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REVIEW

Silently transformable: the many ways bacteria conceal their built-in capacity of genetic exchange

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Abstract Bacteria can undergo genetic transformation by actively integrating genetic information from phylogenetically related or unrelated organisms. The original function of natural transformation remains a subject of debate, but it is well established as a major player in genome evolution. Naturally transformable bacteria use a highly conserved DNA uptake system to internalize DNA and integrate it in their chromosome by homologous recombination. Expression of the DNA uptake system, often referred to as competence, is tightly controlled and induced by signals that are often elusive. Initially thought to be restricted to a few bacterial species, natural transformation increasingly seems widespread in bacteria. Yet, the triggering signals and regulatory mechanisms involved appear diverse and are understood only in a limited set of species. As a result, natural transformation in most bacterial species remains poorly documented and the potential impact of this mechanism on global genetic mobilization is likely underappreciated. Indeed, even when a conserved activator can be identified to artificially induce the expression of the DNA uptake system, the considered species may still remain non-transformable. Recent works indicate that the DNA uptake system is directly subjected to silencing. At least in *Legionella pneumophila* and possibly in other species, a small non-coding RNA prevents expression of the DNA uptake system. Silencing constitutes one more way bacteria control expression of their engine of genetic exchange. It may also be the underlying reason of the undetectable natural transformation of many bacterial species grown under laboratory conditions even though they possess a DNA uptake system.

Keywords HGT · Gene regulation · sRNA · Silencing · Competence

Horizontal gene transfer plays a dominant role in the evolution of prokaryotic organisms (Gogarten and Townsend 2005; Koonin 2016). In bacteria, there are currently three recognized types of horizontal gene transfer (HGT) mechanisms: conjugation, transduction and transformation (Arber 2014). Conjugation and phage transduction involve plasmids, phages or phage-like particles which can be considered selfish replicative elements. HGT events resulting from conjugation and transduction can be seen as a side effect of the propagative behavior of these replicative and parasitic elements. In contrast, natural transformation does not rely on external vehicles or extra-chromosomal elements and is inherent to the species (Johnston et al. 2014). Recent work even suggests that bacteria use this process to rid their chromosome of the "genetic parasites" driving conjugation and transduction (Croucher et al. 2016). Natural transformation is an evolutionarily conserved mechanism, detected in over 80 bacterial species distributed throughout the bacterial domain of the tree of life (Johnston et al. 2014). To undergo transformation, bacteria must become "competent". In this particular physiological state, they express a number of proteins that allow them to internalize exogenous DNA and integrate in their chromosome

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by homologous recombination resulting in permanent acquisition of the DNA sequence and of its features (Johnsborg et al. 2007).

It is commonly accepted that active acquisition of genetic content released by phylogenetically close organisms produce genetic polymorphism and can promote rapid adaptation (Smith et al. 1991). This is well exemplified by the rapid adaptation of *Streptococcus pneumoniae*—the historical model of natural transformation—to antibiotic treatment and its evasion to vaccine strategies (Croucher et al. 2011). However, we see genetic changes resulting from natural transformation through the lens of selection. So our view may be biased toward the observation of HGT events that are ultimately beneficial (or eventually neutral) to their recipients. Yet, all transformation events may not be beneficial and may even be deleterious. Indeed, evidence suggests that genetic transformation can also occur with distantly related and even damaged DNA (Anderson and Seifert 2011; Overballe-Petersen et al. 2013). So depending on the bacteria's biotope, constantly and randomly acquiring genetic material could be as harmful as it could be beneficial, and possibly for this reason competence development is a strictly regulated process in most transformable species (Seitz and Blokesch 2013; Johnston et al. 2014).

Depending on the species, entry into the competence state can be induced or influenced by specific environmental signals, the growth phase and quorum sensing. Until recently, the different regulatory pathways involved in competence development always implicated the production and/or activation of a protein that would allow or enhance the expression of the genes encoding the proteins needed for transformation. These known mechanisms of competence regulation were reviewed quite extensively in Seitz and Blokesch (2013) and Johnston et al. (2014) for Gram-negative and Gram-positive bacteria, respectively. Both reviews showed that, despite the limited understanding of how exactly all the environmental and internal cues that can trigger competence are integrated by the cells, the regulatory pathways all share the presence of a central activator. In the Gram-positive *S. pneumoniae* and *Staphylococcus aureus*, an alternative sigma factor (σ^X and σ^H, respectively) is specifically involved in the transcription of the competence genes (Lee and Morrison 1999; Morikawa et al. 2012). On the other hand, a transcription activator is used in *Bacillus subtilis* (ComK) (Mohan and Dubnau 1990). The transcription co-regulator Sxy in the Gram-negative *Haemophilus influenzae*, and its ortholog TfoX in *Vibrio cholerae*, act as activating cofactors of CRP (cAMP Receptor Protein) to allow its preferential binding on the promoters of competence genes and their transcriptional activation (Macfadyen 2000; Redfield et al. 2005; Blokesch 2012). Furthermore, the central regulator of competence in *V. cholerae* is actually bipartite as the transcription activator QstR also acts directly on a subset of genes essential for transformation (Lo Scrudato and Blokesch 2013).

Thus, except for the presence of a central activator, it seems that the regulatory pathways leading to the development of competence are quite diverse. By contrast, the genes belonging to the competence regulon, and in particular those encoding the DNA uptake system, are widespread and highly conserved, suggesting that most bacteria could be naturally transformable (Claverys and Martin 2003; Johnsborg et al. 2007). Moreover, a great number of bacterial species, including important human and plant pathogens, are known to be naturally transformable under specific conditions and yet lack the canonical competence regulatory systems (e.g., *Helicobacter pylori*, *Campylobacter jejuni*, *Acinetobacter baumannii*, *Ralstonia solanacearum*, *Agrobacterium tumefaciens*) (Johnston et al. 2014). This is also the case for *Legionella pneumophila*, a human pathogen whose evolution is driven by extensive recombination (Gomez-Valero et al. 2011; Sánchez-Busó et al. 2014; David et al. 2016). Although the mechanisms behind such recombination remain elusive, it may be linked to the natural transformability of *L. pneumophila* which was only revealed over two decades after this species was identified (Stone and Kwaik 1999). Initially found to develop under microaerophilic conditions, competence is now clearly detectable when *L. pneumophila* isolates are grown at sub-optimal growth temperature (30 °C) (Buchrieser and Charpentier 2013). Even under these conditions, competence is transient and occurs at the transition from exponential growth to stationary phase. This represents a good example of regulated competence in the absence of the activators previously identified in the historical models of natural transformation.

Two studies revealed that competence in *L. pneumophila* was repressed, directly or indirectly, by three different proteins bearing no similarities to known competence regulators (Sexton and Vogel 2004; Charpentier et al. 2008). We recently showed how one of these repressors directly controls competence development by enabling the post-transcriptional silencing of genes encoding the DNA uptake system (Attaiech et al. 2016). Our experiments indicate that the protein RocC, which carries a FinO/ProQ domain, is an RNA chaperone that performs a function similar to Hfq. In complex with the trans-acting sRNA RocR, they target the 5′ untranslated region (5′UTR) of the mRNAs of the genes encoding the DNA uptake system (*comEA*, *comEC*, *comF*, *comM*), presumably blocking their translation and provoking their degradation (Fig. 1a). Both components are required for this silencing, as the loss of either RocC or RocR is sufficient to cause constitutive competence. Most importantly, a programmed change in expression of RocC triggers competence development when *L. pneumophila* is grown under competence-inducing conditions. By a yet

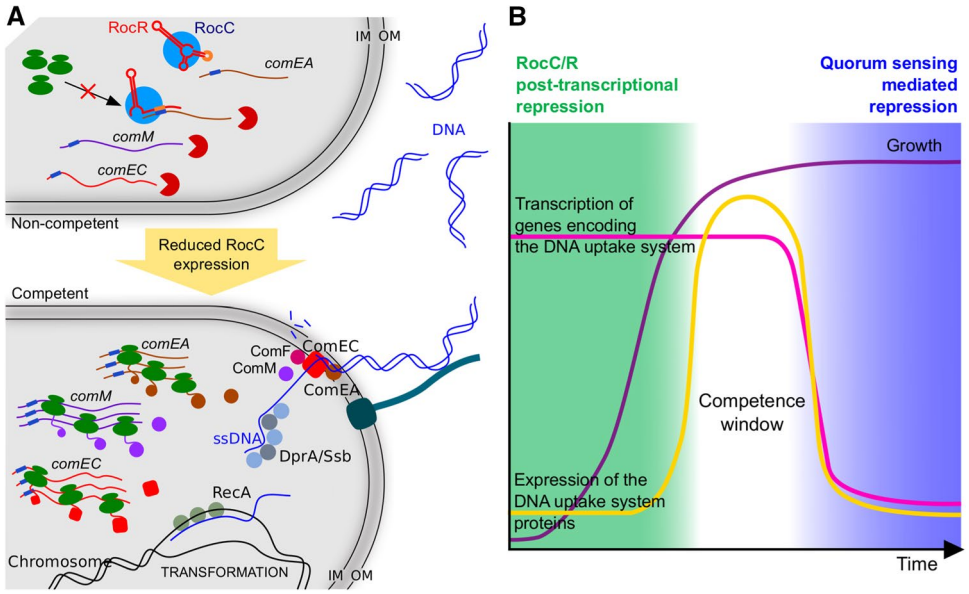

Fig. 1 Competence regulation in *Legionella pneumophila*. **a** Model of the regulated silencing of natural transformation by the RocC/R system during competence development at 30 °C. In the exponential growth phase, RocC stabilizes RocR and the resulting ribonucleoprotein complex can bind the mRNAs of genes of the competence regulon via a conserved 6-nt sequence called RocR box and promote their degradation. At the end of the exponential phase, RocC expression decreases. This triggers the destabilization of RocR and thus the stabilization of the mRNAs of the competence genes. These transcripts are then translated, the DNA uptake system is assembled, and horizontal gene transfer by natural genetic transformation can occur. *IM* inner membrane, *OM* outer membrane. **b** Model of the dual repression of competence during growth of *L. pneumophila* Paris strain at 30 °C. In the exponential phase, genes encoding the DNA uptake system are actively transcribed, but the RocC/R system prevents translation and the mRNAs are destabilized. When RocC/R expression levels decrease, the DNA uptake system is no longer silenced and transformation can occur. When the culture reaches the stationary phase, quorum sensing, via the *lqs* system, represses expression of the DNA uptake system, probably at the transcriptional level. The difference in the timing of the two repression systems allows expression of the DNA uptake system only during a short period, defining a "competence window"

unknown mechanism, RocC becomes less expressed at the end of the exponential phase which results in the decreased expression of RocR, as its stability depends on the presence of the RocC RNA chaperone. This unleashes translation of the mRNAs encoding the proteins of the DNA uptake system. Now protected by the translating ribosomes, these mRNAs are more stable and accumulate, further increasing the expression of the DNA uptake system and allowing transformation (Fig. 1a). Upon entry into the stationary phase, competence is again repressed but not by the RocC/R system, since expression of both components remains low. Competence in stationary phase was shown to be repressed, presumably at the transcriptional level, by the *lqs* quorum sensing system (Kessler et al. 2013). We propose that the transient nature of the competent state in *L. pneumophila* results from two independent repression systems operating at the post-transcriptional level in the exponential phase and at the transcriptional level in post-exponential phase (Fig. 1b). Interestingly, mutants of *rocC* or *rocR* remain constitutively competent in stationary phase, indicating that, in the total absence of this silencing system, the repression activity of the *lqs* system is not strong enough to completely shut down expression of the DNA uptake system. Thus, silencing seems to represent the dominant control of the DNA uptake system with RocC/R acting as the master repressor of competence development in *L. pneumophila*.

The discovery that the DNA uptake system is subjected to silencing in *L. pneumophila* sheds new light on the regulatory mechanisms of competence. Silencing of the DNA uptake system may be at play in other species, and this regulatory layer is not incompatible with the presence of transcriptional activators. In the human pathogen *Listeria monocytogenes*, the DNA uptake system is under the control of the transcriptional activator ComK also found in *B. subtilis* and also involves the alternative sigma factor σ^H (Rabinovich et al. 2012; Liu et al. 2016; Medrano Romero and Morikawa 2016). Despite the ComK and σ^H-dependent increased transcriptional levels of genes encoding the DNA uptake system, *L. monocytogenes* remains refractory to natural transformation, suggesting that the DNA uptake system is not fully operational (Rabinovich et al. 2012; Medrano Romero and Morikawa 2016). Interestingly, a bioinformatics analysis of *L. monocytogenes* genome revealed RliE, an sRNA antisense to *comC* (Mandin et al. 2007). In vitro interaction experiments showed that RliE could not

only bind the 5′UTR of the *comC* mRNA, but also that of *comEA-EB-EC*, *comFA-FC* and *lmo0945* mRNAs (Mandin et al. 2007). These genes encode proteins homologous to proteins participating in the DNA uptake system of *B. subtilis*, so RliE could potentially be implicated in competence regulation. If so, RliE may require an RNA chaperone distinct from the *Legionella* RocC which belongs to a family of domain carrying a FinO/ProQ domain not found in Firmicutes (Attaiech et al. 2016). It is tempting to speculate that, even when ComK and σ^H are activating the transcription of the DNA uptake system encoding genes, RliE may still be blocking their expression at the post-transcriptional level, hence repressing transformability.

Generally, attempts at revealing natural transformation in species presumed non-transformable by expression of the required transcriptional activators have had limited success. For instance, synthetic expression of *B. subtilis* ComK in *Bacillus cereus* (Mirończuk et al. 2008), or overexpression of TfoX in *Aggregatibacter actinomycetemcomitans* (Bhattacharjee et al. 2007) were sufficient to induce competence and transformants were readily obtained. However, most of the times, these strategies fail to induce transformability even though they allow for the transcriptional activation of the DNA uptake system encoding genes. Overexpression of the alternative sigma factor σ^X in *Lactococcus lactis* (Wydau et al. 2006) or in *Streptococcus pyogenes* (Woodbury et al. 2006), or σ^H in *Lactobacillus sakei* (Schmid et al. 2012) or the overexpression of the transcriptional regulator Sxy/TfoX in *Escherichia coli* (Sinha et al. 2009; Sinha and Redfield 2012) all led to a strong induction of several competence genes orthologs, but failed to render the strain transformable. A possible explanation for the absence of detectable transformation is that competence is often evidenced by an increased expression of the DNA uptake system at the transcriptional level. Yet, a silencing mechanism may still be operational, preventing translation of essential components of the DNA uptake system. The presence of sRNA targeting the 5′UTR of *comEA*/*comEC* in distantly related species such as *L. pneumophila* and *L. monocytogenes* suggests that silencing of the natural transformation could be a conserved strategy. Thus, the possibility that the DNA uptake system is generally subjected to sRNA-based silencing should be broadly investigated. We encourage our colleagues to explore the possibility that this overlooked mechanism could be responsible for an unexplained absence of transformation in their model, despite the presence of DNA uptake systems.

The variability of mechanisms involved in competence regulation attest to the plasticity of means by which bacteria regulate gene expression and cope with the necessity to adapt to the multiple environments. Regardless of the different molecular strategies, the end result of competence development is to allow bacteria to acquire extracellular DNA which contributes to their genetic plasticity and is thought to play a central role in their adaptation to their environment. Decades of work on the models of natural transformation have led to an impressive level of understanding of the complex regulation of competence in a few species. Importantly, this helped understand how these species turn on competence when faced with changing and stressful environments such as exposure to antibiotics (Stevens et al. 2011; Slager et al. 2014) and how this impacts their virulence (Blokesch 2015; Lin et al. 2016). This also allowed the design of strategies to prevent natural transformation from occurring during infection, so as to hinder the acquisition of antibiotic resistance (Zhu and Lau 2011). As we come to realize how widespread natural transformation is in bacteria (and in pathogens), it is also clear that we are just beginning to apprehend the variety of ways by which they control their ability to actively acquire genetic material. Deciphering the signaling pathways used to trigger competence is a worthy challenge that, like in *L. pneumophila*, may reveal unsuspected regulatory mechanisms.

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