CHAPTER 7

Summary and general discussion

Parts of this chapter were published as part of a review:

J.S. wrote the review.
The added value of PneumoBrowse and PneumoExpress

*De novo* assembly of *S. pneumoniae* D39V

The project described in Chapter 2, leading to the development of PneumoBrowse, started off with the *de novo* genome assembly of the pneumococcal strain used in our laboratory (D39V). While we expected to see some minor changes (SNPs and indels) in D39V relative to the previously published D39W [1], we were surprised to see several more severe differences. Firstly, we realized that D39V lacks the cryptic plasmid pDP1, found in D39W. Secondly, thanks to the power of long-read sequencing (PacBio), we identified a 162 kb inversion containing the *ter* region and observed a repeat expansion in *pavB* (implications are discussed below). Both of these regions are characterized by the presence of repeat sequences and it is not clear whether these loci in D39W are actually different from those in D39V or that the D39W genome was mis-assembled. However, it is clear that the *ter* region can actually be inverted and that *pavB* does really display a varying number of repeats in different pneumococcal strains (Chapter 2).

PneumoBrowse – The information hub

The number of sequenced genomes with accompanying annotations is increasing very rapidly and will probably continue to do so in the future, as sequencing costs drop further and accuracy keeps improving. While this is a highly valuable development for genomic research, there is also a downside. Instead of simply depositing the sequence information to an appropriate database (e.g. NCBI or EBI), researchers also commonly include an automatically generated genome annotation. Although understandable, there are a few problems with this habit. Firstly, there are countless different tools available to perform automated annotation, making the collection of genome annotations highly inconsistent. Secondly, none of these tools are perfect or up-to-date with current literature. This wild-growth of genome annotations has led the National Center for Biotechnology Information (NCBI) to introduce their Prokaryotic Genome Annotation Pipeline (PGAP) [2] for standardization of the annotations contained in their databases. Although this initiative has certainly improved consistency and therefore facilitated comparisons between strains and organisms, there is no reason to assume that reliability has also been improved. Genes that are wrongly annotated by this specific tool, will now be consistently wrong throughout the database. In our opinion, manual curation is absolutely essential in the pursuit of a full and correct annotation of a specific genome. And while PneumoBrowse (Chapter 2) does not represent the first manually curated pneumococcal genome annotation [3], it does contain a higher level of detail than any bacterial genome annotation we are aware of, including transcript boundaries, transcription regulatory sequences and putative non-coding RNAs. To detect these non-coding RNAs, a novel approach was used, based on fragment size analysis of paired-end
sequenced RNA libraries.

Especially in light of the explosive growth of genome databases, it is challenging to present data in a clear and accessible way. Therefore, we chose to use a visually pleasing, user-friendly genome browser [4] as a platform, rather than just depositing the annotation file to one of the aforementioned databases. The current version of PneumoBrowse represents a starting point and continuous curation is needed to make sure new findings are integrated. Additionally, feedback from the community is important for the identification of potential issues with the current annotation.

Unfortunately, the time needed for proper curation is orders of magnitude larger than the time it takes to sequence a bacterial genome. Therefore, initiatives like PGAP represent a reasonable compromise, but annotations produced by such tools should be regarded as highly preliminary and manually curated annotations take strong precedence.

**Considerations in transcriptome analysis**

The detailed annotation of D39V allows for a more complete analysis of the pneumococcal transcriptome (Chapters 3, 4). Firstly, the expression levels of newly identified features, such as several small non-coding RNAs (sRNAs), can now be studied. Additionally, the interpretation of RNA-seq data largely depends on the available knowledge on the nature and function of gene products. Although the pneumococcal genome still encodes many proteins with unknown function (Chapter 2), this number will continue to decrease as scientists from all over the world bundle their forces to get as full an understanding as possible of this important human pathogen.

An important step forward in prokaryotic transcriptome analysis is our ability to incorporate operon information into the interpretation of observed expression changes (Chapters 2, 3, 4). Most transcriptome studies treat each gene as a separate entity that can be differentially expressed independently of other genes. The presence of global transcriptional regulators and polycistronic transcripts render this an oversimplified interpretation, albeit the best approach in the absence of knowledge on operon structures and regulatory sequence elements. However, the arrival of novel technology has made it possible to obtain a detailed overview of transcript boundaries: transcription start sites and terminators can be determined by techniques such as Cappable-seq [5] and the utilization of paired-end sequencing (Chapter 2), respectively. This knowledge can help identifying which promoter regions are most likely to have an altered activity in the studied conditions, explaining the differential expression of individual genes (Chapters 3, 4). Additionally, precise determination of transcription start sites facilitates the identification of regulatory motifs, such as transcription factor binding sites and RNA switches (Chapters 2, 3, 4). Finally, gene enrichment analysis (GEA) is a common approach to identify
whether specific groups of genes (e.g. metabolic pathways or protein classes) are overrepresented among differentially expressed genes. For want of a better alternative, we, too, used this technique (Chapters 3, 4, 5, 6), but are fully aware of its limitations. The statistics that form the foundation of GEA depend on the assumption that genes behave independently of one another, with the exception of the gene cluster one is testing for. For example, when 10 genes implicated in translation are all upregulated during competence (Chapter 4), typical GEA methods cannot distinguish between the scenario where all 10 genes are part of the same operon (Chapter 4) and the (in this case hypothetical) scenario where each gene is transcribed from a different promoter. It is clear that these two scenarios are not nearly equivalent and new statistical methods are needed to incorporate operon information into GEA tools.

Finally, even when incorporating operon and regulon information, one should be aware of the possibility that the simultaneous differential expression of two genes can also arise through other mechanisms, e.g. involving chromosomal gene order, spatial proximity or differential mRNA degradation (see Chapter 1; [6]). An important example of such a mechanism is the upregulation of oriC-proximal genes in response to DNA replication stress, leading to the activation of competence in S. pneumoniae (Chapter 5).

**PneumoExpress – the hypothesis-generating machine**

In Chapter 3, we presented transcriptome data from 22 infection-relevant conditions. Key parameters (e.g. temperature, carbon source or contact with host cells) were changed to mimic transitions the pneumococcus may undergo within the host or between hosts. While these data are by no means expected to perfectly reflect pneumococcal gene expression levels in the natural habitat, they provide a wealth of qualitative information on the transcriptomic effect that a shift in environmental conditions may have. Furthermore, the diverse nature of the selected conditions (e.g. rich vs. poor medium, competent vs. non-competent cells) allows for a quantitative analysis of correlated gene expression, presented in the form of a co-expression matrix. When the expression of two genes is strongly correlated throughout the studied conditions, there is a good chance that they share a regulatory connection. Importantly, the co-expression matrix cannot provide information on the nature of this connection, but rather is a powerful hypothesis-generating machine. In many cases, the relationship seems clear (e.g. members of the same operon or regulon), but subsequent experiments will have to be performed to investigate the validity of the generated hypotheses.

Several examples were shown in Chapters 2, 3 and 4 that demonstrate the power of PneumoExpress, including the analysis of PyrR-regulated RNA switches (Chapter 2) and the identification of new competence gene briC (Chapters 3, 4; [7]).
Triggering pneumococcal competence

The competence regulon

Competence for genetic transformation in *S. pneumoniae* has been extensively studied for decades and the core regulatory system responsible for its activation and shutdown is rather well-understood. In Chapter 4, we updated the competence regulon. To this end, we built on information from excellent microarray-based studies from nearly 15 years ago [8,9]. We could refine the previously reported regulon using Illumina sequencing data from PneumoExpress (Chapter 3), mapped on the up-to-date D39V genome presented in Chapter 2. Combined with a promoter-based (rather than gene-based) approach to transcriptome analysis (Chapters 2, 4), this gave a completer and more nuanced overview of the competence regulon. Among the regulated genes, we found several sRNAs with unknown function and future experimental work is required to assess the relevance of these novel elements. Furthermore, we confirmed (and refined) several stress response regulons that were previously reported to be activated during competence, including the CiaR and VraR (LiaR) regulons [10,11]. The implications of these are discussed later.

Competence and pneumococcal genome plasticity

In 2006, Prudhomme et al. reported that a variety of antimicrobial compounds could trigger pneumococcal competence [12]. In light of the rapid spread of antibiotic resistance, this was recognized as a troubling phenomenon, where sub-lethal doses of antibiotics could enhance the genomic plasticity of the pneumococcus. Afterwards, Croucher et al. showed in a series of papers (2011–2017) that clinical interventions, over the years, have indeed led to severe changes in the pneumococcal genome [13–15]. Firstly, transformation with exogenous DNA could lead to the acquisition of antibiotic resistance genes (e.g. encoding drug efflux pumps) or to gene modification, where homology-based recombination results in the substitution of one or more bases. For example, a single nucleotide polymorphism (SNP) in *gyrA* or in an analogous region of *parC* confers resistance to fluoroquinolone antibiotics [13,16,17], which are known to induce competence (Chapter 5; [12]). Gram-positive bacteria are more benefited by and show stronger selection for the SNP in *parC*, compared to the one in *gyrA*. Interestingly, however, Gram-negative bacteria display the opposite trend [18] and therefore both resistance alleles may be readily available in the host environment. Due to the resemblance between the relevant regions of these two genes, intragenomic recombination might allow the propagation of such a SNP from *gyrA* to *parC* (or vice versa). It is not certain whether competence would play a role in this process, but the increased level of recombinase RecA during competence (Chapter 4) would render this a plausible hypothesis. A second
manifestation of genomic plasticity is the rapid evolution of surface-exposed pneumococcal proteins [13,15], many of which are commonly considered suitable vaccine targets. Variation of these loci, including the capsule locus (leading to serotype switching), may lead to immune system avoidance. Homologous recombination was thought to be the major force in this process, although phase variation of restriction–modification systems [14,19] and transmission of mobile genetic elements were also described to play a role [15]. Notably, many of the genes encoding variable surface proteins, including several choline–binding proteins (CBPs), are highly repetitive of nature, which might explain the enhanced recombination rate in these loci. In Chapter 2, we reiterated the observation by Lanie et al. that pavB, encoding surface protein PavB, contains several imperfect repeats of approximately 456 basepairs and that the number of these repeats varies between strains [1]. Also in Chapter 2, we added the group of immunogenic pneumococcal histidine–triad proteins (PhtABDE) to the list of variable antigens. Most strikingly, we showed that a 162 kb inversion around the ter region of the genome resulted in a rearrangement of Pht–encoding genes phtB and phtD. Furthermore, we presented evidence of intra– or intergenomic recombination between the different pht genes, including a newly annotated pseudogene, phtF (Chapter 2). Combined with a preliminary inquiry into the variation potential of a vaccine–relevant region of PhtD, these data call for future studies into the sustainability of using these proteins as vaccine targets.

Mechanisms of competence induction

Although the promotion of competence by certain antibiotics and the potential implications were clear, the mechanism(s) by which this inducing effect is brought about remained unknown. In 2011–2012, the group of M. Sebert revealed the mechanism responsible for competence activation by aminoglycoside antibiotics [20,21]. The surface-exposed protease HtrA was shown to degrade the extracellular signaling peptide CSP [21], in addition to misfolded proteins. Since antibiotics from the aminoglycoside class lead to elevated levels of misfolded proteins, HtrA becomes saturated and CSP degradation is reduced. Therefore, the CSP level can, subsequently, exceed the threshold value required for the regulatory positive–feedback loop to overcome counteracting forces and competence to be activated. Interestingly, the gene encoding HtrA is part of the CiaR regulon and, therefore, induced during competence (Chapter 4). The implications of this complication are further discussed later.

HtrA-mediated CSP degradation could not explain how several other antimicrobial compounds, including trimethoprim and fluoroquinolones, triggered pneumococcal competence. In Chapter 5, we presented a second mechanism for competence activation, that did explain competence activation by these compounds. We showed that these antibiotics all, directly or indirectly,
caused DNA replication elongation to stall or slow down. Since, apparently, the initiation of new rounds of replication is not sufficiently repressed in these conditions, dosage of genes close to the origin of replication increases. The fact that both \textit{comAB} and \textit{comCDE} are among these \textit{oriC}-proximal genes explain competence development in response to replication-targeting compounds. Indeed, natural competence development in a strain containing a duplication of \textit{comC} [22] or multiple \textit{comCDE} translocation/duplication strains confirmed the impact of gene dosage shifts.

Finally, we were interested to find out whether other clinically relevant compounds could also induce competence and tested a panel of commonly prescribed antibiotics (\textbf{Chapter 6}). We found that competence was triggered by the beta-lactam aztreonam and by the beta-lactamase inhibitor clavulanic acid. Both of these compounds target penicillin-binding protein 3 (PBP3), resulting in chain formation, contrasting with the typical diplococcal state of \textit{S. pneumoniae}. CSP produced by chain-forming cells is largely detected by the producing cell or chain, leading to more efficient local quorum sensing and earlier activation of competence. At the same time, diffusion into the common CSP pool and, therefore, communication between different chains is significantly reduced, which is reflected by the desynchronization of competence throughout the population and a concomitant extension of the time window in which transformation can take place. We would like to stress here, as discussed in \textbf{Chapter 6}, that this mechanism stays within the definition of quorum sensing and does not suggest the requirement of actual cell–cell contact for the propagation of competence. This is confirmed by experiments from both \textbf{Chapter 6} and Moreno-Gámez et al. [23], where competence in a \textit{ΔcomC} strain could be induced by wild-type cells without direct contact between the cells. Unwillingness to call pneumococcal competence a quorum-sensing system is, in our opinion, based on the false supposition that the ‘quorum’ that is being sensed needs to be a fixed constant. As discussed by us (\textbf{Chapter 6}) and by P. Williams [24], this is not the case and quorum-sensing systems can monitor many parameters of the environment besides overall cell density. In the case of pneumococcal competence, these parameters include, among others, translational fidelity [20], replication state (\textbf{Chapter 5}) and local cell density (\textbf{Chapter 6}).

Together, the three mechanisms described above can explain the effects of all competence-inducing antibiotics that we are aware of. Interestingly, both HtrA-mediated [20,21] and chaining-mediated competence activation (\textbf{Chapter 6}) do not require differential production of any specific transcript or protein. Finally, the gene dosage distribution shift following treatment with replication-targeting compounds does lead to a proportional effect on the transcriptome (\textbf{Chapter 5}). However, most standard transcriptome analysis approaches, including typical gene enrichment analysis, would not be able to
identify such an effect. As discussed in Chapter 1, the role of chromosomal gene order in cell fate determination is still largely neglected and we can only hope that our work will act as a wake-up call. Further considerations regarding the relevance of chromosome architecture can be found at the end of this chapter.

**Competence regulation in the host**

Up to this point, only competence induction in planktonic cells was discussed, simply because these experiments are most easily optimized and reproduced. Furthermore, we usually specifically design our experiments in such a way that cells do not become naturally competent, but competence can be induced by the addition of certain agents. To accomplish this, we utilize the fact that a pneumococcus’ ability to become naturally competent strongly depends on the pH of the growth medium [25]. At ‘high’ pH (~8.0), cells readily become naturally competent, even in the absence of inducing compounds. In contrast, at ‘low’ pH (~6.8), we are not able to induce competence with any compound (except CSP).

One might wonder, therefore, to what extent our findings can be extrapolated to the natural habitat of the pneumococcus, since the natural milieu in which pneumococci reside is much more complex than our laboratory setup. For example, inside the nasopharynx, the pneumococcus co-exists with many different microorganisms, the temperature is lower than in our experiments, bacteria interact with host cells, and different nutrients are available. Furthermore, rather than planktonic, pneumococcal cells primarily grow in complex, mixed biofilms [26,27]. Indeed, Wei and Håvarstein performed transformation assays in biofilms, showing that CbpD-mediated fratricide, during which the pneumococcus exports bacteriocins to kill neighboring cells [28], is required for horizontal gene transfer through pneumococcal competence [29] (Chapter 4).

Both mechanisms for competence induction presented in this thesis (Chapters 5, 6) are already relevant from a fundamental point of view: highlighting both the importance of chromosomal organization and local quorum sensing. However, we would like to make a case that it is likely that each of these mechanisms is also entirely relevant in the natural niche of the pneumococcus. As mentioned above, we use pH to artificially create a situation in which forces counteracting natural competence (e.g. CSP degradation) are perfectly balanced by promoting factors (e.g. CSP production). A small disbalance in this system may result in the population-wide activation of competence. Examples of such disbalances are a reduction of CSP degradation [21], an increase in CSP production (Chapter 5) or a local increase in CSP concentration (Chapter 6). Even though conditions in the nasopharyngeal passage might be vastly different, it seems likely that pneumococci are in a comparable equilibrium state there, such that they will become competent only under certain conditions. The only reasonable alternative to this equilibrium state would be continuous activity of the competent
state, which is illogical for multiple reasons. Firstly, the competence system lays a heavy burden on the cell (also see next section), necessitating the activation of several stress response regulons (Chapter 4; [8]). Secondly, the tight and sophisticated regulation of competence activation and shutdown is unlikely to have evolved if competence were always on. Thirdly, Mostowy et al. showed that recombination events occur at relatively low frequency [30], which fits better with occasional, transient bursts of competence. Although Marks et al. reported higher pneumococcal transformation rates in the mouse nasopharynx [31], we expect that the large inoculum sizes used in their experiments will lead to competence upregulation (related to Chapter 6) and therefore to an overestimation of natural transformation rates. If, in vivo, pneumococcal cells are indeed in a responsive state where small imbalances can trigger competence, all three known mechanisms for competence induction remain valid and antibiotic-induced horizontal gene transfer is a real threat.

The notion that pneumococcal transformation probably mainly takes place in biofilms [29] is also interesting in the context of the third discussed mechanism for competence activation (Chapter 6), where cell chaining causes a switch from global quorum sensing to more efficient, local quorum sensing. In analogy, we would expect competence to be activated when a sufficient number of cells come into close proximity of each other in the early stages of biofilm formation. Indeed, it seems logical for the pneumococcus to only activate competence once the local cell density is high, maximizing its chance of DNA acquisition. Conveniently, as a result, the newly identified early competence gene briC (Chapters 3, 4), which was reported to be required only during the late stages of biofilm formation [7], would be upregulated. Such an integration into the competence regulon of a checkpoint for biofilm formation would prevent the expenditure of resources on biofilm formation in the absence of a sufficient number of pneumococcal cells. However, future studies will have to further explore the potential role of competence in biofilm formation.

The purpose of pneumococcal competence

It remains a matter of debate what the main purpose of competence is, not only in the pneumococcus but in prokaryotes in general [32]. Firstly, and most obviously, transformation represents a powerful tool for the acquisition of new traits and to promote genomic diversity. Secondly, the material that is taken up can be used as nutrients by the competent cell [33]. In some prokaryotes, a third possibility is that competence functions as a general stress response mechanism [34]. The latter hypothesis is especially interesting for S. pneumoniae, which lacks the common bacterial SOS response [35]. Indeed, several stress response regulons are activated during competence, including the CiaR and HrcA regulons (Chapter 4). However, Dagkessamanskaia et al. showed that competence
in a *ciaR* mutant leads to stationary phase autolysis and stronger activation of the HrcA regulon. Together with the notion that none of the reported stress regulons are directly regulated by ComE or ComX, these observations suggest that they are actually activated in response to the stress that is caused by the development of competence. Indeed, it seems rather counterintuitive that the major stress response of the pneumococcus would be mediated by a system that, in itself, constitutes such a heavy burden to the cell. Nevertheless, it might very well be that the activation of competence during stress is beneficial to a cell in specific situations. If that were the case, there may have been some selective pressure to allow competence to be switched on in such conditions. Indeed, data from Engelmoer and Rozen demonstrated an increased survival rate of competent cells over non-competent cells, in the presence of aminoglycosides and DNA-damaging agent mitomycin C (MMC) [36]. On the other hand, we have observed a negative effect of competence on survival rates in the presence of other antimicrobial compounds (not shown).

As a final reflection on the possible role of competence as a general stress response mechanism, we will try to place the three known mechanisms of competence induction in this context. Firstly, DNA damage, such as induced by MMC, may lead to competence activation through a gene dosage increase of *oriC*-proximal genes (*Chapter 5*). Secondly, an increase in the level of misfolded proteins, occupying HtrA and reducing CSP degradation, could be the result of an increased mutation rate [37]. In both of these cases, the production of DNA repair proteins, as well as transformation (*Chapter 4*), all part of the core *com* regulons, might be beneficial to the cell. However, perhaps a more relevant implication of HtrA-mediated CSP degradation is the prevention of competence activation during cell envelope stress [38], since cell envelope stress is thought to activate the CiaR regulon, including *htrA*. Finally, we could not think of any obvious fitness advantage of competence induction during chain formation (*Chapter 6*). In conclusion, although we acknowledge the possibility of a beneficial effect of competence during specific types of stress, we do not believe that competence-mediated activation of stress regulons represents an efficient general stress response.

Interestingly, Croucher et al. put forward a fourth potential purpose of competence, derived from the observation that transformation favors gene deletions over insertions [39]. They show that this preference provides the cells with a defense mechanism against mobile genetic elements (MGEs), including BOX elements, RUPs, SPRITEs and IS elements (see *Chapter 2*). Indeed, they convincingly argue that the frequent disruption by MGEs of genes required for transformation reflects the fact that transformation negatively affects the spread of these elements. However, they do not provide conclusive evidence that the ability to eliminate MGEs from the genome represents the main selective force
leading to the evolution of bacterial competence.

There is no reason to assume a single driving force in the evolution of competence and all proposed roles might have some truth to it. However, based on literature and the work in this thesis, we believe that the most important aspect of pneumococcal competence is simply transformation, thereby generating genetic diversity.

**Implications of gene dosage distribution shifts**

Various aspects of chromosome organization, including chromosome structure and topology, have been described and are increasingly being studied. The growing pool of knowledge on properties related to the spatial organization of genes, including accessibility to transcription-related proteins, spatial colocalization of genes, and mRNA and protein diffusibility, will be very important for the understanding of bacterial gene regulation. However, while not unrelated, chromosomal gene order is yet another aspect that affects the regulatory landscape of a bacterial cell, and has not received as much attention as necessary. As described in Chapter 1, the exact position of a gene on the chromosome determines when, where, and how often its DNA is copied. Although gene order is not critical for cell survival in laboratory conditions [40], the significance of this facet of chromosome organization is emphasized by the several examples that have emerged of regulatory processes that depend on the dynamic copy number fluctuations during DNA replication (Chapter 5; [41–43]). Future research will have to determine how widespread these mechanisms are.

Additionally, regardless of the role of the chromosomal location of a gene under natural circumstances, it is important to keep in mind the potential impact certain experiments will have on copy number distributions in a cell; translocation of genes, antibiotic treatment, nutrient limitation, and other types of stress can each in their own way induce transcriptional changes by affecting gene dosage, either locally or genome-wide. A better understanding and increased awareness of the role of chromosomal gene order in the regulation of key processes is therefore paramount in understanding bacteria, and possibly also archaea, both in nature and in the laboratory.

**Normalization of transcriptome data**

Analysis of transcriptome data is often performed using automated pipelines that do not require much input from the user. The relative ease of use of these techniques has led to large amounts of highly valuable data. To our knowledge, however, none of the existing analysis packages consider the possibility of a genome-wide copy number shift, which is expected in several conditions, including treatment with certain antibiotics (Chapter 5) and changes in growth rate, temperature or replication rate. As a result of such an altered gene dosage pattern around the
chromosome, it may become difficult to set a proper baseline of expression or differential expression; most normalization methods depend, at least in part, either on the assumption that most genes will have an unaltered expression level (e.g. upper-quartile or median normalization), or on the assumption that the total number of transcripts per cell remains roughly the same (e.g. transcripts per million (TPM) [44] as a measure of expression). In case of a global shift in copy number distribution neither of these assumptions is necessarily valid and failing to acknowledge this may lead to overestimation of the number of truly differentially expressed genes, and simultaneously camouflage the changes of interest. The most accurate interpretation of data in these situations depends on the question one is trying to answer, but it is important to be aware of the role copy number changes have in these experiments.

Additionally, normalization methods for quantitative reverse-transcriptase–PCR (qRT–PCR) are usually based on the assumption that certain reference genes will keep a constant expression level in the various conditions that are being compared. This may be, even in the absence of copy number effects, a very dangerous assumption, but is especially so when gene dosage distribution shifts are in play. It is therefore advisable to, in addition to using more than one reference gene, confirm that copy number shifts are not responsible for the observed results. This can be accomplished by a qPCR experiment using chromosomal DNA as a template.

**Copy number effects in synthetic and natural systems**

In Chapter 1, we highlighted the role of copy number fluctuations in bacterial processes. In synthetic constructs the copy number of an integrated DNA sequence can be critical for its proper functioning and in these cases the location of chromosomal integration should be carefully deliberated [45].

Surprisingly, beyond the well-known replication-associated gene dosage, not too many examples of bacterial decision-making are available that have been ascribed to the genomic location of key factors involved. Over the last couple of years, however, several of these examples have emerged (Chapter 5; [41–43]). Combined with the fact that DNA replication is universally present in all living organisms, this is highly suggestive of the possibility that these effects are much more abundant in bacterial biology than currently acknowledged. In fact, archaeal chromosomes typically contain no more than a few replication origins and since bacterial and archaeal chromosomes share several organizational traits [46], some of the discussed mechanisms may very well be active in archaean as well.
References

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