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Natural genetic transformation: prevalence, mechanisms and function

Ola Johnsborg, Vegard Eldholm, Leiv Sigve Håvarstein*

Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway

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Abstract

Studies show that gene acquisition through natural transformation has contributed significantly to the adaptation and ecological diversification of several bacterial species. Relatively little is still known, however, about the prevalence and phylogenetic distribution of organisms possessing this property. Thus, whether natural transformation only benefits a limited number of species or has a large impact on lateral gene flow in nature remains a matter of speculation. Here we will review the most recent advances in our understanding of the phenomenon and discuss its possible biological functions.

Keywords: DNA uptake apparatus; Type IV pili; Competence regulation; Type IV secretion system

1. Introduction

Comparative analyses of prokaryotic genomes show that acquisition of genetic material through lateral gene transfer has been a major driving force in the evolution of these organisms. Exchange of genetic material, however, can only speed up evolution if donors and recipients use the same system to encode, store and process genetic information. Consequently, prokaryotic ''sex'' must have played a significant role in preserving the near universality of the genetic code. In a study of 88 prokaryotic genomes, the percentage of laterally transferred genes was estimated to vary from 0 to 22% in bacterial and 5–15% in archaeal species, respectively [50]. These results, which probably represent an underestimation, show that lateral transfer of DNA, together with mutations, gene loss and duplication of existing genes, shape the genomes of organisms in both domains of life. According to the literature, Bacteria and Archaea use the same mechanisms, i.e. conjugation, transduction, and natural transformation, to acquire exogenous DNA [86,87]. This is undoubtedly true for conjugation and transduction, but whether natural transformation contributes at all to lateral gene transfer in the Archaea is not known at present due to lack of data. In contrast, natural transformation, which is defined as the active uptake and heritable integration of extracellular DNA, has been extensively studied in selected bacterial species such as *Streptococcus pneumoniae*, *Neisseria* spp., *Bacillus subtilis* and *Haemophilus influenzae* [24,30,60,61,120]. Unlike conjugation and transduction, transfer of DNA by natural transformation is initiated by the recipient cell. Thus, while conjugation and transduction rely on extrachromosomal genetic elements promoting their own maintenance and distribution, natural transformation is part of the normal physiology of the competent bacterium and might therefore be considered to be uniquely adapted to the needs of the host.

In this review, we give a brief overview of the phylogenetic distribution of naturally transformable bacteria, summarize current knowledge of the mechanisms behind DNA uptake and release, and discuss how natural transformation might benefit bacteria possessing this property.

* Corresponding author at: Department of Chemistry, Biotechnology and Food Science, Biotechnology Building, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway. Tel.: +47 6496 5883; fax: +47 6496 5901.

E-mail address: sigve.havarstein@umb.no (L.S. Håvarstein).

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2. Phylogenetic distribution of naturally transformable prokaryotes

2.1. The Archaea

To the best of our knowledge, very little experimental data have been put forward to document the presence of natural genetic transformation in the Archaea. Only preliminary experiments describing low transformation frequencies in *Methanococcus voltae* and *Methanobacterium thermoautotrophicum* have been reported [8,114,150]. In neither of these cases was it demonstrated that the transformants obtained were generated by a mechanism corresponding to natural transformation in Bacteria. Furthermore, no follow-up studies have been published to verify and extend the original findings. In general, DNA uptake in competent bacteria requires the presence of type IV pili or pseudopili [26]. Assembly of the pseudopilus requires the same components as the type IV pilus (see below). Archeae produce a flagellum which in many respects is more closely related to type IV pili than to the bacterial flagellum. However, archaeal and bacterial flagella are both rotating structures, while the twitching motility mediated by type IV pili depends on extension and retraction of the pilus [102]. DNA uptake and twitching motility are functionally closely connected, as loss of motility invariably leads to loss of transformability [149]. Since the archaeal flagellum is different from type IV pili with respect to the way it moves, it is unlikely that it forms part of a DNA uptake apparatus similar to one employed by naturally competent bacteria. Thus, judging from the limited data available, the status of the Archaea with respect to natural transformation is still an open question.

2.2. Gram-positive bacteria

The phylum Firmicutes, the low G + C Gram-positives, contains a number of naturally transformable species (Table 1). The two best studied are *S. pneumoniae* and *B. subtilis*, both of which have served as model organisms for research on

Table 1
Naturally competent prokaryotes

Phylum		Species	Reference
Euryarchaeota		*Methanobacterium thermoautotrophicum*	[150]
		Methanococcus voltae	[8]
Deinococcus-Thermus		*Deinococcus radiodurans*[a]	[136]
		Thermus aquaticus	[82]
		Thermus caldophilus	[82]
		Thermus flavus	[82]
		Thermus thermophilus	[82]
Cyanobacteria		*Nostoc muscorum*	[137]
		Synechococcus elongatus[b]	[128,138]
		Synechocystis spp.[c]	[53,85,133]
		Thermosynechococcus elongatus	[107]
Chlorobi		Chlorobium limicola	[108]
		Chlorobium tepidum	[47]
Proteobacteria	Alpha	*Agrobacterium tumefaciens*	[40]
		Methylobacterium organophilum	[104]
		Bradyrhizobium japonicum[d]	[89]
	Beta	*Achromobacter* spp.	[78]
		Eikenella corrodens	[139]
		Kingella denitrificans	[147]
		Kingella kingae	[17,147]
		Neisseria gonorrhoeae	[91]
		Neisseria meningitidis	[22]
		Ralstonia solanacearum	[9]
		Thiobacillus thioparus	[152]
		Thiobacillus sp. strain Y	[152]
	Gamma	Acinetobacter baylyi	[142]
		Acinetobacter calcoaceticus	[75]
		Actinobacillus actinomycetemcomitans	[140]
		Actinobacillus pleuropneumoniae	[15]
		Aggregatibacter aphrophilus[e]	[140]
		Azotobacter vinelandii	[109]
		Cardiobacterium hominis	[139]
		Haemophilus influenzae	[91]
		Haemophilus parainfluenzae	[54]
		Haemophilus parasuis	[11]
		Legionella pneumophila	[134]
		Moraxella spp.	[16,76–78]
		Pseudomonas fluorescens	[40]
		Pseudomonas stutzeri and related species	[20]
		Pseudomonas spp.[f]	[48]
		Vibrio cholerae	[92]
		Vibrio parahaemolyticus	[48]
		Vibrio spp.	[48,73]
	Epsilon	Campylobacter coli	[144]
		Campylobacter jejuni	[144]
		Helicobacter pylori	[101]
Firmicutes		*Bacillus amyloliquefaciens*	[35]
		Bacillus licheniformis	[52]
		Bacillus subtilis	[99]
		Lactobacillus lactis	[66]
		Leuconostoc carnosum	[62]
		Streptococcus pneumoniae	[39]
		Streptococcus mitis	[6]
		Streptococcus oralis	[123]
		Streptococcus crista	[34]
		Streptococcus infantis	[146]
		Streptococcus gordonii	[110]
		Streptococcus sanguinis[g]	[51,71]
		Streptococcus anginosus	[71]
		Streptococcus intermedius	[71]
		Streptococcus constellatus	[71]
		Streptococcus thermophilus[h]	[12]
		Streptococcus bovis	[95]
		Streptococcus mutans	[115]
		Thermoactinomyces vulgaris	[68]
Actinobacteria		*Mycobacterium smegmatis*	[103]
		Streptomyces spp.	[122]

[a] Prev. *Micrococcus radiodurans*.
[b] Prev. *Anacystis nidulans*, *Synechococcus* sp. *PCC 7942* and *PCC 6301*.
[c] Including prev. *Agamenellum quadriplicatum*.
[d] Prev. *Rhizobium japonicum*.
[e] Prev. *Haemophilus aphrophilus*.
[f] Prev. *Vibrio* sp. strain WJT-1C.
[g] Prev. *Streptococcus sanguis*.
[h] Competent when ComX is overexpressed.

natural transformation in Gram-positive bacteria. In both genera, the competence genes can be divided into two groups; those involved in deciding when conditions are right for development of the competent state (early genes) and those required for DNA binding, import and recombination (late genes). The nature of the early genes and the way they control competence development can vary considerably between species, but often involves cell-cell communication with specific peptide pheromones [3,31,74]. In contrast, the late genes are highly conserved in many AT-rich Gram-positive bacteria, even in species traditionally regarded as non-competent [83,90]. Examples of "non-competent" species harboring the late *com* genes are *Lactococcus lactis*, *Lactobacillus plantarum* and *Listeria monocytogenes* [14,80,151]. These species may require unusual conditions for competence development that are difficult to recreate in the laboratory. Alternatively, the sporadic distribution of strains and species found to be naturally competent in the phylum Firmicutes may reflect that this property is frequently lost. Presumably, bacteria experiencing such loss suffer no immediate harm, but might be at a disadvantage in the longer run. It is therefore plausible that many naturally transformable species encompass a substantial number of competence-deficient strains, some of which may regain full transformation proficiency by reverting to the original phenotype.

The prevalence of natural transformation in the phylum Actinobacteria, the high G + C Gram-positives, can only be a matter of speculation at present. So far, one member of this phylum, *Mycobacterium smegmatis*, has been described as naturally transformable [10,103]. In addition it has been reported that radioactive DNA is taken up by a *Streptomyces* strain, but no genetic transformation was demonstrated in this case [122]. Database searches show that the genomes of a number of species belonging to the Actinobacteria contain homologs of late *com* genes such as ComEA and ComEC (personal observation). Since the products of these genes are involved in translocation of DNA across the cytoplasmic membrane in competent bacteria, their presence in Actinobacteria suggests that competence for natural transformation might be more widespread in this phylum than documented in the literature.

2.3. The proteobacteria

Naturally transformable species have been discovered in the alpha, beta, gamma and epsilon subdivisions of proteobacteria (Table 1), indicating that the ancestor of this monophyletic group possessed this property. The best studied proteobacteria, with respect to natural genetic transformation, are the important human pathogens *H. influenzae*, *N. gonorrhoeae* and *N. meningitidis*. Similar to the Gram-positive model organisms *B. subtilis* and *S. pneumoniae*, these species have been subjected to in-depth studies aimed at elucidating structural and functional aspects of natural transformation. A substantial research effort has been devoted to map and assign a function to the components of the molecular motor that translocates DNA across the cell envelope. Most of these studies has been carried out with *Neisseria* spp. and *B. subtilis* as model systems [1,19,24,36,49]. As mentioned above, type IV pili/pseudopili are an essential component of the DNA uptake machinery in virtually all competent bacteria. The exception is the epsilon-proteobacterium *Helicobacter pylori* which relies on a type IV secretion system for uptake of exogenous DNA (Fig. 1) [21,67]. Naturally competent members of the families Neisseriaceae (beta subdivision) and Pasteurellaceae (gamma subdivision) strongly prefer to take up DNA containing their own specific DNA uptake signal sequences (DUS or USS). Thus, it was shown more than twenty years ago that *N. gonorrhoeae* does not take up DNA from *H. influenzae* and vice-versa [91]. Both the 12 bp neisserial DUS sequence and its 9–10 bp USS counterpart in *H. influenzae* appear to be randomly distributed throughout the genomes of these bacteria when viewed on a large scale. At close range, however, there is an overrepresentation of DUS/USS sequences in genome maintenance genes and transcriptional terminators [2,38,129]. Recently, it has become clear that two related but non-identical USS sequences, termed the *H. influenzae* (Hin) and *Actinobacillus pleuropneumoniae* (Apl) types, are present in various species belonging to the Pasteurellaceae. Interestingly, *H. influenzae* and *A. pleuropneumoniae* preferentially take up DNA of the Hin and Apl types respectively, demonstrating that these bacteria have evolved mechanisms to discern between DNA from close and more distant relatives [121]. Some naturally competent proteobacteria, however, such as *Acinetobacter* spp. and *H. pylori*, lack this fascinating DNA-sorting mechanism [38,111,113,126]. It is possible that species belonging to these genera have evolved other ways to discriminate between homologous and foreign DNA.

2.4. The phyla Cyanobacteria and Deinococcus-Thermus

Although, a few species of cyanobacteria have been known for decades to be naturally transformable, relatively few studies have focused on the phenomenon itself (Table 1). Instead, the transformability of some strains, such as the unicellular *Synechocyctis* sp. strain PCC 6803, has been a valuable tool for researchers investigating photosynthesis and phototaxis in cyanobacteria. Nevertheless, it has been shown that dsDNA is converted to the single-stranded form during uptake and that internalization of DNA depends on the presence of type IV pili [81,100].

Thermus thermophilus, which belongs to the extreme thermophilic bacteria, has emerged as the leading model organism for the study of heat-stable proteins. Due to their inherently robust nature, enzymes from *T. thermophilus* are very attractive for various industrial applications. Such proteins are also interesting subjects for basic research as they provide insight into structural factors enhancing protein thermostability and resistance to denaturating agents. Research on the physiology and metabolism of *T. thermophilus* is facilitated by the fact that this species is naturally transformable, making it highly amenable to sophisticated genetic manipulation [63,82]. Using natural transformation to introduce mutations into the chromosome of *T. thermophilus* will, for instance, be a powerful method for assigning functions to the large number of

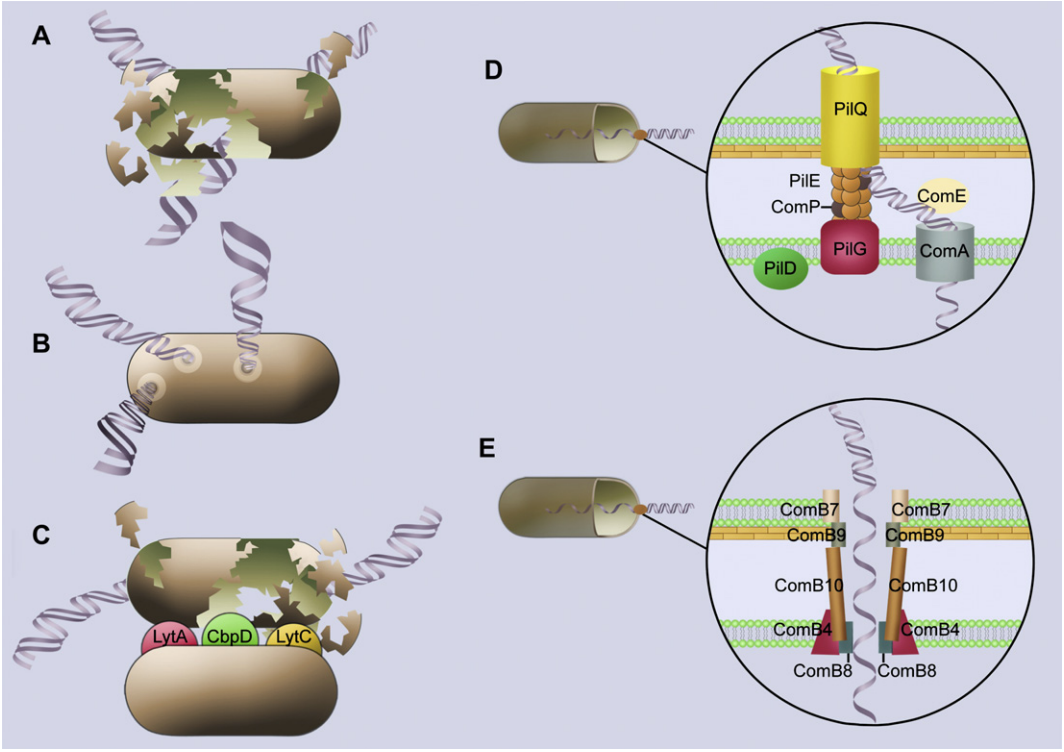

Fig. 1. Mechanisms for release of donor DNA (A, B, C) and uptake of transforming DNA (D, E) in bacteria that are competent for natural transformation. **A**) Passive release of DNA from bacteria that have died from natural causes. **B**) Active release of DNA in *N. gonorrhoeae* mediated by a type IV secretion system. **C**) Capture of DNA by a fratricide mechanism. Competent *S. pneumoniae* cells kill and lyse non-competent sister cells present in the same environment. The competent cells express a set of murein hydrolases (LytA, CbpD, and LytC) that attack the cell wall of non-competent target cells, resulting in cell lysis and release of DNA that can be taken up by the competent cells. **D**) DNA uptake mediated by a type IV pilus system in *N. gonorrhoeae*. The pilins PilE and ComP are assembled to form a competence pseudopilus. Assembly is dependent on the prepilin peptidase PilD and the membrane protein PilG. Double-stranded DNA is channelled across the outer membrane through a pore formed by the PilQ multimer, and periplasmic dsDNA is bound by the membrane-anchored receptor ComE. Then, single-stranded DNA enters the cytoplasm through the channel protein ComA, while the other strand is degraded outside the cytoplasmic membrane. **E**) DNA uptake mediated by a type IV secretion-like system in *H. pylori*. This DNA transport system is formed by multiple protein subunits that assemble into a pore that is predicted to span the cellular envelope. While the classical type IV secretion systems utilize a F-pilus-like fiber for substrate export through the transport pore, it is presently unclear whether such structures are utilized by type IV secretion-like systems involved in DNA import.

unknown ORFs present in this species. Sixteen competence genes located within seven separate transcription units has now been identified in *T. thermophilus*. Through homology searches it has been shown that these *com* genes encode proteins involved in DNA translocation, type IV pilus biogenesis and DNA recombination [46,124]. Comparative genomics and phylogenetic analyses show that acquisition of numerous genes from thermophilic Bacteria and Archaea has helped *T. thermophilus* adapt to high-temperature environments, demonstrating that lateral gene transfer has been the driving force in the evolution of this species [106]. Interestingly, it has recently been found that *T. thermophilus* HB27 takes up DNA from Bacteria, Archaea and Eukarya equally well [127]. The broad substrate specificity of the DNA translocator makes it tempting to speculate that gene acquisition by natural transformation has played a major role in adapting this bacterium to life at extreme thermal conditions.

3. The DNA uptake apparatus

Bacteria that are naturally competent for genetic transformation express a set of proteins dedicated to the uptake and subsequent homologous recombination of transforming DNA. In most Gram-negative bacteria, transport of exogenous DNA through the outer membrane involves type IV pili (Fig. 1) [24,25,141]. Entry of DNA through the outer membrane is probably initiated by pilus retraction, which is achieved by rapid depolymerization of the pilin polymer. Available experimental data indicate that the pilus fiber is closely associated with an outer membrane protein channel formed by the secretin protein PilQ. Structural studies of PilQ suggest that this protein forms a homododecameric structure with a central cavity that serves as a pilus pore. Interestingly, recent results have demonstrated that the PilQ complex has significant DNA binding capacity, suggesting that the PilQ pore could actively participate in transport of DNA into the periplasm [4,32,33,43,49]. The type IV pili of Gram-negative bacteria are multifunctional organelles. In addition to being required for uptake of DNA, they are also involved in bacterial adhesion and twitching motility. They form long filamentous structures that are easily visible under the electron microscope. Apart from a couple of exceptions [64,143], the type IV pili of Gram-positive bacteria have not been observed to protrude from their surface, and they have therefore been named

pseudopili [24,26]. Both pilus types are essentially assembled by the same apparatus from orthologous components [24,26]. In both Gram-positive and Gram-negative bacteria, passage of DNA through the cytoplasmic membrane involves the membrane-anchored dsDNA binding protein ComEA (ComEA in *B. subtilis* and ComE in *N. gonorrhoeae*) and the polytopic membrane protein ComEC (ComEC in *B. subtilis* and ComA in *N. gonorrhoeae*). In *B. subtilis* and *S. pneumoniae*, translocation also depends upon the ATPase ComFA, which presumably produces the energy required to drive single-stranded DNA through the ComEC channel [24]. Single-stranded DNA that has entered the cytoplasm can be integrated into the recipient's genome by RecA-dependent homologous recombination.

4. Regulation of competence development

Naturally transformable species belonging to the genera *Neisseria*, *Deinococcus*, *Acinetobacter*, *Synechococcus* and *Chlorobium* have been reported to be competent throughout the logarithmic growth phase, while others are competent only for short time periods (*S. pneumoniae*) or develop competence at the onset of stationary phase (*B. subtilis*) [112]. With the exception of *N. gonorrhoeae* and *N. meningitidis*, which synthesize their competence proteins in a constitutive manner, it is likely that most naturally transformable bacteria regulate expression of their *com* genes in response to certain cellular and/or environmental signals. In *S. pneumoniae*, induction of competence depends on the secreted competence-stimulating peptide (CSP), its membrane-embedded histidine kinase receptor ComD, and the cognate response regulator ComE [69,70,116,145]. Binding of CSP to ComD results in autophosphorylation of the kinase followed by transfer of the phosphoryl group to the response regulator ComE. As a consequence, the level of phosphorylated ComE (ComE-P) in the cytoplasm increases rapidly, driving the cell into the competent state [28]. ComE-P activates expression of 20 early genes, two of which are identical and encode the alternative sigma factor ComX [84]. ComX has been found to direct the expression of about 60 genes, including those encoding the DNA uptake apparatus [37,117]. In laboratory cultures of *S. pneumoniae*, spontaneous competence induction take place during early exponential growth, whereas the competent state in *B. subtilis* cultures develops at the onset of stationary phase. Regulation of competence for natural transformation in *B. subtilis* is an extremely complex process that integrates a number of different stimuli such as nutritional stress and cell density [61]. A detailed description of this regulatory network is beyond the scope of this review, but we will briefly outline the part involved in cell density control of competence development. Similar to *S. pneumoniae*, *B. subtilis* produces a secreted peptide pheromone, ComX, that stimulates competence development [88]. Unlike pneumococcal CSP, however, ComX is post-translationally modified containing a modified tryptophan residue with a geranyl group [105]. Accumulation of ComX is sensed by the membrane histidine kinase ComP, resulting in the transfer of a phosphate group from the latter to the response regulator ComA. ComA-P acts as a transcriptional activator that turns on transcription of *comS*, which encodes a protein that prevents proteolysis of the ComK competence master regulator. Upon accumulation, ComK, which acts autocatalytically by binding to a region in its own promoter, activates the transcription of the genes required for transformation [29,61]. Both *B. subtilis* and *S. pneumoniae* have competence regulatory systems that are interconnected with other regulatory pathways in the cell. ComA-P activity is, for example, regulated by the proteins RapC and RapF, which inhibit DNA binding of ComA-P. Rap activity is in turn regulated by the secreted pentapeptide pheromones PhrC and PhrF, which upon internalization, inhibit the activity of the Rap proteins [13,118,130]. Transcription of the Phr proteins is under direct control of the alternative sigma factor σ^H, which is regulated in response to signals such as growth phase, external pH and Clp proteolytic complexes. The competence regulatory system in *S. pneumoniae* is influenced by the two-component system CiaRH that has been proposed to monitor cell wall integrity, as well as the stress-related global regulator StkP [56,125]. Integration of competence regulation with other cellular regulators presumably enables these species to finely tune competence development in response to the overall physiological state of the cell.

In general, less is known about competence regulation in Gram-negative than in Gram-positive bacteria. In recent years, however, considerable progress has been made in understanding the factors governing this process in *H. influenzae*. In this bacterium, transfer of actively growing cells to a defined starvation medium (MIV) induces expression of the DNA uptake genes [65,120]. So far, two key regulators, CRP and Sxy, have been found to control competence development under these conditions. CRP, which is homologous to the *E. coli* catabolite activator protein (CAP), functions as a transcriptional activator. The competence genes in *H. influenzae* are preceded by promoter elements that contain a subclass of CRP binding sites known as CRE sites [18,23,120]. CRP-mediated activation of these promoter elements depends on cAMP, which is the allosteric effector of CRP, but also on the gene product of *sxy* [148]. In contrast to *crp*, *sxy* is upregulated by starvation in MIV medium. The molecular mechanisms underlying such regulation of *sxy* are not understood. Neither has the mechanism for Sxy-mediated control of CRP activity been elucidated. So far, it has been demonstrated that Sxy is not necessary for CRP transcription, and the current model involves a mechanism in which Sxy interacts directly with CRP to stabilize the interaction between CRP and DNA at the CRE sites [18]. An *sxy* homologue, $tfoX^{VC}$, has also been demonstrated to be essential for competence development in *Vibrio cholerae* [92]. This species was found to develop a competence phenotype when grown in the presence of chitin, which induces the expression of 41 genes including $tfoX^{VC}$. Microarray analysis revealed that $tfoX^{VC}$ is required for chitin-induced expression of genes predicted to encode a type IV pilus assembly complex and a DNA translocation machinery. Subsequent mutational analysis confirmed the involvement of these genes in transformation. Furthermore,

competence in *V. cholerae* was also found to depend on HapR, which is involved in regulation of biofilm formation and virulence [92]. The expression of *hapR* depends on the stationary-phase sigma factor RpoS, but is also regulated by the cell density regulatory protein LuxO, which represses *hapR* expression at low cell densities [153]. Hence, competence development in *V. cholerae* is triggered by a diverse set of signals, including the growth substrate chitin, stationary phase stress, and cell density (see Fig. 2).

The above examples suggest that bacteria regulate competence development by complex mechanisms that communicate with other regulatory networks in the cell. In order to achieve efficient transformation of a given bacterium in the laboratory, the mechanisms underlying competence development in the respective bacterium must be elucidated. Taking this into account, the list of naturally transformable bacterial species compiled in Table 1 probably represents the tip of the iceberg relative to the actual number of such species present in nature. In the genus *Streptococcus*, for example, the late competence genes, i.e. those required for DNA binding, processing, uptake and recombination, appears to be omnipresent. It is therefore curious that only a fraction of the species comprising this genus have been demonstrated to be naturally transformable under laboratory conditions. This could be due to inappropriate growth conditions and lack of environmental cues required to trigger competence development. This view is supported by the fact that the competent state can be induced in *S. thermophilus* LMG18311 if ComX, the alternative sigma factor controlling the late *com* genes, is overexpressed [12]. Spontaneous competence development, on the other hand, has never been observed for this species under laboratory conditions.

5. Acquisition of transforming DNA

Synthesis of the competence protein machinery would be futile without the simultaneous presence of homologous transforming DNA in close proximity to the competent cell. So far, there is no evidence that bacteria regulate competence development in direct response to the occurrence of free DNA in their surroundings. In fact, several bacterial species, such as *N. gonorrhoeae*, express the competence phenotype constitutively, while others, such as *S. pneumoniae*, *B. subtilis*, and *H. influenzae* spontaneously induce competence development irrespective of the presence of free DNA in the growth environment. Traditionally, it has therefore simply been assumed that dead and lysed bacteria present in the same habitat as the competent cells provide the transforming DNA. While DNA from such sources undoubtedly will contribute to the reservoir of free DNA accessible to a competent bacterium, recent investigations have revealed that some competent bacterial species have evolved molecular mechanisms that probably serve to increase the availability of homologous DNA (Fig. 1).

A horizontally acquired genetic island (GGI) that is found in approximately 80% of all gonococcal isolates has been shown to contain genes encoding a putative type IV secretion system [42]. Such membrane-associated transporter systems, which are ancestrally related to bacterial conjugation systems, have previously been demonstrated to function in the secretion of cellular macromolecules in Gram-negative bacteria. Typically, a type IV secretion system is composed of 8–12 protein subunits including components involved in energy supply, channel formation and pilus biogenesis. Together, the various proteins assemble into a complex that spans the inner and outer membrane of the cell. The mechanism for substrate translocation has not been elucidated. One model predicts that the pilus fiber acts as a piston forcing the substrate through a channel spanning the outer membrane, while an alternative hypothesis suggests that the pilus itself forms the secretion channel [5,21]. Although deletion of gonococcal GGI genes belonging to the putative type IV secretion system did not decrease the level of autolysis, such mutations reduced the release of DNAseI-sensitive DNA from log phase cells, and also greatly reduced the mutant cell's ability to act as donors of transforming DNA [58,59]. Thus, the GGI type IV secretion system appears to be involved in a mechanism for active donation of DNA that contributes to lateral gene transfer between neisserial cells (Fig. 1).

Recent advances in our understanding of the behavior of competent *S. pneumoniae* cells indicate that they might not

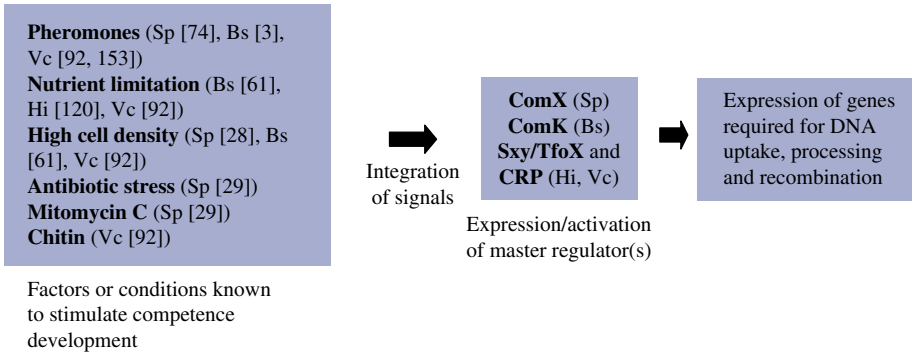

Fig. 2. Overview of factors or growth conditions known to have a stimulatory effect on competence development in *S. pneumoniae* (Sp), *B. subtilis* (Bs), *H. influenzae* (Hi) and *V. cholera* (Vc), the best studied species with respect to regulation of the competent state. A common denominator for competence development in these species appears to be nutritional depletion (Bs, Hi and Vc) or some other form of stress (Sp). In addition, Sp, Bs and Vc all use pheromone-mediated quorum-sensing systems to ensure that competence development takes place at high cell density.

solely rely on taking up DNA from dead bacteria. Competent pneumococci have evolved the ability to lyse non-competent siblings present in the same environment, and this lytic process, termed fratricide, most likely serves as an active DNA acquisition mechanism [57,98,131,132]. In liquid culture, lysis of non-competent cells has been shown to depend on the concerted action of the murein hydrolases CbpD, LytA and LytC [79,98,132]. The key effector CbpD is produced only by the competent attacker cells [79], whereas the autolysins LytA and LytC can be supplied by the attacker and/or the target cells [57]. Thus, CbpD appears to trigger the lytic activity of LytA and LytC in a manner that is lethal to the non-competent target cells but is harmless to the competent attackers. The CbpD-producing cells protect themselves against the action of the activated autolysins by producing an immunity protein, ComM, of unknown function [72]. On agar plates effective cell lysis also depends on the two-peptide bacteriocin CibAB. This bacteriocin is co-expressed with a bacteriocin immunity protein, CibC, which was demonstrated to protect the competent cells from lysis [57]. Transcriptional analysis of the genes involved in pneumococcal fratricide revealed that *comM* is an early gene, whereas *cbpD*, *lytA* and *cibABC* belong to the late genes. The two autolysins are produced by non-competent as well as competent cells, but *lytA* expression is somewhat upregulated in the latter. Regulation of *lytC* expression, on the other hand, is not connected in any way to competence development. Fratricide and competence is tightly coupled processes with respect to regulation and kinetics, and it is therefore reasonable to assume that they are functionally connected as well (Fig. 1) [30,31].

The presence of DNA secretion in gonococci and fratricide in pneumococci seriously question the hypothesis that transforming DNA solely originates from arbitrary passive release of genetic material from dead bacteria. Most likely, the purpose of these DNA release mechanisms is to make gene exchange between related bacteria more efficient. Clearly, elucidation of the biological role of DNA secretion and fratricide will help us to better understand how natural transformation contributes to increasing the fitness of bacteria possessing this property (see next section).

6. Natural transformation − food gathering or gene exchange?

How do bacteria benefit from being competent for natural transformation? This question has been raised and discussed in a number of papers [29,44,119,135], but the answer is still far from clear. Competent bacteria have to bear the cost of replicating and expressing the *com* genes, and run the risk of incorporating defective or harmful genes into their genomes. These costs would undoubtedly lead to loss of the *com* genes if they were not compensated for by specific gains in fitness acting at the species and/or population level. Two by nature very different models have traditionally been put forward to explain the role of natural genetic transformation. One of them, hereafter called the sex hypothesis, postulates that the purpose of natural transformation is to capture genetic material from other cells in order to repair damaged genes, generate genetic diversity and acquire novel traits. The other holds that competent bacteria take up DNA primarily for nutritional purposes [45,119]. It should be pointed out that the two models are not mutually exclusive. Besides, the process may serve somewhat different functions in different species, since the conditions governing competence induction evidently vary between species. In our view, the best way to approach the problem is to study natural transformation in detail in selected organisms and use the information obtained to exclude models that do not receive support from experimental data. *S. pneumoniae* represents one of the best studied bacteria with respect to natural genetic transformation, and we will therefore focus our discussion primarily on this species. In pneumococci, the strongest argument against the DNA as food hypothesis relates to the fate of the incoming DNA. DNA uptake is initiated by the binding of dsDNA to the surface of the competent pneumococcal cell. The bound dsDNA is processed by the endonuclease EndA which, in conjunction with the DNA uptake apparatus, mediates transport of a single strand into the cytoplasm in the 3′ to 5′ orientation [93]. In contrast, the complementary strand is degraded outside the cell [94]. Considering the possibility that DNA is taken up for nutritional purposes, this appears to be a wasteful and inefficient food-gathering mechanism. Immediately after entering the cytoplasm, the ssDNA is found to be tightly associated with proteins [96]. Experimental evidence strongly indicates that this so-called eclipse complex serves to protect the ssDNA from degradation and prepare it for recombination with the recipient's genome. Early work carried out by Morrison and co-workers [97] identified single-strand binding protein B (SsbB) as a likely component of the complex. This protein, which is a paralog of another pneumococcal single-strand binding protein (SsbA) thought to serve housekeeping functions, is encoded by a late *com* gene and is expressed only in competent cells [55]. In addition, incoming ssDNA is protected by the products of the late *com* genes *recA* and *dprA*, as this DNA is degraded immediately in mutants lacking either of these genes [7]. RecA, which is known to play an important role in recombinational DNA repair in bacteria, is without doubt also involved in strand exchange activities during transformational recombination. Another late *com* gene, believed to be essential for effective homologous recombination, has been termed *coiA*. A pneumococcal mutant lacking a functional *coiA* gene accumulates transforming DNA in eclipse intracellularly, but fails to incorporate this DNA into its chromosome [41]. This observation strongly indicates that the competence-specific CoiA protein specifically promotes recombination during natural genetic transformation in the pneumococcus. Thus, despite the fact that most of the DNA taken up is degraded and recycled without being subjected to recombination, the processing of captured DNA is not as would be expected if it was primarily acquired for nutritional purposes. It can therefore safely be concluded that food gathering is not the principal role of natural transformation in *S. pneumoniae*. The same conclusion was reached for *A. calcoaceticus* after it was discovered that competence in this species is

optimally induced when starving cultures are diluted into fresh medium [112]. The finding that competent pneumococci are tuned for recombination with incoming donor DNA, however, is entirely consistent with the sex hypothesis.

Sex in bacteria can be defined as the capture and inheritance of exogenous DNA, i.e. a process that brings together DNA from different sources into a single cell. In contrast to Gram-negative bacteria such as *Neisseria* spp. and *H. influenzae*, which primarily take up DNA containing so-called DNA uptake sequences, competent streptococci will bind and import DNA from any source. Thus, in principle, the source of exogenous DNA for competent pneumococci could be the entire oropharyngal metagenome. In practice, however, pneumococci appear to have developed a mechanism, fratricide, which favors capture of DNA from siblings. Interestingly, recent results show that fratricide also takes place in *Streptococcus mitis*, a close relative of *S. pneumoniae*. In addition, cross-species lysis between *S. mitis* and *S. pneumoniae* has been observed (unpublished data). What does this competence-regulated cell lysis mechanism tell us about the role of natural transformation in *S. pneumoniae* and related naturally competent streptococci such as *S. mitis* and *Streptococcus oralis*? If the mechanism really serves to enhance the efficiency of DNA transfer within and between these species, natural transformation might be advantageous because it stimulates exchange of genetic material between relatively closely related bacteria. Undoubtedly, competent pneumococci will also take up DNA released from more distantly related bacteria that have died and fallen apart from natural causes. But, as such low-homology DNA recombine at low efficiency, and more often are harmful than beneficial to the recipient, it is unlikely that this type of gene exchange represents the selective force preserving a functional transformation machinery in naturally competent pneumococci. Interestingly, the size of pneumococcal genomes seems to be restricted upwards to about 2.2 Mbp. This limits metabolic capacities and environmental adaptability, but reduces the costs of replicating and maintaining a larger genome. However, by being able to capture DNA from their close relatives, pneumococcal strains will have access to a collective gene pool, a so-called supragenome, containing allelic variants and genes not present in their own chromosomes. The worldwide increase in penicillin-resistance observed among clinical isolates of *S. pneumoniae* during the 1990s illustrates how naturally competent pneumococci can benefit from sharing a gene pool with their close relatives. Penicillin resistance in *S. pneumoniae* is caused by alterations in the penicillin binding proteins (PBPs) that reduce these enzymes' affinity for beta-lactam antibiotics. Sequencing of genes encoding such low-affinity PBPs has revealed that they contain mosaic blocks that are highly divergent from the corresponding sequences in sensitive pneumococci. Extensive research on this phenomenon has demonstrated that *S. mitis* and *S. oralis*, members of the indigenous microflora of the upper respiratory tract, are the original donors of the mosaic blocks implicated in penicillin resistance in *S. pneumoniae* [27]. This example clearly shows that pneumococci subjected to external stress can overcome the problem by acquiring new genes or alleles from the supragenome. Presumably, the sharing of a supragenome compensates for the disadvantage of possessing a relatively small genome, and enables pneumococci and their naturally competent relatives to adapt faster to environmental changes.

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