Network rewiring is an important mechanism of gene essentiality change

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Gene essentiality changes are crucial for organismal evolution. However, it is unclear how essentiality of orthologs varies across species. We investigated the underlying mechanism of gene essentiality changes between yeast and mouse based on the framework of network evolution and comparative genomic analysis. We found that yeast nonessential genes become essential in mouse when their network connections rapidly increase through engagement in protein complexes. The increased interactions allowed the previously nonessential genes to become members of vital pathways. By accounting for changes in gene essentiality, we firmly reestablished the centrality-lethality rule, which proposed the relationship of essential genes and network hubs. Furthermore, we discovered that the number of connections associated with essential and non-essential genes depends on whether they were essential in ancestral species. Our study describes for the first time how network evolution occurs to change gene essentiality.

Gene essentiality varies across species and is one of the most dramatic phenotypic changes a gene can undergo. For instance, deletion of MAP kinase kinase1 (Map2k1) did not affect the fitness of yeast, but its loss of function caused embryonic lethality in mouse. In contrast, deletion of serine/threonine-protein kinase ICK caused lethality in yeast but had no apparent phenotypic effect in mouse. Generally, orthologs are considered to deliver the same function in different species. Given that this is not always the case, why and how does essentiality of the same functional gene change between species?

The C-L rule explains that highly connected proteins in a network are more likely to be essential for cell viability. However, a weak correlation between network connections and gene essentiality has led to controversies over the C-L rule. A system-level understanding of how gene essentiality can change will give us a chance to understand the design principles of key biological processes and provide opportunity for predicting important gene functions.

Here, we investigated the mechanisms of gene essentiality changes in the framework of network expansion during evolution. We hypothesized that network rewiring has a significant effect on gene essentiality changes because rewiring of interactions enables genes to be integrated into new pathways and the new interactions can increase the probability of becoming involved in a vital biological process.

Results
Gene essentiality frequently changes during evolution. We found that a significant portion of 2,144 mouse genes with yeast orthologs changed their essentialities between mouse and yeast (Fig. 1a). We arranged the orthologous pairs of yeast and mouse genes into four phenotypic groups based on their changing essentiality patterns. We found 91 genes are essential in both yeast and mouse (E2E), 246 genes are nonessential in yeast but essential in mouse (N2E), 659 genes are essential in yeast but nonessential in mouse (E2N), and 1,149 genes are nonessential in both yeast and mouse (N2N). The list of yeast and mouse gene orthologs and their essentiality measurements can be accessed in Supplementary Table S1.

Increase of network connections explains gene essentiality changes. We hypothesized that the frequent gene essentiality changes we observed are related to interaction rewiring, which allows genes to integrate into, or separate from, important biological pathways. To test this hypothesis, we examined the increase of network connections between yeast and mouse protein-protein interaction (PPI) networks (Fig. 1b). It has been suggested that the number of protein interactions are highly correlated with the complexity of the organism. Protein interactions were measured by experiments from yeast and mouse separately and the network connections between yeast and mouse were compared by ortholog mapping (see Materials and Methods).
all the four classes of essentiality changes increased the average network connections in mouse relative to yeast, but the amount of increase was quite different in the four classes. In particular, N2E genes have the highest increase in network connections, whereas E2N genes have the smallest increase among the four phenotypic groups. The increase in connectivity was most significant in N2E genes compared to all genes \( (p = 6.76 \times 10^{-7}; \text{Fig. 1c}) \), whereas the increase for E2N genes was significantly smaller than the average \( (p = 1.30 \times 10^{-4}) \).

Because of a large evolutionary distance between yeast and mouse, we investigated more species pairs that diverged enough but closer than the distance between yeast and mouse. We found that all genes

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**Figure 1** | Increase in network connections and gene essentiality changes between yeast and mouse. (a) Gene essentiality changes between yeast and mouse. The numbers of essential and nonessential genes of yeast (left) and mouse (right) are presented. (b) A network evolution model describing gene essentiality changes. (c) Network connections of the four phenotypic classes. The first two panels display the average number of network connections in yeast and mouse respectively. Error bars indicate the standard error. The last panel shows the fold increase in the average number of connections in mouse relative to yeast.

**Figure 2** | Comparison of network connections in various species. (a) Increase in network connections by the complexity of organisms. The fold increase in the number of connections relative to yeast is plotted. (b) Increase of network connections between various species pairs. The fold increases of network connections in worm over yeast, chicken over worm, and mouse over chicken were presented.
Figure 3 | Functional enrichment analysis of essentiality changing genes. (a) Comparison Biological processes of N2E, E2N, and N2N genes with those of E2E genes in yeast and mouse. (b) Enrichment of biological processes in the four phenotypic groups. Gene ontology terms that are significantly enriched ($p < 0.001$) in N2E genes are presented. (c) Network connections of Map2k1 in yeast, worm, chicken, and mouse. Interaction partners Map2k1 in yeast (left) and mouse (right) are depicted with the orthologs connected by dotted lines.
gradually increased their network connections in the course of evolution (Fig. 2a) but N2E genes increased network connections fastest among all phenotypic groups from the comparison of closer species (Fig. 2b). These results suggest that essential genes in unicellular organisms that become nonessential in multicellular organisms, fail to rapidly expand their network connections in the course of evolution.

N2E genes have integrated into vital biological pathways. Next we asked whether the increased connections create new connections to core biological functions and thereby increased essentiality. It has been suggested that genes may become essential by participating in core pathways9, but evidence for this hypothesis has heretofore been lacking. We find that new interactions gained from network expansion do tend to cause integration of N2E genes into vital pathways of essential genes (Fig. 3a). Functional enrichment analysis of gene ontology of biological processes (BPs) was carried out for interactions formed by N2E, E2N, N2N, and E2E genes in yeast and mouse (Supplementary Table S2). The analysis reveals that interactions of N2E genes gained from network expansion have dramatically increased their participation in essential BPs of E2E genes. Specifically, in yeast, interactions of N2E genes share 50% of BPs with E2E genes, but in mouse, the fraction sharply increases to 74%. Whereas interactions of E2N genes share 77% of BPs with E2E genes in yeast, the fraction decreases to 59% in mouse.

Many N2E genes become integrated into BPs that are vital for the development of multicellular organisms (Fig. 3b and Table 1). Interactions of N2E proteins are highly enriched in developmental processes where a single misregulation could cause embryonic lethality. For example, the expanded network connections of Map2k1, a N2E gene, are involved in key pathways in multicellular organisms (Supplementary Table S3). Map2k1 participates in placenta development in mouse via newly evolved interactions. It has eight interaction partners in the yeast PPI network, but its network connections increased to 23 in the mouse PPI network (Fig. 3c). Consequently, the deletion of Map2k1 is not lethal in yeast, but causes embryonic lethality in mice2,3. Among the interaction partners of Map2k1 is epidermal growth factor receptor, EGFR, which regulates the epidermal growth factor pathway that is crucial for cell growth and morphogenesis16.

Gene essentiality change is related with protein complex membership. We next asked how N2E genes have quickly increased their network connections at the molecular level. We examined the membership changes of protein complexes between yeast and mouse, and found that N2E genes showed the highest rate of engaging in protein complexes among the four groups (Table S2). The analysis reveals that interactions of N2E genes gained from network expansion have dramatically increased their participation in essential BPs of E2E genes. Specifically, in yeast, interactions of N2E genes share 50% of BPs with E2E genes, but in mouse, the fraction sharply increases to 74%. Whereas interactions of E2N genes share 77% of BPs with E2E genes in yeast, the fraction decreases to 59% in mouse.

Table 1: Developmental processes of N2E genes in mouse

| Developmental process                        | N2E genes                                                                 |
|---------------------------------------------|---------------------------------------------------------------------------|
| blastocyst development                      | Cul3, Smarcb1, Ada, Sp3, Junb                                              |
| mammary gland development                   | Phb2, Atp7b                                                                |
| in utero embryonic development              | Prmt1, Sin3a, Cul3, Slc30a1, Ccnb2, Smarcb1, Msh2, Ube2a, Mapk1, Myo1e, Mecom, Sp3, Ccnb1, Plcg1, Junb, Lsg, Fgfr1, Ada, Hsf1, Map2k1 |
| immune system development                   | Exo1, Msh2, Mrea, Ung, Sp3, Rps19, Slc11a2, Xrc6c, Lsg, Blm, Spdl1, Msh6, G6pdx, Ccnb2, Mih1, Myo1e, Tceai, Ada, Hels, Sod2, Dnaja3 |
| developmental process                        | G6pdx, Ccnb2, Msh2, Myo1e, Mrea, Sp3, Rps19, Slc11a2, Lsg, Blm, Spdl1, Tceai, Ada, Hels, Sod2, Dnaja3 |
| positive regulation of developmental process| Hmg1b, Junb, Lsg, Xrc6c, Fgfr1, Ada, Map2k1, Mapk14                         |
| tube development                             | Phb2, Hmg1b, Timeless, Sp3, Ppp3r1, Ppges3, Fgfr1, Ada                    |
| gland development                            | Phb2, Atp7b, Fgfr1                                                         |
| chordate embryonic development               | Msh2, Ube2a, Phgad, Sp3, Junb, Lsg, Fgfr1, Map2k1, Prmt1, Sin3a, Cul3, Slc30a1, Ccnb2, Smarcb1, Mapk1, Myo1e, Mecom, Ccnb1, Plcg1, Ada, Hsf1, Atm |
| embryonic development ending in birth or egg hatching | Msh2, Ube2a, Phgad, Sp3, Junb, Lsg, Fgfr1, Map2k1, Prmt1, Sin3a, Cul3, Slc30a1, Ccnb2, Smarcb1, Mapk1, Myo1e, Mecom, Ccnb1, Plcg1, Ada, Hsf1, Atm |
| blood vessel development                     | Myo1e, Mapk1, Phk2, Veif1, Junb, Ppp2b, Spdl1, Atp5, Fgfr1, Map2k1, Mapk14 |

Figure 4 | Protein complex membership and evolution of gene essentiality changes. (a) Fraction of genes newly involved in protein complexes are compared in each phenotypic group. (b) Evolutionary rates (dN/dS) of each phenotypic group in yeast. The evolutionary rates (dN/dS) were calculated from nucleotide sequences for 3,392 orthologous open reading frames (ORFs) in four Saccharomyces species including S. cerevisiae, S. paradoxus, S. mikatae, and S. bayanus.
multicellular organisms. This suggests that protein complex membership may be an important mechanism for expanding network connections that can affect gene essentiality changes.

To increase network connections rapidly, N2E genes may have acquired new interaction sites through fast adaptive evolution. To test this possibility, we examined the evolutionary rates of E2E, N2E, E2N, and N2N genes in various yeast species, and discovered that N2E genes have rapidly evolved. Evolutionary rates of yeast genes were calculated as the ratio of nonsynonymous substitutions ($dN$) to synonymous substitutions ($dS$) from the four complete genomes of Saccharomyces species. As shown in Fig. 4b, N2E genes show a rapid evolutionary rate compared to E2E ($p = 5.67 \times 10^{-5}$) and E2N genes ($p = 2.79 \times 10^{-7}$). Interestingly, the evolutionary rates of N2E and N2N genes were similar ($p = 0.82$). The rapid evolutionary rate of N2N genes is probably due to low selective pressure on nonessential genes.

**Discussion**

Having confirmed that network evolution influences gene essentiality changes, we asked how interaction rewiring has impacted the information flow of biological networks. Betweenness centrality is a measure of a node’s centrality in a network equal to the number of shortest paths between all pairs of nodes that pass through that node. Proteins with high betweenness centrality tend to interact with many different functional groups and are important for controlling information flow in the network. We discovered that the betweenness centrality of N2E genes is higher than those of N2N and E2N genes when they have same number of network connections (Fig. 5). Of the four groups, E2E genes have the highest betweenness centrality due to their importance in information flow in PPI network. However, N2E genes showed a dramatic increase in betweenness centrality if they were highly connected (>16 network connections). The increased betweenness centrality affects the functional role of N2E genes by reforming the modular architecture of the PPI network. Although both N2E and N2N genes were nonessential in yeast, the extensive rewiring of network connections for N2E genes in more complex organisms enables them to connect with various functional modules, thereby controlling information flow around newly evolved essential genes.

Our findings on the evolution of networks allow us to firmly reestablish the C-L rule by showing that highly connected genes in a network are indeed more essential when network rewiring is properly considered. The C-L rule has been debated because of an apparent weak correlation between network connection and gene essentiality. We suspected that the poor correlation may have occurred because the evolution of gene essentiality was not considered previously (Fig. 6). According to the C-L rule, essential genes in yeast will have a relatively high connectivity. If rewiring leads it to become nonessential in mouse (E2N), connections will decrease relative to essential mouse genes (see above), but not enough evolutionary time may have occurred to descend to the level of a nonessential gene that was already nonessential in yeast (N2N). Similarly, if a nonessential gene becomes essential in mouse (N2E), then connections are generally added rapidly (see above), but insufficient evolutionary time may have occurred to achieve the connection level of a gene that was already essential in yeast and remained essential in mouse. As shown in Fig. 6, when we only consider genes with conserved essentiality in both yeast and mouse, the correlation between connectivity and essentiality becomes extremely high ($R^2 = 0.97$).

In other words, when we set a common starting point in the connectivity race, essential genes do acquire more connections than nonessential ones.
non-essential genes. Thus, the C-L rule does explain the relationship between gene essentiality and network connection. It also suggests that interaction rewiring should be properly considered for predicting gene essentiality on a genome-wide scale through the mapping of orthologs.

The relationship between gene essentiality changes and the increase of network connections is also true for relatively young genes that are found from either yeast or mouse. Among mouse genes that do not have yeast orthologs, 2,189 were found to be essential (X2E) and 12,207 were nonessential (X2N). We found that X2E has significantly more network connections than X2N in the mouse PPI network (p = 2.16 × 10⁻⁴). Meanwhile, of yeast genes without mouse orthologs, 427 were found to be essential (E2X) and 3,983 were nonessential (N2X). Similarly, E2X were found to have significantly more network connections than N2X (p = 5.33 × 10⁻¹³). These biases of network connections in young genes suggest that genes engaging in more interactions are likely to be essential.

When young genes first arose, they are likely to be nonessential because their ancestral species survived without them and they share network connections with their parental genes. As they underwent interaction rewiring, those that gained more interactions became essential and had more chances to be a member of vital pathways.

To our knowledge, this study highlights for the first time that interaction rewiring is a key to the evolution of gene essentiality. Relating network rewiring with phenotypic changes will improve our understanding of the functional evolution of genes.

Methods

Essential and nonessential genes of yeast and mouse. Phenotype data of mouse gene essentiality database. Nucleic Acids Res 40, D901–906 (2012).

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J.K. and S.K. designed the study. J.K., J.B., S.K. wrote the paper. I.K, S.K.H. analyzed data.

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