Abstract

We showed in this paper that similarity network can be used as an powerful tools to study the relationship of tRNA genes. We constructed a network of 3719 tRNA gene sequences using simplest alignment and studied its topology, degree distribution and clustering coefficient. It is found that the behavior of the network shift from fluctuated distribution to scale-free distribution when the similarity degree of the tRNA gene sequences increase. tRNA gene sequences with the same anticodon identity are more self-organized than the tRNA gene sequences with different anticodon identities and form local clusters in the network. An interesting finding in our studied is some vertices of the local cluster have a high connection with other local clusters, the probable reason is given. Moreover, a network constructed by the same number of random tRNA sequences is used to make comparisons. The relationships between properties of the tRNA similarity network and the characters of tRNA evolutionary history are discussed.

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I. INTRODUCTION

Transfer ribonucleic acid, or tRNA for short, is an important molecule which transmits genetic information from DNA to protein in molecular biology. It has been known that all tRNAs share a common primary, secondary, tertiary structure. Most tRNA sequences have a "CCA" hat in terminus 5' and a polyA tail in terminus 3' in its primary structure. Its secondary structure is represented by a cloverleaf. They have four base-paired stems and a variable stem, defining three stem loops (the D loop, anticodon loop, and T loop) and the acceptor stem, to which oligonucleotides are added in the charging step. Variable loop varies in length from 4 to 13 nt, some of the longer variable loops contain base-paired stems. The tRNAs also share a common three-dimensional shape, which resembles an inverted "L". Though much effort had been put on tRNA research in the past time, little is known about specific features of tRNA that are exclusive to a species, taxa or phylogenetic domain level. With the progress of genome projects, a vast amount of nucleotide sequence data of tRNA is now available, which makes it possible to study the tRNA genes expression for a wide range of organisms.

Recently scientists are trying to find specific feature in genes families by a new tool—complex networks. With the development of techniques on oligonucleotide or cDNA arrays, using gene chips to erect a complicated network and studying its feature and evolution has become a hot subject, and has gained a success. Basically, the networks can be classified into two types in terms of its degree distributions $p(k)$ of nodes: exponential networks and scale-free networks. The former type has a prominent character that although not all nodes in that kind of network would be connected to the same degree, most would have a number of connections hovering around a small, average value, i.e. $k \sim \langle k \rangle$, where $k$ is the number of edges connected to a node and is called degree of the node. The distribution leads to a Poisson or exponential distribution, such as random graph model and small-world model, which is also called homogenous networks. The latter type network has a feature that some nodes act as "very connected" hubs which have very large numbers of connections, but most of the nodes have small numbers of connections. Its degree distribution is a power-law distribution, $p(k) \sim k^{-\gamma}$. It is called inhomogeneous network, or scale-free network.

The tRNA sequences have similarities in sequences and structure, which make it possible to construct networks and use specialized clustering techniques to make classification. The
similarity of tRNA sequences suggests their relationships in evolutionary history. If we consider all the tRNA sequences at present evolve from common ancestor via mutation, the sequence similarity will reveal their evolutionary affiliation. There are lots of tRNA sequences. The similarities of every two of the sequences are different. Lots of data will be dealt with. Since complex network is a good model to describe and study complex relationships, the network model may be useful in this field. In this paper, we constructed a similarity network of 3917 tRNA genes in order to show network model is a powerful tool to study the evolutionary relationships among the tRNA genes. The topology of the network is discussed, the degree distribution and clustering coefficient are considered, and the network constructed by the same number of random tRNA sequences is used to make comparisons.

II. MATERIALS AND METHODS

A. tRNA sequences

Transfer RNA sequence have been collected into database by Sprinzl et al. in 1974. All of our data, 3719 tRNA genes sequences, are retrieved from this database (free available at http://www.uni-bayreuth.de/departaments/biochemie/sprinzl/trna/), which including 61 anticodon subsets, 429 species, and 3 kingdoms: Archaea, Bacteria, and Eucarya. Each tRNA sequence has 99 bases when the variable stem is considered. For convenience of alignment, the absent bases in some positions of the tRNAs are inserted with “blank”. Firstly, we align the tRNA sequences with the same anticodons, and then align all 3719 tRNAs. Since there have been too many conclusions proving that tRNA genes have a high similarity in sequences, the results of the alignment of 3719 tRNA gene sequences will not be listed in detail. We only focus on some prominent characters of the statistics of the alignment.

B. tRNA sequences network

If each base including the inserted “blank” is considered equally, the length of a tRNA is $L = 99$. To align two tRNA sequences, a parameter $s$ is used to depict their similarity degree, which indicates how many bases in the same position of two tRNA gene sequences are identical. For example, if the first bases of two tRNA sequences both are A, one score is
added to \( s \). Obviously, \( 0 \leq s \leq 99 \). Although it is the simplest kind of alignment, as we show later, it gives lots of information of the relationships among tRNA genes. When \( s = 99 \), it means two sequences are matched perfectly. Since the perfectly matched sequences have the the same significance in biology, we take only one of them as a representative. To construct the tRNA similarity network, every sequence is considered as a node. If the alignment score \( s \) of two tRNA sequences is larger than a given similarity degree \( s_0 \), put an edge between the corresponding nodes. Obviously, if \( s_0 \) is small, the nodes will connect closely, and when \( s_0 \) grows larger, the number of connections will decrease.

For comparison, we make a similarity network of the same number of random tRNA genes. To generate the random tRNA genes, every base of the sequences is randomly taken from the four bases (C, G, A and T) and the sequences must confirm to the prototype of the real tRNA, which means the sequences we generate randomly must confirm the secondary structure of tRNA.

III. GRAPH TOOL

Pajek (the Slovene word for spider), a program for large-network analysis [13] (free available at http://vlado.fmf.uni-lj.si/pub/networks/pajek/), was used to map the topology of the network.

IV. RESULTS

A. The topology of network

Figure 1 displays several typical topologies of the similarity network of different kinds of tRNA gene sequences. Figure 1 (a), (b) and (c) are similarity networks constructed by tRNA genes with the same anticodons (CGC, CCA and TGC respectively) and \( S_0 = 60 \). The networks of tRNA genes with the same anticodon identity are highly clustered. Some of them divide into two or more clusters, such as figure 1 (c). Each of the clusters almost entirely connected when \( s_0 \) is small. When \( s_0 \) grows large, the connection number decreases, and the network becomes not so closely connected. Figure 1 (d) is the similarity network of anticodon GTT when \( s_0 = 80 \). As more nodes added in the network, the network becomes more complex. Figure 1 (e), (f) shows the network with a large \( N \) (the number of nodes).
(e) is the network containing anticodons CAT and GCC with $S_0 = 80$, and (f) is the network of all 3420 tRNA sequences with $S_0 = 90$. Small local clusters with the same anticodons get together to form a large cluster, ”very connected” hubs can be observed in the center of the network (figure 1(f)). At a large similarity degree, the scale free property (or power law distribution) emerges, which means a few nodes have a large degree (number of connections), but most nodes have a small degree. To make the figure 1(e) more visualized, we extracted the nodes whose connections number is bigger than 25 to make the figure 2. It also has hubs in the center of the network. Of course, the hubs are smaller. The scale free property is still kept.

The distribution of the connected probability of the networks of the tRNA genes with the same anticodon is shown in table I. The connected probability is defined as the fraction of number of real connections to the largest number of possible connections. In the table it can be found, when $s_0 = 50$, the network is almost entirely connected and most of the connected probabilities are larger than 0.8; when $s_0 = 90$, most of the connected probabilities decrease to one tenth of the former, and some decrease to zero.

Consider the network of random tRNA sequences in the same size. When similarity degree $s_0$ is small, most of the nodes have the same number of connections. When $s_0$ increases, the number of the edges of the network decreases sharply and most of the nodes lose their links; only few of them have two or three edges linked. Table II shows the statistics of the connection numbers of real tRNA similarity network and random tRNA similarity network at different similarity degrees. The table shows that when $s_0 = 50$, the number of the connections of the two networks are very large; and when $s_0 = 90$, both of them drop, but the random one drops more quickly than real one does. The connection number of real tRNA network $n_{real}$ drops from 3434403 ($S_0 = 50$) to 3429 ($S_0 = 90$). The connection number of random tRNA network $n_{random}$ drop from 4321688 ($S_0 = 50$) to 0 when $S_0 = 80$. It shows the real tRNA sequences have more similarity with each other than random ones do. In other words, the real tRNA sequences are not randomly taken. If we consider that the real tRNA genes have evolutionary relationships, the differences between the statistics of real and random tRNA similarity networks shown above can be explained to a certain extent.
B. Degree distributions

It has already been found that networks constructed of the large scale organization of genomic sequence segments display a transition from a Gaussian distribution via a truncated power-law to a real power-law shaped connectivity distribution towards increasing segment size.[14]. The similarity networks of tRNA sequences have similar features. The investigations begin with an important parameter, degree distribution $p(k)$ of the nodes, and the analysis is considered in figure 3.

As observed in Figure 3, with the similarity degree $s_0$ increasing, $p(k)$ behaves more and more similar to power-law distribution. When $s_0 = 50$, degree distribution $p(k)$ of the nodes follows a uninterrupted fluctuated distribution. For those $k < 1088$, $Np(k)$ fluctuate from 1 to 3; and for those $k > 1800$, $Np(k)$ fluctuate from 1 to 9, and the peak of the fluctuation is at $k = 2600$. The mean degree $\langle k \rangle = 2008$, and the maximal degree $k_{\text{max}} = 3052$. When $s_0 = 60$, the peak of the fluctuation deviates to left, at $k = 100$. When $s_0 = 70$, the distribution of $p(k)$ appears a analogous power-law distribution if ignore the minimal value of $k$. For $s_0 > 70$, the distribution transits from a analogous power-law distribution to a real power-law. As shown in figure 3(e), when $s = 90$, the distribution curve fits the power-law perfectly. The fitting result is $p(k) = 0.192k^{-1.636} - 0.006$.

Comparing to the real tRNA gene sequences, the degree distribution of the network of random tRNA sequences, when $s_0 = 50$, is a Gaussion distribution (figure 3(f)). Most nodes have approximately the same degree, $k \approx \langle k \rangle = 2527$; the maximal degree $k_{\text{max}} = 2895$ and the minimal degree $k_{\text{min}} = 2327$. When $s_0 = 60$, the distribution is almost unchanged (figure 3(g)). When $s_0 = 70$, the number of the edges descend sharply with its maximal degree $k = 5$. In figure 3(f), (g), there are lower peaks except the main peaks of the Gaussian distribution. It is possibly because the random tRNA sequences are not generated completely arbitrarily for they must conform to the prototype of the real tRNA.

From above data analysis, we can conclude the real tRNA genes are more self-organized than the random tRNA genes. The power-law distribution means there are a few tRNA genes which behave as "very connected" hubs of the similarity network. Lots of tRNA genes are similar with them in arranging of sequences. If we suppose all the tRNA genes come from common ancestor, it is possible that the "very connected" tRNA genes will have more relationships with the ancestor than other tRNA genes do. In other words, the "very
connected” tRNA genes probably diverge less from ancestral sequences than other tRNA genes do in the evolutionary history. In mathematics, a way to construct a scale-free network is to follow a rule that an added node has much more possibility to connect with a node with a large degree than to connect with a node with a small degree [9]. In the tRNA similarity network, it maybe means the tRNA genes which have small degrees diverged more from ancestor sequences and is less stable than the tRNA genes which have large degrees.

C. Clustering coefficient

If a node connect with \( i \) other nodes and there are \( j \) edges connected within these \( i \) nodes, the clustering coefficient of the original node is defined as

\[
c = \frac{2j}{i(i-1)}
\]

where \( i (i - 1) / 2 \) is the total number of possible connections among \( i \) nodes. Clustering coefficient reflects relationships of the neighbors of a node, and quantifies the inherent tendency of the network to clustering. As shown in Figure 4, the average clustering coefficient \( c_{\text{real}} \) of the real tRNA network is larger than the random one. As \( s_0 \) increase, \( c_{\text{real}} \) decrease. When \( s_0 = 60 \), it approaches a local minimum and experience a little increase and then decreases slowly again. Comparing with the average clustering coefficient of the tRNA network, the average clustering coefficient \( c_{\text{random}} \) of the random network decreases fast while \( s_0 \) increases, when \( s_0 > 70 \), \( c_{\text{random}} \to 0 \). The behavior of the coefficient of two networks is also illustrated in table II. When \( s_0 = 50 \), \( c_{\text{real}} = 0.777367 \), \( c_{\text{random}} = 0.747479 \); when \( s > 70 \), \( c_{\text{random}} \) drops to zero quickly, but \( c_{\text{real}} \) decrease slowly. Once again, we proved the real tRNA genes are not randomly selected. The real tRNA genes have close relationships with each other.

Table III shows the distribution of the average clustering coefficient of 19 tRNA groups which are classified by the possible amino acid-accepting. Some groups contain isoacceptor tRNA which consist of different tRNA species that bind to alternate codons for the same amino acid residue. The tRNA group who carries the amino acid residue named Met is ignored for it contains only one tRNA sequence. Comparing table III with table II we can conclude that the nodes are more likely to connect with the nodes within the same amino acid group. The tRNA similarity network can be classified into several large clusters with the same amino acids. It hints that in tRNA genes evolutionary step is much more likely to
happen within the same amino acid group. The cases that a tRNA gene of certain amino acid evolve to tRNA gene of another amino acid are rare.

V. DISCUSSION

In this paper, we want to show the network model is a powerful tool to study the relationship of tRNA genes. Although some results are not new, such as the real tRNA genes are not random and the relationships among tRNA genes with same anticodon are closer than the relationships among tRNA genes with different anticodons, they are evidences that network model works well for the network model distinguishes these properties clearly. What is more, the tRNA similarity network behaves scale-free properties when \( s_0 \) is large. As we know the scale-free nature is rooted in two generic mechanisms. Firstly scale-free networks describe open systems that grow by the continuous addition of new nodes. Secondly scale-free networks exhibit preferential attachment that means the likelihood of connecting to a node depends on the node’s degree. With these mechanisms, the ”very connected” nodes in scale-free networks usually are added in the network at early time during the growth of the network. It has been found that most recent tRNA genes are evolved from a few common precursors, and these oldest evolutionary sequences, comparing to the recent tRNA genes. Therefore, in tRNA similarity network, the ”very connected” tRNA genes may have diverged less from their ancestors than weakly connected ones.

Most recently, many research conclusions show that genes of related function could behave together as a group in the networks constructed according to their similarity features. In this paper, although we use the simplest alignment, this property can be found. When similarity degree \( s_0 \) is small, nodes of the tRNA genes with the same anticodons are connected to form a local cluster, among them are entirely connected. When \( s_0 \) increases to a large value, a scale-free character emerges that a few nodes compose the core of the network and most of nodes have low links. These observations seem to be perfectly fit to the evolutionary processes of the tRNA genes. On the other hand, the oldest tRNA genes undergo disturbances such as mutation, loss, insertion, or rearrangement etc. during the evolution. Some new tRNA genes are suited for the environment and reserved. So, they have a high similarity to its ancestral sequences. In the network constructed by similarity degree of these tRNA genes, they form local clusters.
An interesting finding of tRNA similarity networks is that some local clusters have high connectivity with the other clusters; or to say, some nodes of one cluster have lots of connections with some nodes of another cluster. See figure 5. It may hint that the evolution relationship of tRNA sequences of two different anticodons. As shown in figure 5(a), the network is of two different anticodons: ACG and CCA. The solid circle nodes are the tRNA genes of ACG, and the hollow circle nodes are the tRNA genes of CCA. In this figure, they mix into one cluster. Figure 5(b) shows that the network of anticodons TAG and TGA. The solid circle nodes are the tRNA genes of TAG, and the hollow circle nodes are tRNA genes of TGA. They appear three clusters in the topology map, and each cluster has some nodes which highly connect with some nodes of other clusters. It shows that although some tRNA genes have different anticodons, they have high similarities in sequences. In evolutionary history, the tRNA genes of one anticodon identity can evolve to tRNA genes of another identity. The above finding may be an evidence of this kind of evolutionary mode. In the other hand, from figure 5(c), the network of the same anticodon GCC split into two cluster. It hints the evolution process of the tRNA genes of same anticodon may diverge in the history. Therefore, there are different modes of evolutionary processes, i.e. evolution within the same anticodon groups and evolution among different anticodon groups. The former may be the main part of tRNA evolution. The later may be the key cases of the interaction among tRNA of different anticodons during the evolution.

For the alignment we used is simply counting the number of cites that are identical, it losts many information in the evolution process. More complicated alignment models may exhibit more details of the relationships among tRNA genes. The content of tRNA database is limited, the numbers of tRNA sequences from different organisms varied largely. Therefore, the biases of taxon samples may influence the topology of the network and the results gotten from the network may not completely reflect the evolution relationship of tRNA genes. It is a limitation of network model that will be improved when more tRNA genes are sequenced. Although we did not get many new results from what we have know about the evolution of tRNA genes, the results contribute as proofs that the network model can work well in the research of relationship of tRNA genes and is a useful tool.
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|     | 50  | 60  | 70  | 80  | 90  |
|-----|-----|-----|-----|-----|-----|
| aac | 0.8182 | 0.8182 | 0.7636 | 0.3636 | 0.1091 |
| aag | 0.8 | 0.6 | 0.6 | 0.1 | 0 |
| aat | 1 | 0.9333 | 0.7333 | 0.1333 | 0.0667 |
| acg | 0.9111 | 0.8537 | 0.4378 | 0.1347 | 0.0604 |
| agc | 1 | 1 | 0.9615 | 0.5513 | 0.2692 |
| agg | 1 | 0.7333 | 0.6667 | 0.5333 | 0.0667 |
|agt | 0.75 | 0.7142 | 0.3929 | 0.0357 | 0.0357 |
| acc | 1 | 0.7 | 0.2 | 0.1 | 0.1 |
| act | 1 | 1 | 1 | 1 | 0 |
| aga | 1 | 1 | 0.7867 | 0.4338 | 0.1691 |
| caa | 0.8842 | 0.6182 | 0.2192 | 0.1035 | 0.0025 |
| cac | 0.9455 | 0.6909 | 0.4727 | 0.2364 | 0.1273 |
| cag | 0.7493 | 0.4431 | 0.1396 | 0.06268 | 0.0256 |
| cat | 0.9034 | 0.486 | 0.1704 | 0.0549 | 0.0194 |
| cca | 0.9874 | 0.8414 | 0.3676 | 0.0985 | 0.037 |
| ccc | 0.8611 | 0.7778 | 0.4722 | 0.0833 | 0 |
| ccg | 0.8182 | 0.8181 | 0.3455 | 0.1636 | 0.1091 |
| cct | 1 | 0.9091 | 0.3818 | 0.0364 | 0 |
| cga | 0.9083 | 0.45 | 0.1167 | 0.0417 | 0.0016 |
| cgc | 1 | 1 | 0.8667 | 0.0667 | 0 |
| cgg | 1 | 1 | 0.6667 | 0.3809 | 0.0476 |
| cgt | 1 | 0.956 | 0.4725 | 0.0549 | 0.011 |
| ctc | 1 | 0.9809 | 0.6 | 0.2857 | 0.0762 |
| ctg | 1 | 0.8053 | 0.3474 | 0.1211 | 0.0947 |
| ctt | 0.7808 | 0.5045 | 0.3649 | 0.1156 | 0.036 |
| gaa | 0.9424 | 0.5709 | 0.2778 | 0.0891 | 0.0151 |
| gac | 1 | 1 | 0.4314 | 0.2026 | 0.1503 |
| gag | 0.9636 | 0.5818 | 0.2909 | 0.0545 | 0 |

TABLE I: The distribution of the connected probability of all 57 anticodons’ tRNAs networks, which have excluded four anticodons for they have too small vertices. The statistic shows that when \( s = 50 \), many networks are complete connection; when \( s = 90 \), the connected probability decreasing sharply, some of the connected probability decrease to zero.
TABLE II: The number of edges and average cluster coefficients of two networks respective to similarity degrees. The number of nodes is 3420. $s_0$: similarity degree; $n$: the number of edges of the network; $c$: average cluster coefficient

| $s_0$ | $n_{real}$ | $n_{random}$ | $c_{real}$ | $c_{random}$ |
|-------|------------|--------------|------------|--------------|
| 50    | 3434403    | 4321688      | 0.777367   | 0.747479     |
| 60    | 994571     | 367845       | 0.541708   | 0.139572     |
| 70    | 142264     | 773          | 0.578806   | 0.000682     |
| 80    | 19453      | 0            | 0.567380   | 0.000000     |
| 90    | 4249       | 0            | 0.286254   | 0.000000     |

TABLE III: The average clustering coefficient of 19 tRNA possible aminoacid-accepting groups’ networks, each network is named using three-letter amino acid abbreviations.

| $s_0$ | 50 | 60 | 70 | 80 | 90 |
|-------|----|----|----|----|----|
| VAL   | 0.838031 | 0.653432 | 0.704976 | 0.61135 | 0.302831 |
| ASH   | 0.868872 | 0.878484 | 0.745679 | 0.306494 | 0.151515 |
| ASP   | 0.924069 | 0.814945 | 0.818599 | 0.637089 | 0.391828 |
| CYS   | 0.928983 | 0.765042 | 0.717865 | 0.637071 | 0.320786 |
| ALA   | 0.738776 | 0.745374 | 0.6728   | 0.481527 | 0.253499 |
| GLN   | 0.865414 | 0.727525 | 0.630352 | 0.338557 | 0.245455 |
| GLU   | 0.929629 | 0.808843 | 0.767439 | 0.559427 | 0.260123 |
| GLY   | 0.888469 | 0.874198 | 0.683965 | 0.540341 | 0.200635 |
| HIS   | 0.93883  | 0.745659 | 0.716469 | 0.677458 | 0.406243 |
| LEU   | 0.921699 | 0.681914 | 0.733301 | 0.609716 | 0.313292 |
| LYS   | 0.722222 | 0.666667 | 0.666667 | 0      | 0      |
| PHE   | 0.877036 | 0.678511 | 0.744765 | 0.621813 | 0.287326 |
| PRO   | 0.97332  | 0.848348 | 0.546192 | 0.447778 | 0.18366 |
| SER   | 0.856441 | 0.666374 | 0.649365 | 0.542714 | 0.175925 |
| STOP  | 0.758638 | 0.707049 | 0.731592 | 0.57147  | 0.217136 |
| THR   | 0.93964  | 0.831404 | 0.6121   | 0.585127 | 0.309603 |
| TRP   | 0.9265   | 0.789019 | 0.754142 | 0.581435 | 0.225642 |
| TYR   | 0.932773 | 0.779707 | 0.684642 | 0.558649 | 0.306018 |
| ARG   | 0.805026 | 0.834632 | 0.632049 | 0.375894 | 0.147186 |
FIG. 1: the topology of the network. (a), (b), (c), (d)) are the topology of network of the same anticodons. The three capital letters are the three anticodons subsets of tRNA genes, (a): CGC, $S_0 = 60$, $N = 6$, $P = 1.0$; (b): CCA, $S_0 = 60$, $N = 150$, $P = 0.8414$; (c): TGC $S_0 = 60$, $N = 215$, $P = 0.5892$ (d): GTT $S_0 = 80$, $N = 145$, $P = 0.028$). (e), (f) are the topology of network of different anticodons. (e): $S_0 = 80$, $N = 304$, $P = 0.04$, network of CAT and GCC; (f): $S_0 = 90$, $N = 3420$, $P = 0.0034$. $S_0$ is the similarity degree; $N$ is the number of the nodes; $P$ is the connection probability.
FIG. 2: The topology of network which extract the nodes which degree $k \geq 25$ from figure 1 (f).

$S_0 = 90$
FIG. 3: (a), (b), (c), (d), (e) are the degree distribution of the tRNA gene sequences network, $N=3420$; The line in (e) is power law fitting of the data. The formula is $p(k) = 0.192k^{-1.036} - 0.006$. (f), (g) are the degree distribution of the random tRNA sequence network, $N=3420$. 
FIG. 4: The distribution of the clustering coefficient of the two networks according to their similarity degree.
FIG. 5: Cluster of network of tRNA genes of different anticodons. They are segments of the topology of 3420 tRNA genes. (a) Composing 96 vertices and 97 edges, similarity degree $S_0$ is 60, contain anticodons: ACG (solid circle) and CCA (hollow circle); (b) Composing 226 vertices and 227 edges, similarity degree $S_0$ is 60, contain anticodons: TAG (solid circle) and TGA (hollow circle).