Dynamic miRNA-TF-mRNA circuits in mouse lung development

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***Abstract*—Genes, transcription factors (TF), microRNAs (miRNA) are well-known to have important regulating roles in dynamic biological processes. In the last years, many studies have been devoted to the elucidation of transcriptional or post-transcriptional regulating activities of TFs or miRNAs, respectively. However, very limited attempts have been made to consider the dynamic characteristics of miRNA-TF-mRNA circuits, which are the biological network motifs considering miRNAs, TFs and genes as a whole in the complicated biological procedures like mouse lung development. Here we propose to mine miRNA-TF-mRNA circuits related to the mouse lung development by integrating TF-mRNA, miRNA-mRNA, TF-miRNA, and time-course expression data, and to further analyze the variations of these circuits in different stages of the lung development. To our best knowledge, this is the first time to take transcriptional and post-transcriptional information together to describe the mouse lung development. Our preliminary results show that miRNA-TF-mRNA circuits vary in different stages of the lung development and play different roles.**

***Keywords*—miRNA-TF-mRNA circuit; mouse lung development; miRNA; mRNA; TF**

I. INTRODUCTION

Although the factors involved in lung development have not been fully understood, biologists have indeed found that certain elements existing in the biological processes play important roles. In molecular biology, gene expression profile analysis across different lung development stages shows a series of novel transcription factors, target genes, temporal regulation, and candidate regulatory pathways observed during lung development [1, 2]. As known, the development of the mammalian lung contains six stages such as embryonic, pseudoglandular, canalicular, saccular, alveolar, and maturation of the microvasculature [3]. The embryonic stage begins from about 9 to 12 days, pseudoglandular stage is from about 12 to 15 days, canalicular stage is from about 15 to 17 days, saccular and alveolar appear after 17 days, and the maturation of the microvasculature stage is just the adult lung[1]. In these different stages of lung development, the involved regulatory elements should include both common and specific ones.

It is well known that transcription factors (TFs) and microRNAs (miRNAs) are key regulators for gene expression regulation in higher eukaryotes. For a protein-coding gene to be expressed, it must first be transcribed. In transcription, the code of the gene's DNA is converted into a complementary code as an mRNA molecule. The mRNA molecule then participates in the second phase: translation. In translation, the code of the mRNA is converted into an amino acid sequence of a protein [4]. While, miRNAs are a class of small RNAs (21–24 nt) that can regulate the expression of target genes at the post-transcriptional level. Based on the pairing of miRNAs and their target sites on corresponding mRNA, the complexes can inhibit translation by either degrading of the mRNA, or by blocking translation without degrading the targets[5].

There have been many studies focusing on investigating the functions of miRNAs or TFs, respectively. For examples, Dong *et al*. have used statistical and computational approaches to analyze the dynamical regulating patterns of miRNAs on mRNAs for lung development based on the expression values [6]. Bandyopadhyay *et al*. have collated the differential expression patterns of specific miRNAs in cancer tissues and obtain a global perspective of miRNA dysregulation in multiple cancer types [7]. There are also some researches focusing on miRNA network in the different species or tissues [8-10]. In addition, there are lots of work considering the regulatory relationships or regulation network of TFs and target genes [11]. In fact, many computational tools have been proposed to predict the regulatory relations between miRNA and mRNA, such as mirConnX [12], TargetScan [13], EMBL [14], PicTar [15], and Miranda [16], or to predict the regulatory relations between TF and mRNA, such as TRANSFAC [17, 18], JASPAR[19], and TRRD[20]. However, very limited attempts have been made to consider the regulatory combinations of TFs, miRNAs and mRNAs (genes), especially in the lung development, which is obviously not enough to understand the molecular mechanism of the complicated biological processes involved in organism development.

Therefore, in this paper, we propose to detect the triple relations among miRNAs, TFs and mRNAs to identify the non-trivial regulatory combinations in the lung development of mouse. We further analyze the combinations across the lung development and find out stage-specific combinations. Our results give a systematic view for understanding the alteration of transcriptional or post-transcriptional regulatory factors and their roles involved in the mouse lung development.

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II. METHOD

In this paper, we define the triple relations among miRNAs, TFs and mRNAs as *circuits*. A circuit is a network where transcriptional and post-transcriptional sub-network information is fused together in order to propose and recognize non trivial regulatory combinations. The general circuit network structure is shown in Figure 1. Briefly, the circuit contains the non-trivial combinations of TF-miRNA, TF-mRNA and miRNA-mRNA pairs related to a specific biological procedure (as lung development in this work).

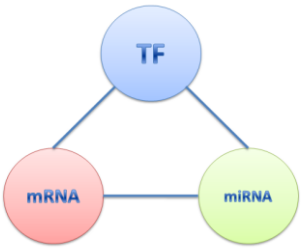

Figure 1. The network structure of a circuit

According to above definition, we propose a framework to find the circuits and investigate their variations across the lung development by integrating several data resources, whose flow chart is shown in Figure 2 and introduced clearly in following sub-sections.

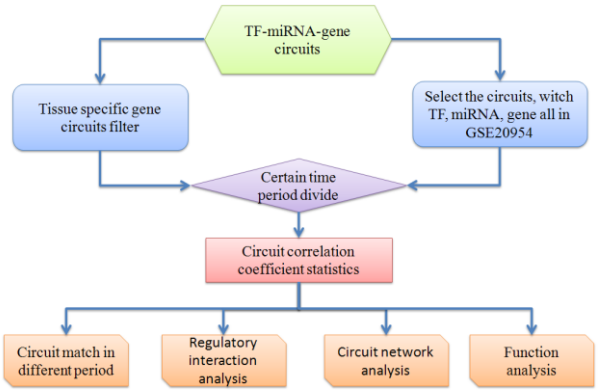

Figure 2. Flow chart of analysis procedure on dynamic miRNA-TF-mRNA circuit

A. Data resources

(1) Time series data related to the lung development: GSE21053 [6]. It contains seven time points of gene expression data GSE20954 and miRNA expression data GSE20152, both measured from two mouse samples at time points embryo day 12 (E12), embryo day 14 (E14), embryo day 16 (E16), embryo day 18 (E18), postnatal day 2 (P2), postnatal day 10 (P10), and postnatal day 30 (P30). Since we cannot find the corresponding protein data of TFs at present, we just made use of the gene expression values of the TFs' coding genes instead.

(2) The lung specific genes: we extract the lung-specific genes from the tissue-specific gene database TiSGeD [21]. By setting SPM [21] threshold as 0.3, 511 lung-specific genes are determined, 455 of which have gene expression values in GSE20954 and thus are used for further study.

(3) Pair-wise relations: the miRNA-gene pairs are extracted from TargetScan [13] and miRanda [22]; TF-gene pairs are from Tred [23] and KEGG [24]; miRNA-TF pairs are from circuitDB [25]. Note that we also extract some miRNA-gene and TF-gene pairs from circuitDB [25].

B. Extration of miRNA-TF-mRNA circuit candidates

Based on several data resources, we extract the candidates of miRNA-TF-mRNA circuits by the procedure shown in Figure 3.

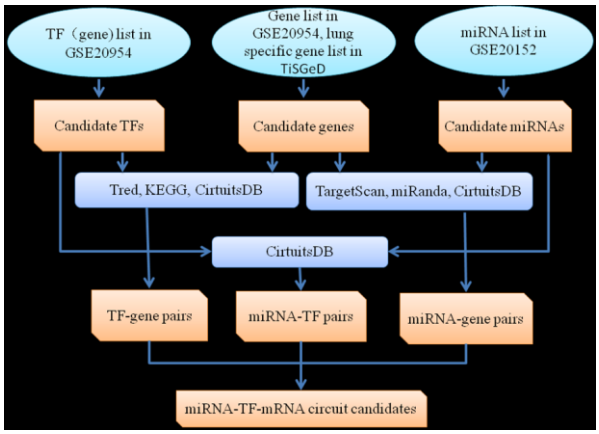

Figure 3. Extracting miRNA-TF-mRNA circuit candidates

First, we collect all lung related miRNAs, TFs and genes from several data resources. Concretely, we extract the miRNAs from the miRNA expression data GS20152, TFs from the mRNA expression data GS20954 (we just use the coding genes to represent the TFs in this work). The genes belonging to both GS20954 and lung-specific gene set in TiSGeD database are selected as the lung related genes.

Then, we detect the pair-wise relations among genes, TFs and miRNAs, as described above. All the pairs are combined into triple relations as the potential miRNA-TF-mRNA circuits.

As a result, we have found 880 circuit candidates, containing 227 genes, 21 TFs and 62 miRNAs in total.

C. Detecting the circuits related to lung development

In order to detect the non-trivial circuits that are related to the lung development, we calculate the significance of each of the three pair-wise relations in the same circuit candidate by using Pearson correlation coefficient.

Suppose two nodes in the pair-wise relation be i and j, (i, j are two vectors of expression data along a period of time points), then the Pearson correlation coefficient $R(i,j)$ can be computed as:

$$R(i,j)=\frac{\mathrm{E}[(i-\mu_i)*(j-\mu_j)]}{\sqrt{E[(i-u_i)*(i-\mu_i)]E[(j-\mu_j)*(j-\mu_j)]}}$$ Wh

ere E is the mathematical expectation and μ_i is the mean of vector i.

We use the permutation test to evaluate the significance of the correlation and set the p-value threshold as 0.05. That is, the pair-wise relation is considered to be significant in case its p-value is less than 0.05. Only when all of the three pair-wise

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relations in a combination are significant, this corresponding candidate can be regarded as a circuit at the specific period of time.

III. RESULTS AND DISCUSSIONS

Based on the 880 collected circuit candidates, we investigate regulatory combinations of TFs and miRNAs across the lung development.

By two-way clustering the genes across seven time points of the lung development, we have found that the lung development of mouse can basically be divided into two stages (shown in Figure 4), stage 1: time points 1-3, and stage 2: time points 4-7. It is noticeable that time points 1-4 correspond to embryo days 12, 14, 16 and 18, while time points 5-7 correspond to postnatal days 2, 10 and 30. However, time point 4 is clustered with time points 5-7 together instead with time points 1-3. Thus we guess the molecular mechanism of pseudoglandular and canalicular of lung (embryo days 12-17) are very different with that of saccular and alveolar (⩾embryo day 17) which continues until a period of time after birth (postnatal days 2, 10, and 30). Moreover, we have found that in the same cluster, gene profiles at point 7 (postnatal day 30) is different with those in other three points, which may be due to the fact that postnatal day 30 corresponds to the maturation of the microvasculature stage.Therefore, we only consider time points 1-3 and 4-6 of the lung development and call them as early and late stages, respectively, in the following analysis.

Accordingly, we mine the early and late circuits in the lung development, and obtain 85 (14 miRNAs, 7 TFs and 40 genes) and 23 (11 miRNAs, 6 TFs and 16 genes) circuits, respectively. To see how the circuits change across the lung development, we also mine the circuits involved in the whole six time points of the lung development (191 circuits containing 25 miRNAs, 10 TFs and 94 genes).

The circuits overlap among early, late and whole stages is shown in Figure 5, which illustrates that there are different circuits involved in different stages. This means the role of TF or miRNA within the regulatory circuits would be changed greatly during the lung development and must be studied in a dynamical way.

A. *Dynamic circuits in the lung development*

The circuits in the early and late stages of the lung development are shown in Figure 6. We can see there are different circuits involved in different stages. For examples, at the early period, the target genes of miRNA mir-200 are Anxa3, Fas, Frmd4a, Limch1, Lrp4, Mal2, Mamdc2, Pdgfra, Pls3, Rabgap1l, Slc39a8, Sox17, Spnb2, Sumo1, Tmtc2; But at the late period, the target genes of miRNA mir-200 are changed to Hmbox1, Itgb1, Phactr2.

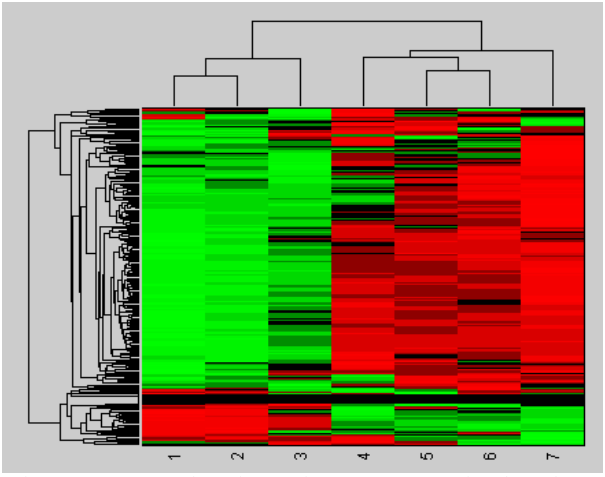

Figure 4. Two-way clustering on time course expression data of 113 genes.

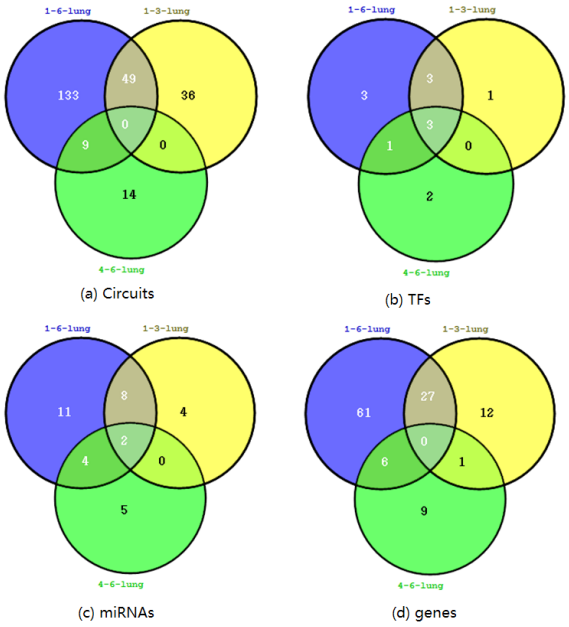

Figure 5. Overlap of circuits, miRNAs, TFs and genes in different lung development stages

Of course, some miRNAs or TFs actually play same or different roles in both stages. We plot their gene expression profiles in Figure 7.

(1) TF overlap (Figure 5(b)): We have found that there are three TFs, BACH2, IRF1, MYC, participated in the early and late stages. BACH2 is down-regulated in the early stage and up-regulated in the late stage; and IRF1 shows up-regulated while MYC shows down-regulated across all the time points (Figure 7(a)). Noted, MYC amplification is shown to be a prognostic marker of patients with early-stage lung adenocarcinoma [26].

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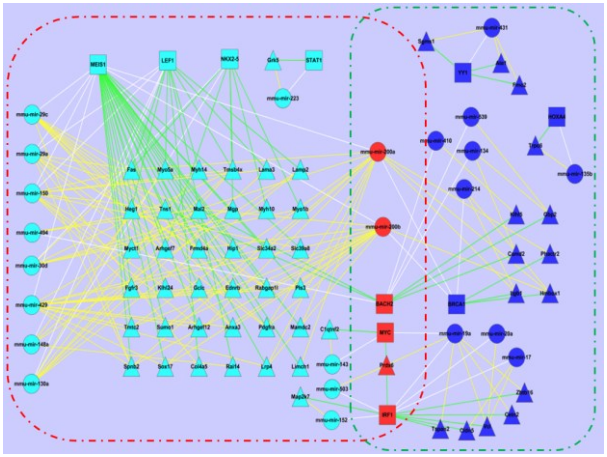

Figure 6. Circuits in early and late stages of the lung development (circuits in red and green rectangles are of early and late stages respectively; and circle, square and triangle nodes stand for miRNAs, TFs and genes)

(2) miRNA overlap (Figure 5(c)): We have found that there are two miRNAs participated in both the early and late stages: mmu-mir-200a and mmu-mir-200b. They show similar expression profiles along six time points of the lung development: first down-regulated and then up-regulated (Figure 7(b)). It is known that, the mir-200 family members have been shown to play an important role in fibrotic lung diseases, may be a therapeutic approach in treating pulmonary fibrotic diseases [27].

(3) gene overlap (Figure 5(d)): We have found that there is one common gene, Prdx6, occurring in the early and late stages (Figure 7(c)). It has been shown that the deletion of Prdx6 exaggerates lipopolysaccharide (LPS)-induced acute lung injury with increased oxidative stress [28].

B. Dynamic regulatory interaction of miRNA-TF-mRNA circuits in different lung development stages

To analyze the dynamic regulations in different stages, we construct the circuit network of the common TFs (BACH2, IRF1, MYC), miRNAs (mmu-mir-200a, mmu-mir-200b) and gene (Prdx6) in different lung development stages (as shown in Figure 8).

From Figure 8, we can see that gene Prdx6 is involved in one circuit in the early stage (IRF1~mmu-mir-503~Prdx6), while involved in the other two circuits (MYC~mmu-mir-19a~Prdx6, IRF1~mmu-mir-19a~Prdx6) in the late stage. Obviously, IRF1 regulates gene Prdx6 in both early and late stages, while it also regulates gene Map2k7 in the early stage and regulates Cetn2, Tspan2, Vcl, Zbtb16, Cldn5 in the late stage, by cooperating with miRNAs mmu-mir-19a, mmu-mir-17, mmu-mir-20. We have also found that the transcription factor BACH2 can collaborate with different miRNAs to co-regulate different genes in the early and late stages of the lung development.

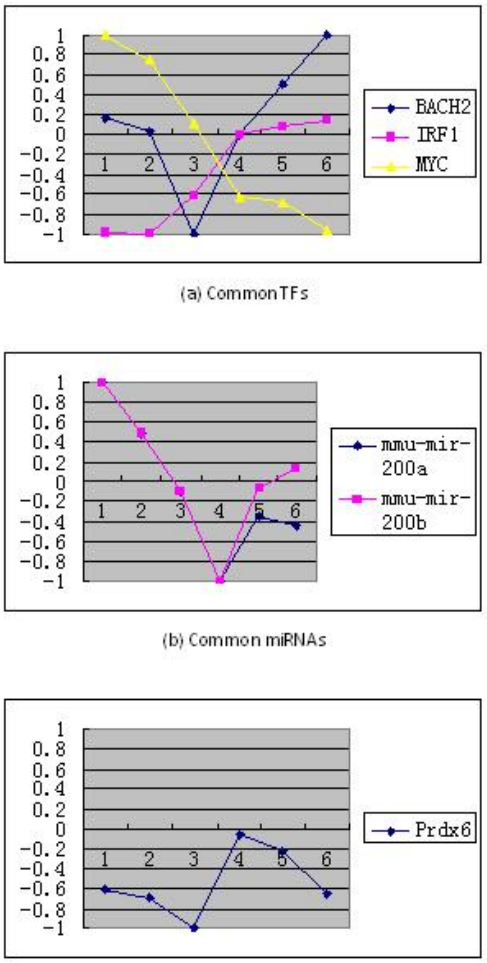

Figure 7. Common TFs, miRNAs and genes involved in two different stages of lung development

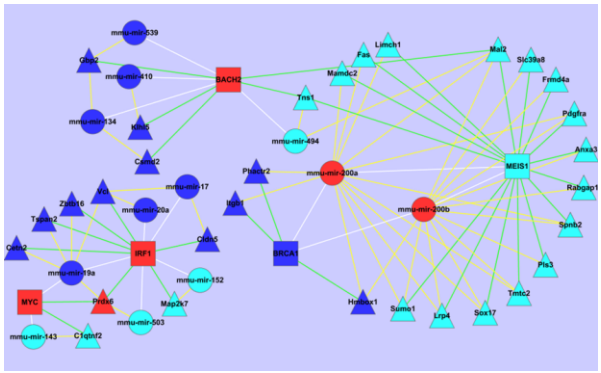

Figure 8. The circuit network in different stages with common nodes.

C. miRNA-TF-mRNA circuit induced functional specificity in Lung development

In this study, we have found that the miRNA and the TF usually dynamically execute regulation during different stages of mouse lung development. In particular, the associated genes usually display negative regulation in different stages (seeing Figure 4). To computationally explore the potential functional relevance of dynamically regulated genes by TF and miRNAs

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during the lung development, we have employed biological process and pathway analysis.

Table 1 shows all the enriched biological processes with P-value<0.05, using the DAVID tool [29]. At the Early period, the top three GO term: GO:0006793 GO:0006796 and GO:0016310 are phosphorus related function. As known, inorganic phosphate (Pi) plays a critical role in diverse cellular functions and low Pi affects the lung development of mice by disturbing protein translation [30]. The GO:0051094 (positive regulation of developmental process) and GO:0035023 (regulation of Rho protein signal transduction) are also very relevant to the lung development. As known, Rho protein plays a significant role in inhibiting lung development [31] while the ROCK2 plays a major role in the formation of the gas exchange units and as a sensor accelerated lung development [32, 33].

Table 2 shows all the enriched pathways with P-value<0.05, using the DAVID tool [29]. The pathways indicated in bold are associated with lung. For mmu04810, Actin cytoskeleton play a role in human pulmonary artery ECS [34]. For mmu00740, lung remodeling induced by exposure of total parenteral nutrition to ambient light is due to the interaction between vitamin C and peroxides generated by the exposure of riboflavin to light [35]. For mmu04060, the analysis of lung adenocarcinoma tissue specimens demonstrated that the genes involved in these biological pathways had high rates of over-expression [36]. For mmu00230, metabolic changes in the lung as a result of ventilation-induced lung injury are reflected by an increased level of purine in the bronchoalveolar lavage fluid and that purine may, thus, serve as an early marker for ventilation-induced lung injury [37]. For mmu00533, Keratan sulfate biosynthesis, the pathways are associated with prostate cancer and small cell lung cancer[38].

IV. CONCLUSION

In this study, we have collected the existing regulation interactions related to mouse lung development: TF and miRNA, TF and mRNA, miRNA and mRNA, and built a lung-tissue specific data set of miRNA-TF-mRNA circuits. We have used Pearson correlation coefficient to measure the rationality of the existence of such circuits and analyzed them in different stages of the mouse lung development. The results have showed that the relevant transcriptional or post-transcriptional factors and their roles involved in the mouse lung development are both changed greatly in different stages. Therefore, the miRNA-TF-mRNA circuits and its analysis can be used in wide translational biomedicine studies.

ACKNOWLEDGMENT

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TABLE 1. TOP 10 GO BIOLOGICAL PROCESS TERMS IN DIFFERENT PERIOD

Period	NO	Term	P-value
Early	1	GO:0006793~phosphorus metabolic process	5.40E-05
	2	GO:0006796~phosphate metabolic process	5.40E-05
	3	GO:0016310~phosphorylation	1.21E-04
	4	GO:0045596~negative regulation of cell differentiation	2.60E-04
	5	GO:0051056~regulation of small GTPase mediated signal transduction	3.32E-04
	6	GO:0006468~protein amino acid phosphorylation	5.28E-04
	7	GO:0035023~regulation of Rho protein signal transduction	5.30E-04
	8	GO:0007167~enzyme linked receptor protein signaling pathway	7.96E-04
	9	GO:0022604~regulation of cell morphogenesis	0.001564
	10	GO:0051094~positive regulation of developmental process	0.002006
Late	1	GO:0006928~cell motion	7.08E-05
	2	GO:0016477~cell migration	0.001009
	3	GO:0030030~cell projection organization	0.002595
	4	GO:0017148~negative regulation of translation	0.002614
	5	GO:0010558~negative regulation of macromolecule biosynthetic process	0.00269
	6	GO:0010608~posttranscriptional regulation of gene expression	0.002856
	7	GO:0032268~regulation of cellular protein metabolic process	0.003146
	8	GO:0031327~negative regulation of cellular biosynthetic process	0.003426
	9	GO:0048870~cell motility	0.003474
	10	GO:0051674~localization of cell	0.003474

TABLE 2. PATHWAY TERMS IN DIFFERENT PERIOD, PVALUE<0.05

Period	No	Term	P-value
Early	1	mmu04512:ECM-receptor interaction	0.005104
	2	mmu04810:Regulation of actin cytoskeleton	0.006057
	3	mmu04115:p53 signaling pathway	0.014693
	4	mmu05200:Pathways in cancer	0.028372
	5	mmu00740:Riboflavin metabolism	0.030496
	6	mmu04060:Cytokine-cytokine receptor interaction	0.036709
Late	1	mmu00230:Purine metabolism	0.011819
	2	mmu00533:Keratan sulfate biosynthesis	0.013904

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