobacter pylori*. *PLoS Pathog.* **6**, e1001026 (2010).
125. Baltrus, D. A. & Guillemin, K. Multiple phases of competence occur during the *Helicobacter pylori* growth cycle. *FEMS Microbiol. Lett.* **255**, 148–155 (2006).
126. Higgins, D. A. *et al.* The major *Vibrio cholerae* autoinducer and its role in virulence factor production. *Nature* **450**, 883–886 (2007).
127. Chen, X. *et al.* Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* **415**, 545–549 (2002).
128. Stephenson, S., Mueller, C., Jiang, M. & Perego, M. Molecular analysis of Phr peptide processing in *Bacillus subtilis*. *J. Bacteriol.* **185**, 4861–4871 (2003).
129. Lanigan-Gerdes, S., Dooley, A. N., Faull, K. F. & Lazazzera, B. A. Identification of subtilisin, Epr and Vpr as enzymes that produce CSF, an extracellular signalling peptide of *Bacillus subtilis*. *Mol. Microbiol.* **65**, 1321–1333 (2007).
130. Lanigan-Gerdes, S. *et al.* Identification of residues important for cleavage of the extracellular signaling peptide CSF of *Bacillus subtilis* from its precursor protein. *J. Bacteriol.* **190**, 6668–6675 (2008).
131. Henke, J. M. & Bassler, B. L. Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. *J. Bacteriol.* **186**, 6902–6914 (2004).
132. Neiditch, M. B. *et al.* Regulation of LuxPQ receptor activity by the quorum-sensing signal autoinducer-2. *Mol. Cell* **18**, 507–518 (2005).
133. Ng, W. L. & Bassler, B. L. Bacterial quorum-sensing network architectures. *Annu. Rev. Genet.* **43**, 197–222 (2009).
134. Lenz, D. H. *et al.* The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in *Vibrio harveyi* and *Vibrio cholerae*. *Cell* **118**, 69–82 (2004).
135. Halfmann, A., Kovacs, M., Hakenbeck, R. & Bruckner, R. Identification of the genes directly controlled by the response regulator CiaR in *Streptococcus pneumoniae*: five out of 15 promoters drive expression of small non-coding RNAs. *Mol. Microbiol.* **66**, 110–126 (2007).
136. Schnorpfeil, A. *et al.* Target evaluation of the non-coding csRNAs reveals a link of the two-component regulatory system CiaRH to competence control in Streptococcus pneumoniae R6. *Mol. Microbiol.* **2**, 334–349 (2013).
137. Stevens, K. E., Chang, D., Zwack, E. E. & Sebert, M. E. Competence in *Streptococcus pneumoniae* is regulated by the rate of ribosomal decoding errors. *mBio* **2** e00071-11 (2011).
138. Cassone, M. *et al.* The HtrA protease from *Streptococcus pneumoniae* digests both denatured proteins and the competence-stimulating peptide. *J. Biol. Chem.* **287**, 38449–38459 (2012).
139. Foster, P. L. Stress responses and genetic variation in bacteria. *Mutat. Res.* **569**, 3–11 (2005).
140. Inamine, G. S. & Dubnau, D. ComEA, a *Bacillus subtilis* integral membrane protein required for genetic transformation, is needed for both DNA binding and transport. *J. Bacteriol.* **177**, 3045–3051 (1995).
141. Barany, F., Kahn, M. E. & Smith, H. O. Directional transport and integration of donor DNA in *Haemophilus influenzae*. *Proc. Natl Acad. Sci. USA* **80**, 7274–7278 (1983).
142. Méjean, V. & Claverys, J. P. Use of a cloned fragment to analyze the fate of donor DNA in transformation of *Streptococcus pneumoniae*. *J. Bacteriol.* **158**, 1175–1178 (1984).
143. Mell, J. C., Hall, I. M. & Redfield, R. J. Defining the DNA uptake specificity of naturally competent *Haemophilus influenzae* cells. *Nucleic Acids Res.* **40**, 8536–8549 (2012).
144. Claverys, J. P. & Håvarstein, L. S. Cannibalism and fratricide: mechanisms and raisons d'être. *Nature Rev. Microbiol.* **5**, 219–229 (2007).
145. Claverys, J. P., Martin, B. & Håvarstein, L. S. Competence-induced fratricide in streptococci. *Mol. Microbiol.* **64**, 1423–1433 (2007).
146. Johnsborg, O., Eldholm, V., Bjornstad, M. L. & Håvarstein, L. S. A predatory mechanism dramatically increases the efficiency of lateral gene transfer in *Streptococcus pneumoniae* and related commensal species. *Mol. Microbiol.* **69**, 245–253 (2008).
147. Aas, F. E., Lovold, C. & Koomey, M. An inhibitor of DNA binding and uptake events dictates the proficiency of genetic transformation in *Neisseria gonorrhoeae*: mechanism of action and links to type IV pilus expression. *Mol. Microbiol.* **46**, 1441–1450 (2002).
148. Redfield, R. J. *et al.* Evolution of competence and DNA uptake specificity in the Pasteurellaceae. *BMC Evol. Biol.* **6**, 82 (2006).
149. Frye, S. A., Nilsen, M., Tonjum, T. & Ambur, O. H. Dialects of the DNA uptake sequence in *Neisseriaceae*. *PLoS Genet.* **9**, e1003458 (2013).
150. Blokesch, M. & Schoolnik, G. K. The extracellular nuclease Dns and its role in natural transformation of *Vibrio cholerae*. *J. Bacteriol.* **190**, 7232–7240 (2008).
151. Finkel, S. E. & Kolter, R. DNA as a nutrient: novel role for bacterial competence gene homologs. *J. Bacteriol.* **183**, 6288–6293 (2001).
152. Palchevskiy, V. & Finkel, S. E. *Escherichia coli* competence gene homologs are essential for competitive fitness and the use of DNA as a nutrient. *J. Bacteriol.* **188**, 3902–3910 (2006).
153. Sun, D. *Etude de la transformation plasmidique naturelle d'Escherichia coli et des ses relations éventuelles avec la compétence programmée pour la transformation génétique et la compétence dite nutritionnelle.*Thesis, Univ. P. Sabatier, Toulouse, France (2011).

154. Blomqvist, T., Steinmoen, H. & Håvarstein, L. S. Natural genetic transformation: a novel tool for efficient genetic engineering of the dairy bacterium *Streptococcus thermophilus*. *Appl. Environ. Microbiol.* **72**, 6751–6756 (2006).
155. Morikawa, K. *et al.* A new staphylococcal sigma factor in the conserved gene cassette: functional significance and implication for the evolutionary processes. *Genes Cells* **8**, 699–712 (2003).
156. Schmid, S., Bevilacqua, C. & Crutz-Le Coq, A. M. Alternative sigma factor σ^H activates competence gene expression in *Lactobacillus sakei*. *BMC. Microbiol.* **12**, 32 (2012).
157. Opdyke, J. A., Scott, J. R. & Moran, C. P. Jr. A secondary RNA polymerase sigma factor from *Streptococcus pyogenes*. *Mol. Microbiol.* **42**, 495–502 (2001).
158. Woodbury, R. L., Wang, X. & Moran, C. P. Jr. Sigma X induces competence gene expression in *Streptococcus pyogenes*. *Res. Microbiol.* **157**, 851–856 (2006).
159. Mashburn-Warren, L., Morrison, D. A. & Federle, M. J. The cryptic competence pathway in *Streptococcus pyogenes* is controlled by a peptide pheromone. *J. Bacteriol.* **194**, 4589–4600 (2012).
160. Thorne, C. B. & Stull, H. B. Factors affecting transformation of *Bacillus licheniformis*. *J. Bacteriol.* **91**, 1012–1020 (1966).
161. Koumoutsi, A. *et al.* Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in *Bacillus amyloliquefaciens* strain FZB42. *J. Bacteriol.* **186**, 1084–1096 (2004).
162. Kovacs, A. T., Smits, W. K., Mironczuk, A. M. & Kuipers, O. P. Ubiquitous late competence genes in *Bacillus* species indicate the presence of functional DNA uptake machineries. *Environ. Microbiol.* **11**, 1911–1922 (2009).
163. Mironczuk, A. M., Kovacs, A. T. & Kuipers, O. P. Induction of natural competence in *Bacillus cereus* ATCC14579. *Microb. Biotech.* **1**, 226–235 (2008).
164. Cameron, A. D. & Redfield, R. J. Non-canonical CRP sites control competence regulons in *Escherichia coli* and many other γ-proteobacteria. *Nucleic Acids Res.* **34**, 6001–6014 (2006).
This study identifies a subset of CRP-binding sites that depend on the Sxy competence-regulating cofactor for transcriptional activation, which led to the finding that *sxy* regulons are induced during competence in *H. influenzae* and Gammaproteobacteria.
165. Sinha, S., Cameron, A. D. & Redfield, R. J. Sxy induces a CRP-S regulon in *Escherichia coli*. *J. Bacteriol.* **191**, 5180–5195 (2009).
166. Sinha, S. & Redfield, R. J. Natural DNA uptake by *Escherichia coli*. *PLoS ONE.* **7**, e35620 (2012).
167. Shimodaira, H. & Hasegawa, M. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* **16**, 1114–1116 (1999).

Acknowledgements
The authors thank Y. Quentin for his help with phylogenetic analysis and the construction of the species tree. The authors apologize to researchers whose work could not be specifically cited owing to space limitations.

Competing interests statement
The authors declare no competing interests.

FURTHER INFORMATION
Sun, D. Thesis (reference 153): https://www-lmgm.biotoul.fr/uk/equipes/grpclav/Sun_2011.html

SUPPLEMENTARY INFORMATION
See online article: S1 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF