

Genomic Arrangement of Regulons in Bacterial Genomes

Han Zhang^{1,2,9}, Yanbin Yin^{1,3,9}, Victor Olman¹, Ying Xu^{1,3,4*}

1 Computational Systems Biology Laboratory, Department of Biochemistry and Molecular Biology and Institute of Bioinformatics, University of Georgia, Athens, Georgia, United States of America, **2** Department of Automation and Intelligent Science, College of Information Technical Science, Nankai University, Tianjin, China, **3** BioEnergy Science Center, United States of America, **4** College of Computer Science and Technology, Jilin University, Changchun, Jilin, China

Abstract

Regulons, as groups of transcriptionally co-regulated operons, are the basic units of cellular response systems in bacterial cells. While the concept has been long and widely used in bacterial studies since it was first proposed in 1964, very little is known about how its component operons are arranged in a bacterial genome. We present a computational study to elucidate the organizational principles of regulons in a bacterial genome, based on the experimentally validated regulons of *E. coli* and *B. subtilis*. Our results indicate that (1) genomic locations of transcriptional factors (TFs) are under stronger evolutionary constraints than those of the operons they regulate so changing a TF's genomic location will have larger impact to the bacterium than changing the genomic position of any of its target operons; (2) operons of regulons are generally not uniformly distributed in the genome but tend to form a few closely located clusters, which generally consist of genes working in the same metabolic pathways; and (3) the global arrangement of the component operons of all the regulons in a genome tends to minimize a simple scoring function, indicating that the global arrangement of regulons follows simple organizational principles.

Citation: Zhang H, Yin Y, Olman V, Xu Y (2012) Genomic Arrangement of Regulons in Bacterial Genomes. PLoS ONE 7(1): e29496. doi:10.1371/journal.pone.0029496

Editor: Jonathan H. Badger, J. Craig Venter Institute, United States of America

Received: October 5, 2011; **Accepted:** November 29, 2011; **Published:** January 3, 2012

Copyright: © 2012 Zhang et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: DOE of the US, BioEnergy Science Center grant (DE-PS02-06ER64304) NSF of the US, DEB-0830024. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: xyn@bmb.uga.edu

⁹ These authors contributed equally to this work.

Introduction

Regulons are the basic units of cellular response systems in bacterial cells, and represent a most basic concept in bacterial studies. A bacterial *regulon* is a group of operons that are transcriptionally co-regulated by the same regulatory machinery, consisting of *trans* regulators (transcription factors or simply TFs) and *cis* regulatory binding elements in the promoters of the operons they regulate. Operationally, a regulon contains operons regulated by one same transcription factor. Since the term *regulon* was first proposed in 1964 [1], 173 regulons have been fully or partially identified in *E. coli* K12 [2] and numerous more in other bacteria e.g. *B. subtilis*. Loosely speaking, regulons can be categorized into two classes: local and global regulons, with the former corresponding to regulons consisting of only a few component operons and the latter having a relatively large number of operons [3]. While the functionalities of the known regulons have been well studied, very little is known about how regulons are organized in a bacterial genome. The only published related work is by Janga *et al.* [4], which found that for small regulons, TFs tend to be located close to their targets (TGs).

We present a computational study here to elucidate the organizational principles of regulons in a bacterial genome. We have carried out our study on *E. coli* K12 and also on *B. subtilis* str. 168 to demonstrate the generality of the results. Our key findings are (1) operons of each regulon tend to form a few closely located clusters along with genome; (2) TFs are under stronger evolutionary constraints than their TGs; and (3) the global

arrangement of the component operons of all the (known) regulons in a genome tend to minimize a simple scoring function.

Results and Discussion

We have examined all the 3,684 regulatory relationships between TFs and their TGs in RegulonDB [2], involving 173 TFs and 729 TGs forming 173 regulons. We assigned genes to operons based on the operon information in the DOOR database (14) (<http://csbl1.bmb.uga.edu/OperonDB>) of *E. coli* K12. Among these regulatory relationships, 105 TFs are self-regulated; 123 (71%) of the 173 TFs regulate more than one TG; 411 (56%) of the 729 TGs are regulated by more than one TF; and 131 (18%) TGs are also TFs so they are regulated by upper-stream TFs while regulating downstream targets in the global transcription regulation network.

Operons in a regulon tend to form clusters in terms of their genomic locations

Intuitively we would expect that operons in a regulon should stay close to each other in a genome to facilitate efficient co-regulation, which was used earlier to explain the formation of operons [5]. To test if this is indeed the case, we examined the distribution of the distances between two neighboring operons within a regulon, measured as the (smallest) number of operons between the two operons (we do not consider the orientations of operons). We noted that 523 (32%) of the 1,624 such distances in the 173 regulons are less than two (and 47% less than 10), as

shown in Figure 1A, suggesting that the component operons in a regulon tend to cluster together, although they may form multiple clusters. This remains to be true for all large regulons, which are defined as regulons with more than 5 component operons in this study. For example, *crp*, the largest regulon in *E. coli*, consists of 230 operons, 86 of which (37%) have distances less than two. As a control, we have checked a similar distance distribution calculated over 173 artificial regulons by randomly grouping *E. coli* K12 operons. Figure 1B shows the distance distribution, which is clearly very different from the one in Figure 1A. Similar observations were made when studying the regulons of *B. subtilis* (Figure 1C and 1D).

We hypothesize that operons in each cluster of each regulon encode enzymes participating in the same metabolic pathway. To check for this, we consider a (maximal) list of operons of a regulon forms an *operon cluster* if the maximum distance between each pair of neighboring operons in this list is less than five. Using this definition, we obtained 353 operon clusters (85% from large regulons with size >5 as defined above), 242 of which each has at least two operons mapped to some SEED metabolic pathways

(<http://www.theseed.org/>) [6]. Among them, 191 (79%) clusters have at least two operons participating in the same SEED pathway. Interestingly among these 191 clusters, 158 (83%) have all their mapped operons participating in the same SEED pathways. We noted that all these results do not change substantially when we adjust the distance cutoff from 5 to any integer between 2 and 7 when defining an operon cluster (Table S1). This suggests that each operon cluster generally consists of genes working in the same metabolic pathways. Similar observation (Table S2) was made when studying the *B. subtilis* regulons.

Genomic locations of TFs are under stronger constraints than those of TGs

It has been observed that small regulons tend to have their TFs located close to their TGs [4], suggesting that the genomic location of a TF is under strong constraints from its TGs (meaning TG's locations). A natural question is if a TG is under strong constraints from its TFs. We note that 56% TGs are regulated by more than one TF in *E. coli* K12. From Figure 2A, we conclude that there is no significant difference (Wilcoxon test P -value = 0.31) between

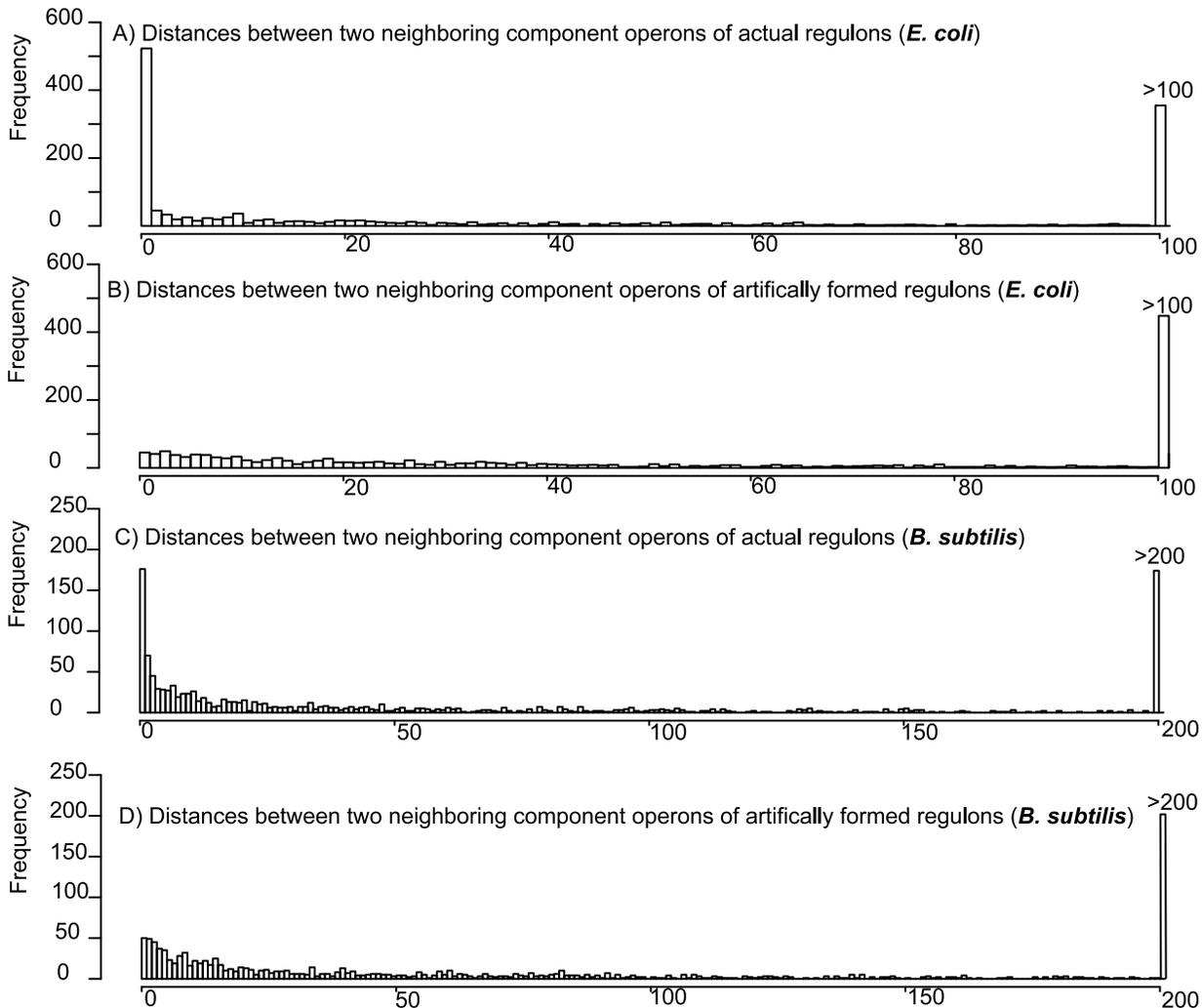


Figure 1. Component operons of regulons tend to be clustered. The distance between two neighboring operons within a regulon is defined as the number of operons between the two operons. The bin width of the histogram is 1. (C) and (D) are similar to (A) and (B), respectively, but are for *B. subtilis*.

doi:10.1371/journal.pone.0029496.g001

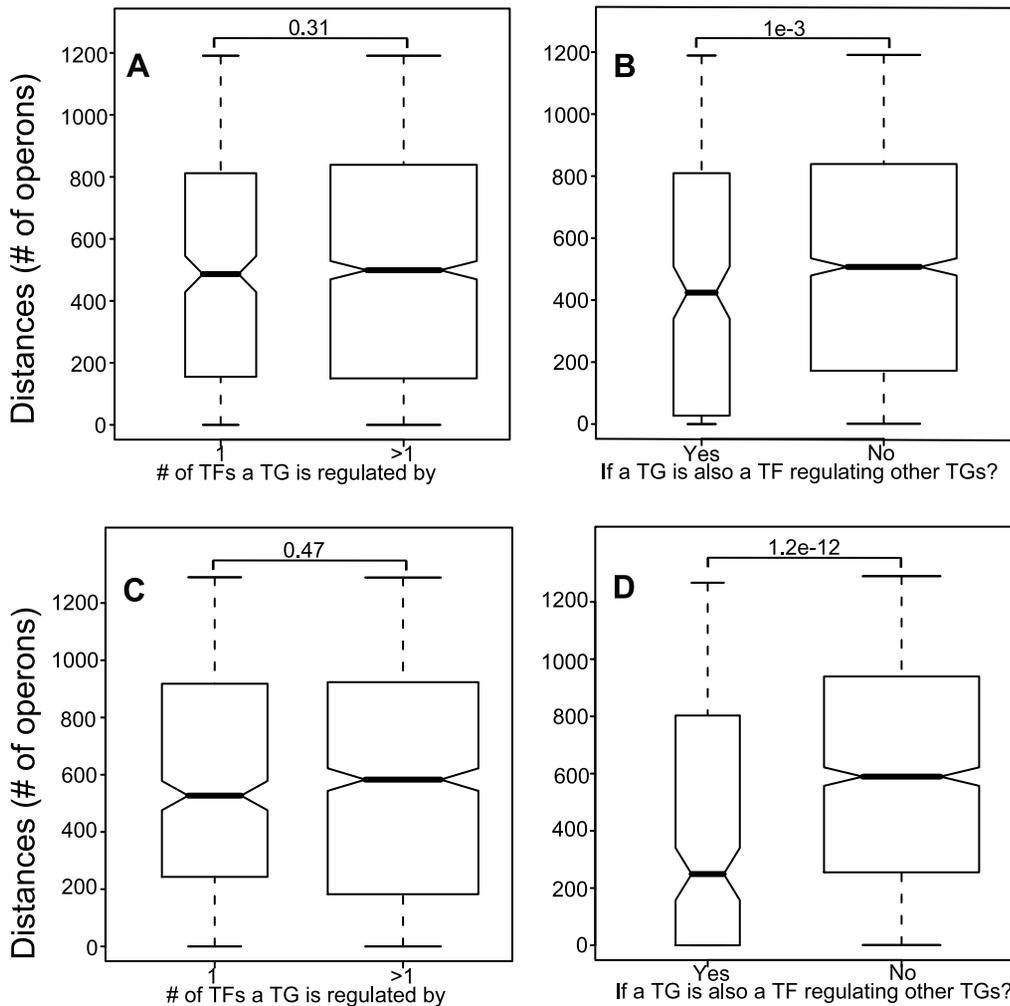


Figure 2. TFs are under stronger constraints than TGs. (A) All TGs are categorized into two groups, TGs regulated by one TF and TGs regulated by multiple TFs. The distributions of the distances (y axis) from TGs to their TFs are shown as box-plots. (B) All TGs are categorized into two groups, TGs that regulate other TGs, and TGs that do not regulate other TGs. The distributions of the distances (y axis) from TGs to their upstream TFs are shown as box-plots. Throughout this paper, the distance between a TF-TG pair is defined as the number of operons between the two operons. (C) and (D) are similar to (A) and (B), respectively, but are for *B. subtilis*. P-values of Wilcoxon tests are shown between two neighboring boxes. doi:10.1371/journal.pone.0029496.g002

TGs regulated by one regulator and those regulated by multiple regulators in terms of the distances to their TFs, meaning that an operon does not have to stay close to its regulator even if it is only controlled by one regulator. This is not surprising because the regulator may control many targets, some of which might be close to the regulator while others may not. This finding is opposed to what we found for TFs controlling a small number of operons (Figure S1), suggesting that the genomic locations of *TGs* are generally under less constraints than those of *TFs*.

To further study this, we divided all TGs into two groups, TGs that are also TFs regulating downstream TGs and TGs that are not TFs. Figure 2B shows that the first group of TGs tends to locate significantly closer (Wilcoxon test P -value = $1e-3$) to their regulators than the second group, directly suggesting that TGs are under stronger constraints than ordinary TGs from their upstream regulators if they are TFs themselves controlling further downstream targets. This is possibly due to the need for such TGs to have faster reaction time to send the regulatory signal down across the regulatory network.

We have also performed all the analyses for *B. subtilis* str. 168 using data from DBTBS [7] database. The results are shown in Figure 2C and 2D and remain as significant as observed in *E. coli*, strongly suggesting that the observations made above are independent of which bacteria we use and hence may apply to all bacterial organisms in general.

Global genomic arrangement of regulons

All these observations led to our main hypothesis that the genomic locations of the component operons of all the regulons encoded in a genome are determined by some global organizational principle. Specifically we hypothesize that the global genomic arrangement of regulons tends to minimize the following function based on our preliminary study:

$$D = \sum_{i=1}^N d_i$$

where N is the total number of regulons encoded in a genome and d_i represents the total distance between the genomic location of the TF and all the TGs of the i^{th} regulon. Note that a similar formula has been used in our recent study on the genomic arrangements of metabolic pathways [8].

We have used the following procedure to demonstrate that the D value of all the known regulons encoded in the *E. coli* genome is significantly smaller than those of the vast majority of alternatively arranged genomes. Specifically, we have considered one million permutations of the genomic locations of $X\%$ of operons (both TFs and TGs) of *E. coli* K-12, for $X=10, 20, \dots, 100$ (see **Methods**). Figure 3A shows the D value distributions for different percentages of reshuffled locations of operons for the *E. coli*

genome. We can clearly see that the current genomic arrangement of operons of *E. coli* K12 has a lower D value (the vertical dash line) than the vast majority of the D values of the reshuffled genomes, which is also supported by statistical tests (all P-values < 0.05 , see Table S3). It is also interesting to see that reshuffling TFs increases D values considerably more than reshuffling TGs (Figure 3B, P-value < 0.05), consistent with our observation made based on Figure 2 that TFs are under stronger constraints than TGs in terms of their genomic locations.

For each known regulon in *E. coli* K12, we have also arbitrarily selected the same number of operons from the pool of all operons covered by the known regulons to form an artificial regulon, and do this for every known regulon. Again, we see the D value of the

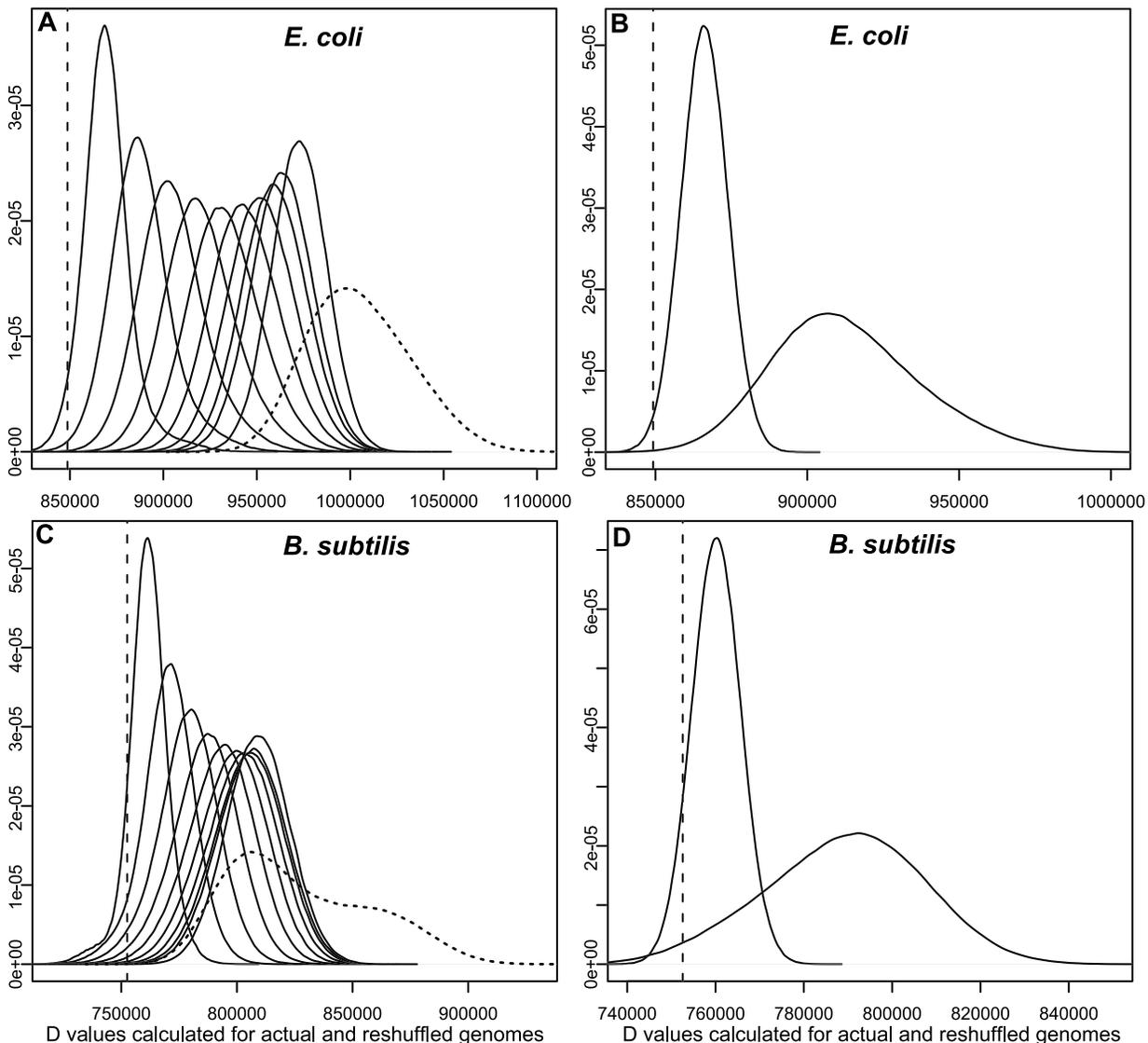


Figure 3. The distributions of D values calculated for the actual and reshuffled genomes. The x-axis represents the D values, and the y-axis is the frequency (density). In (A) each curve is calculated using one million permutations of the current arrangement of the operons in a genome under a specified constraint. Ten D distributions are calculated, with each distribution calculated allowing $X\%$ of operons randomly selected among all the operons under consideration and being randomly permuted, with $X=10, 20, \dots, 100$, respectively, where the ten curves from left to right are consistent with the order of X . The vertical dash line shows the D value for the current arrangement of the operons in the *E. coli* K12 genome. We also conducted permutations using a second manner, i.e. artificially forming regulons and calculating D values for permuted genomes. The result is shown as a dotted curve. (B) A comparison between the D distributions when randomly permuting 100 TGs (curve on the left) versus randomly permuting 100 TFs (curve on the right) in the genome of *E. coli* K-12. (C) and (D) are similar to (A) and (B), respectively, but are for *B. subtilis*. doi:10.1371/journal.pone.0029496.g003

real genome (vertical dashed line) is significantly smaller than those of genomes with artificially formed regulons (the dotted curve in Figure 3A).

To ensure that our observations hold for other bacterial genomes in general, we have checked the observation made in this section on all the 160 known regulons of *B. subtilis*, using the same procedure on *E. coli* genome and the results are as shown in Figure 3C and 3D (see also Table S3), which are clearly highly similar to those shown in Figure 3A and 3B.

This work presents a systematic study of the genomic arrangement of regulons in terms of their organization in a bacterial genome. We made a number of interesting observations related to the organizational principles of regulons in a bacterial genome, namely (1) transcription factors of regulons are under strong constraints from their regulatory targets while TGs do not seem to be under strong constraints from their TFs; (2) regulons tend to form operon clusters, each of which tend to consist of operons encoding the same metabolic pathway; and (3) the genome tends to minimize the overall distance between the TFs and their TGs across all regulons encoded in the genome. We believe that all the observations are mostly due to the need by the cell to efficiently transcribe the relevant genes. Janga et al suggested that TFs of large regulons usually have high expression levels and presumably get to their targets through diffusion, and this might be the reason that they do not need to locate close to their targets. For small regulons, TFs are simply located closely to their targets which should be evolutionarily favored. For larger regulons consisting of multiply clustered operons, the three dimensional packing of the chromosome needs to be considered. It is likely that these organizational principles, along with a few others including genomic organization of metabolic pathways [8], the selfish operon model [9] and the nucleoid compaction [10,11,12,13,14], collectively determine the local and the global organization of all bacterial genes in a genome [15].

Materials and Methods

Date sources

The genome of *E. coli* K-12 MG1655 was downloaded from <ftp://ftp.ncbi.nih.gov> as of 01/14/2009. All the predicted operons for the organism were downloaded from the DOOR [16] database at <http://csbl1.bmb.uga.edu/OperonDB>. All regulons data of *E. coli* K-12 MG1655 and of *B. subtilis* str. 168 were downloaded from the RegulonDB [17] and from the DBTBS [17] database, respectively, as of 03/2010.

Operon shuffling

For each reshuffled genome, the D value defined in the formula was calculated for $X = 10, 20, \dots, 100$; X is the percentage of

operons to be reshuffled (i.e. their genomic locations are permuted). The following two-step procedure was conducted to randomly shuffle a specified fraction (X%) of operons. We first randomly select operons among all operons of the *E. coli* genome for 10,000 times and then randomly permute their locations 100 times for each specific selection of the 10,000. So we do a total of one million permutations and calculate the D value distribution over the million rearranged genomes.

Supporting Information

Figure S1 Box plots of the distance distribution of operons (TGs) to their regulators (TFs). (A) is for *E. coli* and (B) is for *B. subtilis*. P-values of Wilcoxon tests are shown between two neighboring boxes. (EPS)

Table S1 The number of operon clusters participating in the same SEED pathway under different distance cutoffs (*E. coli*). The first column represents the distance cutoff used to define a cluster. The second column is the number of clusters having at least two operons mapped to some SEED metabolic pathways. The third column is the number of clusters having at least two operons participating in the same SEED pathway. The fourth column is the number of clusters having all their mapped operons participating in the same SEED pathways. Regulons with at least two operons are considered. (DOC)

Table S2 The number of operon clusters participating in the same SEED pathway under different distance cutoffs (*B. subtilis*). See Table S1 legend for details. Note there are significantly less operons in *B. subtilis* than in *E. coli* that are mapped to the SEED pathways. This makes the numbers in Table S2 are much smaller than those in Table S1. (DOC)

Table S3 Statistical tests of curves in Figure 3. The ‘skewness’ and ‘kurtosis’ columns are calculated to test if the curves in Figure 3 are normal distribution. ‘skewness’ closer to 0 and ‘kurtosis’ closer to 3 indicates close to normal distribution. The ‘P-value’ column is calculated to test if the curves are significantly larger than the vertical dash line, indicating that the permuted genomes have significant larger D values than the actual genomes. (DOC)

Author Contributions

Conceived and designed the experiments: YY YX HZ. Performed the experiments: HZ YY. Analyzed the data: YY HZ. Contributed reagents/materials/analysis tools: HZ YY VO. Wrote the paper: YY YX HZ.

References

1. Maas WK (1964) Studies on the Mechanism of Repression of Arginine Biosynthesis in *Escherichia Coli*. II. Dominance of Repressibility in Diploids. *J Mol Biol* 8: 365–370.
2. Gama-Castro S, Jimenez-Jacinto V, Peralta-Gil M, Santos-Zavaleta A, Penaloza-Spinola MI, et al. (2008) RegulonDB (version 6.0): gene regulation model of *Escherichia coli* K-12 beyond transcription, active (experimental) annotated promoters and Textpresso navigation. *Nucleic Acids Res* 36: D120–124.
3. Martinez-Antonio A, Collado-Vides J (2003) Identifying global regulators in transcriptional regulatory networks in bacteria. *Curr Opin Microbiol* 6: 482–489.
4. Janga SC, Salgado H, Martinez-Antonio A (2009) Transcriptional regulation shapes the organization of genes on bacterial chromosomes. *Nucleic Acids Res* 37: 3680–3688.
5. Jacob F, Monod J (1961) Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol* 3: 318–356.
6. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang HY, et al. (2005) The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. *Nucleic Acids Res* 33: 5691–5702.
7. Sierro N, Makita Y, de Hoon M, Nakai K (2008) DBTBS: a database of transcriptional regulation in *Bacillus subtilis* containing upstream intergenic conservation information. *Nucleic Acids Res* 36: D93–96.
8. Yin Y, Zhang H, Olman V, Xu Y (2010) Genomic arrangement of bacterial operons is constrained by biological pathways encoded in the genome. *Proc Natl Acad Sci U S A* 107: 6310–6315.
9. Lawrence JG, Roth JR (1996) Selfish operons: horizontal transfer may drive the evolution of gene clusters. *Genetics* 143: 1843–1860.
10. Carpentier AS, Torresani B, Grossmann A, Henaut A (2005) Decoding the nucleoid organisation of *Bacillus subtilis* and *Escherichia coli* through gene expression data. *BMC Genomics* 6: 84.
11. Postow L, Hardy CD, Arsuaga J, Cozzarelli NR (2004) Topological domain structure of the *Escherichia coli* chromosome. *Genes Dev* 18: 1766–1779.

12. Wright MA, Kharchenko P, Church GM, Segre D (2007) Chromosomal periodicity of evolutionarily conserved gene pairs. *Proc Natl Acad Sci U S A* 104: 10559–10564.
13. Jeong KS, Ahn J, Khodursky AB (2004) Spatial patterns of transcriptional activity in the chromosome of *Escherichia coli*. *Genome Biol* 5: R86.
14. Allen TE, Price ND, Joyce AR, Palsson BO (2006) Long-range periodic patterns in microbial genomes indicate significant multi-scale chromosomal organization. *PLoS Comput Biol* 2: e2.
15. Rocha EP (2008) The organization of the bacterial genome. *Annu Rev Genet* 42: 211–233.
16. Mao F, Dam P, Chou J, Olman V, Xu Y (2009) DOOR: a database for prokaryotic operons. *Nucleic Acids Res* 37: D459–463.
17. Salgado H, Gama-Castro S, Peralta-Gil M, Diaz-Peredo E, Sanchez-Solano F, et al. (2006) RegulonDB (version 5.0): *Escherichia coli* K-12 transcriptional regulatory network, operon organization, and growth conditions. *Nucleic Acids Res* 34: D394–397.