Evidence of a universal power law characterizing the evolution of metabolic networks

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Metabolic networks are known to be scale free but the evolutionary origin of this structural property is not clearly understood. One way of studying the dynamical process is to compare the metabolic networks of species that have arisen at different points in evolution and hence are related to each other to varying extents. We have compared the reaction sets of each metabolite across and within 15 groups of species. For a given pair of species and a given metabolite, the number $\Delta k$ of reactions of the metabolite that appear in the metabolic network of only one species and not the other is a measure of the distance between the two networks. While $\Delta k$ is small within groups of related species and large across groups, we find its probability distribution to be $\sim (\Delta k)^{-\gamma'}$ where $\gamma'$ is a universal exponent that is the same within and across groups. This exponent equals, up to statistical uncertainties, the exponent $\gamma$ in the scale free degree distribution $\sim k^{-\gamma}$. We argue that this, as well as our finding that $\Delta k$ is approximately linearly correlated with the degree $k$ of the metabolite, is evidence of a ‘proportionate change’ process in evolution. We also discuss some molecular mechanisms that might be responsible for such an evolutionary process.

Metabolic networks are known to have a wide, scale free distribution of the degree of connectivity of their metabolites \cite{1,2}, $P(k) \sim k^{-\gamma}$ (for a review see \cite{3}). The exponent $\gamma$ has been found to have a value close to 2.2 across all species of organisms that have been studied \cite{4} even though the sets of metabolites and reactions in metabolic networks vary quite substantially across organisms. This structure suggests that some universal process is responsible for the evolution of metabolic networks; however at the present time the nature of this process is not clearly understood. A comparative study of the metabolic networks of organisms that are at varying distances from each other on the evolutionary ladder can shed light on this process. Here we report on such a study that reveals some universal features of this dynamical process.

For growing networks, such as the internet, a preferential attachment of new nodes to higher degree nodes \cite{4} as well as a proportionate change mechanism \cite{5} whereby nodes with higher degree experience proportionately higher changes in degree has been proposed to account for the scale free structure of the network. The latter process can lead to robust exponents \cite{6}. The metabolic network, however, is not a growing network like the internet; during the course of evolution the network has changed without a substantial change in the number of metabolites. Furthermore, so far no concrete evidence has been presented for a preferential attachment or proportionate change process during its evolution. Here we also present evidence for a proportionate change process in metabolic network evolution.

We downloaded a database of metabolic networks of 107 organisms \cite{7}. This contains organisms from all three kingdoms: eukaryotes, prokaryotes and archaea, arranged in 15 groups including animals, plants, fungi, proteobacteria, firmicutes, and others. Organisms in different groups are evolutionarily distant, while those in the same group are relatively close by. We selected one species from each group (typically the one having the largest number of metabolites) and compared the metabolic networks of all 15 species pairwise (105 pairs of distant species). We also compared specific pairs of nearby species (within the same group).

The metabolic network of a given species of organisms is the set of catalysed chemical reactions that can take place in the organism through which it converts ‘food molecules’ into certain other types of molecules needed by the organism. The above database contains a list of 5275 metabolic reactions, each reaction characterized by its chemical equation and whether it is reversible or not. In particular, for every reaction the list of metabolites participating in the reaction (i.e.,

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the set of molecules that are reactants and products of the reaction) is available. For each reaction and species in the database, information is provided as to whether the reaction is present in the metabolic network of that species. The metabolic network of a given species typically contains several hundred reactions and participating metabolites.

For a given pair of species, say A and B, consider the union M of the set of metabolites present in each metabolic network. For every metabolite in M, we now define a measure of the distance between the two networks. Consider a metabolite in M, and let $R_A$ and $R_B$ be the set of reactions in the metabolic network of A (B) in which this metabolite participates. The number $k_A$ ($k_B$) of reactions in $R_A$ ($R_B$) is the degree of the metabolite in the species A (B). Here we consider only the undirected degree of a metabolite, i.e., we do not distinguish whether the metabolite participates as a reactant or a product. Reversible reactions (forward and reverse pair) in which a metabolite participates are treated as a single reaction for calculating its degree. If a metabolite occurs in only one of the species (say, A) and not the other (B), then $R_B$ is the null set and $k_B = 0$.

Consider the reactions in $R_A \cap R_B$. The reactions in $R_A \cap R_B$ represent the links of the metabolite that are common to both species, and hence $k_{AB}$, the size of this set, is a measure of how much the reaction set of this metabolite has remained ‘conserved’ in the evolution leading to species A and B from their last common ancestor. Similarly, set $(R_A \cup R_B)\setminus(R_A \cap R_B)$, that is, the set of reactions in $R_A \cup R_B$ that are not in $R_A \cap R_B$, or equivalently those reactions of this metabolite that are in one network but not the other, measures the divergence between the two networks. The size of the latter set will be referred to as the divergence of the reaction sets of this metabolite between species A and B, and will be denoted $\Delta k$. Note that $\Delta k$ is different from the magnitude of $k_A - k_B$. For example $R_A$ and $R_B$ can be different sets of reactions with the same number of reactions in which case $k_A - k_B = 0$ while $\Delta k \neq 0$. $\Delta k$ is a measure of the difference between the two networks that takes into account the identity of reactions and not just their number.

We computed the degree distribution $P(k)$ for each of the 15 organisms as well as the ‘divergence probability distribution’ $Q(\Delta k)$ for each of the 105 pairs. By definition, for any pair (A, B),

$$Q(\Delta k) = n(\Delta k)/|M|,$$

where $|M|$ is the number of metabolites in M and $n(\Delta k)$ is the number of metabolites in M whose divergence of reaction sets between A and B is equal to $\Delta k$. The divergence probability distribution for pairs of distant organisms is shown in Fig. 1 and compared with the degree distribution. The figure shows that $Q(\Delta k) \sim (\Delta k)^{-\gamma'}$ with $\gamma' = \gamma$ up to statistical uncertainties in both the exponents. That the degree distribution of two species follows the power law $P(k) \sim k^{-\gamma}$ is a statement of the present structure of the two metabolic networks. This is in no way implies that $Q(\Delta k)$ should also follow a power law with the same exponent. The latter is a distinct statement about the dynamical process that leads to the present structure. That the $Q(\Delta k)$ distribution has the same form for all 105 pairs of distant species considered reflects a universal property of the evolutionary process.

A comparison of distant species reveals features of the evolutionary process over long time scales. In order to study the process over short time scales we compared nearby species (that were in the same group). The result of three such comparisons is shown in Fig. 2. As expected, for each pair of nearby species the absolute divergence is smaller than for distant species. This is evident from the fact that the $Q(\Delta k)$ curves are well below the $P(k)$ curves in Fig. 2, in contrast to Fig. 1 where they are much closer, and that larger values of $\Delta k$ are absent in Fig. 2. However, it can be seen that the $Q(\Delta k)$ still follows a power law with the same exponent as before. This suggests that this feature of the evolutionary process is also valid over short evolutionary time scales.

We explored the relationship between $\Delta k$ for a metabolite across a pair of species, and its degree in each of those species. In particular, one can ask for the conditional probability $P(\Delta k|k)$ for a metabolite to have a reaction set divergence $\Delta k$ across a pair of species, given that its average degree in the two species is $k$. We found a positive and approximately linear correlation between $\Delta k$ and the degree of a metabolite (Fig. 3). This is evidence of a ‘proportionate change’ type process in the evolution of metabolic networks.

This provides insight into why the exponents $\gamma'$ and $\gamma$ might be equal or very close. For, let us assume for the moment a perfect correlation between $\Delta k$ and $k$, i.e., $P(\Delta k|k) \sim \delta(\Delta k - f(k))$, or, equivalently, that $\Delta k = f(k)$ for some fixed one-to-one function $f$, and also that $P(k) \sim k^{-\gamma}$. Then the statement $f(k) \sim k^\alpha$ is equivalent to the statement $Q(\Delta k) \sim (\Delta k)^{-\gamma'}$, with $\gamma' = \gamma/\alpha$. In particular $\alpha = 1$ implies $\gamma' = \gamma$ and vice versa. However this is not a complete explanation because, as is evident from Fig. 3, there is stochasticity in the relation between $\Delta k$ and $k$, and not perfect correlation.

What kind of molecular process can give rise to this linear correlation between $\Delta k$ and degree of a metabolite? A reaction is catalyzed by an enzyme to which the reactant molecules bind at specific sites in a 3-dimensional geometry. The structure of the enzyme is determined by the gene or genes that code for it, and genes evolve via several mechanisms, including random mutations and gene duplication followed by divergence
through independent mutations in both the copies. A metabolite with high degree binds to several enzymes that catalyze its reactions. If a gene corresponding to one of these enzymes mutates in a manner that disturbs the binding site of this metabolite on the enzyme, the corresponding reaction could be lost. The more enzymes the metabolite binds to, the proportionately higher is the probability of losing its reactions through random mutations. On the other hand if the gene duplicates and diverges, that can introduce a new enzyme to which it binds and hence a new reaction for this metabolite to participate in. Large degree metabolites have a larger pool of interacting enzymes whose genes can duplicate, and hence if genes duplicate randomly, the number of new reactions a given metabolite participates in is also expected to be positively correlated with its degree. Thus the same mechanisms, namely gene mutations and duplication-divergence, that have been considered as mechanisms for proportionate change and preferential attachment in protein interaction networks [10], could operate for metabolic networks also.

Acknowledgements: We thank S.N. Bose National Centre for Basic Sciences, Kolkata, and Centre for High Energy Physics, IISc, Bangalore for infrastructure and hospitality where part of this work was done. Shalini and A.S. acknowledge a Junior Research Fellowship from UGC and CSIR, respectively.

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[8] In logarithmic binning of k, for example, the rth bin (r = 1, 2, . . .) is plotted at (x, y) = (kr, Pr), where kr = 2r−1, Pr = nr/(Nkr), nr is the number of metabolites with degree in the range kr ≤ k < 2kr, and N is the total number of metabolites in the network.
[9] We remark that given the statistical uncertainty in the exponents, our results are consistent with a value of α slightly different from 1 and γ′ slightly different from γ.
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Figure captions

Fig. 1. The probability distribution of the divergence of reaction sets Q(Δk) for evolutionarily distant organisms compared with the degree distribution P(k) of those organisms. The blue curve (joining the blue triangles) in each figure gives the divergence distribution for the corresponding set of species. For that curve the x-axis of the figure represents Δk and the y-axis represents Q(Δk). The other curves give the degree distribution for those species. For these curves the x-axis represents k and the y-axis represents P(k). Both axes are on a logarithmic scale in all figures. k and Δk are binned logarithmically [8]. Figs. 1a-c compare species from the three kingdoms pairwise, namely the eukaryote Homo sapiens, the prokaryote Escherichia coli and the archaea Methanosarcina mazei. The P(k) curves for these organisms are given by red dots, green squares and brown hexagons, respectively. (a) Comparison between P(k) for H. sapiens (red dots), P(k) for E. coli (green squares) and Q(Δk) across these two species (blue triangles). (b) A similar comparison of H. sapiens and M. mazei. (c) A similar comparison of E. coli and M. mazei. (d) An average taken over 15 species each drawn from a different group in the database. For a given metabolite, the average k over the 15 species and the average Δk across the 105 pairs of species are computed and binned logarithmically. The cyan rhombuses represent P(k) and blue triangles Q(Δk) in Fig. 1d. The blue lines in Figs. 1a-d are consistent with Q(Δk) ∼ (Δk)−γ′. The least square fit value of the slope γ′ ± standard error arising from the scatter of the points plotted in the figures is (a) 2.16 ± 0.13, (b) 2.21 ± 0.10, (c) 2.23 ± 0.08, (d) 2.13 ± 0.13. The values of the exponent in P(k) ∼ k−α are γ = 2.21 ± 0.09 (red), 2.19 ± 0.11 (green), 2.18 ± 0.12 (brown), and 2.07 ± 0.11 (cyan). For each of the 15 organisms considered, γ ranges from 2.09 to 2.21 with mean ± standard deviation = 2.16 ± 0.05, while for each of the 105 pairs γ′ ranges from 2.09 to 2.37 with a mean of 2.17 ± 0.04.

Fig. 2. Q(Δk) and P(k) for evolutionarily closeby species within the same group. Conventions are the same as for Fig. 1a-c, except that the individual species are different. Though Δk values are smaller for closeby species
as compared to distant species, \(Q(\Delta k)\) nevertheless seems consistent with a power law with the same exponent as \(P(k)\). Fig. 2a compares two eukaryotes, both yeasts, *Saccharomyces cerevisiae* (\(\gamma = 2.09 \pm 0.10\), green), and *Schizosaccharomyces pombe* (\(\gamma = 2.18 \pm 0.14\), pink), and \(\gamma'\) is found to be 2.28 ± 0.10 (blue). Fig. 2b compares two prokaryotes, both proteobacteria, *E. coli* (2.19 ± 0.11, green) and *Salmonella typhimurium* (2.17 ± 0.11, pink); \(\gamma' = 2.18 \pm 0.11\) (blue). Fig. 2c compares two archaea, *Pyrococcus horikoshi* (2.25 ± 0.10, green) and *Pyrococcus furiosus* (2.17 ± 0.09, pink); \(\gamma' = 2.28 \pm 0.16\) (blue).

**Fig. 3.** Positive and approximately linear correlation between the divergence of the reaction set of a metabolite and the degree of the metabolite. (a) Scatter plot (on a linear scale) of the average \(\Delta k\) of a metabolite across the 105 pairs of species versus its average degree across the 15 (distant) species. The lone point on the extreme right is a single highly connected metabolite, the hydrogen ion. The plot appears approximately linear with some stochasticity. (b) The same on a logarithmic scale where metabolites are placed in logarithmic bins according to their average degree, and the average \(\Delta k\) for a bin is computed by averaging over all 105 pairs of organisms for a given metabolite and then averaging over all metabolites in the bin. The slope of the least square fitted straight line ± the standard error of the deviation of points in the figure from the fit is 1.08 ± 0.03. (c) \(\Delta k\) versus degree of a metabolite for three pairs of distant species. The three pairs of species chosen are the same as in Figs. 1a-c. For each pair of species \((A, B)\), the x-axis represents \(k_{\text{max}} = \max(k_A, k_B)\) (the larger of the two degrees of the metabolite in the two species). The slopes of the three lines are 1.09 ± 0.03 (green) for *H. sapiens* and *E. coli*; 1.08 ± 0.02 (pink) for *H. sapiens* and *M. mazei*; and 1.03 ± 0.02 (cyan) for *E. coli* and *M. mazei*. (d) \(\Delta k\) versus \(k_{\text{max}}\) of a metabolite for three pairs of closeby species. The three pairs of species chosen are the same as in Figs. 2a-c. These have a larger scatter than distant species. The slopes of best fit lines are 1.03 ± 0.07 (green) for *S. cerevisiae* and *S. pombe*; 0.97 ± 0.12 (pink) for *E. coli* and *S. typhimurium*; and 1.07 ± 0.08 for *P. horikoshi* and *P. furiosus*. 
Fig. 2
Fig. 3