In and out – Contribution of natural transformation to the shuffling of large genomic regions

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Short title: Competence for DNA shuffling

Abstract

Naturally competent bacteria can pull free DNA from their surroundings. This incoming DNA can serve various purposes, ranging from acting as a source of nutrients or DNA stretches for repair to the acquisition of novel genetic information. The latter process defines the natural competence for transformation as a mode of horizontal gene transfer (HGT) and led to its discovery almost a century ago. However, although it is widely accepted that natural competence can contribute to the spread of genetic material among prokaryotes, the question remains whether this mode of HGT can foster the transfer of larger DNA regions or only transfers shorter fragments, given that extracellular DNA is often heavily fragmented. Here, I outline examples of competence-mediated movement of large genomic segments. Moreover, I discuss a recent proposition that transformation is used to cure bacteria of selfish mobile genetic elements. Such a transformation-mediated genome maintenance mechanism could indeed be an important and underappreciated function of natural competence.

Introduction

It has long been understood that prokaryotic chromosomes are often mosaics with stretches of horizontally acquired foreign DNA integrated into their genomes. The well-studied bacterium *Escherichia coli* strain K12, for example, contains 12.8% recognizably foreign DNA, whereas the cyanobacterium *Synechocystis* (strain PCC6803) contains an even higher percentage of foreign DNA (16.6%; [1]). With the advent of whole (bacterial) genome sequencing, large-scale studies are now possible that can further evaluate horizontally moving genetic material. For example, Kloeckges *et al.* constructed a network of genes that are shared among 329 proteobacterial genomes because many representative proteobacteria have now been sequenced [2]. The authors reported significant genome plasticity for the γ -proteobacteria, which showed the “most chameleon-like evolutionary characteristics” [2]. As expected, free-living species seemed to have undergone HGT more frequently compared to their endosymbiotic relatives. It was also suggested that roughly three quarters of genes have moved horizontally at least once in the past [2]. This finding and the fact that transient genes are often involved in specific adaptation processes [3] are consistent with the “public good hypothesis for the evolution of life on Earth” [4]. This hypothesis suggests that “segments of DNA are seen as public goods, available for all organisms to integrate into their genomes” and that they constitute a “pangenome of accessory genes that can be drawn on as needed” [3]. However, acquisition of novel and horizontally moving DNA might come at a cost for the recipient cell. Therefore, why is horizontal gene transfer (HGT) maintained in prokaryotes? In this review, I summarize how one mode of HGT, natural competence for transformation, might contribute to the spread of larger genomic regions (e.g., including genomic islands) and antibiotic resistance. Moreover, I discuss a new concept that was recently proposed by Croucher and colleagues, namely, that transformation might play a crucial role in rescuing bacterial cells from selfish mobile genetic elements (MGEs) [5].

Horizontal gene transfer in bacteria

DNA moves between different bacterial strains or species via mechanisms of HGT [6]. Three major modes of HGT exist: bacterial mating through conjugation, transfer of DNA from one bacterium to another through bacteriophages (transduction) and the acquisition of free DNA from the environment using competence-induced DNA-uptake machinery. Indeed, the latter process, natural competence, is a widely distributed physiological state in which absorbed DNA can be homologously recombined into the genome in case sufficient sequence similarity between donor and acceptor DNA exists. Such recombination ultimately transforms the recipient cell (for some excellent reviews see [7,8])(Fig. 1).

Although HGT, including natural competence, contributes to bacterial evolution and adaptation to new niches, it is also a dangerous process. First, the incorporation of novel genetic material can harm cells if the genetic information is deleterious. Indeed, it has been argued that freely available DNA can hardly ever be beneficial because such DNA is mostly derived from less fit organisms that did not survive selective pressure [9]. Second, external DNA can contain mobile genetic elements (MGEs) such as prophages and integrative conjugative elements (ICE). MGEs encode functions that promote their own transfer and often interfere with host cell physiology. Finally, newly acquired genetic material can itself burden the overall cell physiology. Indeed, the potential consequences of horizontally acquired DNA include the disruption of genomic features (though some elements have evolved a site-specific integration strategy for smooth integration; reviewed by [10]), the sequestration of cellular housekeeping machineries and the limitation of cellular resources (e.g., the accompanying cost for transcription and translation of new genes and system-level effects due to rewiring of regulatory pathways), among others [11]. Therefore, many prokaryotes have evolved strategies to limit the incorporation of new genetic material.

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78 **Defense strategies against foreign DNA and xenogeneic silencing**

79 Defense strategies against horizontally moving genetic material include the degradation of
80 foreign DNA through endonucleases as part of restriction-modification systems through
81 recognition of DNA methylation status [12] or through clustered, regularly interspaced, short
82 palindromic repeat (CRISPR) systems, which are an adaptive immune system of prokaryotes
83 that recognizes DNA stretches in a sequence-specific manner [13]. In addition, the silencing
84 of horizontally acquired DNA regions by the heat-stable nucleoid structuring protein H-NS
85 often occurs in bacteria and is referred to as xenogeneic silencing [14]. Hence, it appears that
86 there is an arms race between the infections of bacterial cells by parasitic MGEs and the
87 corresponding defense strategies that limit the horizontal spread of genetic material and its
88 expression.

89 Acquisition of horizontally moving DNA as a result of phage transduction or plasmid
90 conjugation is not under the direct control of the recipient host. Thus, the evolution of
91 defense mechanisms against such ‘HGT attacks’ is expected. How does this work in the case
92 of natural transformation? Natural competence for transformation is fully operated by the
93 recipient bacterium and is often tightly regulated [15]. Thus, if transformation would be
94 detrimental for the cell, the ability to enter natural competence would not be maintained over
95 evolutionary time scales. Moreover, it is undeniable that naturally competent bacteria have
96 evolved mechanisms to protect incoming DNA from degradation. For example, competent
97 bacteria decorate incoming DNA with the DNA processing protein A (DprA) to shield it
98 from cellular defensive processes [16]. Moreover, when *S. pneumoniae*, for instance, enters
99 competence, it co-induces the enzyme DpnA, which methylates the incoming ssDNA [17].
100 This renders the newly acquired DNA resistant to dsDNA-specific restriction-modification
101 endonucleases (or restrictases) after this foreign ssDNA has been recombined into the

bacterial genome. It is therefore likely that natural competence as a recipient-driven HGT process truly aims to acquire new genetic material.

Acquisition of large genomic region islands by natural transformation

Johnston *et al.* recently demonstrated that the presence of the methylase DpnA is critical for the spread of pathogenicity islands in *S. pneumoniae*, including the capsule locus [17] (Table 1). Interestingly, the exchange of capsule loci among pneumococcal strains initially led to the discovery of natural transformation, based on a set of seminal experiments performed by Frederick Griffith in 1928 ([18] and recently illustrated by [19]). The horizontal transfer of such capsule-encoding genomic islands frequently occurs and is known as serotype switching; this process contributes to the development of escapees against pneumococcal conjugate vaccines [20,21]. Similar serotype or serogroup conversion processes also occur in other naturally competent bacteria such as *Vibrio cholerae* [22,23] (Table 1). In the latter case, the expansion of serogroup-converted strains can be driven by human- (adaptive immunity) and non-human-derived (environmental predators such as bacteriophages; [23]) selective pressure.

Additional work on *V. cholerae* provided evidence for the transfer of other genome segments, which, in this study, encoded metabolic enzymes [24]. A similar transfer event was described for a glucose catabolism locus in competent *Campylobacter coli* [25] (Table 1). Furthermore, whole genome sequencing analyses of naturally transformed *Haemophilus influenzae* and *Helicobacter pylori* indicated the transfer of larger DNA regions with maximal sizes of ~8 kb and ~14kb, respectively [26,27](Table 1). It should be noted that most of the above-mentioned studies made use of purified DNA as transforming material, which could negatively bias the overall length of the transforming material through the purification process of the nucleic acid. Hence, natural transformation can indeed efficiently

transfer larger DNA regions, including genomic islands, from one bacterium to another (Fig. 1) and future studies are expected to test the generality of these findings. Identifying such horizontal movements is rather difficult because transformation does not leave behind a “genetic fingerprint,” such as repeat regions, integrase genes, and other hallmarks of prophages, ICE elements or integrons. Furthermore, natural transformation might contribute to the spread of already integrated MGEs, independent of their normal mode of transfer, as experimentally demonstrated for non-mobilizable integrated cholera toxin prophages [28]. Thus, while not obvious at first glance, natural transformation might contribute to the movement of genomic islands and, ultimately, to evolution and the emergence of new pathogens (Fig. 1).

Does natural competence contribute to the spread of antibiotic resistance?

Natural transformation can likely lead to the transfer of any part of the chromosome. The question therefore arises whether natural competence also contributes to or even drives the spread of antibiotic resistances. Answering this question is important, as antibiotic resistances are on the rise and considered an immediate threat to human health, which was even addressed by the United Nations at their general assembly in 2016. Indeed, the fear of a 'post-antibiotic' era is greater than ever, and it was recently stated that “many researchers now consider horizontal transmission to be the primary driver of antibiotic resistance” [29].

The most problematic bacteria with growing multidrug resistance in hospital settings are the so-called “ESKAPE” pathogens, which include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species [30]. In addition to these ESKAPE pathogens, the Centers for Disease Control and Prevention (CDC) published a report in 2013 that outlines the top 18 drug-resistant threats to the US. In this report, and on the CDC webpage [31], the pathogens are

divided into three threat categories: urgent, serious, and concerning. A similar list of 12 antibiotic-resistant priority pathogens (classified based on critical, high, and medium priority) was recently announced by the World Health Organization [32]. Upon closer inspection, it appears that several of the organisms on these two lists are known or suspected to be naturally transformable (Fig. 2). It will therefore be important to better study the link between natural transformability and antibiotic resistances. Indeed, Domingues *et al.* recently showed that natural transformation facilitates the spread of gene cassettes, transposons, and integrons in *Acinetobacter baylyi*. The incorporation of the horizontally transferred DNA occurred through various transposition events as well as through homologous and heterologous recombination [33]. It should also be noted here that although they frequently are of low efficiency [34], many naturally competent bacteria are also able to take up plasmid DNA and maintain them as self-replicating entities. Natural competence can therefore also foster the spread of plasmid-encoded antibiotic resistances without the need for cell-to-cell contact-mediated conjugation. Such HGT events could therefore have major consequences on the spread of resistance in hospital settings because the common disinfection strategies are unlikely to sufficiently reduce traces of DNA.

Natural transformation as a means to rescue genomes from MGEs

The benefits of natural transformation or, more precisely, the ability to take up free genetic material from the surroundings has been extensively discussed in the past [35-39]. The main arguments proposed in this context were that freshly acquired DNA can serve nutritional purposes ('DNA for food'), can be used to acquire new genetic information ('DNA for evolution') or, alternatively, can serve in the template-directed repair of damaged genetic material ('DNA for repair'). The latter argument was based on the findings that certain antibiotics and other DNA damaging compounds induce natural competence in some

bacterial species [40-43], especially those that lack canonical SOS response systems. Moreover, naturally competent *S. pneumoniae* cells commit fratricide (that is, killing their siblings), thereby increasing the pool of clonal transforming DNA that can serve repair purposes [44]. Natural transformation might therefore contribute to genome maintenance in these (and possibly other) organisms (Fig. 1).

A recent study by Croucher *et al.* added an exciting new perspective on the role that transformation might have on genome maintenance, namely, that natural transformation aids in freeing bacterial genomes from selfish MGEs, which might have entered the organism by any mode of HGT (e.g., conjugation, transduction, or transformation) [5](Fig. 1). Natural transformation is based on double homologous recombination, for which only short stretches of highly homologous DNA are required, without any interference by in-between genetic material. This feature allows the integration of new genetic islands, as alluded to above. However, it can also lead to the expulsion of DNA regions, a fact that has been partially neglected in the past. Croucher and colleagues argued that this is especially true for short DNA fragments, which have a higher probability to be devoid of (large) MGEs [5]. Indeed, while DNA is abundant in most environments, it is often highly fragmented [45,46], making the presence of DNA stretches of larger size or those that include MGEs rather unlikely. Recent work from our group, however, provided evidence that naturally competent *V. cholerae* does not solely rely on freely available (fragmented) DNA, which is most likely derived from unfit bacteria. Instead, this organism actively increases the pool of transforming material through kin-discriminated neighbor predation using its type VI secretion system as a molecular killing device [47]. It will be interesting to test whether such a coupling of contact-dependent neighbor predation and DNA uptake fosters the transfer of longer DNA fragments, as heavy fragmentation might be prevented in this context. If this were the case, this mechanism could even contribute to the spread of MGEs instead of (or in addition to) leading

to the curing from MGEs.

Inactivation of natural competence by selfish mobile genetic elements

While natural competence has been predicted for more than 80 prokaryotic species [8], some of these bacteria, including the ‘lab pet’ bacterium *Escherichia coli*, have not yet been shown to naturally induce this physiological state. Moreover, loss of the ability to induce natural competence and/or to absorb extracellular DNA also frequently occurs and can mask natural transformability in bacterial species despite the presence of core competence proteins such as those that encode the DNA uptake machinery [7,48]. Such a loss of natural transformability can be based on the cessation of competence gene expression or the uncontrolled production of extracellular nucleases that degrade the transforming material. Both of these effects have been demonstrated in quorum-sensing (QS) mutants of *V. cholerae*, as the latter lack a functional QS regulator that serves both as a co-inducer of natural competence and as a repressor of an extracellular/periplasmic nuclease, Dns [49-51].

Notably, recent *V. cholerae* isolates from Haiti were found to be barely transformable, despite their QS proficiency [52]. Through analysis of such strains, Dalia *et al.* demonstrated that the cause of this lack of transformation is an MGE-encoded extracellular nuclease (IdeA) [53](Table 2). IdeA possesses 52% identity and 64% similarity to the QS-repressed indigenous nuclease Dns of *V. cholerae* and, accordingly, prevented transformation in the same manner as Dns [49,53]. In the Haitian isolates, the *ideA*-carrying MGE is the 97.7-kb ICE VchInd5, which was also frequently observed in *V. cholerae* isolates from Bangladeshi patients [53]. However, the prevalence of this ICE element in Bangladeshi isolates significantly dropped in recent years, rendering *V. cholerae* strains transformable once more. Interestingly, the first identified ICE of *V. cholerae*, SXT [54], also contains a nuclease-encoding ORF, with 93% identity and 96% similarity to IdeA (this study; see Table 2),

despite its overall different genetic organization compared to VchInd5 [55]. This further highlights the link between the ability of ICE elements to inhibit natural transformation in *V. cholerae* and might have contributed to the maintenance of the SXT element over many years [56]. In addition to the example of the ICE-encoded nuclease in *V. cholerae* [53], Croucher *et al.* highlighted, or *in silico* predicted, a plethora of other naturally competent bacteria, which are thought to have lost their transformability due to the integration of MGEs into key competence genes [5] (Table 2).

Conclusion

That the “ability to delete selfish mobile genetic elements is an important function of transformation” is a novel hypothesis put forward by Croucher and colleagues [5]. This idea will certainly foster new *in vitro* evolution experiments. Moreover, current developments that allow sequencing of whole genomes based on long reads will contribute to properly assembled bacterial genomes so that the location and number of MGEs can be unambiguously identified. Such an analysis will be required to confirm or refute the finding that naturally transformable *Streptococci* had significantly fewer prophages than non-transformable strains of the same genus [5]. While the main benefit of DNA uptake by naturally competent bacteria might very well be that absorbed DNA can serve as a nutrient source, for genome repair, or to acquire variant alleles, a (potentially unselected) side effect might indeed be the movement of larger genomic regions among competent bacteria. Answering the question whether natural transformation primarily contributes to the uptake and integration of large genomic segments or whether its primary role is to rescue the genome from selfish MGEs or both will be of prime importance to better address the spread of antibiotic resistances. Indeed, if natural transformation would allow efficient removal of antibiotic resistance clusters from naturally competent bacteria, competence induction might

eventually be considered for the treatment of otherwise-untreatable multi-drug resistant pathogens (if competence is maintained in the latter). More research is therefore required to elucidate the competence trigger of hospital-acquired pathogens, including those highlighted in Figure 2.

Acknowledgments

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References

1. Ochman H, Lawrence JG, Groisman EA: **Lateral gene transfer and the nature of bacterial innovation.** *Nature* 2000, **405**:299-304.
2. Kloesges T, Popa O, Martin W, Dagan T: **Networks of gene sharing among proteobacterial genomes reveal differences in lateral gene transfer frequency at different phylogenetic depths.** *Mol. Biol. Evol.* 2011, **28**:1057-1074.
3. Booth A, Mariscal C, Doolittle WF: **The Modern Synthesis in the Light of Microbial Genomics.** *Annu. Rev. Microbiol.* 2016, **70**:279-297.
4. McInerney JO, Pisani D, Baptiste E, O'Connell MJ: **The Public Goods Hypothesis for the evolution of life on Earth.** *Biol. Direct* 2011, **6**:41.
5. Croucher NJ, Mostowy R, Wymant C, Turner P, Bentley SD, Fraser C: **Horizontal DNA Transfer Mechanisms of Bacteria as Weapons of Intragenomic Conflict.** *PLoS Biol.* 2016, **14**:e1002394.
6. McInerney JO, Cotton JA, Pisani D: **The prokaryotic tree of life: past, present... and future?** *Trends Ecol. Evol.* 2008, **23**:276-281.
7. Chen I, Dubnau D: **DNA uptake during bacterial transformation.** *Nat. Rev. Microbiol.* 2004, **2**:241-249.
8. Johnston C, Martin B, Fichant G, Polard P, Claverys JP: **Bacterial transformation: distribution, shared mechanisms and divergent control.** *Nat. Rev. Microbiol.* 2014, **12**:181-196.
9. Redfield RJ: **Evolution of bacterial transformation: is sex with dead cells ever better than no sex at all?** *Genetics* 1988, **119**:213-221.
10. Touchon M, Bobay LM, Rocha EP: **The chromosomal accommodation and domestication of mobile genetic elements.** *Curr. Opin. Microbiol.* 2014, **22**:22-29.
11. Baltrus DA: **Exploring the costs of horizontal gene transfer.** *Trends Ecol. Evol.* 2013, **28**:489-495.

12. Vasu K, Nagaraja V: **Diverse functions of restriction-modification systems in addition to cellular defense.** *Microbiol. Mol. Biol. Rev.* 2013, **77**:53-72.
13. Marraffini LA: **CRISPR-Cas immunity in prokaryotes.** *Nature* 2015, **526**:55-61.
14. Singh K, Milstein JN, Navarre WW: **Xenogeneic Silencing and Its Impact on Bacterial Genomes.** *Annu. Rev. Microbiol.* 2016, **70**:199-213.
15. Seitz P, Blokesch M: **Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria.** *FEMS Microbiol. Rev.* 2013, **37**:336-363.
16. Mortier-Barriere I, Velten M, Dupaigne P, Mirouze N, Pietrement O, McGovern S, Fichant G, Martin B, Noirot P, Le Cam E, et al.: **A key presynaptic role in transformation for a widespread bacterial protein: DprA conveys incoming ssDNA to RecA.** *Cell* 2007, **130**:824-836.
17. Johnston C, Martin B, Granadel C, Polard P, Claverys JP: **Programmed protection of foreign DNA from restriction allows pathogenicity island exchange during pneumococcal transformation.** *PLoS Pathog.* 2013, **9**:e1003178.
18. Griffith F: **The significance of pneumococcal types.** *J. Hyg.* 1928, **27**:113-159.
19. Blokesch M: **Natural competence for transformation.** *Curr. Biol.* 2016, **26**:3255.
20. Croucher NJ, Harris SR, Fraser C, Quail MA, Burton J, van der Linden M, McGee L, von Gottberg A, Song JH, Ko KS, et al.: **Rapid pneumococcal evolution in response to clinical interventions.** *Science* 2011, **331**:430-434.
21. Wyres KL, Lamberts LM, Croucher NJ, McGee L, von Gottberg A, Linares J, Jacobs MR, Kristinsson KG, Beall BW, Klugman KP, et al.: **Pneumococcal capsular switching: a historical perspective.** *J Infect Dis* 2013, **207**:439-449.
22. Hall RH, Khambaty FM, Kothary M, Keasler SP: **Non-O1 *Vibrio cholerae*.** *Lancet* 1993, **342**:430.
23. Blokesch M, Schoolnik GK: **Serogroup conversion of *Vibrio cholerae* in aquatic reservoirs.** *PLoS Pathog.* 2007, **3**:e81.
24. Miller MC, Keymer DP, Avelar A, Boehm AB, Schoolnik GK: **Detection and transformation of genome segments that differ within a coastal population of *Vibrio cholerae* strains.** *Appl. Environ. Microbiol.* 2007, **73**:3695-3704.
25. Vorwerk H, Huber C, Mohr J, Bunk B, Bhujji S, Wensel O, Sproer C, Fruth A, Flieger A, Schmidt-Hohagen K, et al.: **A transferable plasticity region in *Campylobacter coli* allows isolates of an otherwise non-glycolytic food-borne pathogen to catabolize glucose.** *Mol. Microbiol.* 2015, **98**:809-830.
26. Mell JC, Shumilina S, Hall IM, Redfield RJ: **Transformation of natural genetic variation into *Haemophilus influenzae* genomes.** *PLoS Pathog.* 2011, **7**:e1002151.
27. Bubendorfer S, Krebs J, Yang I, Hage E, Schulz TF, Bahlawane C, Didelot X, Suerbaum S: **Genome-wide analysis of chromosomal import patterns after natural transformation of *Helicobacter pylori*.** *Nat Commun* 2016, **7**:11995.
28. Udden SMN, Zahid MSH, Biswas K, Ahmad QS, Cravioto A, Nair GB, Mekalanos JJ, Faruque SM: **Acquisition of classical CTX prophage from *Vibrio cholerae* O141 by El Tor strains aided by lytic phages and chitin-induced competence.** *Proc. Natl. Acad. Sci. USA* 2008, **105**:11951-11956.
29. Cannon B: **Microbiology: Resistance fighters.** *Nature* 2014, **509**:S6-8.
30. Rice LB: **Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE.** *J Infect Dis* 2008, **197**:1079-1081.
31. Centers for Disease Control and Prevention (CDC). **Biggest Threats.** http://www.cdc.gov/drugresistance/biggest_threats.html 2013.
32. WHO: **Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics.** News release by the WHO

- (<http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/>)
2017.
33. Domingues S, Harms K, Fricke WF, Johnsen PJ, da Silva GJ, Nielsen KM: **Natural transformation facilitates transfer of transposons, integrons and gene cassettes between bacterial species.** *PLoS Pathog.* 2012, **8**:e1002837.
 34. Saunders CW, Guild WR: **Pathway of plasmid transformation in *Pneumococcus*: open circular and linear molecules are active.** *J. Bacteriol.* 1981, **146**:517-526.
 35. Michod RE, Wojciechowski MF, Hoelzer MA: **DNA repair and the evolution of transformation in the bacterium *Bacillus subtilis*.** *Genetics* 1988, **118**:31-39.
 36. Claverys JP, Prudhomme M, Martin B: **Induction of competence regulons as a general response to stress in gram-positive bacteria.** *Annu. Rev. Microbiol.* 2006, **60**:451-475.
 37. Engelmoer DJ, Rozen DE: **Competence increases survival during stress in *Streptococcus pneumoniae*.** *Evolution* 2011, **65**:3475-3485.
 38. Ambur OH, Engelstadter J, Johnsen PJ, Miller EL, Rozen DE: **Steady at the wheel: conservative sex and the benefits of bacterial transformation.** *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 2016, **371**: 20150528.
 39. Redfield RJ: **Do bacteria have sex?** *Nat. Rev. Genet.* 2001, **2**:634-639.
 40. Prudhomme M, Attaiech L, Sanchez G, Martin B, Claverys JP: **Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*.** *Science* 2006, **313**:89-92.
 41. Slager J, Kjos M, Attaiech L, Veening JW: **Antibiotic-induced replication stress triggers bacterial competence by increasing gene dosage near the origin.** *Cell* 2014, **157**:395-406.
 42. Dorer MS, Fero J, Salama NR: **DNA damage triggers genetic exchange in *Helicobacter pylori*.** *PLoS Pathog.* 2010, **6**:e1001026.
 43. Charpentier X, Kay E, Schneider D, Shuman HA: **Antibiotics and UV Radiation Induce Competence for Natural Transformation in *Legionella pneumophila*.** *J. Bacteriol.* 2011, **193**:1114-1121.
 44. Claverys JP, Havarstein LS: **Cannibalism and fratricide: mechanisms and raisons d'etre.** *Nat. Rev. Microbiol.* 2007, **5**:219-229.
 45. Overballe-Petersen S, Harms K, Orlando LA, Mayar JV, Rasmussen S, Dahl TW, Rosing MT, Poole AM, Sicheritz-Ponten T, Brunak S, et al.: **Bacterial natural transformation by highly fragmented and damaged DNA.** *Proc. Natl. Acad. Sci. USA* 2013, **110**:19860-19865.
 46. Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D: **Release and persistence of extracellular DNA in the environment.** *Environ. Biosafety Res.* 2007, **6**:37-53.
 47. Borgeaud S, Metzger LC, Scrignari T, Blokesch M: **The type VI secretion system of *Vibrio cholerae* fosters horizontal gene transfer.** *Science* 2015, **347**:63-67.
 48. Matthey N, Blokesch M: **The DNA-Uptake Process of Naturally Competent *Vibrio cholerae*.** *Trends Microbiol.* 2016, **24**:98-110.
 49. Blokesch M, Schoolnik GK: **The extracellular nuclease Dns and its role in natural transformation of *Vibrio cholerae*.** *J. Bacteriol.* 2008, **190**:7232-7240.
 50. Lo Scrudato M, Blokesch M: **The regulatory network of natural competence and transformation of *Vibrio cholerae*.** *PLoS Genet.* 2012, **8**:e1002778.
 51. Seitz P, Blokesch M: **DNA transport across the outer and inner membranes of naturally transformable *Vibrio cholerae* is spatially but not temporally coupled.** *mBio* 2014, **5**:e01409-14.

52. Katz LS, Petkau A, Beaulaurier J, Tyler S, Antonova ES, Turnsek MA, Guo Y, Wang S, Paxinos EE, Orata F, et al.: **Evolutionary dynamics of *Vibrio cholerae* O1 following a single-source introduction to Haiti.** *MBio* 2013, **4**.
53. Dalia AB, Seed KD, Calderwood SB, Camilli A: **A globally distributed mobile genetic element inhibits natural transformation of *Vibrio cholerae*.** *Proc. Natl. Acad. Sci. USA* 2015, **112**:10485-10490.
54. Waldor MK, Tschäpe H, Mekalanos JJ: **A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in *Vibrio cholerae* O139.** *J. Bacteriol.* 1996, **178**:4157-4165.
55. Spagnoletti M, Ceccarelli D, Rieux A, Fondi M, Taviani E, Fani R, Colombo MM, Colwell RR, Balloux F: **Acquisition and evolution of SXT-R391 integrative conjugative elements in the seventh-pandemic *Vibrio cholerae* lineage.** *MBio* 2014, **5**.
56. Mutreja A, Kim DW, Thomson NR, Connor TR, Lee JH, Kariuki S, Croucher NJ, Choi SY, Harris SR, Lebens M, et al.: **Evidence for several waves of global transmission in the seventh cholera pandemic.** *Nature* 2011, **477**:462-465.
57. Rabinovich L, Sigal N, Borovok I, Nir-Paz R, Herskovits AA: **Prophage excision activates *Listeria* competence genes that promote phagosomal escape and virulence.** *Cell* 2012, **150**:792-802.
58. Renda BA, Chan C, Parent KN, Barrick JE: **Emergence of a Competence-Reducing Filamentous Phage from the Genome of *Acinetobacter baylyi* ADP1.** *J. Bacteriol.* 2016, **198**:3209-3219.
59. Beaber JW, Hochhut B, Waldor MK: **Genomic and functional analyses of SXT, an integrating antibiotic resistance gene transfer element derived from *Vibrio cholerae*.** *J. Bacteriol.* 2002, **184**:4259-4269.
60. Loessner MJ, Inman RB, Lauer P, Calendar R: **Complete nucleotide sequence, molecular analysis and genome structure of bacteriophage A118 of *Listeria monocytogenes*: implications for phage evolution.** *Mol. Microbiol.* 2000, **35**:324-340.
61. Borezee E, Msadek T, Durant L, Berche P: **Identification in *Listeria monocytogenes* of MecA, a homologue of the *Bacillus subtilis* competence regulatory protein.** *J. Bacteriol.* 2000, **182**:5931-5934.
62. Buchrieser C, Rusniok C, Kunst F, Cossart P, Glaser P, Listeria C: **Comparison of the genome sequences of *Listeria monocytogenes* and *Listeria innocua*: clues for evolution and pathogenicity.** *FEMS Immunol. Med. Microbiol.* 2003, **35**:207-213.
63. Jorth P, Whiteley M: **An evolutionary link between natural transformation and CRISPR adaptive immunity.** *MBio* 2012, **3**.
64. Liu F, Zhu Y, Yi Y, Lu N, Zhu B, Hu Y: **Comparative genomic analysis of *Acinetobacter baumannii* clinical isolates reveals extensive genomic variation and diverse antibiotic resistance determinants.** *BMC Genomics* 2014, **15**:1163.
65. Redfield RJ, Findlay WA, Bosse J, Kroll JS, Cameron AD, Nash JH: **Evolution of competence and DNA uptake specificity in the Pasteurellaceae.** *BMC Evol. Biol.* 2006, **6**:82.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

Annotations:

Croucher NJ, Mostowy R, Wymant C, Turner P, Bentley SD, Fraser C: **Horizontal DNA Transfer Mechanisms of Bacteria as Weapons of Intragenomic Conflict**. *PLoS Biol.* 2016, **14**:e1002394.

** This outstanding study poses a new hypothesis, namely, that an important function of transformation is to delete parasitic genetic elements.

Baltrus DA: **Exploring the costs of horizontal gene transfer**. *Trends Ecol. Evol.* 2013, **28**:489-495.

** An excellent and comprehensive review of the identification of HGT-mediated fitness costs and how they could interfere with the evolutionary potential of HGT.

Johnston C, Martin B, Granadel C, Polard P, Claverys JP: **Programmed protection of foreign DNA from restriction allows pathogenicity island exchange during pneumococcal transformation**. *PLoS Pathog.* 2013, **9**:e1003178.

** This work provides convincing evidence that the competence-induced methylase DpnA of *Streptococcus pneumoniae* plays an important role in protecting incoming transforming DNA from degradation. The authors also show that such protection is essential for the horizontal spread of large genomic islands (e.g., >20 kb).

Domingues S, Harms K, Fricke WF, Johnsen PJ, da Silva GJ, Nielsen KM: **Natural transformation facilitates transfer of transposons, integrons and gene cassettes between bacterial species**. *PLoS Pathog.* 2012, **8**:e1002837.

* This study shows that naturally transformable *Acinetobacter baylyi* can acquire antibiotic resistance traits from integron-carrying strains of other bacterial species. The integration of the incoming DNA occurred via transposition and homologous and heterologous recombination. The authors concluded that natural transformation facilitates transfer of genetic elements between bacterial species.

Slager J, Kjos M, Attaiech L, Veening JW: **Antibiotic-induced replication stress triggers bacterial competence by increasing gene dosage near the origin**. *Cell* 2014, **157**:395-406.

* In this study, the authors demonstrate that DNA replication-targeting antibiotics induce natural competence in *Streptococcus pneumoniae* due to increased copy number of origin-proximal competence genes.

Overballe-Petersen S, Harms K, Orlando LA, Mayar JV, Rasmussen S, Dahl TW, Rosing MT, Poole AM, Sicheritz-Ponten T, Brunak S, et al.: **Bacterial natural transformation by highly fragmented and damaged DNA**. *Proc. Natl. Acad. Sci. USA* 2013, **110**:19860-19865.

* The authors described the ability of naturally competent *Acinetobacter baylyi* to acquire and incorporate fragmented DNA stretches, including ancient DNA from a 43,000-year-old woolly mammoth bone.

Borgeaud S, Metzger LC, Scrignari T, Blokesch M: **The type VI secretion system of *Vibrio cholerae* fosters horizontal gene transfer.** *Science* 2015, **347**:63-67.

** In this study, the authors revealed that naturally competent *Vibrio cholerae* increases the pool of transforming DNA through kin-discriminated neighbor predation. The concomitant production of the molecular killing device (a type VI secretion system) and the competence-induced DNA-uptake machinery fosters the direct transfer of genetic material.

Dalia AB, Seed KD, Calderwood SB, Camilli A: **A globally distributed mobile genetic element inhibits natural transformation of *Vibrio cholerae*.** *Proc. Natl. Acad. Sci. USA* 2015, **112**:10485-10490.

** In this study, the authors determined why current Haitian isolates of *V. cholerae* are non-transformable. This non-transformability is caused by the production of an extracellular/periplasmic nuclease, which is encoded on a mobile genetic element.

502 **Table 1. Examples of transformation-mediated transfers of larger DNA regions.**

Bacterial species	Transferred DNA region (size)	Reference
<i>Streptococcus pneumoniae</i>	Capsule-encoding genomic region (size not determined at that time)	[18]
<i>Vibrio cholerae</i>	O-antigen cluster (>42kb)	[23]
<i>Vibrio cholerae</i>	Regions encoding metabolic operons (max 44.9 kb; 22.7 kb on average)	[24]*
<i>Vibrio cholerae</i>	Cholera toxin prophage (~7 kb)	[28]
<i>Haemophilus influenzae</i>	Different regions of chromosome (mean length ~8.1 kb)	[26]*
<i>Streptococcus pneumoniae</i>	Capsule-encoding genomic region (>21kb)	[17]*
<i>Campylobacter coli</i>	Glucose catabolism locus <i>glc</i> (>8.7kb)	[25]*
<i>Helicobacter pylori</i>	Different regions of chromosome (max. ~13kb)	[27]*

* Purified genomic DNA served as transforming material.

505 **Table 2. Competence and transformation inhibitory mechanism of MGEs.[#]**

Species	Type of MGE	Mechanism of inhibition	Reference
<i>Experimentally demonstrated</i>			
<i>Vibrio cholerae</i> (O1 El Tor)	ICE (VchInd5)	Production of nuclease (<i>ideA</i>)	[53]
<i>Listeria monocytogenes</i> (10403S)	Prophage (Φ10403S)	Interruption of gene encoding competence master regulator (<i>comK</i>)	[57]
<i>Acinetobacter baylyi</i> ADP1	Prophage (CRAΦ)	Reactivated phage reduces competency	[58]
<i>In silico predicted</i>			
<i>Vibrio cholerae</i> (O139; strain MO10)	ICE	Production of nuclease (MO10_03105 / s62 gene [59])	This study
<i>Listeria monocytogenes</i>	Prophage (A118)	Interruption of gene encoding competence master regulator (<i>comK</i>)	[60]
<i>Listeria monocytogenes</i> (LO28)	Prophage	Interruption of gene encoding competence master regulator (<i>comK</i>)	[61]
<i>Listeria innocua</i> (Clip11262)	Prophage	Interruption of gene encoding competence master regulator (<i>comK</i>)	[62]
<i>Aggregatibacter actinomycetemcomitans</i> (HK1651)	MGE	Interruption of competence gene (<i>comM</i>)	[63]
<i>Acinetobacter baumannii</i>	MGE (AbaR-like resistance islands or GI _{BJ4})	Interruption of competence gene (<i>comM</i>)	[64]
<i>Mannheimia succiniciproducens</i>	MGE (17kb in size)	Interruption of competence gene (<i>comM</i>)	[65]
<i>Streptococcus pneumoniae</i> (670-6B)	Prophage	Interruption of gene encoding structural competent of DNA-uptake pilus (<i>comYC</i>)	[5]
<i>Streptococcus mutans</i> (NLML9)	Prophage	Interruption of gene encoding structural competent of DNA-uptake pilus (<i>comYC</i>)	
<i>Streptococcus parauberis</i> (KRS-02083)	Prophage	Interruption of gene encoding structural competent of DNA-uptake pilus (<i>comYC</i>)	
<i>Lactococcus lactis</i> (KLDS 4.0325)	Prophage	Interruption of gene encoding structural competent of DNA-uptake pilus (<i>comYC</i>)	
<i>Streptococcus equi</i>	Prophage (φSeq3)	Interruption of late competence gene (<i>comFA</i>)	

<i>Streptococcus suis</i> (89–590)	Prophage	Interruption of competence gene (<i>comFA</i>)	
<i>Bacillus cereus</i> (VD214)	Prophage	Interruption of competence gene (<i>comFA</i>)	
<i>Listeria monocytogenes</i> (10403S)	Prophage	Interruption of gene encoding competence master regulator (<i>comK</i>)	
<i>Bacillus cereus</i> (VD214)	Prophage	Interruption of competence gene (<i>comFA</i>)	
<i>Bacillus thuringiensis</i> (serovar tolworthi)	Prophage	Interruption of competence gene (<i>comFA</i>)	
<i>Enterococcus faecalis</i> (RMC65)	Prophage	Interruption of competence co-regulated gene (<i>radC</i>)	
<i>Acinetobacter baumannii</i> (1598530)	MGE	Interruption of competence gene (<i>comM</i>)	
<i>Mannheimia haemolytica</i> (USMARC-185)	MGE	Interruption of gene encoding an ortholog of a competence protein (<i>comM</i>)	
<i>Francisella philomiragia</i> (FAJ)	MGE	Interruption of gene encoding an ortholog of a competence protein (<i>comM</i>)	
<i>Pseudomonas syringae</i> (BRIP34881)	MGE	Interruption of gene encoding an ortholog of a competence protein (<i>comM</i>)	

506 # Information mostly derived from ref [5]

Figure legends

Fig. 1. Natural transformation of bacterial cells through the integration of foreign

DNA leads to genome maintenance and evolution. Naturally competent bacteria can integrate transforming DNA into their genome, thereby repairing damaged DNA (red spike) (1) or deleting MGEs (shown by a stretch of red arrows) (2). Both processes contribute to genome maintenance. Alternatively, incoming DNA can encode new genetic information and replace indigenous genes (3) or insert genomic islands (shown by a stretch of green arrows) (4) into the recipient genome, thereby fostering evolution.

Fig. 2. Correlation between drug resistance and natural transformability. Top 18 drug-resistant threats to the United States according to the CDC (**a**) and priority list of antibiotic-resistant pathogens according to the WHO (**B**). Names appearing in bold mark species with at least some conserved competence genes (e.g., core genes encoding parts of the DNA-uptake complex). Underlined names indicate species for which natural competence and transformation have been experimentally demonstrated. Schemes adapted from the CDC and WHO webpages [31,32].

Figure 1

Figure 2

(a)

URGENT	<i>Clostridium difficile</i> Carbapenem-resistant Enterobacteriaceae (CRE) Drug-resistant <i>Neisseria gonorrhoeae</i>
SERIOUS	<u>Multidrug-resistant <i>Acinetobacter</i></u> <u>Drug-resistant <i>Campylobacter</i></u> Fluconazole-resistant <i>Candida</i> (fungus) Extended spectrum β -lactamase producing Enterobacteriaceae (ESBLs) Vancomycin-resistant Enterococcus (VRE) Multidrug-resistant <i>Pseudomonas aeruginosa</i> Drug-resistant Non-typhoidal <i>Salmonella</i> Drug-resistant <i>Salmonella</i> Typhi Drug-resistant <i>Shigella</i> <u>Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)</u> <u>Drug-resistant <i>Streptococcus pneumoniae</i></u> Drug-resistant tuberculosis (MDR and XDR)
CONCERNING	<u>Vancomycin-resistant <i>Staphylococcus aureus</i> (VRSA)</u> Erythromycin-resistant <i>Streptococcus</i> Group A Clindamycin-resistant <i>Streptococcus</i> Group B

(b)

CRITICAL	<u><i>Acinetobacter baumannii</i></u> , carbapenem-resistant <i>Pseudomonas aeruginosa</i> , carbapenem-resistant <i>Enterobacteriaceae</i> , carbapenem-resist, 3 rd generation cephalosporin-resistant
HIGH	<i>Enterococcus faecium</i> , vancomycin-resistant <i>Staphylococcus aureus</i> , methicillin-resistant, vancomycin intermediate and resistant <u><i>Helicobacter pylori</i></u> , clarithromycin-resistant <u><i>Campylobacter</i></u> , fluoroquinolone-resistant <i>Salmonella</i> spp., fluoroquinolone-resistant <i>Neisseria gonorrhoeae</i> , 3 rd generation cephalosporin-resistant, fluoroquinolone-resistant
MEDIUM	<u><i>Streptococcus pneumoniae</i></u> , penicillin-non-susceptible <i>Haemophilus influenzae</i> , ampicillin-resistant <i>Shigella</i> spp., fluoroquinolone-resistant

Graphical abstract