Cell Research (2009) 19:793-795.

www.nature.com/cr

LETTER TO THE EDITOR

RLEdb: a database of rate-limiting enzymes and their regulation in human, rat, mouse, yeast and *E. coli*

Cell Research (2009) **19**:793-795. doi: 10.1038/cr.2009.61; published online 26 May 2009

Dear Editor,

Rate-limiting enzymes, because of their relatively low rates of catalysis, are essential for flux control in metabolic pathways [1, 2]. They themselves are often extensively regulated, such as by transcription factors and post-translational modifications, and thus play the important role of linking metabolic pathways to gene expression regulatory networks and signal transduction networks. Identification and classification of rate-limiting enzymes and their regulators are the first steps towards understanding their roles in metabolic flux control. However, currently this information is scattered in the literature and, to date, there has been no systematic analysis. A comprehensive and well-annotated database of rate-limiting enzymes, with an emphasis on the regulation of these enzymes, may provide a useful resource for biochemists and molecular biologists. Here, we present the first literature-based Rate-Limiting Enzymes database (RLEdb), covering 383 rate-limiting enzymes in five organisms, including human, rat, mouse, yeast and *E. coli*, and their detailed regulatory information. RLEdb is freely available at http://rle.cbi.pku.edu.cn.

This comprehensive collection of rate-limiting enzymes in RLEdb was curated from published literature in the following four steps: (1) All known enzyme names and enzyme code were extracted from the IntEnz database; (2) PubMed was searched using the keywords "rate-limiting enzyme" together with each enzyme name and enzyme code; (3) The retrieved abstracts were grouped using the Related Articles function in Entrez (This allowed us to quickly and easily assess if and how the searched enzyme name or code is related to rate-limiting enzymes and provided cross-checking between different publications); (4) The abstracts were read manually to collect the rate-limiting enzymes and other related information such as organisms. We focused on five organisms and collected 383 rate-limiting enzymes in total, including 148 in human, 107 in rat, 97 in mouse, 16 in yeast and 15 in *E. coli*, respectively. For best accuracy, we did not assume that a rate-limiting enzyme in one organism is also a rate-limiting enzyme in another. For example, cyclooxygenases were reported to be not rate-limiting in guinea pigs [3], although many reports confirmed their rate-limiting role in human.

Next we retrieved and integrated extensive functional information about the rate-limiting enzymes from public databases such as GeneRifs [4], Uniprot [5], KEGG-Ligand 44.0 [6], BRENDA 7.1 [7], and HPRD [8]. For example, 1476 protein-protein interaction pairs involving rate-limiting enzymes were integrated from the BioGRID [9], HPRD [8], and BIND [10] databases. Other information includes chromosome localization, subcellular localization, tissue expression, protein functional domain, gene ontology assignments, and known disease association.

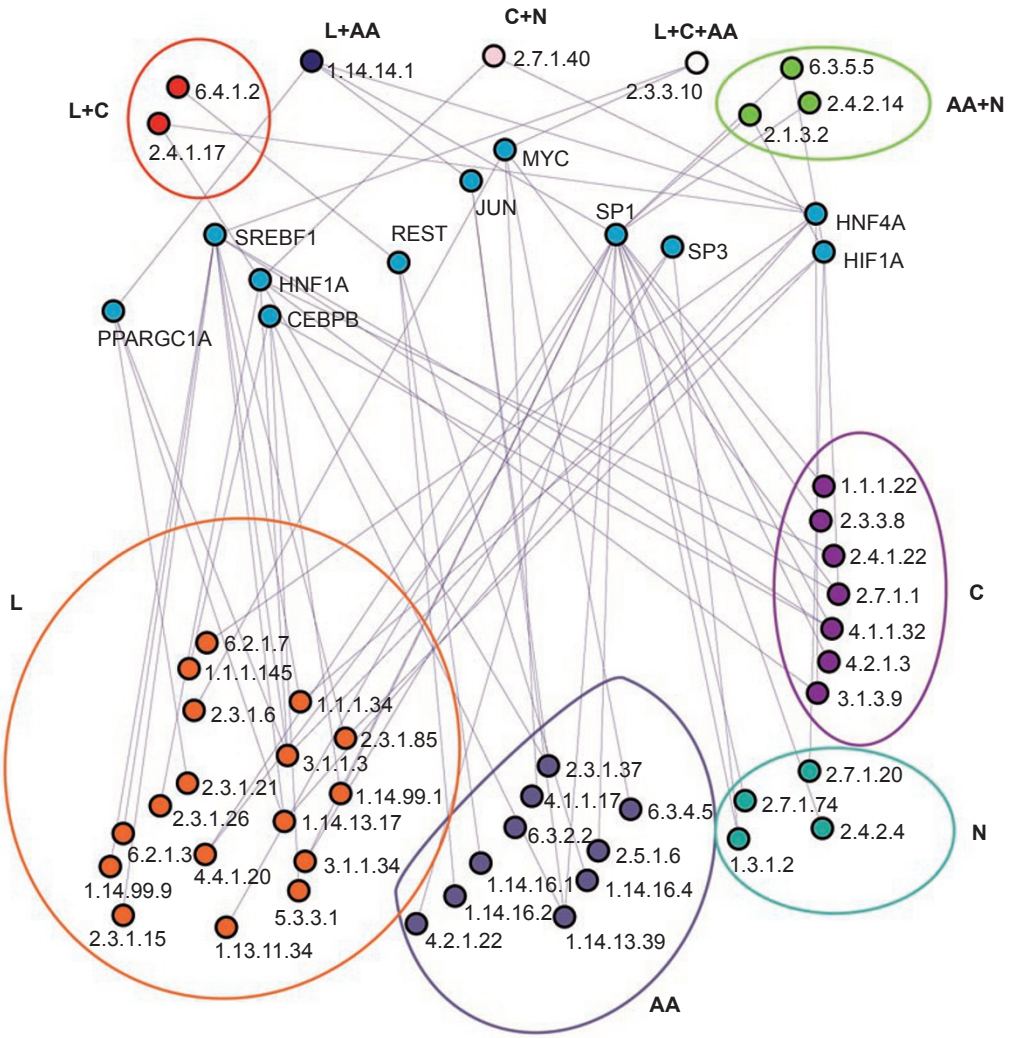
In particular, we collected information on the regulation of the rate-limiting enzymes. We focused on three classes of regulators: transcription factors, post-translational modifications (especially phosphorylation), and regulatory inhibitors (such as allosteric and feedback effectors). Reviewing the literature on these particular rate-limiting enzymes showed that there are 40 transcription factors that commonly regulate metabolic pathways, such as the *SREBP* family, *HNF4alpha* and *PPARalpha*. For post-translational modifications we not only collected information from the literature, but also integrated data from PhosphoSite [11] and SwissProt databases [5]. Enzyme inhibitor information was extracted from BRENDA 7.1 [7], and a similar semi-automatic method was used to convert the available text information about inhibitors to KEGG compound identifiers as described in a previous study [12]. In addition, we filtered out those inhibitors that can not be produced by enzymes *in vivo*.

All data and information in RLEdb were stored in a MySQL relational database on a Linux server. Web-based queries to the database were implemented in Perl scripts running in an Apache environment. RLEdb allows users to query by enzyme or gene, to browse by regulatory information, KEGG pathway maps, tissue expression, subcellular localization and chromosome number, or to run

BLAST searches against the sequences in RLEdb. Bulk download files are also provided. A feedback page was implemented to collect comments on existing records or suggestions of new rate-limiting enzymes from users.

A quick analysis of the data in RLEdb revealed interesting findings. For instance, only two rate-limiting enzymes in human were not related to diseases; this may be a consequence of their central role in the control of metabolic flux and metabolic regulation. A total of 95 rate-limiting enzymes were involved in 478 regulatory pairs with upstream transcription factors, creating a bridge between the transcription regulatory networks and metabolic networks. The regulatory relationship between a few common human transcription factors and the rate-limiting enzymes they regulate is shown in Figure 1.

RLEdb is the first high-quality resource on rate-limiting enzymes and their regulation. By integrating diverse functional data in one place, RLEdb can assist biochemists and biologists to study not only each individual rate-limiting enzyme, but also the broader molecular networks. As more data and information become available, we will maintain and update RLEdb quarterly using our automated retrieval and annotation pipeline followed by manual quality control.



L: Enzymes in lipid metabolism
C: Enzymes in carbohydrate metabolism
AA: Enzymes in amino acid metabolism
N: Enzymes in nucleotide metabolism

Figure 1 The regulatory pairs between 11 common human transcription factors and 46 rate-limiting enzymes they regulate. The 11 transcription factors are represented with their gene symbol. All 46 enzymes are represented with their enzyme code. Enzymes involved in the same pathway group are marked and included in circles with the same color.

Acknowledgments

This work was supported by Hi-Tech Research and Development Program of China (863 Programs) (2006AA02Z334, 2006AA02Z314, 2006AA02A312, 2007AA02Z165), and National Basic Research Program of China (973 Program) (2006CB910404, 2007CB946904).

Min Zhao[1], Xin Chen[1], Ge Gao[1], Louis Tao[1], Liping Wei[1]

[1]*Center for Bioinformatics, National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing 100871, China*

Correspondence: Liping Wei
Tel: +86-10- 6275-5206; Fax: +86-10-6275-2438
Email: weilp@mail.cbi.pku.edu.cn

References

1 Bucher Th, Russmann W. Gleichgewicht und Ungleichgewicht im System der Glykolyse. *Angewandte Chemie* 1963; **75**:881-893.
2 Newsholme EA, START C. Regulation in Metabolism. John Wiley and Sons Ltd, 1973.
3 Schellenberg JC, Kirkby W. Production of prostaglandin F2 alpha and E2 in explants of intrauterine tissues of guinea pigs during late pregnancy and labor. *Prostaglandins* 1997; **54**:625-638.
4 Mitchell JA, Aronson AR, Mork JG, *et al*. Gene indexing: characterization and analysis of NLM's GeneRIFs. *AMIA Annu Symp Proc* 2003; **2003**:460-464.
5 Matys V, Kel-Margoulis OV, Fricke E, *et al*. TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. *Nucleic Acids Res* 2006; **34** (Database issue):D108-D110.
6 Kanehisa M, Goto S, Hattori M, *et al*. From genomics to chemical genomics: new developments in KEGG. *Nucleic Acids Res* 2006; **34** (Database issue):D354-D357.
7 Chang A, Scheer M, Grote A, Schomburg I, Schomburg D. BRENDA, AMENDA and FRENDA the enzyme information system: new content and tools in 2009. *Nucleic Acids Res* 2008; **37**(Database issue):D588-D592.
8 Prasad TS, Goel R, Kandasamy K, *et al*. Human Protein Reference Database--2009 update. *Nucleic Acids Res* 2008; **37**(Database issue): D767-D772.
9 Breitkreutz BJ, Stark C, Reguly T, *et al*. The BioGRID Interaction Database: 2008 update. *Nucleic Acids Res* 2008; **36** (Database issue):D637-D640.
10 Willis RC, Hogue CW. Searching, viewing, and visualizing data in the Biomolecular Interaction Network Database (BIND). *Curr Protoc Bioinformatics* 2006; Chapter 8:Unit 8.9.
11 Gnad F, Ren S, Cox J, *et al*. PHOSIDA (phosphorylation site database): management, structural and evolutionary investigation, and prediction of phosphosites. *Genome Biol* 2007; **8**:R250.
12 Gutteridge A, Kanehisa M, Goto S. Regulation of metabolic networks by small molecule metabolites. *BMC Bioinformatics* 2007; **8**:88.