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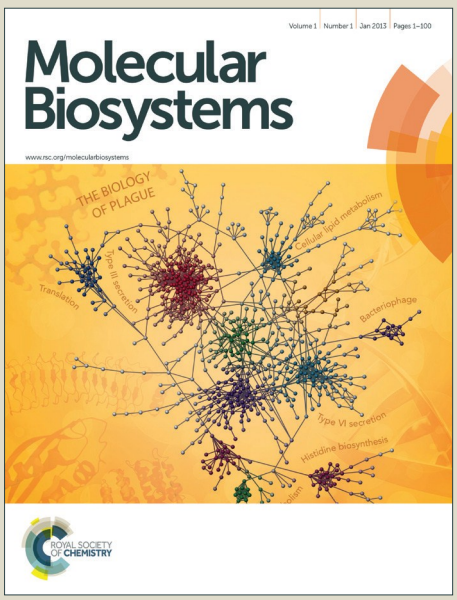

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Revisiting Demand Rules for Gene Regulation

Mahendra Kumar Prajapat**, Kirti Jain**, Debika Choudhury, Nikhil Raj, & Supreet Saini*

Department of Chemical Engineering

Indian Institute of Technology Bombay, Mumbai - 400076, India

*Corresponding Author Email: saini@che.iitb.ac.in, Phone: 91 22 25767216, Fax: 91 22 2572 6895

** Authors contributed equally to the work.

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Abstract

Starting with Savageau's pioneering work regarding demand rules for gene regulation from 1970s, here, we choose the simplest transcription network and ask: How does the cell choose a particular regulatory topology from all available possibilities? According to the demand rules, a cell chooses an activator based regulation of a target if the target protein is required for most of the time. On the other hand, if the target protein is only required sporadically, its control tends to be via a repressor-based regulatory topology. We study the natural distribution of topologies at genome, systems, and micro-level in *E. coli* and observe deviations from demand rules. Analyzing regulation of amino acid biosynthesis, transport, and carbon utilization in *E. coli* and *B. subtilis*, and comparing choice of topology with demand, we observe an alternate pattern emerging. Simulations of networks are used to help explain the natural distribution of topologies in nature. Overall, our results indicate that choice of topology is drawn randomly from a pool of all networks which satisfy the dynamic requirements of the cell, as dictated by physiology. In short, our results suggest that the cell picks "whatever works".

Introduction

A critical feature of all living organisms is the ability to tune behavior in response to stimuli.[1-5] The most widespread and well-understood mode of this tuning is transcription, which enables cells to modulate gene expression in response to cues. Looking at the simplest transcription network, where a regulator R, in presence or absence of signal, controls expression of a target T - different possibilities emerge. Control of the target might be via positive or negative regulation. When we consider the fact that most transcription factors in *E. coli* are also auto-regulators, six possible topologies emerge (Figure 1).[3, 4, 6-10] In this study, we seek to answer the following question: Among all the available regulatory designs, how does a cell pick one to control target expression?

In a series of papers in the 1970s, Savageau proposed "demand rules for gene regulation",[11-16] according to which, a target T is positively regulated (Figure 1A-C) if, in the organism's natural habitat, T is required for a high fraction of time. On the other hand, if the target is only required sporadically, it tends to be regulated negatively (Figure 1D-F).[12, 13] Evidence for demand rules was provided as conformity in regulation of sugar utilization enzymes in *E. coli* with the demand rules.[11, 12] In 2006, Alon et. al. provided a functional explanation for demand rules.[17] They argued that positive regulation for a frequently needed target T ensured erroneous binding of other transcription factors to the promoter was minimized. Alon et. al. demonstrate and propose, in a later report,[18] that such an approach for gene regulation acts as an insulator of the promoter regions, preventing erroneous transcription.

However, demand rules raise a few interesting questions. Active control, as proposed by the demand rules, will increase the demand of regulators in the cell. The cost associated with production of additional regulators for control is likely detrimental for cellular growth.[19-21] In addition, demand rules seem contrary to the concept of genetic robustness, which focuses on loss of fitness due to mutations acquired by an individual.[22] How then do we reconcile these seemingly opposite logics? In a 2009 report, Hwa et. al. have, via a theoretical framework, demonstrated that the choice of mode of gene regulation could be biased for or against demand rules, and is dictated by population size and the time scale of environmental evolution.[23] Their framework remains to be experimentally tested though. An alternate approach can be to examine response of different topologies to cues. The response can be quantified in terms of parameters like time of response, response to noise, and cost of control.[3, 24-28] However, questions like whether, over physiologically relevant range of

biochemical parameter values, there are inherent qualitative differences in the response that can be generated by different topologies remain unanswered.

In this work, we perform simulations of the simplest transcriptional network (Figure 1), and compare our results with the natural distribution of regulatory interactions among topologies in *E. coli*. We revisit some of the results proposed by Savageau and study in detail the control of sugar utilization and amino acid biosynthesis & transport in *E. coli.* Finally, we characterize the role of control cost in dictating fitness of a cell. Put together, our results indicate several deviations from demand rules exist. Our results indicate that choice of a topology for gene expression control is likely chosen randomly from all available topologies which satisfy the dynamic demands of physiology associated with a particular cellular function.

Experimental Procedure and Mathematical Analysis

Regulatory interactions in E. coli

The Regulator-Target (R-T) interaction dataset for the transcriptional regulatory networks was acquired from RegulonDB.[29] There are 197 transcription factors reported in RegulonDB, of which, seven are listed twice, individually, as well as in dimeric form with another protein. We have considered 190 unique regulators for our study, and their interactions with targets have been classified in two ways; (i) between regulator protein and target gene and (ii) between regulator protein and promoter (all genes in an operon). This resulted in 4970 interactions in Regulator-Target gene classification (Sheet S1 in Excel) and 2139 interactions in Regulator-Promoter classification (Sheet S2 in Excel). Out of 190 transcription factors, seven are global regulators (CRP, H-NS, Lrp, IHF, ArcA, Fis, and FNR) and control around 51% of all genes in *E. coli*.[30] Excluding interactions of global regulators, there are 2625 interactions in regulator-target gene class and 1176 interactions in regulator-promoter class. Multiple transcription factors feeding into a promoter were categorized into more than one topology, depending on the nature of interaction of the target gene with each interacting transcription factor.

Distribution of R-T interactions among six topologies

On the basis of the specific roles in cellular physiology, the interactions were further classified into six functional sub-groups, as reported in *EcoCyc*.[31] For each functional sub-group, all the involved target genes were identified and distributed among six topologies. Biosynthesis pathways for amino acids and degradation pathways for carbohydrate were studied further in detail.

Biosynthesis and transport pathways of amino acids: For biosynthesis of an amino acid, we considered regulation of only target genes, which play a role in biosynthesis (Sheet S3 in Excel) of that particular amino acid and its transport (Sheet S4 in Excel) only.

Frequency of occurrence of amino acids: Frequency of each of the 20 amino acids from coding region of *E. coli* DH10β genome was calculated to estimate the relative demand of all amino acids in *E. coli* (Sheet S5 in Excel). The availability in the intestine of an amino acid was normalized by its demand (as per the relative expression of proteins in *E. coli*) to get estimate of the real availability of an amino acid.

Sugar utilization and transport: Genes encoding for enzymes involved in metabolism of a particular carbon source until the metabolic branch merges with another in the network were considered in our analysis (Sheet S6 in Excel). This was done to ensure that regulation of only those enzymes which are exclusively involved in utilization of a particular carbon source are analyzed. The interactions between the identified genes and their regulators (R-T) have been distributed across the defined topologies. In addition, genes involved in transport of sugars were also analysed in same way (Sheet S7 in Excel).

Mathematical analysis of the six topologies

Mathematical model for each topology was formulated by writing ordinary differential equations, and simulating deterministically. The three differential equations for each topology describe the rate of change in inactive regulator, R, the rate of change in active regulator, R* via signal and the rate of change in target, T, as described in Supplement text.

Definition of parameter space. To analyze the six topologies, networks were generated with different sets of biochemical parameters. To choose parameter values and range, physiologically observed values of all parameters was analyzed and the resultant space was called parameter space (Figure S1A). From parameter space, the red region represents commonly observed values reported in literature,[32-38] biased towards exhibiting limited diversity in dynamics. In this work, we chose the "unbiased region" (blue) from parameter space to explore all possible dynamics.[32-38]

Generation of networks from a topology. The model of topologies B, C, E, and F consists of nine parameters whereas of each from A and D consists of seven parameters. Inactive regulator, R and active regulator, R* were assumed to be degraded at equal rate. The ratio of association constant to dissociation constant of signal to regulator was 100 and assumed to be constant in all topologies. The choice of this ratio was found to be close to experimental observations in many natural systems. Additionally, in our analysis, we found that changing this parameter had the least effect on dictating performance of a network.

It was also assumed that all topologies regulate same type of target T but just differ in control mechanism. Hence, we did not vary the parameters of association and dissociation of signal to regulator and degradation of target T. We generated 14641 (11^4) networks for topology A & D by varying only four parameters and 15625 (5^6) networks for topologies B, C, E, and F by varying only six parameters. Such an approach was recently adopted by Ma and co-workers in the context of analysis of adaptation in biochemical networks.[39] We also performed simulations with log-normal parameter distributions across the range of values.

However, different parameter ranges do not qualitatively affect our analysis. We also performed the simulations by defining parameter sets using Latin Hypercube method. In addition, we performed simulations using basal expression of the target T as non-zero. The results from all these simulations are as shown in Figure S1.

Calculation of performance indicators. Each network was deterministically simulated using ODE45 in MATLAB.[40, 41] Dynamic simulation of each network in both transitions from OFF to ON and from ON to OFF state was performed. To carry the simulation steps, we first simulated each network from an initial state of no regulator or target molecules, to ON state. The steady state in the ON state was used as an initial condition to study transition from ON to OFF state. The steady state from the OFF state was then used to study transition from ON to OFF state. The dynamics of each network was recorded and performance indicators (as given below) for each network were calculated. The following five criteria were defined as indicators of performance of a network.

a. **Steady state expression of T protein** was calculated in ON and OFF state.
b. **Response time:** time (t_{50}) to reach 50% of the total change in steady state expression of T.
c. **Cost of response:** amount of R to produce or remove unit T,
d. **Switchability:** steady state T in ON condition divided by steady state T in OFF condition.
e. **Sensitivity:** how sensitive is each topology to varying signal strength? Response level at steady state and response time were calculated in switching OFF condition to ON and vice versa.

Definition of "High Performance Box". A box in the performance indicator space defining networks with robust response, low cost, and fast dynamics was named as "High Performance Box". Networks with steady state expression of T $\geq$ 1 Arbitrary Units (A.U.) in ON state, with a minimum switchability factor of 1.4; activation time (t_{50}) $\leq$ 694 (A.U.) and deactivation time $\leq$ 788 (A.U.); and cost of activation and cost of deactivation $\leq$ 2 (A.U.) were considered to define the outer edges of the "High Performance Box". Networks outside of the "Performance Box" were assumed to be more costly, exhibiting minimal expression of target protein T, or/and slow responding networks, and hence excluded from our analysis. The precise definition of the "High Performance Box" does not qualitatively change our results. As shown later in the paper, we note that on relaxing (making the "High Performance Box" bigger) or constraining (making the "High Performance Box" smaller), the relative number of parameter sets in the "High Performance Box" do not change qualitatively.

Evolutionary experiments

E. coli grown overnight in LB at 37ºC with shaking was sub-cultured (1:100) in tubes containing 1ml M9 media, 1% casamino acids and a sugar source. The tubes either contained 0.4% arabinose or 0.35% glucose with 0.05% arabinose. The cultures were grown for 24 hours at 37ºC, and propagated daily by sub-culturing 1:100 into fresh M9 media containing respective sugars for 3000 generations. The last strains from each lineage was transformed with plasmid based promoter fusions of arabinose metabolic genes (*araB* (PEC3876-98156236)), from Thermo Scientific *E. coli* promoter collection (PEC3877). Fluorescence (488/525nm) and absorbance (600nm) values were measured in a Tecan microplate reader (Infinite M200 PRO).

Results

At the genome scale, E. coli chooses topologies differentially for control of gene expression

To understand the "logic" behind choice of topology for gene expression control, we enumerated all regulatory interactions in *E. coli*, and classified them in one of the six topologies as shown in (Figure 1).[29] We note that there is no qualitative difference in the number of interactions which are controlled via positive (~49.6%) or negative regulation (~50.4%) (Figure 2A). The number of interactions belonging to topologies [A-C] or [D-F] again remain qualitatively equal to each other if we consider the regulator – operon as one interaction (instead of regulator-gene) (Figure 2B). Including global regulators (and their regulons) in the enumeration yields similar results (Figure S2(A-B)).

However, the R-T frequency distribution in *E. coli* changes qualitatively when we analyze the number of interactions in each of the six topologies in (Figure 1). As represented in (Figure 2C), among the six topologies, F is over-represented. This is followed by topologies A, B, C, and D, with no statistically significant difference between them. Last, topology E is the least represented (~5% of all interactions). We observe the same general trend when we define one interaction as regulator R controlling a promoter, instead of a gene (Figure 2D). On including the global regulators in the analysis, a slightly different picture emerges, where topologies C and F are the most represented (as most global regulators auto-regulate themselves), followed by topologies B, D, A, and E (which is again under represented) (Figure S2(C-D)).

Overall, our analysis suggests that *E. coli* prefers certain regulatory arrangements over others. What are the factors that dictate this choice? Various possibilities exist, including, demand rules,[11-16] error-minimization,[17] or minimizing cost of control.[19-21] To understand the differences between the frequencies of the six topologies, we study and analyze their distribution at two different scales. At the first level, we analyze frequency distribution of regulatory arrangements at a systems level, where a system is defined as sum of all interactions which serve the cell towards a broad common function (such as carbohydrate utilization, or stress response) . For example, all regulatory interactions which control amino acid biosynthesis were classified as a system. At the second level, we analyze in detail the demand and corresponding regulatory design at a micro level. Here by micro level, we mean, for example, analysing regulatory interactions which dictate biosynthesis of a particular amino acid (as against a general analysis of control of all topologies involved in

amino acid biosynthesis). As another example, we place all metabolism related regulatory interactions at the systems level, and regulatory interactions which define control of catabolism of a particular sugar as an example of a micro level.

Differential choice of topology at a systems scale

To analyze frequency distribution of topologies in further detail, we separated R and T interactions in *E. coli* into six functional sub-groups, namely: (a) amino acid transport and metabolism, (b) sugar metabolism and energy production, (c) coenzyme transport and metabolism, (d) inorganic transport and metabolism, (e) cell division and nucleotide metabolism, and (f) stress response (Figure 3). In almost all groups, distribution of number of interactions among A-C and D-F is statistically identical to the distribution observed at the genome scale in *E. coli*. Moreover, when analyzed individually, we note that in each of the six classifications, topology E is under-represented, and topology F is over-represented. In addition, the frequency distribution in all six groups is statistically similar to the one observed globally in *E. coli* (Figure 3A-F). We revisit the reason and nature of this distribution later in this manuscript. It is not wholly surprising that topology F is over-represented in nature. Negative auto-regulation is known to speed up response in cellular systems,[9, 25] and both topologies C and F possess that architecture. However, subtle differences exist. While topology C speeds up response when the system transitions from an OFF to an ON state, topology F speeds up cellular response in transition from ON to OFF state. Could other similar dynamic criteria explain the differential use of topologies in *E. coli*?

Revisiting Demand Rules for Gene Regulation

Next, we analyse demand rules at a micro scale in *E. coli* and *B. subtilis.* Application of demand rules require that we quantify demand for a protein in the environment that the bacterium has evolved in. We do this using two different examples, as discussed below.

In the first example, we study amino acid biosynthesis & transport in *E. coli,* primarily present in mammalian intestine. While *E. coli* has the ability to synthesize all 20 amino acids, because of their unequal presence in the intestine, not all amino acids are required equally by the bacterium.[47, 48] The demand for amino acids is further biased by the number of codons for each amino acid in the *E. coli* coding region, and the relative expression of each protein in the *E. coli* genome. To get a better estimate of the relative demand for each amino acid, we use data published by Sunney Xie and co-workers in 2009.[52] In this study, the authors report absolute levels of expression of more than a 1000 proteins in *E. coli*, thus providing data for demand for amino acids. In our analysis, we identified the biosynthesis pathway(s) which are uniquely dedicated to synthesis of a particular amino acid only,[31]

analysed regulation of each gene in the pathway(s), and classified regulation as positively or negatively regulated topologies.

Amino acid biosynthesis and transport are cellular functions with inversely related demand. For instance, if an amino acid is not present in the surroundings (resulting in low demand for transporters), the biosynthetic demand would be high, and vice versa. In (Figure 4A), the x-axis represents amino acids in increasing availability in *E. coli* habitat, while the y-axis gives the fraction of all regulatory interactions, controlling biosynthesis and transport of that amino acid, belonging to topologies A-C. Our analysis shows that regulation of both biosynthesis and transport exhibit a statistically insignificant correlation with increasing availability. This is contrary to the demand rules. Adherence to the demand rules would have meant that transporters of abundant amino acids are regulated by A-C topologies, and biosynthetic genes for such amino acids are primarily regulated by D-F topologies. The reverse would have held true for scarcely available amino acids. The same results hold on including interactions involving global regulators or when we normalize demand by including number of codons for an amino acid in our analysis (Figure S3 (A-C)). All results show statistically insignificant relationship against demand rules. Additionally, we performed a similar analysis for the soil bacterium *B. subtilis*, and found no correlation between choice of topology and availability of an amino acid in the surroundings (Figure S4 (A & C)).[49, 50]

In the second example, we focus on metabolism of sugars preferred by *E. coli* in its natural habitat.[51] Based on their abundance, we obtained the relative demand for carbohydrates in the intestine.[51] For our analysis, we only considered part of metabolism which exclusively deals with catabolism of that particular carbon source only. The genes encoding the respective enzymes and their regulators were studied, and classified into activator- or repressor-based topologies. Our results indicate that regulation of enzymes involved in carbon utilization is independent of the availability of the sugar (Figure 4B). A similar statistically insignificant result was obtained on including global regulators (Figure S3D). In case of carbon utilization, the expression of transporter genes should be positively correlated with expression of genes involved in catabolism. However, our analysis shows that the choice of topology does not seem linked with availability of the carbon source (Figures 4B and S3D). Similar analysis was performed for carbon utilization in *B. subtilis* and no statistical correlation was observed between choice of topology and demand for product of gene of interest (Figure S4 (B & D)).[49, 50] As shown in (Table 1), the Spearman correlation coefficients between demand and fraction of [A-C] topologies controlling expression show weak statistical significance, at best.

Overall, our results indicate deviations from Savageau's demand rules. Rather, looking at our results from the micro level analysis, it seems that choice of topology is made independent of demand. A major difference in our and Savageau's analysis is that we consider all regulatory interactions controlling cellular functions, while Savageau's work only accounted for the key regulator involved in a particular cellular process, for example, AraC for arabinose catabolism.[11, 13]

Speculative Evolutionary Experiment to Note Switch in Mode of Regulation

As a speculative test of the demand rules, we performed long-term experiments where we fed 0.2% arabinose as a sole carbon source to *E. coli* for 3000 generations. In parallel, we also grew *E. coli* in 0.2% glucose and 0.005% arabinose. The culture grown on arabinose had high demand for arabinose utilization genes, whereas the culture with small amounts of arabinose would only express from *araBAD* operon when out of glucose - thus creating differential demand for the *araBAD* gene products. AraC, when bound to arabinose, is known to be an activator of the *araBAD* operon. In absence of arabinose, AraC is known to be a repressor of the *araBAD* operon. This dual regulation can be observed experimentally in wild type *E. coli.* On altering demand for the *araBAD* gene products, the dual regulation can still be observed in both (with high, and low demand for *araBAD* gene products) the strains (Figure 5). In a relatively short span of 3000 generations, no switch in mode of regulation was observed, though the absolute levels of expression were different in the two strains, and had evolved from the parent wild-type *E. coli*. Our efforts in searching in literature for experimental evidence for switch in mode of regulation in response to demand were unsuccessful.

If demand rules do not explain choice of topology, then how can we understand the differences in frequency distribution of the six topologies we are analysing? To answer this, we perform simulations of the six topologies and characterize their performance as described in the next section.

Simulations to quantify performance of networks across topologies

We define a list of factors that best define performance of a regulatory circuit. These include (a) steady state target expression, (b) time of response, (c) control cost, (d) ability to be effectively switched ON and OFF, and (e) sensitivity towards environmental signal (see methods for more details). We hypothesize that these five indicators define performance of a genetic network, and hence simulate all six topologies and quantify performance. However, network dynamics are dictated by the values of the associated biochemical parameters. To account for biases introduced by parameters, we simulated about 90,000 networks (~15,000

networks in each topology). The choice of parameters for these networks was taken from a from a range. Each network was simulated using ODE45 in MATLAB, and transition from OFF to ON and ON to OFF tracked.

Because of our choice of parameters from a parameter space, many networks are "dead" (steady state target expression less than one); have infinite cost; or have physiologically unviable dynamics. Among the six topologies, topology E has the greatest percent of these "dead" networks (data not shown). For analysis, we considered only those networks which express target T and are able to effectively switch ON/OFF. In addition, we impose limits on the time of activation (& deactivation) and control cost. Placing these constraints allows us to define a "High Performance Box". In rest of the article, we only consider networks which lie within this "High Performance Box", unless otherwise stated. A frequency count of the networks with positive and negative control of T shows that the two are identical (Figure 6A), consistent with the global distribution in *E. coli* (Figure 2 (A-B)).

Frequency distribution of the six topologies shows that, just as in *E. coli* (Figure 2 (C-D)), topology E is under-represented, and topology F, most represented in the "Performance Box" (Figure 6B). Hence, our simulations suggest, and we speculate that distribution of a topology in a cell is proportional to the frequency of the topology in the "High Performance Box" (p-value = 0.276, thus rejecting the null hypothesis that the natural and "High Performance Box" distributions are statistically different). Changing the dimensions of the "High Performance Box" does not alter the frequency distribution of the topologies (Figure 7). We note, however, that there are subtle differences in the distribution of the networks among the six topologies between our computational results and the *E. coli* distribution. We hypothesize that these differences in distributions are due to the inherent differences in the dynamic features of the six topologies (Figure S5-9).

One interesting qualitative difference in the performance of the six topologies in shown in Figure S7. For all networks in “High Performance Box”, change in steady state target response (∆Target) in both switching ON (blue) and OFF (red) against change in amount of total regulators (∆Regulator) at steady state were plotted. Total regulators include both active and inactive form of regulator. Positive value of ∆Target indicates the switching of a network from OFF to ON condition whereas negative value indicates switching from ON to OFF condition. Positive value of ∆Regulator indicates that a particular switching needs production of more regulators and negative value of ∆Regulator indicates regulators need to be degraded for that particular switching.

Intuitively, networks in topologies A-C should offer qualitatively similar ΔTarget-ΔRegulators profile. Likewise, networks in topologies D-F should have similar ΔTarget-ΔRegulators profile qualitatively. However, as shown in (Figure S7), we note that topologies A and D, topologies B and F, and topologies C and E offer qualitatively similar ΔTarget-ΔRegulator profile. In topologies A and D, due to absence of feedback, ΔTarget values for all networks are plotted at almost zero change in total regulators. The ΔTarget-ΔRegulator plane in shows that topologies C and E offer dynamics where production of regulators is required to switch the network OFF and degradation of regulators is required to switch the network ON. On other hand, topologies B and F offer a dynamics where they require production of regulators to switch the network ON and degradation of regulators during switching network OFF. In other words, it suggests that topologies C and E are expensive in switching OFF whereas topologies B and F are expensive in switching ON. In (Figure S7), we show that how economic a control in a particular topology is to maintain a certain response level at steady state. Our analysis (Figure S7) suggests that topologies A-C and topologies D-F are qualitatively similar in showing maintenance cost dynamic. However, topology B and C, for some networks, require high number of regulators to maintain target response in ON and OFF conditions respectively. Again, only topology E, in both ON and OFF conditions, remains expensive by requiring high number of regulators to maintain response level at steady state. In terms of control of switching and maintaining response level, topology F is suited for most of the cellular functions in the cell whereas topology E stands the most expensive.

Discussion

Transcription networks are of interest from a number of perspectives like structure, topology, dynamics, and evolution.[3, 24, 37, 56-58] Despite significant effort in trying to understand dynamic features of topologies – an open question remains. Why does a cell choose a particular topology over the others?

Demand rules provide an insight into this question. However, our analysis reveals that the agreement with demand rules is rather limited. What then could be the additional determinants? Dynamically, as our analysis shows, there are several subtle differences across topologies. Our simulations and comparison with natural distribution of topologies in *E. coli* suggests that, given a performance criteria, the frequency distribution among the six topologies in *E. coli*, and our simulations is identical. The simplest explanation from this analysis regarding choice of topology could be that a network is perhaps randomly picked out from a group that satisfies the demands of performance. Or simply, we speculate that the cell picks "whatever works".

In a 2009 study, Hwa et. al. demonstrated that different modes of regulation lead to qualitatively different patterns of protein levels when cells are grown in conditions supporting different growth rates.[59] They demonstrate that constitutively and positively controlled genes exhibit a decrease in steady state with growth rate, negatively regulated genes can exhibit a weakly negative or a strongly positive correlation between protein levels and growth rate. Could additional considerations like maintenance of protein levels at a constant levels, independent of growth rate, be a selective force for certain physiological roles?

Here, we describe simulations of the simplest regulatory topology. In terms of increasing the complexity of the network structures we can explore, our simulation approach very rapidly approaches saturation. In addition, combinatorial inputs of multiple regulators into one promoter remain unanswered and unexplored. These additional interactions would make the possible range of dynamic behaviour much more complex and richer, but at the same time computationally intractable.

Additional Files

(i) Supplement_Data- It contains supporting data in excel format.

(ii) Supplement Figures and Methods.

Author Contributions

KJ: All RegulonDB analysis. MKP: All Simulation work. DC: Evolutionary Experiment. NR: analysis on relative amino acid demand data. MKP, KJ, SS: Conceived the study. MKP, KJ, SS: Wrote the paper.

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Competing Interest

The authors declare that no competing interests exist.

Figure Caption

Figure 1. Showcase of six topologies derived by interactions between a regulator R (blue) and a target, T (Pink). In topologies A, B, and C, in presence of the appropriate environmental or cellular signal, expression of target T is controlled positively by an induced regulator (R*). In contrast, target expression in topologies D, E, and F is under repression by an un-induced regulator (R), and the repression is relieved under appropriate conditions.

Figure 2. Frequency distribution of R-T interactions in *E. coli*. All **(A** and **C)** Regulator-Gene interactions **(B** and **D)** Regulator-Promoter interactions in *E. coli*, without including global regulators, were calculated from RegulonDB and then distributed **(A** and **B)** among activator- [A-C] and repressor-based [D-F] topologies and **(C** and **D)** among the six distinct topologies.

Figure 3. Division of R-T interactions among six functional groups. All Regulator-Promoter interactions in *E. coli* were classified in six functional groups. For each functional group without including global regulators, frequency of activator-based topologies [A-C] roughly equals repressor-based topologies [D-F]. Among individual topologies, F is over-represented and E is under-represented in all six functional groups.

Figure 4. Correlation between demand and activator-based control. (A) X-axis represents amino acids in increasing availability to *E. coli*, and y-axis represents fraction of regulatory interactions in the activator-based topologies for each amino acid biosynthesis regulon (blue) and its transport (red). **(C)** X-axis represents carbon sources in increasing order of preference to *E. coli* in the intestine, and y-axis, the fraction of regulatory interactions in the activator-based topologies controlling expression of metabolic genes involved in utilization (blue) of each sugar and its transport (red).

Figure 5. Long-term experiment to track changes, if any, in mode of regulation. P_{araBAD} expression in strain grown in 0.4% arabinose for 3000 generations (called Strain 1) and strain grown in 0.35% glucose and 0.05% arabinose for 3000 generations (called Strain 2). WT refers to MG1655. *ΔaraC* strain refers to the mutant created by knocking out *araC* from the parent evolved strain.

Figure 6. Frequency distribution of R - T interactions from simulations. (A) Frequency distributions of networks belonging to the activator-based [A-C] and repressor-based [D-F] topologies in the "Performance Box" and **(B)** Percent frequency of networks belonging to each of six topologies in the "Performance Box".

Figure 7. Frequency distribution of R-T interactions from simulations. A fraction of networks belonging to each of six topologies in the "Performance Box". **(A** and **B)** represent fraction of networks in transient performance boxes when the boundaries of nominal performance box have been tightened up, **(C)** represents the most constrained performance box, **(D** and **E)** represent fraction of networks in transient performance boxes when the boundaries of nominal performance box have been relaxed, **(F)** represents the most relaxed performance box. In each transient performance box, the fractional distribution changes by number but not by the pattern.

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Figure 1

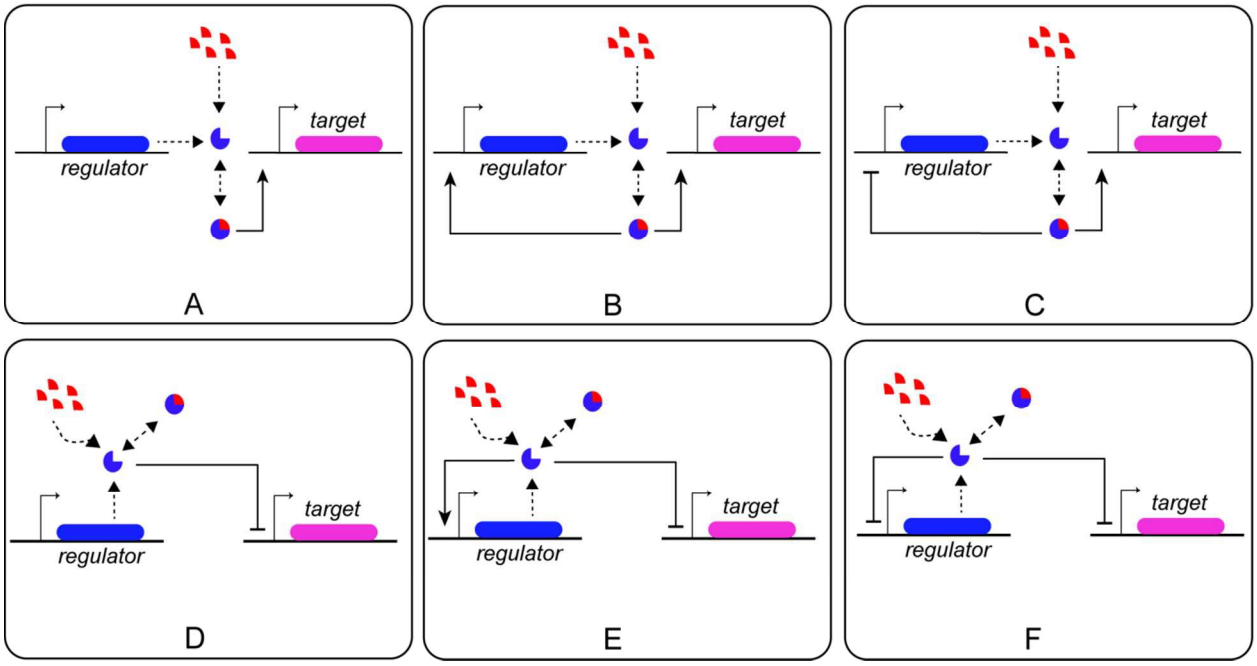

Figure 2

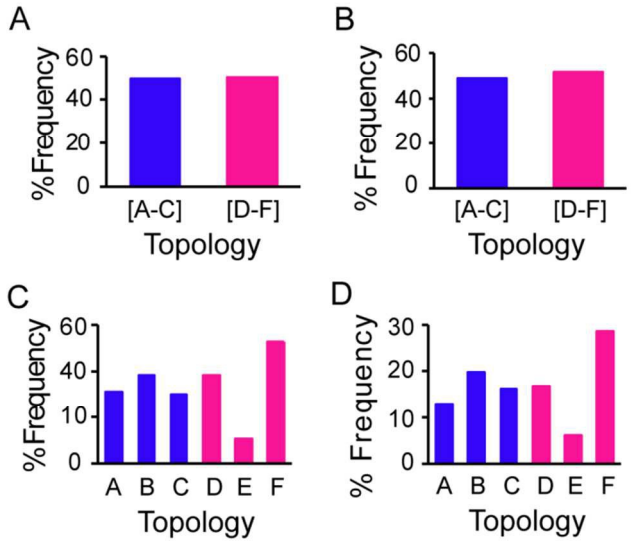

Figure 3

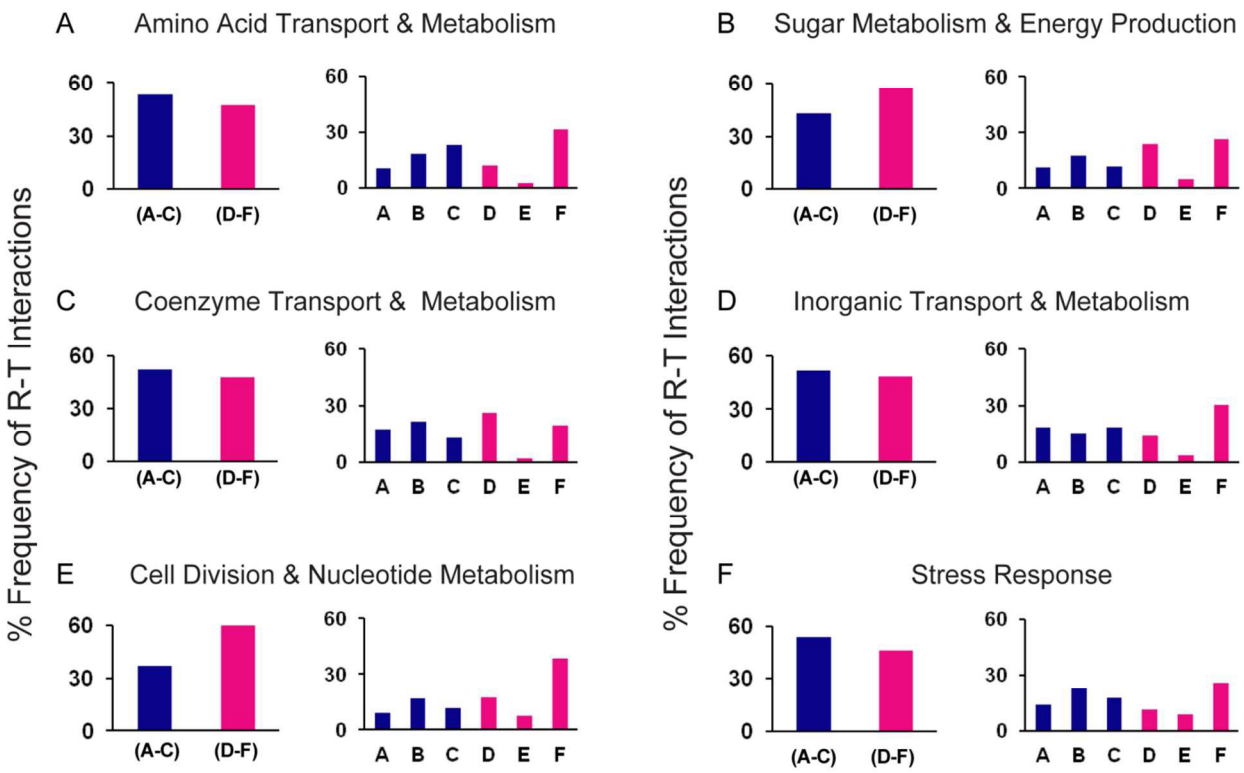

Figure 4

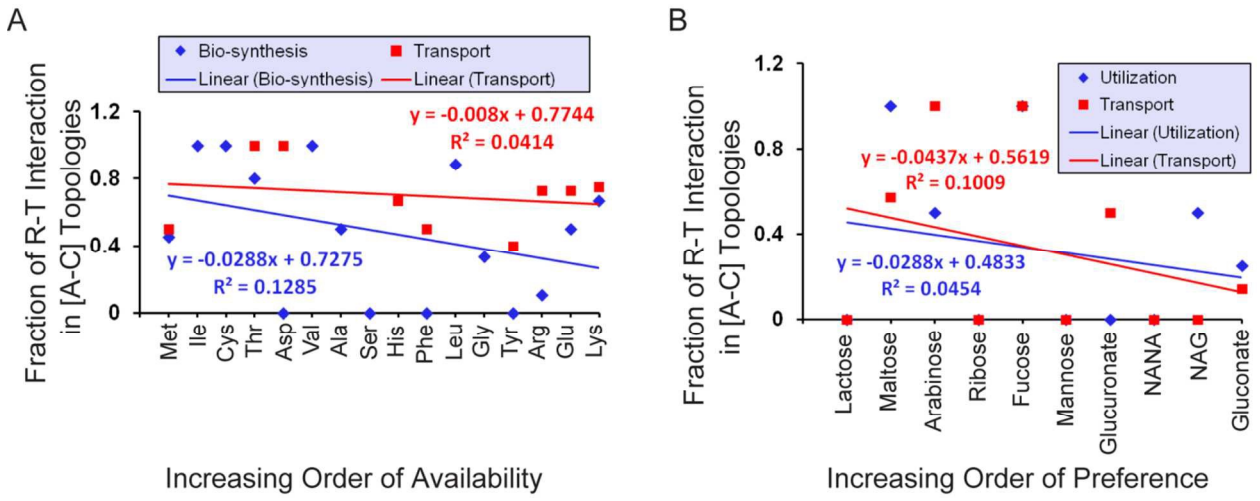

Figure 5

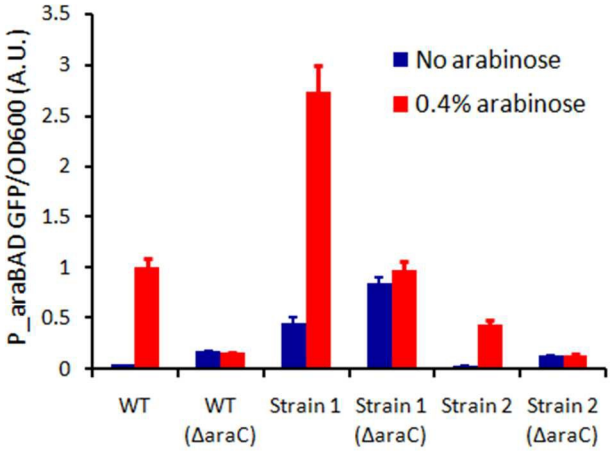

Figure 6

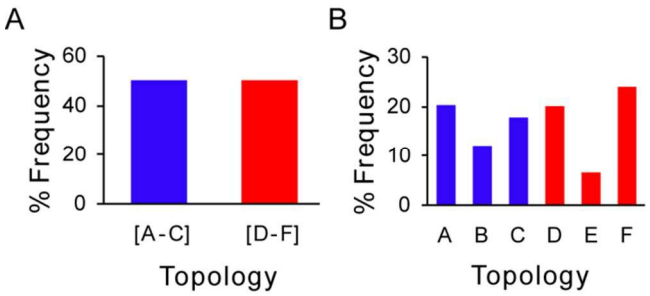

Figure 7

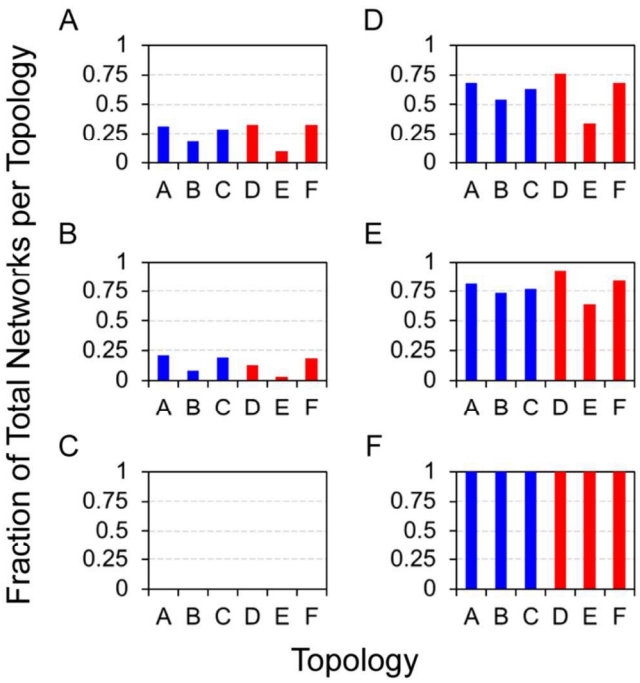

Table 1: Speakman's Rank Correlation between demand and fraction of regulatory interactions belonging to topologies [A-C]

	Sugar		Amino Acid	
	Utilization	**Transport**	**Biosynthesis**	**Transport**
E. coli				
Without Global Regulator	-0.097	-0.212	-0.304	-0.046
With Global Regulator	-0.02	-0.206	-0.145	-0.527
Without Global Regulator (Normalized with genome demand)	-	-	-0.323	0.0
With Global Regulator (Normalized with genome demand)	-	-	-0.334	0.085
B. subtilis				
Without Global Regulator	0	-	-0.183	-
With Global Regulator	0	-	-0.217	-