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ORIGINAL RESEARCH

Inference of Self-Regulated Transcriptional Networks by Comparative Genomics

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Abstract: The assumption of basic properties, like self-regulation, in simple transcriptional regulatory networks can be exploited to infer regulatory motifs from the growing amounts of genomic and meta-genomic data. These motifs can in principle be used to elucidate the nature and scope of transcriptional networks through comparative genomics. Here we assess the feasibility of this approach using the SOS regulatory network of Gram-positive bacteria as a test case. Using experimentally validated data, we show that the known regulatory motif can be inferred through the assumption of self-regulation. Furthermore, the inferred motif provides a more robust search pattern for comparative genomics than the experimental motifs defined in reference organisms. We take advantage of this robustness to generate a functional map of the SOS response in Gram-positive bacteria. Our results reveal definite differences in the composition of the LexA regulon between Firmicutes and Actinobacteria, and confirm that regulation of cell-division inhibition is a widespread characteristic of this network among Gram-positive bacteria.

Keywords: comparative genomics, transcription factor, LexA, transcriptional network, SOS response, motif discovery

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Introduction

The ability to uncover and decipher transcriptional regulation systems constitutes an invaluable tool in molecular biology[1,2] and represents a major challenge in bioinformatics.[3–5] Transcriptional regulation is mediated mainly by a subset of proteins known as transcription factors (TF) that bind DNA and can either hinder (repressors) or promote (activators) the formation of an open complex by the RNA-polymerase holoenzyme.[6] Transcription factors recognize a relatively small set of sites collectively known as the binding or sequence motif,[7] often represented using sequence logos.[8] The semi-specific recognition of binding sites by their cognate transcription factors allows implementing computational tools for the discovery and detection of transcription binding motifs and sites.[1] Motif discovery methods focus on the identification of overrepresented patterns in groups of sequences to generate a motif description.[3,9] Conversely, site search algorithms take in a motif description and use pattern matching techniques to search for putative sites on DNA sequences.[9,10] Scanning genomic sequences in this way leads to a noisy but informative reconstruction of transcriptional regulatory networks, which can be later validated by in vitro and in vivo methods,[11–13] or linked to other sources of information in order to reconstruct regulatory networks.[1,14–16]

In the past, numerous studies have exploited comparative genomics approaches to analyze the composition and conservation of transcriptional regulatory networks or regulons. These studies can yield important insights into several facets of transcriptional regulatory networks that are difficult to approach experimentally. By assessing the spread of a given regulatory signal, for instance, one can infer the ancestry and biological relevance of a regulatory mechanism.[17,18] Similarly, the analysis of the genetic makeup of a regulon across different species can shed light into its core evolutionary conserved components and reveal previously unidentified regulon members.[11,12,19] Comparative genomics approaches to regulatory network analysis rely on the basic notion that regulons are composite entities that aggregate four different elements: the regulatory TF, a biological function, the network of regulated genes and the motif recognized by the TF. Most comparative genomics approaches to regulon analysis make the implicit assumption that both the regulatory TF and its biological function are preserved. Motif discovery techniques relying on comparative genomics typically assume also that both the network of genes and the TF-binding motif are preserved in order to apply phylogenetic footprinting techniques to enhance motif discovery.[20,21] In contrast, regulon analysis techniques based on site search assume only conservation in the TF-binding motif and seek to elucidate variations in regulon composition.[1,11,12,17–19,22]

Forfeiting the requirement of network conservation makes site search-based analyses of regulatory networks by comparative genomics implicitly dependent on an initial description of the TF-binding motif. This description is typically based on a model organism in which a substantial number of sites[12,17,19] or regulated genes[17,18] is known from previous experimental work. In the latter case, a motif discovery tool is applied to gene promoter regions to generate a candidate motif to start the multi-genome search. More recently, mutational analysis of a single site has been proposed to construct a viable TF-binding motif.[13] Still, these strategies require the generation or availability of previous experimental knowledge in a model organism. This is inconvenient because this model organism can sometimes be relatively distant from the clade of interest, casting doubts on the underlying hypothesis of TF-binding motif conservation.

Many prokaryotic transcriptional networks can be described in terms of the single-input module (SIM) paradigm or as variations and elaborations of this basic configuration.[23,24] In this connection paradigm, a single regulator controls the temporal activation of several cis-regulated genes.[24] It has been observed previously that the master transcription factor of a SIM is often self-regulated,[23] and that self-regulation is even more prevalent in repressor-based SIMs.[25] The bacterial SOS response is a well-known example of self-regulated SIM regulatory network.[26] The SOS response regulates a variable number of genes that are under direct transcriptional control of the LexA repressor.[27] In *Escherichia coli*, where the SOS response was originally described, LexA recognizes a 16 bp-long palindromic motif (CTGT-N8-ACAG). LexA dimers bind tightly to instances of this motif in the promoter region of 30 operons, regulating the activity of up to 40 genes involved in DNA repair, translesion synthesis (TLS) and regulation of cell division.[22,28] In the advent of DNA damage, the recombination protein RecA acquires an active state and is able to induce self-catalytic cleavage of LexA dimers, de-repressing the SOS network.[29,30] Explicit regulation of *recA* and self-regulation of the *lexA* gene ensures that repression is restored rapidly after DNA damage has been addressed.[26]

The LexA protein has been shown to recognize an unusually large repertoire of binding motifs across the Bacteria domain, with more than 15 distinct motifs described to date.[27] This variety in binding motifs is associated with substantial diversity in regulon composition, which has been mapped in some bacterial classes through comparative genomics approaches.[11,22] The evidence compiled thus far through experimental and in silico techniques suggests that there is a small set of genes persistently regulated by LexA in most bacteria.[27] This core LexA regulon comprises the *lexA* and *recA* genes, and is often complemented by a multiple gene cassette (*imuA*-*imuB*-*dnaE*2) involved in mutagenesis.[31] Recent work has analyzed the composition of the LexA regulon in two Gram-positive species (the actinobacterium *Corynebacterium glutamicum*[32] and the Firmicute *Listeria monocytogenes*),[33] complementing previous work in other Gram-positive species (*Bacillus subtilis* and *Mycobacterium tuberculosis*)[34,35] and providing a multifaceted view of the Gram-positive LexA regulon.

The ever-growing abundance of genomic and metagenomic data ensures that, within a given phylogenetic group, many sequences encoding orthologs of the same transcription factor will be readily available. By coupling the assumption of self-regulation to that of motif conservation, one can theoretically forgo the need for experimental knowledge in a model organism. Motif discovery algorithms can be applied to the upstream region of the orthologous genes encoding the transcription factor of interest in order to generate a candidate motif to conduct site search-based analysis of a simple transcriptional network. Taking advantage of the recent availability of experimental data on the SOS transcriptional network in several Gram-positive bacteria, here we provide proof of concept for this approach and we compare it against the conventional method based on extension from a single experimental model organism. Our results reveal that this approach is powerful enough to generate de novo transcriptional network maps, which can be used for functional annotation. Furthermore, we show that the use of a phylogenetically-broad sampling base for motif discovery can yield robust motifs for site search, generating more consistent results than the conventional methodology. We also show that the necessary steps of this approach can be extended to other transcriptional repressors and tool suites. Finally, we use this approach to generate for the first time a systematic mapping of the core LexA regulon in Gram-positive bacteria. This map reveals distinct patterns of LexA regulon composition between Firmicutes and Actinobacteria and supports the notion that cell-division inhibition is persistently regulated by the SOS response in these bacterial groups.

Algorithms and Datasets

Identification of transcription factor homologs

Homologs for the master transcription factor of the genetic system under analysis were identified as best bidirectional BLAST hits[36,37] on a balanced set of genomes from the clade of interest. This set of genomes was generated by selecting at least one, and no more than two, species for every major genus within the clade under study. The intent of this strategy was to maximize coverage while avoiding biases in representation due to the uneven distribution of sequencing projects among genera, which could distort the ensuing motif discovery process. Species were selected using the Integrated Microbial Genomes (IMG) system of the Joint Genome Institute[38] and homologues of the transcription factor were identified as best bidirectional BLAST hits using the protein sequences of well-established homologs for each phylum/class analyzed and a minimum e-value of 10^{-20} on the IMG BLASTP service (http://img.jgi.doe.gov/).

Motif discovery

For each of the identified transcription factor gene homologues, the region 250 bp upstream of the predicted translation start site, which is known to harbor most promoter elements in bacteria,[39] was extracted using the IMG export service. These 250 bp regions were then fed into the MEME service of National Biomedical Computation Resource (http://meme.nbcr.net/) using any number of occurrences for a single motif, a predefined 10–20 bp motif length and otherwise standard parameters. Previous work has shown that most bacterial transcription factors target motifs in the 10–20 bp range[40] and a variable number of occurrences is required to factor in the multiplicity of binding sites described for many bacterial promoters.[41] Whenever the best motif identified by MEME was found to be a palindrome, motif discovery was repeated on a single strand with the palindrome-only option set to refine the model. Alternatively, motif discovery on these same regions was performed using the PhyloGibbs Online service (http://www.phylogibbs.unibas.ch/) using default parameters. A basic phylogeny was estimated with the WUR CLUSTALW server (http://www.bioinformatics.nl/tools/clustalw.html) using the protein sequences of the transcription factor for the CLUSTALW alignment[20,42] and provided to the PhyloGibbs Online service as a Newick-formatted tree file. Because PhyloGibbs does not allow for variable motif input, we conservatively set motif width to the maximum value used for the MEME experiments (20 bp). For the purposes of the comparative genomics analysis, the model was further refined by using the MEME-inferred motif to search again the upstream regions with FITOM (see below) looking for additional binding sites. Search results with scores greater than two standard deviations below the mean of the MEME-inferred collection were considered putative binding sites and added to an expanded collection used as the standard in subsequent genomic searches.

Binding site search

In silico searches of putative binding sites were performed with FITOM[10] and xFITOM[43] (http://compbio.umbc.edu/software). These programs take in a collection of known sites, from which they derive a Position-Specific Frequency Matrix (PSFM). Different scoring methods based on information theory can then be applied to search a given target sequence and the programs annotate results based on the proximity of candidate sites to gene regulatory regions. The searches reported here were all conducted using the sequence information content (R_i) scoring method[44] and otherwise default parameters for FITOM/xFITOM. Searches were based on several collections of experimentally validated binding sites or on collections of binding sites inferred through motif discovery with MEME.[45] For each collection the search threshold was adjusted to eight standard deviations below the mean score for the sites present in the collection, in order to accommodate progressive threshold decrease down to six standard deviations below the mean in the comparative genomics approach (see below).

Benchmarking of the site search process was performed using collections of experimentally validated sites[32–35] as reference for different genomes. For any given genome, searches were run using different collections of sites to define the search motif. Receiver Operating Characteristic (ROC) curves were then generated by plotting the percentage of experimentally validated sites (true positives) with respect to the percentage of non-experimentally validated sites (false positives) detected by the search process when using different thresholds. ROC-curves are shown only for the high-specificity thresholds typically used in site search.

Genome sequences

A set of representative species from a phylogenetic group of interest was selected to perform the comparative genomics analyses. Representative species were chosen to include those in which binding sites for the transcription factor of interest had been experimentally reported as well as species with available reference sequences in the NCBI RefSeq database comprising all major orders within the group, while including a relatively low total number of species to allow detailed analyses of results. Genome sequences for all the selected species were downloaded from the NCBI GenBank database.

Experimental datasets

Collections of LexA-binding sites for *B. subtilis*, *M. tuberculosis*, *L. monocytogenes* and *C. glutamicum* were compiled from experimentally validated sites reported in the literature[32–35] and standardized to a length of 18 bp by searching on the reference genome and expanding, if necessary, the original site.

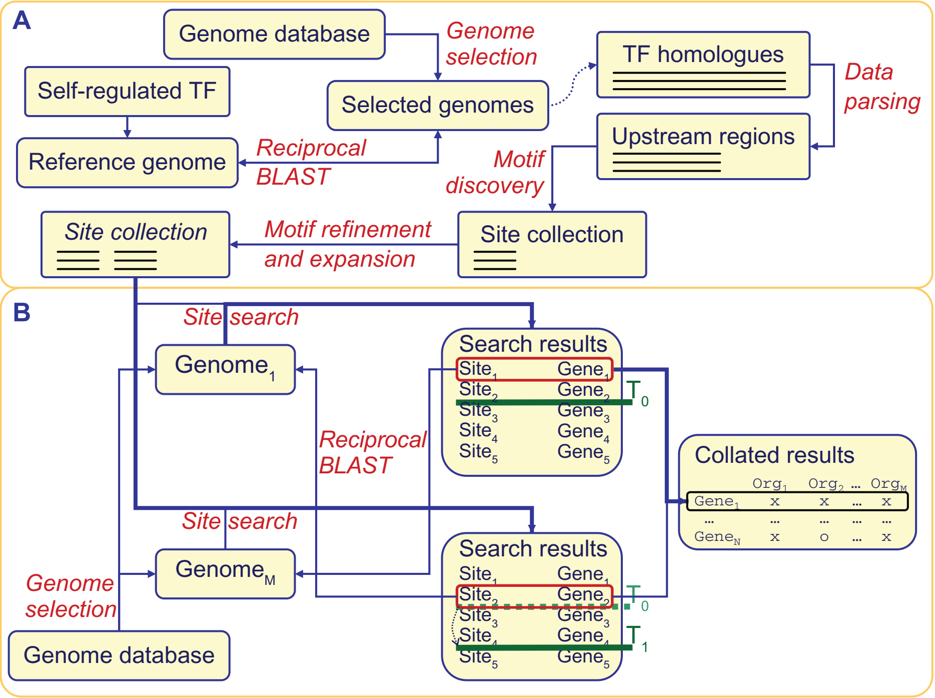
Comparative genomics analysis

To perform the comparative genomics approach, searches with the inferred binding motif were carried out using xFITOM on all genome sequences selected for analysis. Search results were then parsed sequentially, going from systematically high to low scoring sites across all genome results files. A threshold of two standard deviations below the average for the inferred binding motif was applied initially to select candidate sites. For each candidate site, homologues of the putatively regulated genes were identified as best reciprocal BLAST hits against the other selected bacterial genomes. Identified homologues were then mapped back to the corresponding results file. Whenever a gene was found to be putatively regulated in a new genome, the threshold for that particular gene was decreased by one additional standard deviation, down to a maximum of six deviations below the mean when putative evidence of regulation had been established in five or more species. This lower threshold was chosen because it identified binding sites in 95% of the gene upstream regions used for LexA motif discovery. A gene was considered to be putatively regulated if binding sites meeting the above criteria were located upstream of its orthologs in at least two different species.[46] The complete process for parsing search results files, identifying gene homologues as best reciprocal BLAST hits and assessing putative regulation (Fig. 1) was automated using custom Perl scripts. The validity of this comparative genomics approach was qualitatively assessed using the RegPredict Regulon Inference service (http://regpredict.lbl.gov/) with default parameters.[47]

Results and Discussion

Generation of the gram-positive LexA-binding motif

This work explores the feasibility of applying two simple assumptions regarding a predicted transcription factor (self-regulation and motif conservation) to take advantage of the availability of genomic and meta-genomic data in order to yield a first-order map of its transcriptional network. As a test case, we use the LexA protein of Gram-positive bacteria, for which there is comprehensive experimental data available on several organisms. An obvious first step



	Org$_1$	Org$_2$	…	Org$_M$
Gene$_1$	x	x	…	x
…	…	…	…	…
Gene$_N$	x	o	…	x

Figure 1. Schematic representation of the comparative genomics approach used in this work. (**A**) Motif discovery. Self-regulation is assumed for the transcription factor of interest, which is identified univocally in a particular genome. A uniform sample of genome sequences from a given phylogenetic group is selected and multiple homologues of the transcription factor are identified in these genome sequences as best-bidirectional BLAST hits using the TF of interest as the starting query. The upstream sequences of the genes coding for the TF homologues are retrieved and a motif discovery algorithm is applied to them. The resulting best motif model is refined by exploiting its palindromic nature and the collection of sites that compose it is expanded by re-searching the gene upstream regions for additional sites. (**B**) Comparative genomics.

Notes: The expanded site collection is used in subsequent genome-wide searches against a selected subset of genome sequences. Results on each genome are first filtered with an initial threshold (T_0), which is revised ($T_1 \ldots T_N$) when further instances of regulation are discovered for a given gene. Genes showing instances of regulation for more than one species are reported as putative elements of the TF regulon.

in pursuing this goal is to generate a valid candidate TF-binding motif to initiate the search procedure in target genomes. Based on the above assumptions, a candidate TF-binding motif can be obtained by applying a motif discovery algorithm to the promoter region of homologues of the transcription factor under analysis. Here we make extensive use of the standard motif discovery algorithm MEME,[45] but the same kind of analysis may be performed with motif discovery algorithms that incorporate phylogenetic information.[20,21,48]

To identify the LexA-binding motif, we selected a representative sample of 67 Gram-positive genomes (Supplementary data 1) and we identified LexA homologues as best-bidirectional BLAST hits using the *Bacillus subtilis* and *Mycobacterium tuberculosis* LexA proteins as queries. This resulted in the identification of 58 *lexA* homologues (Supplementary data 2), the upstream region of which was used for motif discovery. Default motif discovery with MEME on these 58 promoter regions yields two canonical Gram-positive LexA-binding motifs of lengths 18 and 16 bp, respectively, reported independently as the best- and second best-scoring motifs (Fig. 2A). The first motif is reported in only 38 of the 58 sequences and the second in only 32 sequences. In order to obtain a more generic motif, we expanded the best-scoring motif by conducting a conservative site search on all 58 sequences. This led to a final (expanded) collection of 71 sites distributed among 47 sequences (Fig. 2B) that was used subsequently for site search in the comparative genomics approach (Supplementary data 3). Applying a less conservative search threshold revealed that most of the *lexA* promoter sequences that were not represented in this expanded collection contained several putative weak sites in tandem configuration (Supplementary data 4).

We assessed the dependency of the motif discovery approach on the number of sequences by randomly sampling the 58 promoter regions in groups of 48, 24, 12, 6 and 3 sequences and using either the MEME or PhyloGibbs motif discovery services. Even with the more restrictive parameter settings of PhyloGibbs, the LexA-binding motif was identified routinely using as few as 6 sequences, with motif discovery stabilizing fully at 48 sequences (Supplementary data 5 and Supplementary data 6). We also performed a qualitative analysis of the generality of the motif discovery approach by applying it to other self-regulated transcription factors in different phyla. We analyzed three additional transcriptional repressors (Rex, HrcA and TyrR) in, respectively, the Actinobacteria, the Firmicutes and the Gamma Proteobacteria, and we were able to infer the reported experimental motifs for all three,[49–51] indicating that this type of analysis can be extended to other transcriptional systems (Supplementary data 7, Supplementary data 8, Supplementary data 9).

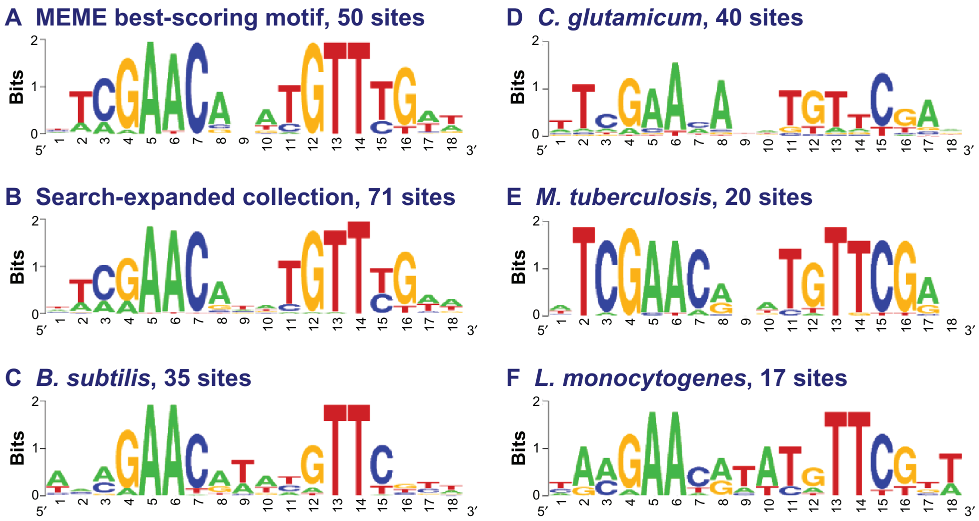


Figure 2. LexA-binding motifs. (**A**) Best-scoring motif reported by MEME based on 50 sites, (**B**) search-expanded motif encompassing 71 sites, (**C**) motif from experimental sites in *B. subtilis*, (**D**) motif from experimental sites in *C. glutamicum*, (**E**) motif from experimental sites in *M. tuberculosis* and (**F**) motif from experimental sites in *L. monocytogenes*.

Search performance of the LexA-binding motif

Conventional approaches to regulatory network analysis by comparative genomics typically exploit experimental data, either in the form of binding site collections or known regulated genes, in a single reference species.[11,12,17–19] A foreseeable problem with this approach is the progressive unreliability of the experimental motif as the phylogenetic distance between source and target species increases. Here we decided to evaluate the impact of this effect on a phylogenetically broad group of bacteria (Gram-positive bacteria) and we analyzed whether our approach, based on a single-gene multi-species derived motif, might also be subject to a similar effect.

We used published experimental results on the composition of the LexA regulatory network in four different Gram-positive bacterial species (the Firmicutes *B. subtilis* and *L. monocytogenes*, and the Actinobacteria *M. tuberculosis* and *C. glutamicum*) to benchmark the search efficiency of each of the four experimental collections, plus the MEME-derived collections, on each bacterial genome.[32–35] The ROC curves shown in Figure 3 demonstrate that phylogenetic proximity does have a substantial impact on search efficiency. In all four genomes, search efficiency drops drastically when using a Firmicutes-derived motif on an Actinobacteria genome and vice versa. At the high specificities typically used for reliable site search (0.9995 specificity), sensitivity decreases by 60% on average when searching with an experimentally known motif defined in one group on a genome belonging to the other. In contrast, the automatically-derived motifs yield search efficiencies that are much closer (13% average decrease for the expanded motif) to those obtained with collections experimentally defined in the same group the searched genome belongs to (Fig. 3). The results also suggest that the expansion of the initial motif identified by MEME generates a slightly noisier motif that systematically improves search efficiency.

Differences in the specific shape of the Gram-positive LexA-binding motif have been noticed

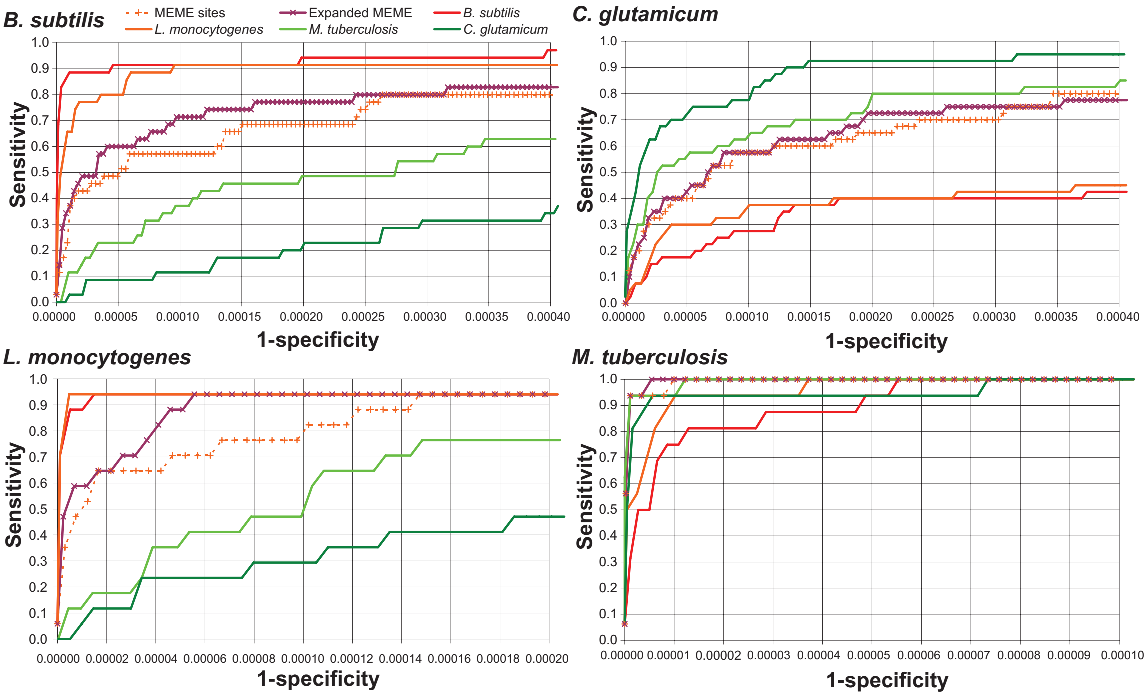


Figure 3. ROC curves for search efficiency with experimentally-validated and MEME-derived collections on four different genomes corresponding to the Firmicutes (left) and Actinobacteria (right).

Notes: Sensitivity corresponds to the fraction of experimentally validated binding sites detected by the search algorithm. Specificity is the fraction of the rest of genomic positions reported by the search algorithm. Only the high-specificity region of the ROC curve is shown to illustrate the differences between the different collections used to define the search motif on different genomes. Expanded ROC curves showing a larger segment of specificity are reported as Supplementary data 10.

before,[32,33,35] but their specific evolutionary relationship and their impact on search efficiency had not been assessed directly. Our results show that differences in the LexA-binding motif of Gram-positive bacteria stem mainly from the evolutionary split between Firmicutes and Actinobacteria. Furthermore, the ROC curves demonstrate that the differences observed among LexA-binding motifs have a definite impact on search efficiency. The sequence logos shown in Figure 2 illustrate how the MEME-inferred motif combines traits of both the Firmicutes (dominance of dyad central positions) and Actinobacteria (importance of spacer and adjacent positions) that allow it to perform well on both phyla. These results thus support the use of a phylogenetically broad sample for motif discovery when conducting comparative genomics analysis, as this leads to a generic motif that can achieve competitive search efficiencies in all target genomes.

Comparative genomics of the gram-positive SOS network

Having benchmarked the efficiency of the MEME-derived collections, we used the expanded collection to perform a comparative genomics analysis of the SOS regulon of Gram-positive bacteria using 11 representative species from the Firmicutes (*B. subtilis* str. 168, *Clostridium acetobutylicum* ATCC 824, *Enterobacter faecalis* V583, *Listeria monocytogenes* serotype 4b str. CLIP 80459, *Staphylococcus aureus* subsp. aureus Mu50) and the Actinobacteria (*Acidothermus cellulolyticus* 11B, *Corynebacterium glutamicum* ATCC 13032, *Leifsonia xyli* subsp. xyli str. CTCB07, *M. tuberculosis* H37Rv, *Nocardia farcinica* IFM 10152 and *Streptomyces griseus subsp. griseus* NBRC 13350). As expected, the conservative nature of the comparative genomics approach leads to a considerable decrease in sensitivity. For instance, only 9 operons, out of 15 experimentally described, are identified as putatively regulated in *L. monocytogenes*. The loss of sensitivity is compensated by a dramatic increase in specificity. Remarkably, all the genes and operons predicted as LexA-regulated in this study have been experimentally validated as members of the SOS response in at least one organism (Table 1). Furthermore, we obtain similar results when using the RegPredict comparative genomics service (Supplementary data 12). This allows us to reliably extend the results of the comparative genomics analysis onto those species for which we lack experimental knowledge.

Only two genes, *recA* and *lexA*, are consistently detected as putatively LexA-regulated, but putative LexA-binding sites can also be detected upstream of genes coding for translesion synthesis polymerases in nearly all species. This is in agreement with the hypothesis of a conserved core SOS regulon that extends beyond *recA* and *lexA* to include TLS as a primary component of the SOS response.[22,27,52–54] In this regard, it is interesting to note that SOS-induced TLS is apparently taken up by two different mechanisms in Firmicutes and Actinobacteria. In agreement with the experimental data available for individual organisms, the former appear to rely on the polymerase IV (encoded by *dinB*) and a polymerase V ortholog (encoded by *uvrX*),[33,34,55] while the latter exploit error-prone nature of the second α-subunit of the DnaE polymerase (encoded by *dnaE*2).[32,35,54] In addition, our analysis suggests that in the Actinobacteria the TLS activity of DnaE2 is quite often coordinated with expression of the mutagenic the *imuA*-*imuB* operon, which has been shown to be involved in DNA damage-inducible mutagenesis in other bacterial classes.[56] This result is consistent with the identification of SOS regulated polycistronic units encompassing *imuA*, *imuB* and *dnaE*2 across the Bacteria domain.[31]

The comparative analyses of search efficiency reveal a consistent phylogenetic split between Firmicutes and Actinobacteria at the LexA-binding motif level (Figs. 2 and 3). This phylogenetic divide is also clearly visible in the repertoire of repair genes regulated by the SOS response in both clades (Table 1). The Firmicutes, for instance, maintain LexA-regulation of the excision repair *uvrBA* operon, a canonical element of the *E. coli* SOS regulon. In contrast, LexA-regulation of *uvrB* and *uvrA* is absent in the Actinobacteria, where the *uvrBA* operon organization has been disrupted. The lack of LexA regulation for the excision repair system in Mycobacteria has been noticed before, even though these and other repair genes area induced by DNA damage.[57] Our findings indicate that lack of LexA regulation for *uvrA* and *uvrB* is the norm among Actinobacteria, leaving open the question of how, if at all, these genes are regulated by DNA damage in this clade.[58]

Table 1. Composition of the LexA regulon in Gram-positive bacteria.

	Gene	Reference	Firmicutes					Actinobacteria					
			Bsu	Cac	Efa	Lmo	Sau	Ace	Cgl	Lxy	Mtu	Nfa	Sgr
Core	*lexA*	32–35,55	■	■	■	■	■	■	■	■	■	■	■
	recA	32–35,55	■	■	■	■	■	■	■	■	■	■	■
TLS	*dinB*	33,34	■		■	■	□	□	□	□	□	■	
	uvrX	33,55	□	□	■	■	■						
	dnaE2	35						□	■		■	□	□
	imuA	32,35						■	■		■	■	
DNA repair	*parE*	34,55	■		■	□	■						■
	pcrA	33,34	■	□	□	■	□	□	□	□	□	□	□
	uvrB	33,34,55	■	■	■	■	■	□	□	□	□	□	□
	yhaO	33	■		■	■	■						
	ruvC	32,35						■	□	□	■	■	□
	splB	35		□				■		■	■	■	■
	alk	35									■	■	■
	whiB2	35						□	□	□	■	■	□
Cell division	*yneA*	33,34	■			■							
	hyp1	55					■						
	divS	32							■				
	Rv2719c	35									■		
	hyp$_2$											■	
	lysM$_D$							■		■			

Notes: Known or predicted LexA regulation is indicated by filled squares, whereas an open square denotes lack of putative LexA-binding sites. Empty cells indicate that a given gene is absent from a particular genome. A list of references in which SOS regulation has been experimentally verified is provided for each gene. Genes are sorted according to the following categories. A richer version of this table, with gene annotation information and with the sequence and location of identified LexA-binding sites is available as supplementary data (Supplementary data 11).
Abbreviations: Core, core regulon members, TSL, Translesion synthesis, DNA repair, genes involved in DNA repair, and Cell division, genes involved in cell division suppression. Species abbreviations are as follows: Bsu, *B. subtilis*; Cac, *C. acetobutylicum*; Efa, *E. faecalis*; Lmo, *L. monocytogenes*; Sau, *S. aureus;* Ace, *A. cellulolyticus*; Cgl, *C. glutamicum*; Lxy, *L. xyli*; Mtu, *M. tuberculosis*; Nfa, *N. farcinica*; Sgr, *S. griseus*.

In contrast with the Actinobacteria, the Firmicutes appear to lack regulation of another hallmark of the *E. coli* SOS regulon: the Holliday junction complex encoded by the *ruvAB* operon. In this case, the absence of LexA regulation is associated with the absence of the *ruvC* gene, which heads the *ruvCAB* operon in Actinobacteria. Regarding the clear-cut phylogenetic split between both groups when analyzing regulon organization, it is also worth noting that genes regulated by LexA only in the Actinobacteria (eg, *splB*, *alk*, *ruvC*, *whiB2*) are usually absent in the Firmicutes. In contrast, genes under LexA regulation only in the Firmicutes (*yhaO*, *uvrB*, *pcrA*) are typically present, but not regulated by LexA, in the Actinobacteria. This is consistent with a RecA-independent mechanism of DNA damage-induction in the Actinobacteria, which has already been shown to coordinate the expression of several DNA repair genes in the Mycobacteriaceae.[57]

Regulation of cell division by the SOS response

In *E. coli*, the SOS response regulates cell division by blocking the formation of the FtsZ ring via the product of the *sulA* gene.[59] Later research has shown that in many bacterial species the *sulA* gene is frequently found in an operon with *lexA*,[31,46] providing a straightforward means for its regulation by the SOS response. In 2003, the protein encoded by the *B. subtilis yneA* gene, which forms a divergent gene pair with *lexA*, was shown to inhibit cell division upon induction of the SOS response.[60] This finding was remarkable because YneA is structurally different and phylogenetically unrelated to SulA. More recently, the products of two additional genes forming a divergent gene pair with *lexA* (Rv2719c in *M. tuberculosis* and *divS* in *C. glutamicum*) have also been shown to suppress cell division upon induction of the SOS response.[61,62] Both the *B. subtilis* YneA and the *M. tuberculosis* Rv2719c contain a peptidoglycan-binding LysM domain that has been shown to be necessary for suppression of cell division by YneA, but not by Rv2719c.[62,63] In contrast, the *C. glutamicum* DivS does not contain a peptidoglycan-binding LysM domain, indicating that these three proteins interfere with cell division in different ways.

Our analysis suggests that this trend towards regulation of cell division by the SOS response through divergent pairing of a cell division suppressor with *lexA* is most likely a defining trait of Gram-positive bacteria. Beyond the above cases and the *L. monocytogenes yneA* ortholog,[33] the comparative genomics analysis identifies two additional genes containing a LysM domain (Acel_1478 in *A. cellulolyticus* and Lxx15870 in *L. xyli*) divergently paired with *lexA*. These two genes do not present significant sequence similarity with either *yneA* or Rv2719c. However, the presence of a conserved LysM domain and the conservation of synteny (Fig. 4) suggest that their function is likely to be preserved. Synteny is also maintained in the *N. farcinica* NFA_37990 and *S. aureus* SAV1340 genes, but neither presents a conserved domain. Nonetheless, given that three independent mechanisms for cell-division suppression have already been suggested for genes paired divergently with *lexA*, the potential role of these two genes in cell division should be an interesting target for experimental analysis.

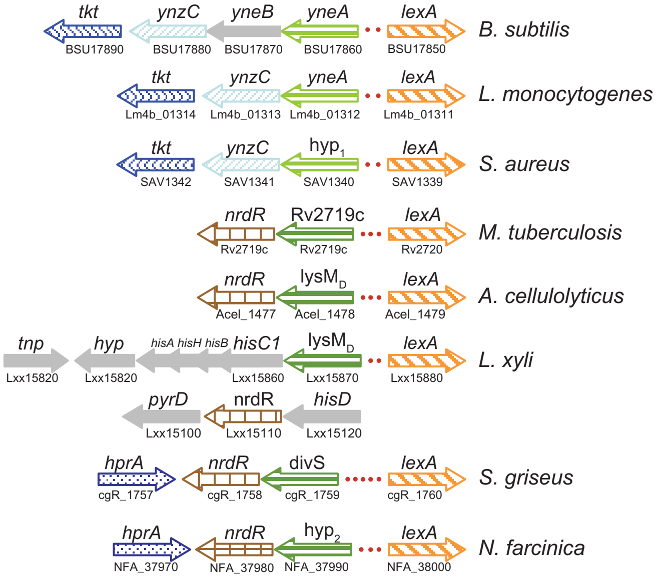


Figure 4. Schematic representation of the genomic region encompassing the *lexA* gene and divergently paired putative and known (*yneA*, *divS*, Rv2719c) cell division inhibitors.

Notes: Genes are represented by arrows. Solid grey arrows indicate non-conserved genes. Red circles denote LexA-binding sites. NCBI GenBank accession numbers are provided relevant genes. The figure illustrates the conservation of synteny in both Firmicutes and Actinobacteria. In *L. xyli*, a recent genetic rearrangement involving a transposase gene (*tnp*) and the *his* operon has visibly displaced *nrdR* from the vicinity of the LysM domain-containing protein encoded by Lxx15870. The *divS* and *nrdR* genes, as well as *yneA*, *yneB* and *ynzC*, are known to constitute operons.[32,60]

Conclusion

This work analyzes the feasibility of exploiting basic assumptions of simple transcriptional networks in order to infer regulatory motifs from the vast amount of genomic and meta-genomic data available, and to reconstruct regulatory networks through comparative genomics using the inferred motif. Our results provide proof of concept for this approach using the SOS regulatory network of Gram-positive bacteria as a test case, paving the way for the development of automated methods that make use of the overabundance of sequence data for de novo inference of simple regulatory networks. Furthermore, benchmarking against experimental data suggests that inferred motifs may yield more robust search patterns. The analysis of the SOS response in Gram-positive bacteria shows clear differences in the composition of the LexA regulon between the two main groups of Gram-positive bacteria and reinforces the notion that part of the DNA repair machinery of the Actinobacteria is regulated independently of LexA. Finally, our study suggests that regulation of cell-division by the SOS response is prevalent in Gram-positive bacteria, providing further evidence of convergent evolution of this trait and pointing to interesting candidates for experimental research.

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Author Contributions

Conceived and designed the experiments: IE. Analysed the data: JPC, FM, JRT, IE. Wrote the first draft of the manuscript: IE. Contributed to the writing of the manuscript: JPC, IE. Agree with manuscript results and conclusions: JPC, FM, JRT, IE. Jointly developed the structure and arguments for the paper: JPC, JRT, IE. Made critical revisions and approved final version: JPC, IE. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

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References

1. Babu MM. Computational approaches to study transcriptional regulation. *Biochem Soc Trans*. Aug 2008;36(Pt 4):758–65.
2. Minchin SD, Busby SJ. Analysis of mechanisms of activation and repression at bacterial promoters. *Methods*. Jan 2009;47(1):6–12.
3. Bailey TL. Discovering sequence motifs. *Methods Mol Biol*. 2008;452:231–51.
4. Tompa M, Li N, Bailey TL, et al. Assessing computational tools for the discovery of transcription factor binding sites. *Nat Biotechnol*. Jan 2005;23(1): 137–44.
5. Das M, Dai H-K. A survey of DNA motif finding algorithms. *BMC Bioinformatics*. 2007;8(Suppl 7):S21.
6. Ptashne M. Regulation of transcription: from lambda to eukaryotes. *Trends Biochem Sci*. Jun 2005;30(6):275–9.
7. D'Haeseleer P. What are DNA sequence motifs? *Nat Biotechnol*. Apr 2006; 24(4):423–5.
8. Schneider TD, Stephens RM. Sequence logos: a new way to display consensus sequences. *Nucleic Acids Res*. Oct 25, 1990;18(20):6097–100.
9. Mrazek J. Finding sequence motifs in prokaryotic genomes—a brief practical guide for a microbiologist. *Brief Bioinform*. Sep 2009;10(5):525–36.
10. Erill I, O'Neill MC. A reexamination of information theory-based methods for DNA-binding site identification. *BMC Bioinformatics*. 2009; 10(1):57.
11. Erill I, Jara M, Salvador N, Escribano M, Campoy S, Barbe J. Differences in LexA regulon structure among Proteobacteria through in vivo assisted comparative genomics. *Nucleic Acids Res*. 2004;32(22):6617–26.
12. Rodionov DA, Gelfand MS, Hugouvieux-Cotte-Pattat N. Comparative genomics of the KdgR regulon in Erwinia chrysanthemi 3937 and other gamma-proteobacteria. *Microbiology*. Nov 2004;150(Pt 11):3571–90.
13. Wang X, Gao H, Shen Y, Weinstock GM, Zhou J, Palzkill T. A high-throughput percentage-of-binding strategy to measure binding energies in DNA-protein interactions: application to genome-scale site discovery. *Nucleic Acids Res*. Sep 2008;36(15):4863–71.
14. Das D, Pellegrini M, Gray JW. A primer on regression methods for decoding cis-regulatory logic. *PLoS Comput Biol*. Jan 2009;5(1):e1000269.
15. Balleza E, Lopez-Bojorquez LN, Martinez-Antonio A, et al. Regulation by transcription factors in bacteria: beyond description. *FEMS Microbiol Rev*. Jan 2009;33(1):133–51.
16. Babu MM, Lang B, Aravind L. Methods to reconstruct and compare transcriptional regulatory networks. *Methods Mol Biol*. 2009;541:163–80.
17. Makarova KS, Mironov AA, Gelfand MS. Conservation of the binding site for the arginine repressor in all bacterial lineages. *Genome Biol*. 2001;2(4): RESEARCH0013.
18. Panina EM, Mironov AA, Gelfand MS. Comparative genomics of bacterial zinc regulons: enhanced ion transport, pathogenesis, and rearrangement of ribosomal proteins. *Proc Natl Acad Sci U S A*. Aug 19, 2003;100(17): 9912–7.
19. Tan K, Moreno-Hagelsieb G, Collado-Vides J, Stormo GD. A comparative genomics approach to prediction of new members of regulons. *Genome Res*. Apr 2001;11(4):566–84.
20. Siddharthan R, Siggia ED, van Nimwegen E. PhyloGibbs: a Gibbs sampling motif finder that incorporates phylogeny. *PLoS Comput Biol*. Dec 2005; 1(7):e67.
21. Blanchette M, Schwikowski B, Tompa M. Algorithms for phylogenetic footprinting. *J Comput Biol*. 2002;9(2):211–23.
22. Erill I, Escribano M, Campoy S, Barbe J. In silico analysis reveals substantial variability in the gene contents of the gamma proteobacteria LexA-regulon. *Bioinformatics*. Nov 22, 2003;19(17):2225–36.
23. Shen-Orr SS, Milo R, Mangan S, Alon U. Network motifs in the transcriptional regulation network of Escherichia coli. *Nat Genet*. May 2002;31(1): 64–8.
24. Alon U. Network motifs: theory and experimental approaches. *Nat Rev Genet*. 2007;8(6):450–61.
25. Stekel DJ, Jenkins DJ. Strong negative self regulation of prokaryotic transcription factors increases the intrinsic noise of protein expression. *BMC Syst Biol*. 2008;2:6.
26. Walker GC. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. *Microbiol Rev*. Mar 1984;48(1):60–93.
27. Erill I, Campoy S, Barbe J. Aeons of distress: an evolutionary perspective on the bacterial SOS response. *FEMS Microbiol Rev*. Nov 2007;31(6): 637–56.
28. Fernandez De Henestrosa AR, Ogi T, Aoyagi S, et al. Identification of additional genes belonging to the LexA regulon in *Escherichia coli*. *Mol Microbiol*. Mar 2000;35(6):1560–72.
29. Sassanfar M, Roberts JW. Nature of the SOS-inducing signal in *Escherichia coli*. The involvement of DNA replication. *J Mol Biol*. Mar 5, 1990;212(1): 79–96.
30. Little JW. Mechanism of specific LexA cleavage: autodigestion and the role of RecA coprotease. *Biochimie*. Apr 1991;73(4):411–21.
31. Erill I, Campoy S, Mazon G, Barbe J. Dispersal and regulation of an adaptive mutagenesis cassette in the bacteria domain. *Nucleic Acids Res*. 2006;34(1):66–77.
32. Jochmann N, Kurze A-K, Czaja LF, et al. Genetic makeup of the Corynebacterium glutamicum LexA regulon deduced from comparative transcriptomics and in vitro DNA band shift assays. *Microbiology*. 2009; 155(5):1459–77.
33. van der Veen S, van Schalkwijk S, Molenaar D, de Vos WM, Abee T, Wells-Bennik MHJ. The SOS response of Listeria monocytogenes is involved in stress resistance and mutagenesis. *Microbiology*. 2010;156(2):374–84.
34. Au N, Kuester-Schoeck E, Mandava V, et al. Genetic composition of the *Bacillus subtilis* SOS system. *J Bacteriol*. Nov 2005;187(22):7655–66.

35. Davis EO, Dullaghan EM, Rand L. Definition of the mycobacterial SOS box and use to identify LexA-regulated genes in *Mycobacterium tuberculosis*. *J Bacteriol*. Jun 2002;184(12):3287–95.
36. Altschul SF, Madden TL, Schaffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. Sep 1, 1997;25(17):3389–402.
37. Fuchsman CA, Rocap G. Whole-genome reciprocal BLAST analysis reveals that planctomycetes do not share an unusually large number of genes with Eukarya and Archaea. *Appl Environ Microbiol*. Oct 2006;72(10):6841–4.
38. Markowitz VM, Korzeniewski F, Palaniappan K, et al. The integrated microbial genomes (IMG) system. *Nucleic Acids Research*. 2006; 34(Suppl 1):D344–8.
39. Huerta AM, Collado-Vides J. Sigma70 promoters in Escherichia coli: specific transcription in dense regions of overlapping promoter-like signals. *J Mol Biol*. Oct 17, 2003;333(2):261–78.
40. Wunderlich Z, Mirny LA. Different gene regulation strategies revealed by analysis of binding motifs. *Trends Genet*. Oct 2009;25(10):434–40.
41. Barnard A, Wolfe A, Busby S. Regulation at complex bacterial promoters: how bacteria use different promoter organizations to produce different regulatory outcomes. *Curr Opin Microbiol*. Apr 2004;7(2):102–8.
42. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*. Nov 11, 1994;22(22):4673–80.
43. Bhargava N, Erill I. xFITOM: a generic GUI tool to search for transcription factor binding sites. *Bioinformation*. 2010;5(2):49–50.
44. Schneider TD. Information Content of Individual Genetic Sequences. *Journal of Theoretical Biology*. 1997;189(4):427–41.
45. Bailey TL, Elkan C. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proc Int Conf Intell Syst Mol Biol*. 1994;2: 28–36.
46. Abella M, Campoy S, Erill I, Rojo F, Barbe J. Cohabitation of two different *lexA* regulons in *Pseudomonas putida*. *J Bacteriol*. Dec 2007;189(24): 8855–62.
47. Novichkov PS, Rodionov DA, Stavrovskaya ED, et al. RegPredict: an integrated system for regulon inference in prokaryotes by comparative genomics approach. *Nucleic Acids Res*. Jul 2010;38(Web Server issue): W299–307.
48. Neph S, Tompa M. MicroFootPrinter: a tool for phylogenetic footprinting in prokaryotic genomes. *Nucleic Acids Res*. Jul 1, 2006;34(Web Server issue): W366–8.
49. Pittard AJ, Davidson BE. TyrR protein of Escherichia coli and its role as repressor and activator. *Mol Microbiol*. Jul 1991;5(7):1585–92.
50. Brekasis D, Paget MS. A novel sensor of NADH/NAD+ redox poise in Streptomyces coelicolor A3(2). *Embo J*. Sep 15, 2003;22(18):4856–65.
51. Reischl S, Wiegert T, Schumann W. Isolation and analysis of mutant alleles of the Bacillus subtilis HrcA repressor with reduced dependency on GroE function. *J Biol Chem*. Sep 6, 2002;277(36):32659–67.
52. Galhardo RS, Do R, Yamada M, et al. DinB upregulation is the sole role of the SOS response in stress-induced mutagenesis in Escherichia coli. *Genetics*. May 2009;182(1):55–68.
53. Paes da Rocha R, Paquola AC, Marques M do V, Menck CF, Galhardo RS. Characterization of the SOS regulon of *Caulobacter crescentus*. *J Bacteriol*. 2008;190:1209–18.
54. Boshoff HI, Reed MB, Barry CE 3rd, Mizrahi V. DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in Mycobacterium tuberculosis. *Cell*. Apr 18, 2003;113(2):183–93.
55. Cirz RT, Jones MB, Gingles NA, et al. Complete and SOS-mediated response of *Staphylococcus aureus* to the antibiotic ciprofloxacin. *J Bacteriol*. Jan 2007;189(2):531–9.
56. Galhardo RS, Rocha RP, Marques MV, Menck CF. An SOS-regulated operon involved in damage-inducible mutagenesis in *Caulobacter crescentus*. *Nucleic Acids Res*. 2005;33(8):2603–14.
57. Rand L, Hinds J, Springer B, Sander P, Buxton RS, Davis EO. The majority of inducible DNA repair genes in *Mycobacterium tuberculosis* are induced independently of RecA. *Mol Microbiol*. Nov 2003;50(3):1031–42.
58. Yang M, Gao C, Cui T, An J, He ZG. A TetR-like regulator broadly affects the expressions of diverse genes in Mycobacterium smegmatis. *Nucleic Acids Res*. Oct 5, 2011. [Epub ahead of print.]
59. Bi E, Lutkenhaus J. Cell division inhibitors SulA and MinCD prevent formation of the FtsZ ring. *J Bacteriol*. Feb 1993;175(4):1118–25.
60. Kawai Y, Moriya S, Ogasawara N. Identification of a protein, YneA, responsible for cell division suppression during the SOS response in *Bacillus subtilis*. *Mol Microbiol*. Feb 2003;47(4):1113–22.
61. Ogino H, Teramoto H, Inui M, Yukawa H. DivS, a novel SOS-inducible cell-division suppressor in Corynebacterium glutamicum. *Mol Microbiol*. Feb 2008;67(3):597–608.
62. Chauhan A, Lofton H, Maloney E, et al. Interference of Mycobacterium tuberculosis cell division by Rv2719c, a cell wall hydrolase. *Mol Microbiol*. Oct 2006;62(1):132–47.
63. Mo AH, Burkholder WF. YneA, an SOS-induced inhibitor of cell division in Bacillus subtilis, is regulated posttranslationally and requires the transmembrane region for activity. *J Bacteriol*. Jun 2010;192(12):3159–73.

Supplementary Data

Supplementary data 1

Representative genomes from Gram-positive species selected to identify *lexA* homologs. The IMG taxon ID, species name and genome status are provided for each genome.

Supplementary data 2

Homologs of *lexA* identified through best-reciprocal BLAST hit in the selected species. The table displays basic gene and protein information, as well as the –250 bp upstream sequence for each gene.

Supplementary data 3

Text file containing the collection of 71 LexA-binding sites generated by expansion of the initial MEME motif using site search on the upstream region of indentified *lexA* homologs.

Supplementary data 4

Table of putative LexA-binding sites located in the promoter region of the 58 *lexA* upstream regions. Multiple instances of weak (from 3 down to 6 standard deviations below the mean; 11.37–5.45 bits) sites for a single promoter are highlighted.

Supplementary data 5

Tables summarizing five independent MEME motif discovery runs on randomly sampled subsets of the collection of 58 *lexA* homologues upstream sequences (Supplementary data 2), for decreasing subset sizes of 48, 24, 12, 6 and 3 sequences.

Supplementary data 6

Tables summarizing five independent PhyloGibbs motif discovery runs on randomly sampled subsets of the collection of 58 *lexA* homologues upstream sequences (Supplementary data 2), for decreasing subset sizes of 48, 24, 12, 6 and 3 sequences.

Supplementary data 7

Results for MEME motif discovery on homologues of the TyrR repressor in the Gamma-Proteobacteria. The selected list of species and upstream regions for motif discovery are provided together with the three best results of the motif discovery process.

Supplementary data 8

Results for MEME motif discovery on homologues of the Rex repressor in the Actinobacteria. The selected list of species and upstream regions for motif discovery are provided together with the three best results of the motif discovery process.

Supplementary data 9

Results for MEME motif discovery on homologues of the HrcA repressor in the Firmicutes. The selected list of species and upstream regions for motif discovery are provided together with the three best results of the motif discovery process.

Supplementary data 10

ROC curves for search efficiency with experimentally-validated and MEME-derived collections on four different genomes corresponding to the Firmicutes (left) and Actinobacteria (right). Sensitivity corresponds to the fraction of experimentally validated binding sites detected by the search algorithm. Specificity is the fraction of the rest of genomic positions reported by the search algorithm.

Supplementary data 11

Table of indentified LexA-binding sites. The regulated gene name, product and GenBank locus number, as well the score, sequence, strand and distance to the gene translation start site are provided for all identified LexA-binding sites.

Supplementary data 12

Results table for the comparative genomics analysis of the LexA regulon in Gram-positive bacteria with the RegPredict service, using the expanded MEME collection as the search motif and otherwise default parameters. Accession numbers indicate the presence of homologs in each species. Predicted LexA regulation is indicated colored boxes, which denote, respectively, the presence of a strong, weak or multiple LexA site, or regulation inferred from predicted membership in a regulated operon. Empty cells indicate that a given gene is absent from a particular genome. Species abbreviations are as follows: Bsu—*B. subtilis*, Cac—*C. acetobutylicum*, Efa—*E. faecalis*, Lmo—*L. monocytogenes*, Sau—*S. aureus*, Ace—*A. cellulolyticus*, Cgl—*C. glutamicum*, Lxy—*L. xyli*, Mtu—*M. tuberculosis*, Nfa—*N. farcinica* and Sgr—*S. griseus*. Subsequent pages provide details concerning the regulated genes and the identified sites.