Topological transition in bacterial protein-protein interaction networks ruled by gene conservation, essentiality and function

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Protein-protein interaction (PPI) networks are the backbone of all processes in living cells. In this work we investigate how gene conservation, essentiality and functional repertoire are reflected in the connectivity $k$ of the corresponding proteins in the PPI networks of 42 evolutionarily unrelated bacterial species. We investigate three issues: i) the degree distribution and densities of the PPI subnetworks of essential and nonessential genes; ii) how the conservation of genes, measured both by the evolutionary retention index (ERI) and by evolutionary pressure (as represented by the ratio $K_a/K_s$) is related to the degree of the corresponding protein in the PPI network; iii) how $k$ of the PPI networks selects functional repertoires, as represented by the Clusters of Orthologous Proteins (COGs). We show that conservation, essentiality and functional specialisation of the genes control in a universal way the topology of the emerging bacterial PPI networks. Noteworthy, a phase transition is observed such that, for $k \geq 40$, bacterial PPI network are mostly populated by genes that are conserved, essential and which, in most cases, belong to the COG J, a category of genes which is related to ribosomal functions and to the processing of genetic information.

INTRODUCTION

To operate the biological activities of a cell, proteins work in association with other proteins, possibly assembled in large complexes. Hence, knowing the interactions of a protein is important to understand its cellular functions. Moreover, a comprehensive description of the stable and transient protein-protein interactions (PPIs) within a cell would facilitate the functional annotation of all gene products, and provide insight into the higher-order organization of the proteome [1, 2]. Several methodologies have been developed to detect PPIs, and have been adapted to chart interactions at a proteome-wide scale. These methods, that combine different technologies with complementary experiments and computational analyses, were shown to generate high-confidence PPI networks, enabling to assign proteins to functional categories [3, 4].

The statistical study of bacterial PPIs over several species (meta-interactomes) has brought important knowledge about protein functions and cellular processes [5, 6]. In this work we focus on 42 bacterial genomes, which are not strictly related among themselves. Our aim is to shed light on the relationship between conservation, essentiality and functional annotation at the genetic level with the connectivity patterns of the PPI networks. We extend previous observations which have suggested a strong correlation between codon bias and the topology of PPI networks one the one side, and between codon bias and gene conservation/essentiality on the other [7, 8].

Individual genes in the genome contribute differentially to the survival of an organism. According to their known functional profiles and based on experimental evidences, genes can be divided into two categories: essential and nonessential ones [9, 10]. Essential genes are not dispensable for the survival of an organism in the environment it lives in [10, 11]. Nonessential genes are those which are dispensable [12], being related to functions that can be silenced without compromising the survival of the phenotype. Naturally, each species has adapted to one or more evolving environments and, plausibly, genes that are essential for one species may be not essential for another one.

It has been argued many times that essential genes are more conserved than nonessential ones [13–17]. The term “conservation” has however at least two meanings. On the one hand, a gene is conserved if orthologous copies are found in the genomes of many species, as measured by the Evolutionary Retention Index (ERI) [9, 18]. On the other hand, a gene is (evolutionarily) conserved when it is subject to a purifying evolutionary pressure which disfavors mutations. This pressure is quite usually measured by $K_a/K_s$, the ratio of the number of nonsynonymous substitutions per nonsynonymous site to the number of synonymous substitutions per synonymous site. In this second meaning a conserved gene is, in a nutshell, a slowly evolving gene [13, 19].

In this work we show that bacterial PPI network display an interesting topological-functional transition, ruled by $k$ and with a threshold above $k \geq 40$. Proteins with high PPI network connectivities (hubs) likely correspond to genes that are conserved and essential. Conversely, genes that correspond to hub proteins in the PPI network are likely to be essential and conserved. We have also observed that below the threshold the functional repertoire is heterogeneous, whereas above the threshold there is a quite strict functional specialisation.
Organisms Abbr. RefSeq STRING n
Agrobacterium tumefaciens (fabrum) agtu NC_003062 176299 2765
Aquifex aeolicus VF5 aqae NC_000918 224324 1497
Bacillus subtilis 168 basu NC_000964 224308 4175
Brucella melitensis bv. 1 str. 16M brme NC_003317.1 224914 2059
Burkholderia pseudomallei K96243 bups NC_006350 272560 3398
Buchnera aphidicola Sg uid57913 busg NC_004061 198804 546
Burkholderia thailandensis E264 buth NC_007651 271848 3276
Caulobacter crescentus cacr NC_011916 565050 3885
Campylobacter jejuni caje NC_002163 192222 1572
Chlamydia trachomatis D/UW-3/CX chtr NC_000117.1 272561 894
Clostridium acetobutylicum ATCC 824 clac NC_003030.1 272562 3602
Deinococcus radiodurans R1 dera NC_001263.1 243230 2629
Escherichia Coli K-12 MG1655 esco NC_000913.3 511145 4004
Francisella novicida U112 frno NC_008601 401614 1719
Haemophilus influenzae Rd KW20 hain NC_000907.1 71421 1610
Listeria monocytogenes EGD-e limo NC_003210.1 169963 2867
Mycoplasma genitalium G37 myge NC_000008 243273 475
Mycoplasma pneumoniae M129 mypn NC_000012.1 272634 648
Mycoplasma pulmonis UAB CTIP mput NC_002771 272635 782
Neisseria gonorrhoeae FA 1090 uid57611 nego NC_002946 242231 1894
Porphyromonas gingivalis ATCC 33277 pogi NC_0010729 431947 2089
Pseudomonas aeruginosa UCBPP-PA14 psae NC_008463 208963 5892
Ralstonia solanacearum GMI1000 raso NC_003295.1 267608 3436
Rickettsia prowazekii str. Madrid E ripr NC_000996.3 272947 8433
Salmonella enterica serovar Typhi saen NC_004631 209261 4352
Shewanella oneidensis MR-1 shon NC_004347 211586 4065
Sphingomonas wittichii RW1 spwi NC_009511 392499 4850
Staphylococcus aureus N315 stau NC_002745.2 158879 2582
Staphylococcus aureus NCTC 8325 stau NC_007795 93061 2767
Streptococcus pyogenes NZ131 stpy NC_011375 471876 1700
Streptococcus sanguinis stsa NC_000009 388919 2270
Synechocystis sp. PCC 6803 ssp NC_000911.1 1148 3179
Thermotoga maritima MSB8 thma NC_000853.1 243274 1858
Treponema pallidum Nichols trpa NC_000919.1 243276 1036
Vibrio cholerae N16961 vich NC_002505 243277 2534
Xylella fastidiosa 9a5c xyfa NC_002488 160492 2766

TABLE I. Summary of the selected bacterial dataset. Organism name, abbreviation, RefSeq, STRING code, size of genome (number of genes n). Genomes annotated in the Database of Essential Genes (DEG) are highlighted with bold fonts.

RESULTS

Degree distribution of PPI networks

We start by studying the degree distributions $P(k)$ observed in bacterial PPIs. We first recall that such a distribution was found to be scale-free in E. Coli [7], meaning that the corresponding PPI network features a large number of poorly connected proteins, and a relatively small number of highly connected hubs. In order to assess the generality of this observation, we compute $P(k)$ for each genome of Table I (plots are reported in figures S2-S3 of the Supplementary Information). Note that, despite the fact that the PPIs of the different bacteria have different sizes and densities, their average connectivity and the support of their $P(k)$ are very similar (see figure S1 in the Supplementary Information). Thus, we can superpose all the considered bacterial degree distributions without the need to normalise the support of each $P(k)$. When doing so, we observe two distinct regimes (see figure 1). For low values of $k$, the distribution has a scale-free shape $P(k) \propto k^{-\gamma}$. This result is consistent with previous findings for yeast, worm and fly [20] and for
co-conserved PPIs in some bacteria [21]. For higher values of \( k \) however the distribution deviates from a power law, and a bump emerges, with a Gaussian-like shape. Interestingly, this feature is almost undetectable taking individual species alone as E. Coli (see fig 2 in supporting materials of Dilucca et al. [7]) but clearly emerges by enriching the statistics with more bacterial species. The bump that, notably, emerges for \( k \geq 40 \) is reasonably due to the contribution of proteins belonging to complexes [22]. Indeed, if we consider the separate contribution of essential and nonessential genes to the \( P(k) \) (for DEG-annotated genomes), we see that the superposed peak is present only in the degree distribution of essential genes. Moreover, the degree distributions for essential and nonessential genes are well separated and the average degree is systematically higher for essential genes than for nonessential ones—consistently with previous findings [20].

![Graph showing probability distribution \( P(k) \) for the number of connections \( k \) of each protein, averaged over the bacterial species considered in Table I. Inset: \( P(k) \) for essential (E) and nonessential (NE) genes, averaged over DEG-annotated genomes. Note that the average degree is higher for essential genes than for nonessential ones, and the two probability distributions are quite distinct. The low \( k \) region of the curve can be represented by a power law with \( \gamma \sim 2.2 \).](image)

**PPI connectivity and gene conservation**

We now investigate whether there is a correlation between the degree of conservation (as measured by ERI) of genes and the connectivity \( k \) of the corresponding proteins in PPI networks. Figure [2] shows that highly connected proteins are also highly conserved among the bacterial species we consider, that constitute a reasonably wide sample of different evolutionary adaptations. This observation is a strong signature of the existence of an invariant structure of conserved hubs in all bacterial PPI networks. Indeed, we observe the existence of a kind of phase transition for bacterial proteins with \( k \geq 40 \), which have ERI close to 1 almost surely, and are thus highly conserved among the species. Proteins that are less connected, on the contrary, have a wide range of ERI values. Interestingly, as shown in the inset of figure [2], the fluctuations in ERI as a function of \( k \) abruptly decrease for connectivities above the threshold.

We then look at the evolutionary pressure exerted on genes whose proteins have different connectivities. The graph in figure [3] shows the ratio \( K_a/K_s \) for groups of genes binned by the connectivity \( k \) of the corresponding proteins, for all the 42 bacterial species considered here. We see that the more connected proteins correspond to genes which are subject to an increasing purifying evolutionary pressure. Indeed, values of the \( K_a/K_s \) systematically decrease until they become zero, as a function of \( k \). This result point to the fact that the more proteins are connected in the PPI networks the more the genes that code them are subject to a purifying evolutionary pressure.

**PPI and Essentiality**

To further investigate the relationship between gene essentiality and protein connectivities, we consider DEG-annotated genomes and classify interactions between proteins (links) making references to the essentiality of the
FIG. 2. Average ERI values of bacterial genes as a function of the degrees $k$ of the corresponding proteins, for all the considered genomes. Error bars are standard deviations of ERI values associated to a given $k$ value. Inset: amplitude of the error bar ($\Delta$ERI) as a function of $k$.

FIG. 3. Dependence of selective pressure in terms of $K_a/K_s$ for a gene on the degree $k$ of the corresponding protein, for all the considered genomes.

corresponding genes. We distinguish three sets of links: $ee$ (linking proteins from two essential genes), $\bar{e}\bar{e}$ (two nonessential genes) and $e\bar{e}$ (linking proteins from an essential gene and a nonessential one). We then compute the density of these sets of links respectively as:

$$
\rho_{ee} = \frac{|ee|}{\frac{1}{2}E(E-1)}, \quad \rho_{\bar{e}\bar{e}} = \frac{|\bar{e}\bar{e}|}{\frac{1}{2}NE(NE-1)}, \quad \rho_{e\bar{e}} = \frac{|e\bar{e}|}{\frac{1}{2}E \cdot NE},
$$

(1)

where $E$ and $NE$ denote the number of essential and nonessential genes, respectively (self-connections are excluded in our analysis). Such densities are then compared with the overall density of the network—restricted to genes classified
as either essential or nonessential:

\[ \langle \rho \rangle = \frac{|ee| + |\bar{e}\bar{e}| + |\bar{e}e|}{\frac{1}{2}(E + NE)(E + NE - 1)}. \] (2)

We use the ratios \( r_{ee} = \rho_{ee}/\langle \rho \rangle \), \( r_{\bar{e}\bar{e}} = \rho_{\bar{e}\bar{e}}/\langle \rho \rangle \) and \( r_{e\bar{e}} = \rho_{e\bar{e}}/\langle \rho \rangle \) to assess the level of connectivity of the subnetworks with respect to the overall connectivity. Table II shows that subnetworks of essential genes are far denser than the overall networks, and that, in general, essential and nonessential genes tend to form network components that are weakly interconnected. This happens because many essential genes encode for ribosomal proteins, which in turn are localized in the ribosome so that they have a major probability to interact [23]. Figures S4-S5 of the Supplementary Information display such network features for each individual species.

| Organisms | \( r_{ee} \) | \( r_{\bar{e}\bar{e}} \) | \( r_{e\bar{e}} \) |
|-----------|--------------|----------------|--------------|
| basu      | 44.46        | 0.80           | 0.11         |
| bath      | 20.07        | 0.76           | 0.25         |
| bups      | 6.21         | 0.83           | 0.27         |
| buth      | 18.69        | 0.70           | 0.22         |
| cacr      | 18.40        | 0.70           | 0.15         |
| caje      | 3.65         | 0.82           | 0.32         |
| esco      | 2.91         | 0.88           | 0.31         |
| frno      | 9.84         | 0.52           | 0.18         |
| hain      | 1.65         | 1.15           | 0.27         |
| hepy      | 2.91         | 0.78           | 0.38         |
| myge      | 1.42         | 0.29           | 0.08         |
| mypu      | 3.42         | 0.22           | 0.12         |
| mytu      | 8.09         | 0.78           | 0.23         |
| pogi      | 11.03        | 0.41           | 0.21         |
| psae      | 9.85         | 0.92           | 0.16         |
| saen      | 28.80        | 0.81           | 0.12         |
| shon      | 6.50         | 0.64           | 0.16         |
| spwi      | 15.47        | 0.74           | 0.22         |
| stau      | 23.05        | 0.58           | 0.23         |
| staum     | 21.89        | 0.64           | 0.16         |
| stpy      | 9.30         | 0.73           | 0.23         |
| stsa      | 30.65        | 0.61           | 0.22         |
| vich      | 8.37         | 0.81           | 0.19         |

TABLE II. Relative density values \( r \) for PPI subnetworks between essential genes (\( r_{ee} \)), between nonessential genes (\( r_{\bar{e}\bar{e}} \)) and between essential and nonessential genes (\( r_{e\bar{e}} \)), for each DEG-annotated bacterial genome.

PPI connectivity and functional specialization

For each PPI network, we define the conditional probability that a protein with degree \( k \) belong to a given COG as:

\[ P(\text{COG}|k) = \frac{P(k|\text{COG})P(\text{COG})}{P(k)}, \] (3)

where \( P(k) \) is the degree distribution in the PPI network, \( P(\text{COG}) \) is the frequency of that COG in the proteome, and \( P(k|\text{COG}) \) is the degree distribution restricted to that COGs. Figure 4 shows the COG spectrum as a function of \( k \) over all bacteria species considered. Interestingly, we again note a marked transition. Below \( k \approx 40 \) the COG spectrum is quite heterogeneous: genes corresponding to proteins with low connectivity are spread over several COGs which correspond to different functions (see Table IV). Genes whose proteins are highly connected \( (k \gtrsim 40) \) are instead mainly concentrated in COG J, which encompasses translation processes and ribosomal functions. There are yet a handful of outliers, hubs with connectivity between 57 and 62, that belong to COG I (related to lipid transport and metabolism), K and L (which, together with J, define the functional class of information storage and processing). The list of these outliers is reported in Table III.
FIG. 4. Probability distribution $P(COG|k)$ of belonging to a given COG for proteins with degree $k$, over all considered genomes. Proteins with low connectivity have a very heterogeneous COG composition, whereas, those with high $k$ basically belong only to COG J.

### Table III. List of outlier proteins (not belonging to COG J) with connectivity around $k = 60$ in figure 4.

| $k$ | COG | Gene | Protein |
|-----|-----|------|---------|
| 57  | 1250I | paaH | 3-hydroxyadipyl-CoA dehydrogenase, NAD-dependent, acyl-CoA synthetase |
| 0365I | paaH | 3-hydroxyadipyl-CoA dehydrogenase, NAD-dependent |
| 58  | 0222J | rplL | 50S ribosomal subunit protein L7/L12 |
| 0335J | rplS | 50S ribosomal subunit protein L19 |
| 0267J | rpmG | 50S ribosomal subunit protein L33 |
| 0365I | acs | acetyl-CoA synthetase |
| 59  | 0183J | paaJ | 3-oxoadipyl-CoA3-oxo-5,6-dehydrosuberyl-CoA thiolase |
| 1960I | ydiO | putative acyl-CoA dehydrogenase |
| 0183I | atoB | acetyl-CoA acetyltransferase |
| 60  | 0197J | rplP | 50S ribosomal subunit protein L16 |
| 0088J | rplD | 50S ribosomal subunit protein L4 |
| 0197J | rplP | 50S ribosomal subunit protein L16 |
| 0087J | rpmC | 50S ribosomal subunit protein L3 |
| 1960I | aidB | putative acyl-CoA dehydrogenase |
| 61  | 0085K | rpoB | RNA polymerase, beta subunit |
| 0202K | rpoA | RNA polymerase, alpha subunit |
| 62  | 0087J | rplC | 50S ribosomal subunit protein L3 |
| 0052J | rpsB | 30S ribosomal subunit protein S2 |
| 2965L | PriB | ribosomal replication protein |

DISCUSSION

Topological analysis of biological networks, such as protein-protein interaction or metabolic networks, has demonstrated that structural features of network subgraphs are correlated with biological functions [24, 25]. For instance, it was shown that highly connected patterns of proteins in a PPI are fundamental to cell viability [26].

In this work we have shown the existence of a topological-functional transition in bacterial species, ruled by the connectivity of proteins in the PPI networks. The threshold in $k$ of the transition is likely to be located between $k = 40$ and $k = 50$. Proteins that have connectivities above the transition are mostly coded by genes that are conserved (as measured both by ERI and $K_a/K_s$) and essential. Moreover the functional repertoire above the threshold focuses mainly on the COG J category with just a few interesting hubs belonging to GOGs I, K AND L.

Indeed, the PPI network of each bacterial species is characterized by a highly connected core of conserved ribosomal
proteins, the components of multi-subunit complexes whose corresponding genes are MOSTLY essential [21, 27] and code for supra-molecular complexes, that pile up in the bump we have observed in the $P(k)$ distribution (figure 1).

We believe that the observations we have presented here can have implications both for the prediction of gene essentiality, based on the knowledge of PPI networks, and for the prediction of interactions between proteins, based on genetic information [28, 29].

It is interesting to note that the results we have presented here are consistent with a previous study based on inferred bacterial co-conserved networks based on phylogenetic profiles [21]. The coupled flows of information from the genetic level up to the proteomic level and vice-versa should be further systematically investigated, taking into account archaeal and prokaryotic genomes in the search for emerging multi-layer structures that could offer basic theoretical grounds for clinical and systemic applications, for instance related to antimicrobial resistances [30–33].

METHODS

In this work we consider a set of 42 selected bacterial genomes (that we have previously investigated [8] see Table I). Nucleotide sequences were downloaded from the FTP server of the National Center for Biotechnology Information (ftp://ftp.ncbi.nlm.nih.gov/genomes/archive/old_genbank/Bacteria/) [34].

Protein-Protein Interaction Networks

PPIs are obtained from the STRING database (Known and Predicted Protein-Protein Interactions, https://string-db.org/) [35]. In STRING, each interaction is assigned with a confidence level or probability $w$, evaluated by comparing predictions obtained by different techniques [36–38] with a set of reference associations, namely the functional groups of KEGG (Kyoto Encyclopedia of Genes and Genomes) [39]. In this way, interactions with high $w$ are likely to be true positives, whereas, a low $w$ possibly corresponds to a false positive. As usually done in the literature, we consider only interactions with $w \geq \Theta$ and select a stringent cut-off $\Theta = 0.9$ that allows for a fair balance between coverage and interaction reliability (see for instance the case of *E. Coli* [7]).

We denote by $k$ the degree (number of connections) associated to each proteins in each PPI network after the thresholding procedure. Note also that after applying the cut-off we are left, for each network, with a number of isolated proteins (i.e., with no connections) that grows as $\sqrt{n}$ (where $n$ is the number of proteins in the genome). These proteins are not considered in the network analysis and are regarded as a kind of statistical noise.

Gene Conservation

We use the Evolutionary Retention Index (ERI) [9] as a first way of measuring conservation of a gene. The ERI of a gene is the fraction of genomes (among those reported in Table I) that have at least an ortholog of the given gene. Then, a low ERI value means that a gene is specific, common to a small number of genomes, whereas high ERI is a characteristic of highly shared, putatively universal and essential genes.

We also make reference to another notion of gene conservation. Conserved genes are those which are subject to a purifying, conservative evolutionary pressure. To discriminate between genes subject to purifying selection and genes subject to positive selective Darwinian evolution, we use a classic but still widely used indicator, the ratio $K_a/K_s$ between the number of nonsynonymous substitutions per nonsynonymous site ($K_a$) and the number of synonymous substitutions per synonymous site ($K_s$) [19]. Conserved genes are characterized by $K_a/K_s < 1$. We have used $K_a/K_s$ estimates by Luo *et al.* [15] that are based on the method by Nej and Gojobori [40].

Gene Essentiality

We used the Database of Essential Genes (DEG, www.essentialgene.org) [15], which classifies a gene as either essential or nonessential on the basis of a combination of experimental evidence (null mutations or trasposons) and general functional considerations. DEG collects genomes from Bacteria, Archea and Eukarya, with different degrees of coverage [41, 42]. Of the 42 bacterial genomes we consider, only 23 are covered—in toto or partially—by DEG, as indicated in Table I.
Clusters of orthologous proteins

We use the rough but still widely adopted functional annotation given by the database of orthologous groups of proteins (COGs) from Koonin’s group, available at http://ncbi.nlm.nih.gov/COG/ [13, 14]. We consider 15 functional COG categories (see Table IV), excluding the generic categories R and S for which functional annotation is too general or missing.

| COG ID | Functional classification |
|--------|--------------------------|
| J      | Translation, ribosomal structure and biogenesis |
| K      | Transcription |
| L      | Replication, recombination and repair |
| D      | Cell cycle control, cell division, chromosome partitioning |
| T      | Signal transduction mechanisms |
| M      | Cell wall/membrane/envelope biogenesis |
| N      | Cell motility |
| O      | Post-translational modification, protein turnover, chaperones |
| C      | Energy production and conversion |
| G      | Carbohydrate transport and metabolism |
| E      | Amino acid transport and metabolism |
| F      | Nucleotide transport and metabolism |
| H      | Coenzyme transport and metabolism |
| I      | Lipid transport and metabolism |
| P      | Inorganic ion transport and metabolism |

TABLE IV. Functional classification of COG clusters.

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Topological transition in bacterial protein-protein interaction networks ruled by conservation, essentiality and function of genes

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SUPPLEMENTARY INFORMATION

FIG. S1. Relation between genome size $n$ and average degree $\langle k \rangle \pm \sigma_k$ (upper panel) and density $\rho$ (bottom panel) of the corresponding PPI network for the set of bacterial species reported in Table 1 of the main text.
For DEG-annotated genomes, the inset shows the contribution of essential (red) and nonessential (blue) genes.

FIG. S1. Degree distribution $P(k)$ (part 1) of the PPI networks for the bacterial species reported in Table 1 of the main text. For DEG-annotated genomes, the inset shows the contribution of essential (red) and nonessential (blue) genes.
FIG. S1. Degree distribution $P(k)$ (part 2) of the PPI networks for the bacterial species reported in Table 1 of the main text. For DEG-annotated genomes, the inset shows the contribution of essential (red) and nonessential (blue) genes.
FIG. S1. Adjacency matrices (part 1) of the PPI networks for the DEG-annotated bacterial species reported in Table 1 of the main text. In each matrix, genes are ordered according to the degree of the corresponding protein in the network, in descending order from left to right and from bottom to top. Links among essential genes correspond to red-colored dots, those among nonessential (and non-annotated) genes to blue-colored dots, and those between essential and nonessential (plus non-annotated) genes to a violet-colored dot.
FIG. S1. Adjacency matrices (part 2) of the PPI networks for the DEG-annotated bacterial species reported in Table 1 of the main text. In each matrix, genes are ordered according to the degree of the corresponding protein in the network, in descending order from left to right and from bottom to top. Links among essential genes correspond to red-colored dots, those among nonessential (and non-annotated) genes to blue-colored dots, and those between essential and nonessential (plus non-annotated) genes to a violet-colored dot.