戊糖磷酸途径中关键酶的系统进化树构建及蛋白质结构初探

——第9组

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Outline

- Background
- Protein structure analysis
- Phylogenetic tree construction and analysis
- Summary

Background

糖类化合物是光合作用的产物，也是生物界分布最广、数量最多的有机化合物。

糖类的生物学功能

- 生物体的结构成分：纤维素、半纤维素、果胶等；
- 生物体的主要能源物质
- 生物体重要的储能物质：淀粉、糖元
- 合成代谢的重要原料和中间产物
- 细胞识别的信息分子：糖蛋白

Background

糖类的分解代谢途径

- 糖酵解（EMP）
- 三羧酸循环（TCA cycle）
- 戊糖磷酸途径（PPP）

Background

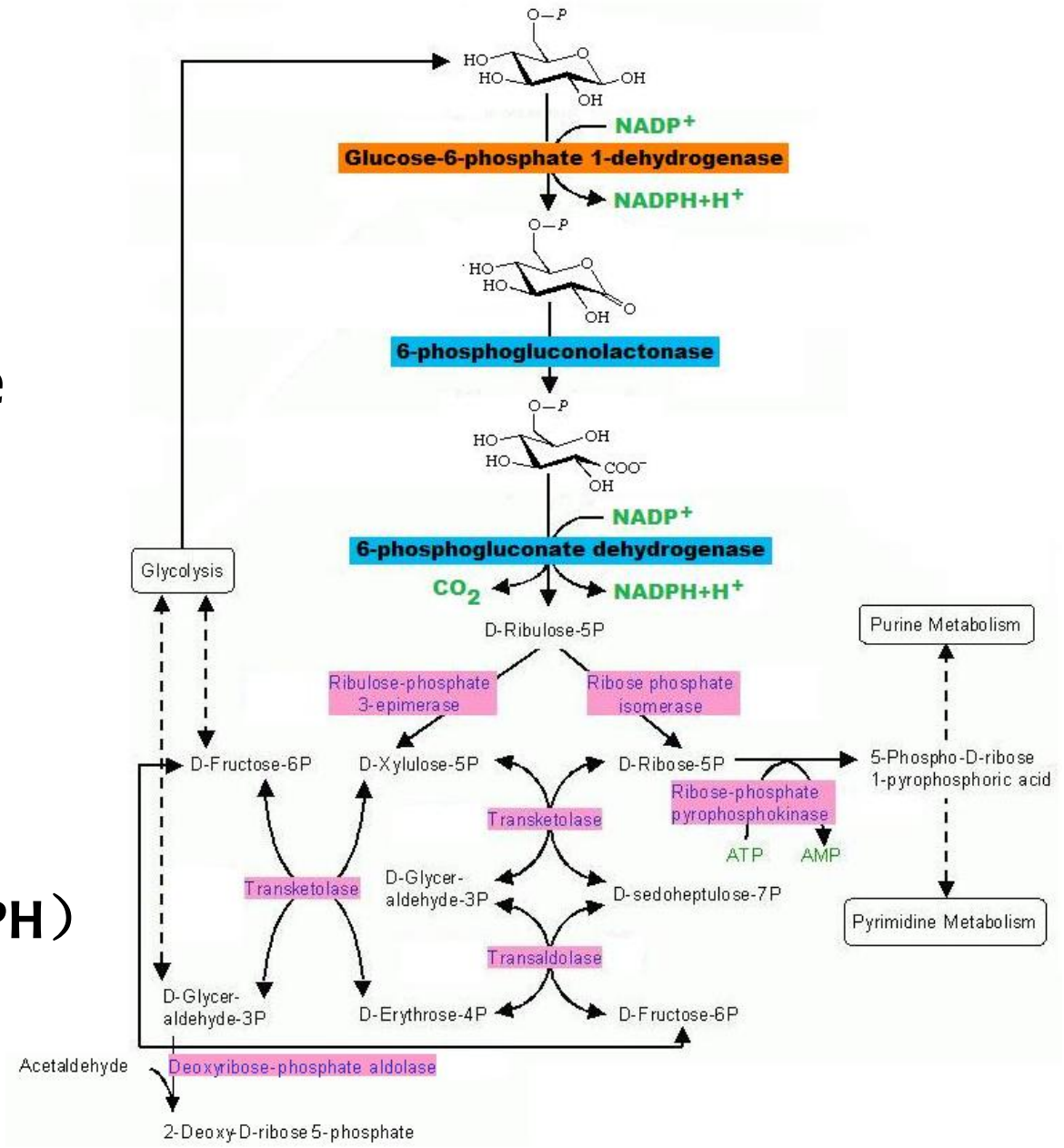
戊糖磷酸途径

pentose phosphate pathway,PPP

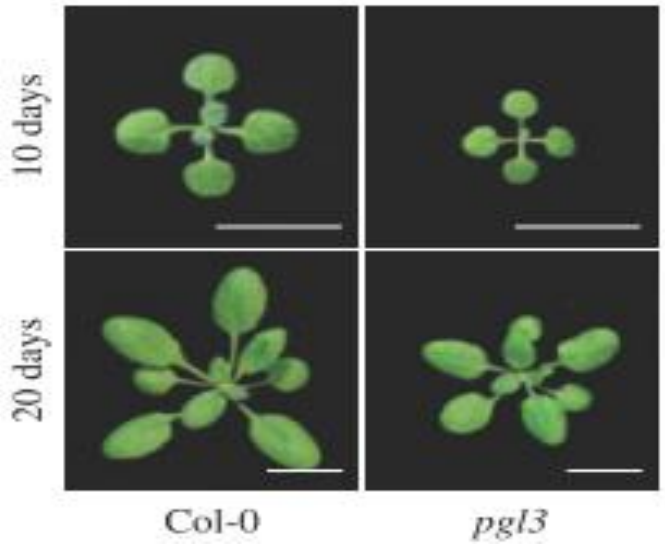
- 不可逆的氧化阶段
- 可逆的非氧化阶段

主要生物学功能

- 提供还原力（NADPH）
- 提供碳骨架



phenotype of *pgl3* mutant

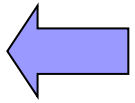


Xiong et al., 2009

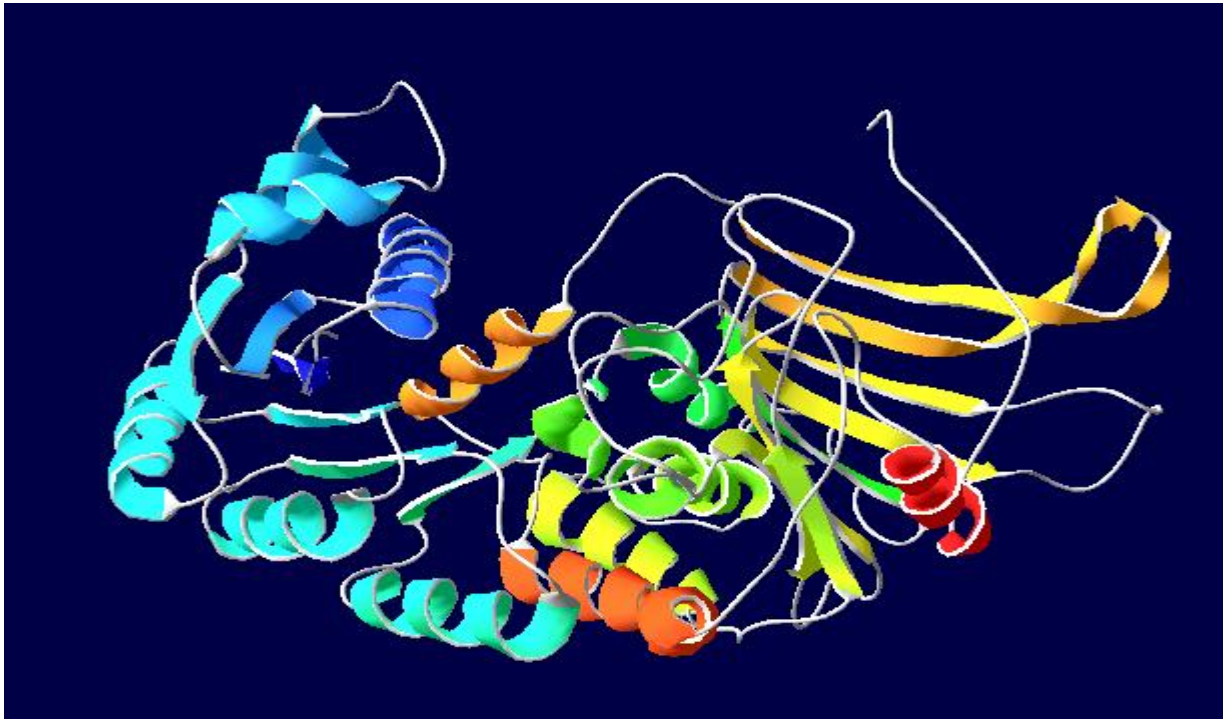
- a dramatic decrease in plant size
- a significant increase in total glucose-6-phosphate dehydrogenase activity
- a marked decrease in cellular redox potential
- constitutive pathogenesis-related gene expression and enhanced resistance to *Pseudomonas syringae pv. maculicola* and *Hyaloperonospora arabidopsidis*

general information of G6PDH in *Arabidopsis*

Protein name	Glucose-6-phosphate 1-dehydrogenase 1
abbreviation	G6PD1/G6PDH1
EC number	1.1.1.49
Gene name	APG1
Protein family	glucose-6-phosphate dehydrogenase family
reaction	D-glucose 6-phosphate + NADP+ = 6-phospho-D-glucono-1,5-lactone + NADPH
Subunit structure	Homo dimer
Subcellular localization	plastids
motif	Transit peptide
ligand	NADP
NADPBinding site	99,131
Substrate Binding site	264,268



3-D structure of G6PDH of human



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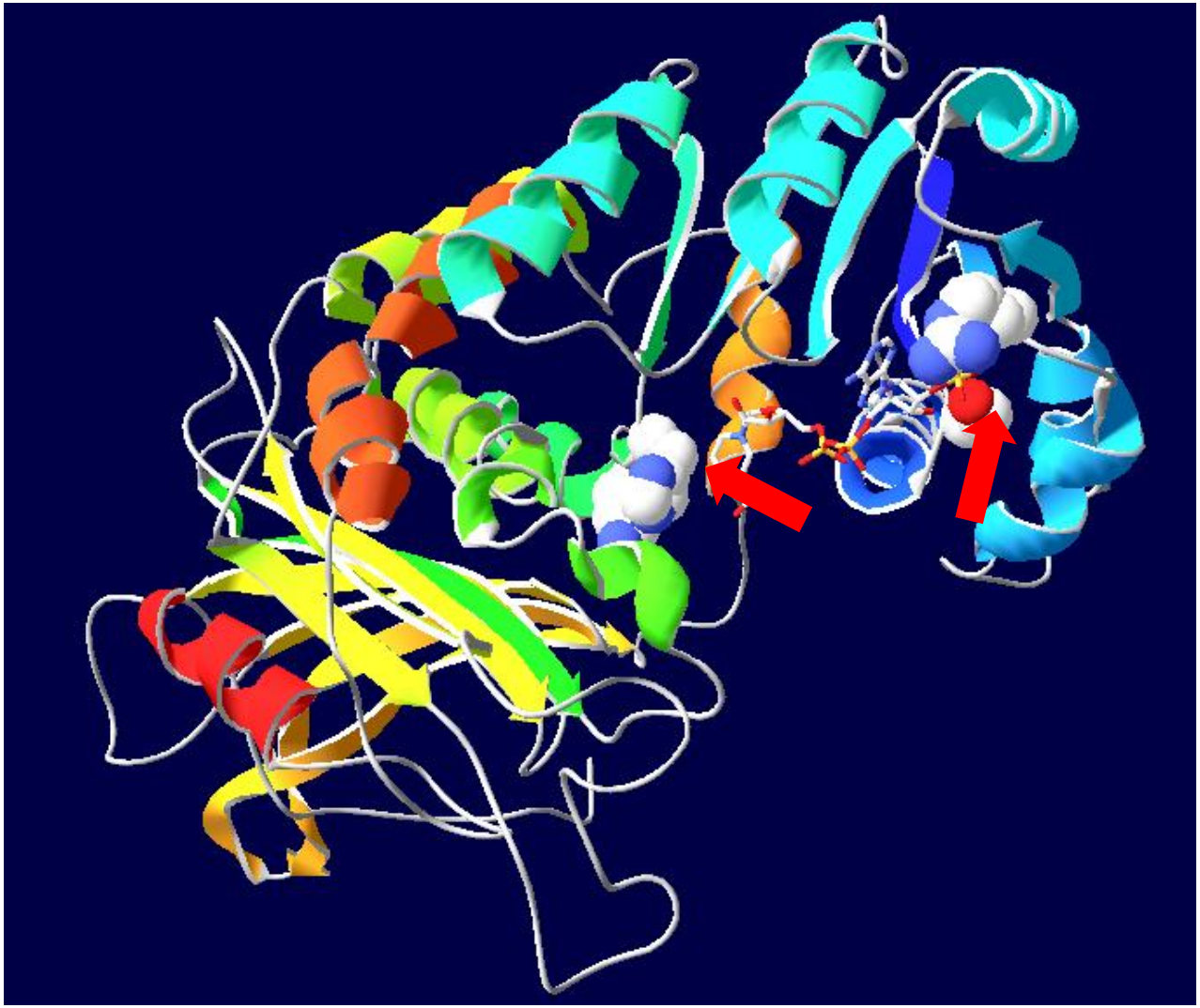
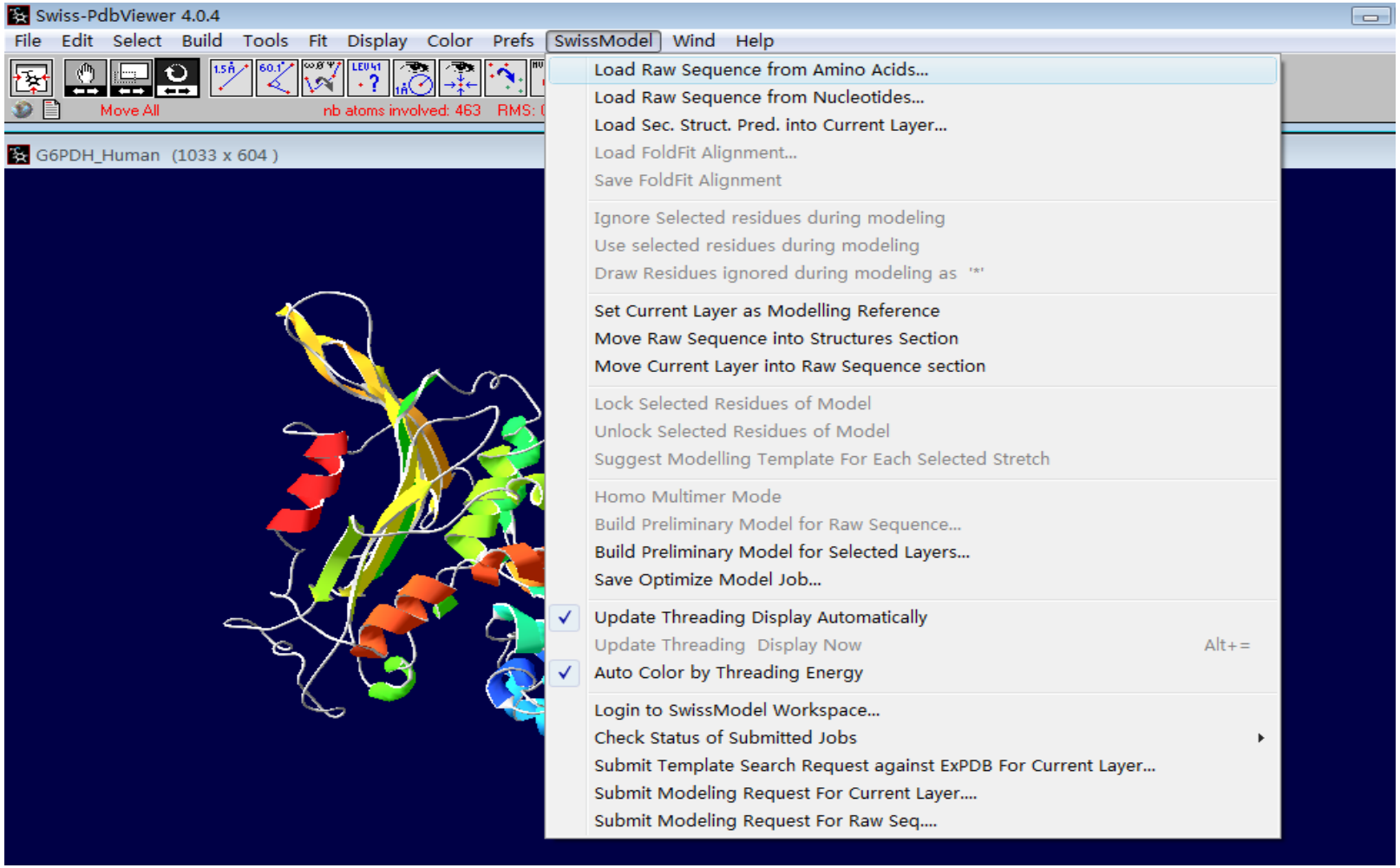


利用Pdbviewer软件中的Swiss-model工具进行拟南芥G6PDH蛋白3-D结构模拟



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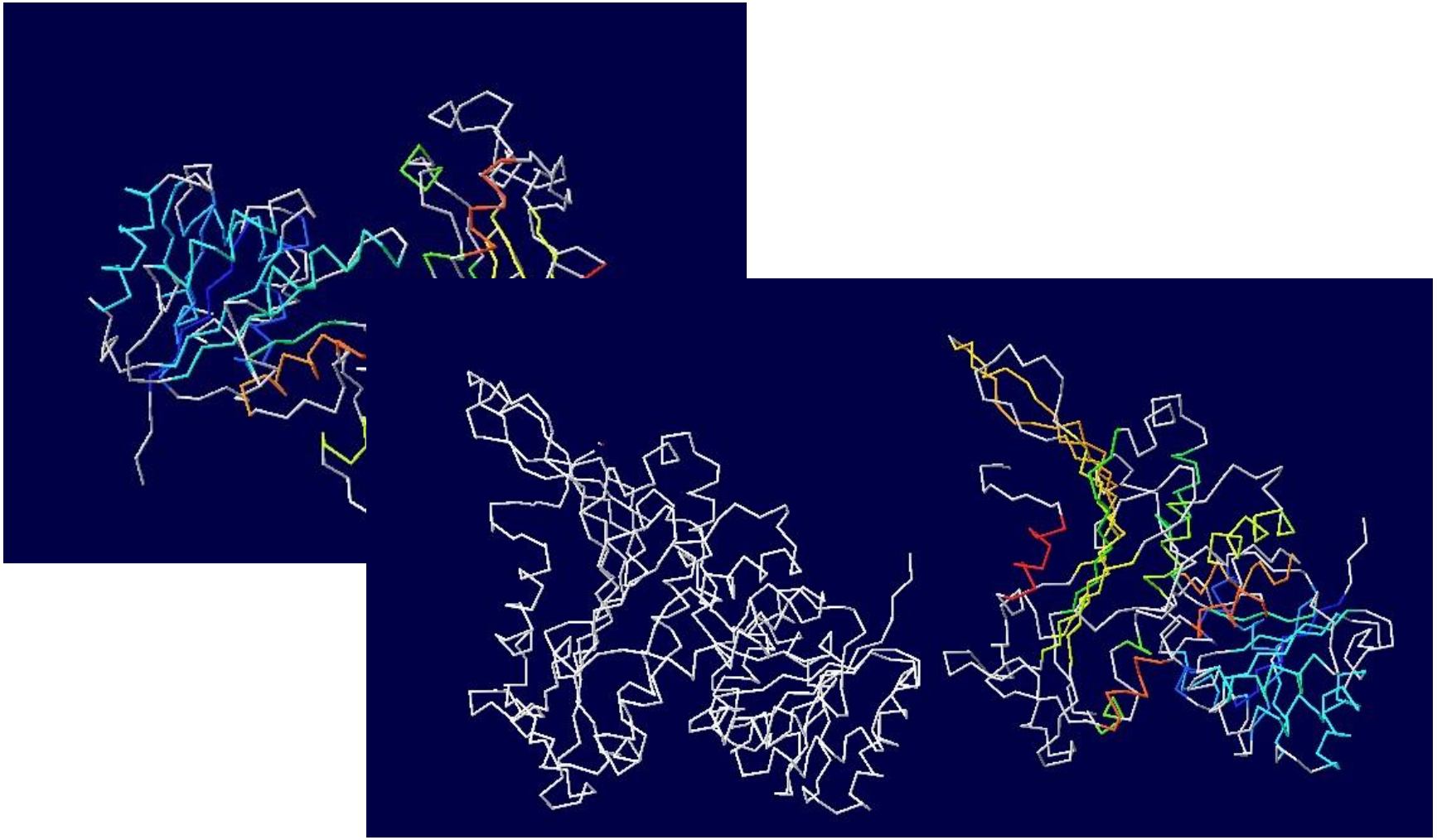

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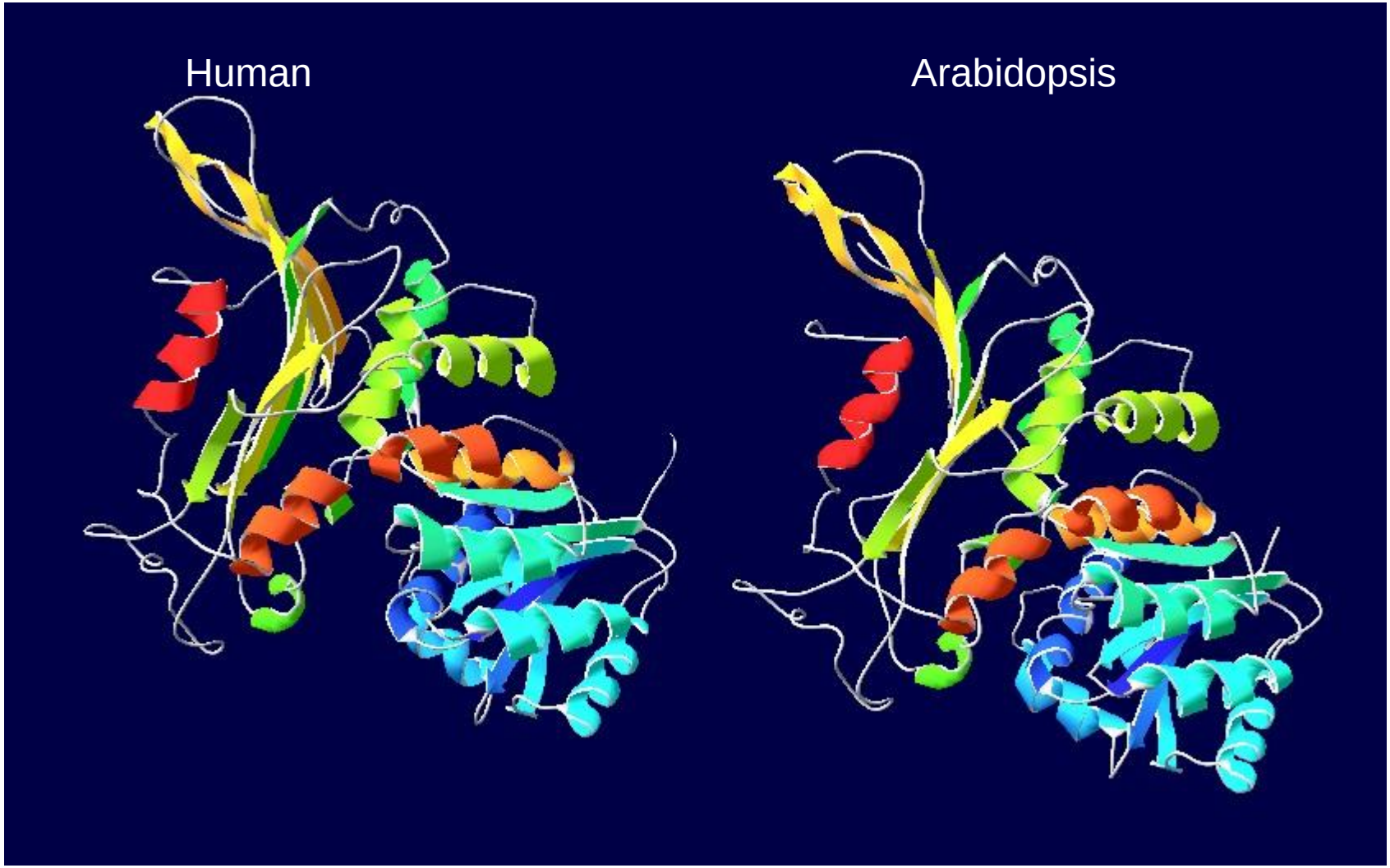


利用UniProt和NCBI获取目标序列

- *Escherichia coli*
- *Bacillus subtilis*

- *Saccharomyces cerevisiae*
- *Schizosaccharomyces pombe*

- *Homo sapiens*
- *Mus musculus*
- *Rattus norvegicus*
- *Drosophila melanogaster*
- *Caenorhabditis elegans*

- *Arabidopsis thaliana*
- *Nicotiana tabacum*
- *Solanum tuberosum*

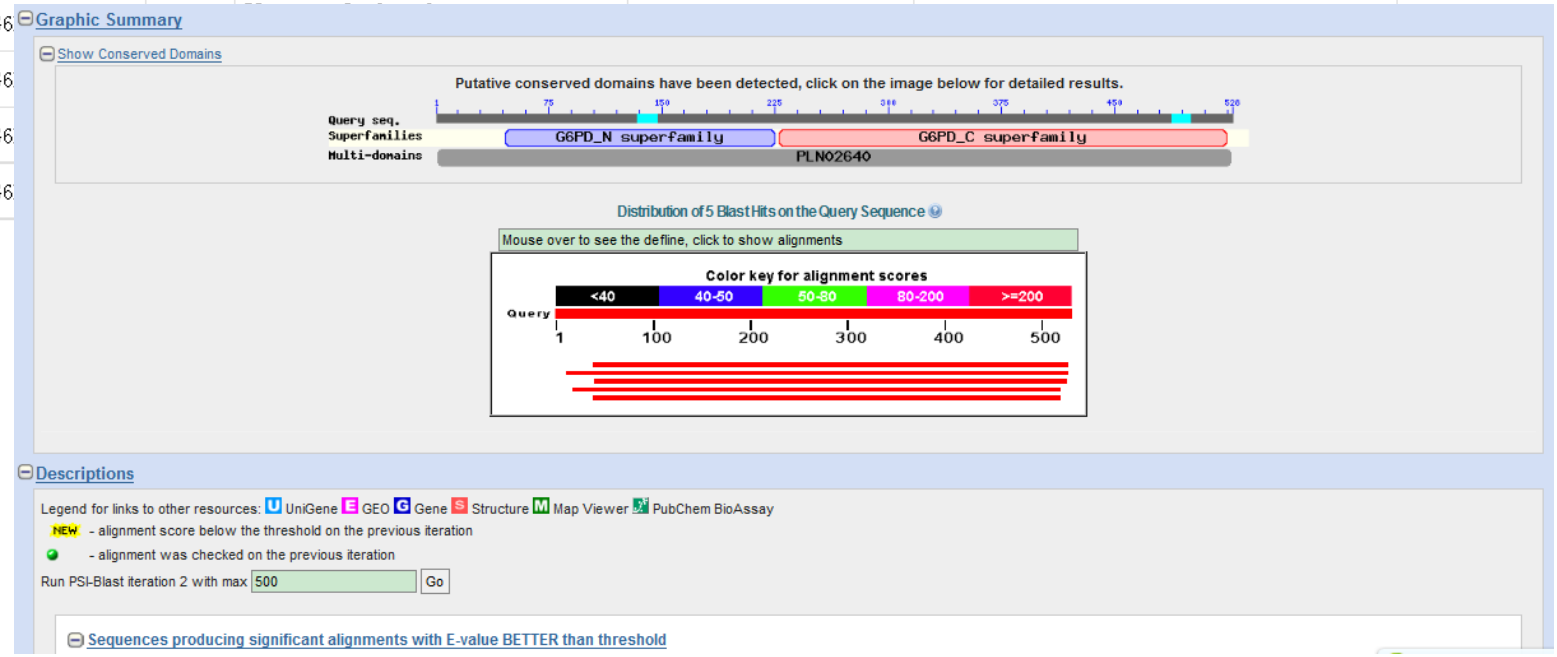
- *Oryzea sativa*
- *Sorghum bicolor*

利用UniProt和NCBI获取目标序列

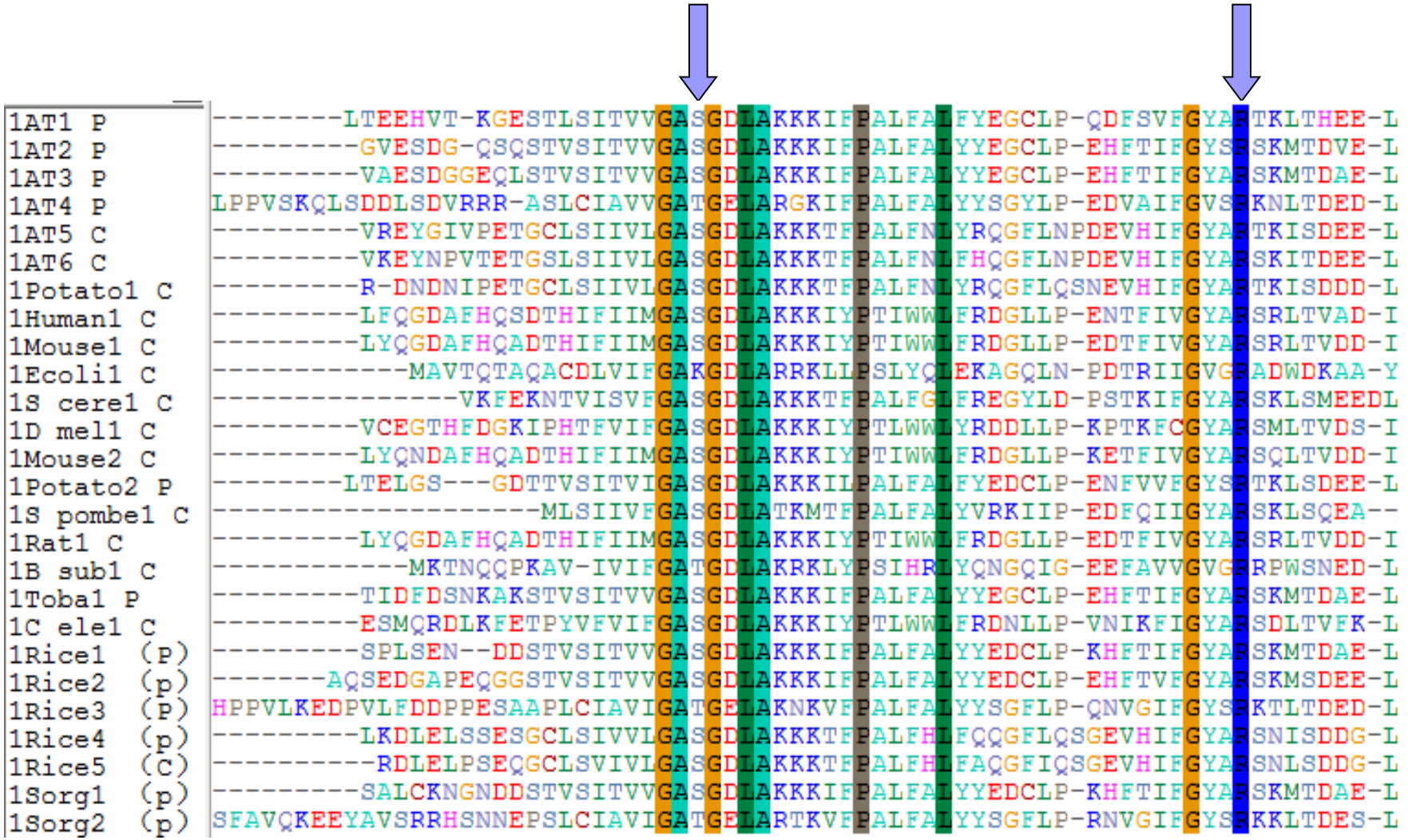
Results Customize

› Show only reviewed (76) ★ (UniProtKB/Swiss-Prot) or unreviewed (4,045) ★ (UniProtKB/TrEMBL) entries
› Restrict term "glucose 6 phosphate" to protein family (3,607), gene ontology (3,935), protein name (4,111)
› Restrict term "1 dehydrogenase" to gene ontology (3), protein name (4,119)
› Restrict term ""glucose 6 phosphate"" to pathway
› Restrict term ""1 dehydrogenase"" to pathway

	Entry	Entry name	Status	Protein names	Gene names	Organism
☐	P11413	G6PD_HUMAN	★	Glucose-6-phosphate 1-dehydrogenase	G6PD	Homo sapiens (Human)
☐	P11411	G6				
☐	P11412	G6				
☐	Q00612	G6				
☐	P0AC53	G6				



利用BioEdit软件中的ClustalW工具进行多序列比对



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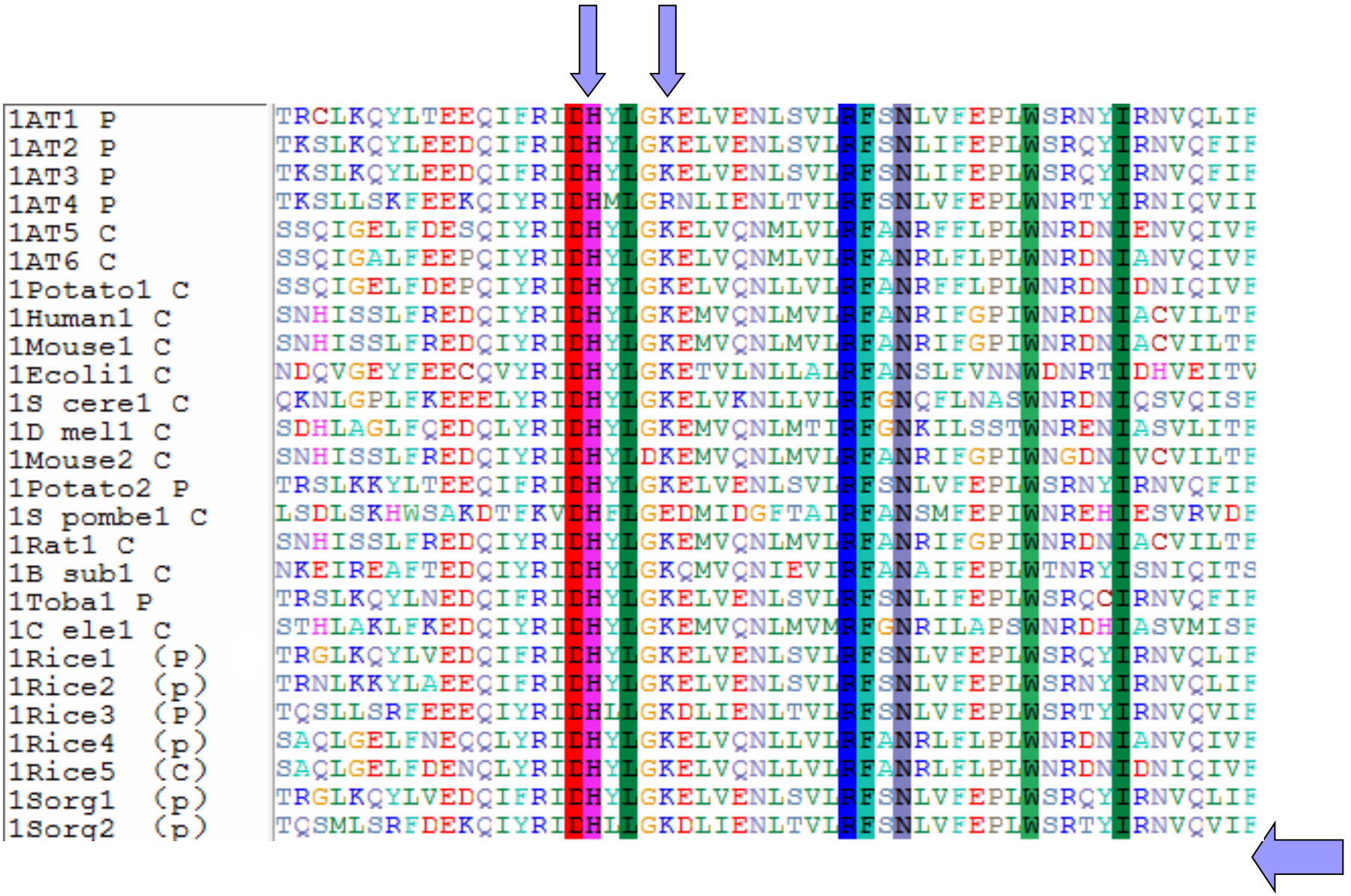
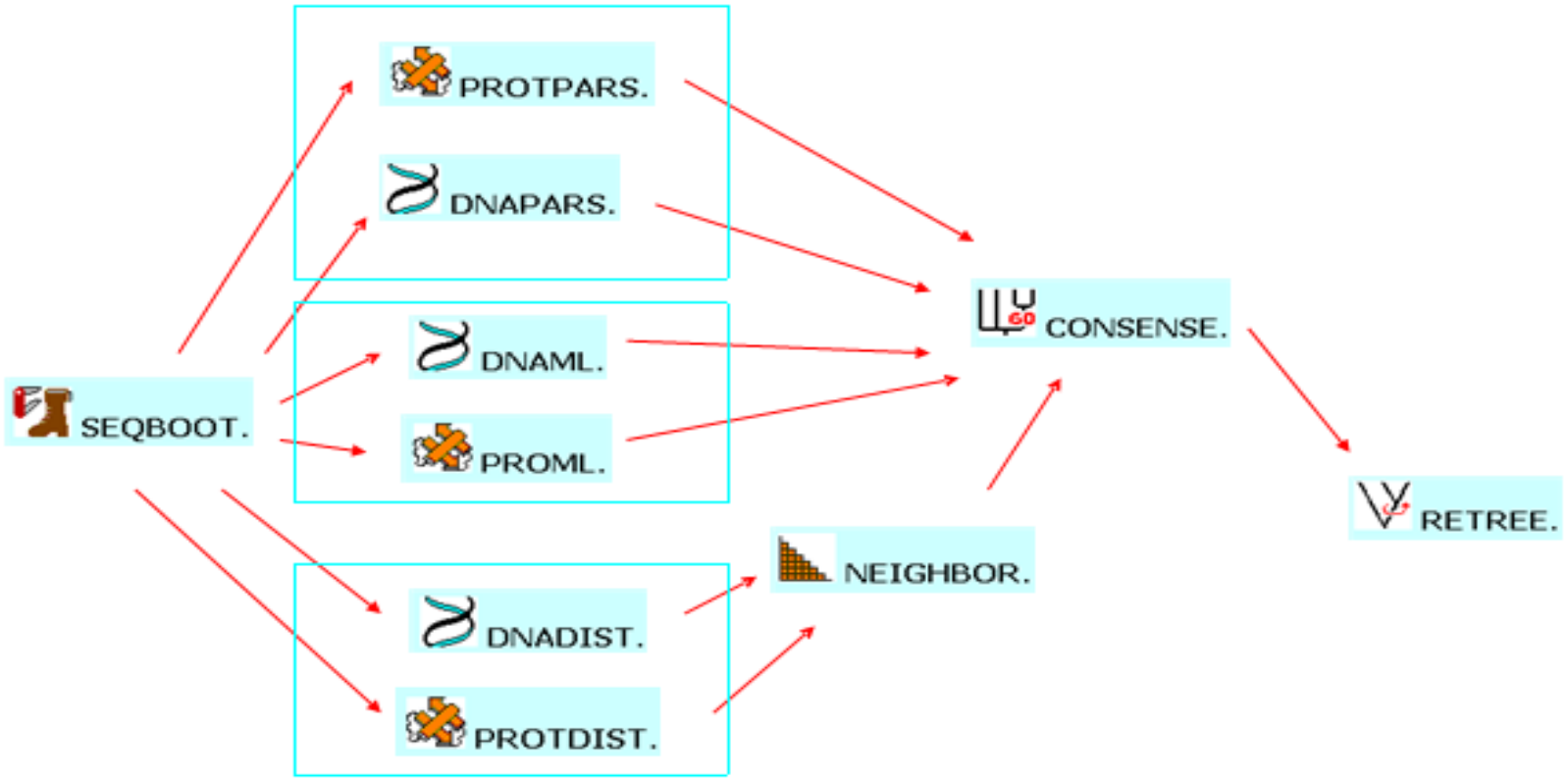
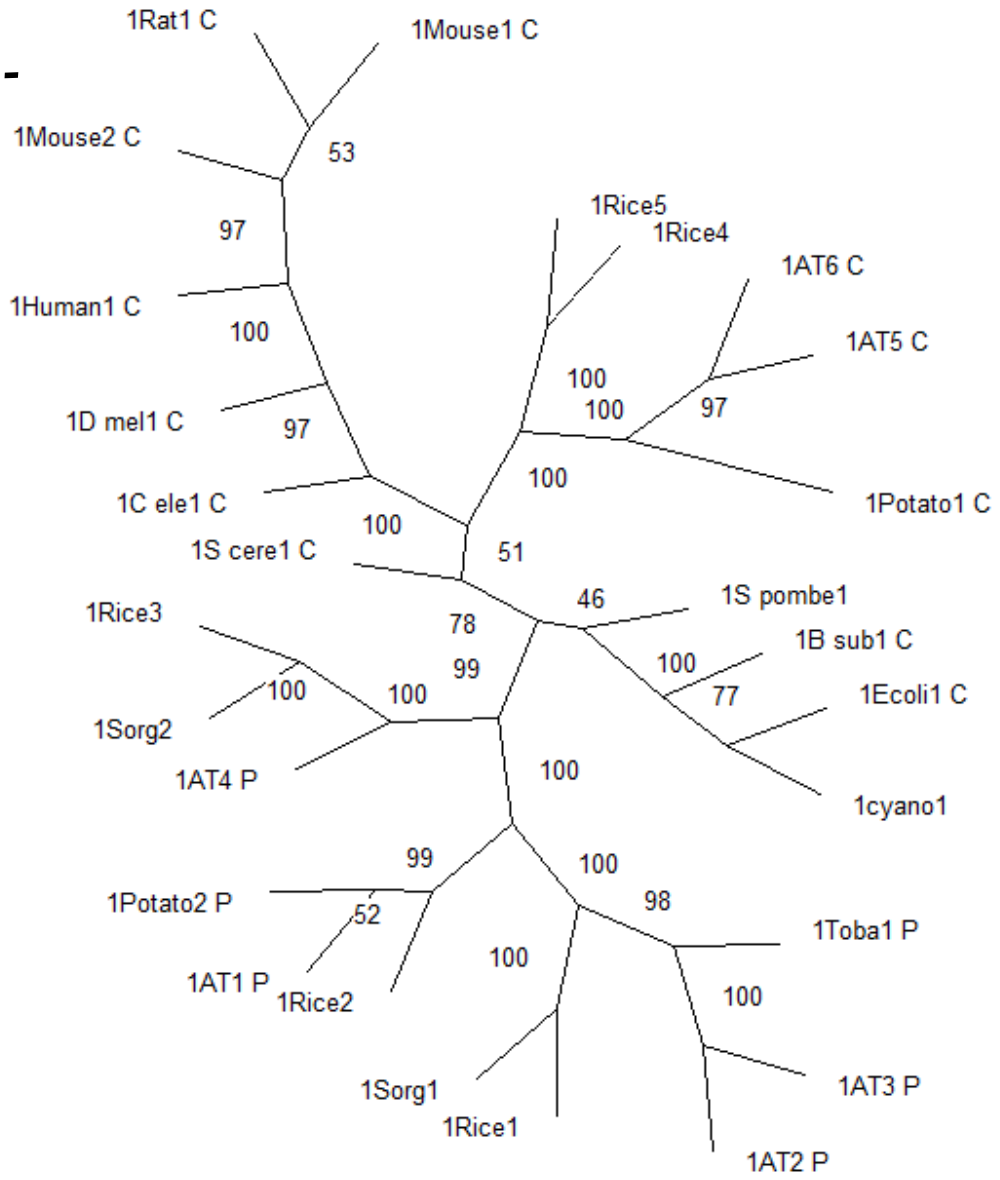


利用Phylip软件构建系统发育树的流程



Kang Juqing's lecture，2011

Glucose-6-phosphate 1-dehydrogenase



利用Phylip软件构建的不同生物中G6PDH的无根系统发育树

TargetP 1.1 Server

TargetP 1.1 predicts the subcellular location of eukaryotic proteins. The location assignment is based on the predicted presence of any of the N-terminal presequences: chloroplast transit peptide (**cTP**), mitochondrial targeting peptide (**mTP**) or secretory pathway signal peptide (**SP**).

For the sequences predicted to contain an N-terminal presequence a potential cleavage site can also be predicted.

NOTE 1: TargetP uses ChloroP and SignalP to predict cleavage sites for **cTP** and **SP**, respectively.

NOTE 2: The method has been tested on *A. thaliana* and *H. sapiens* sets; see the results.

New: the paper about using TargetP and other protein subcellular localization prediction methods:

Locating proteins in the cell using TargetP, SignalP, and related tools
Olof Emanuelsson, Søren Brunak, Gunnar von Heijne, Henrik Nielsen
Nature Protocols **2**, 953-971 (2007).

is now again available for download - please click here.

Instructions | Output format | Article abstract | Data sets

SUBMISSION

Paste a single sequence or several sequences in FASTA format into the field below:

Submit a file in FASTA format directly from your local disk:

浏览...

Organism group
- (•) Non-plant
- ( ) Plant

Prediction scope
- [ ] Perform cleavage site predictions

Cutoffs
- (•) no cutoffs; winner-takes-all (default)
- ( ) specificity >**0.95** (predefined set of cutoffs that yielded this specificity on the TargetP test sets)
- ( ) specificity >**0.90** (predefined set of cutoffs that yielded this specificity on the TargetP test sets)
- ( ) define your own cutoffs (0.00 - 1.00): **cTP**: 0.00 **mTP**: 0.00 **SP**: 0.00 **other**: 0.00

Submit | Clear fields

CENTER FOR BIOLOGICAL SEQUENCE ANALYSIS ■ TECHNICAL UNIVERSITY OF DENMARK DTU

TargetP1.1软件预测结果

CENTERFORBIOLOGICALSEQUENCEANALYSIS CBS

TargetP 1.1 Server - prediction results

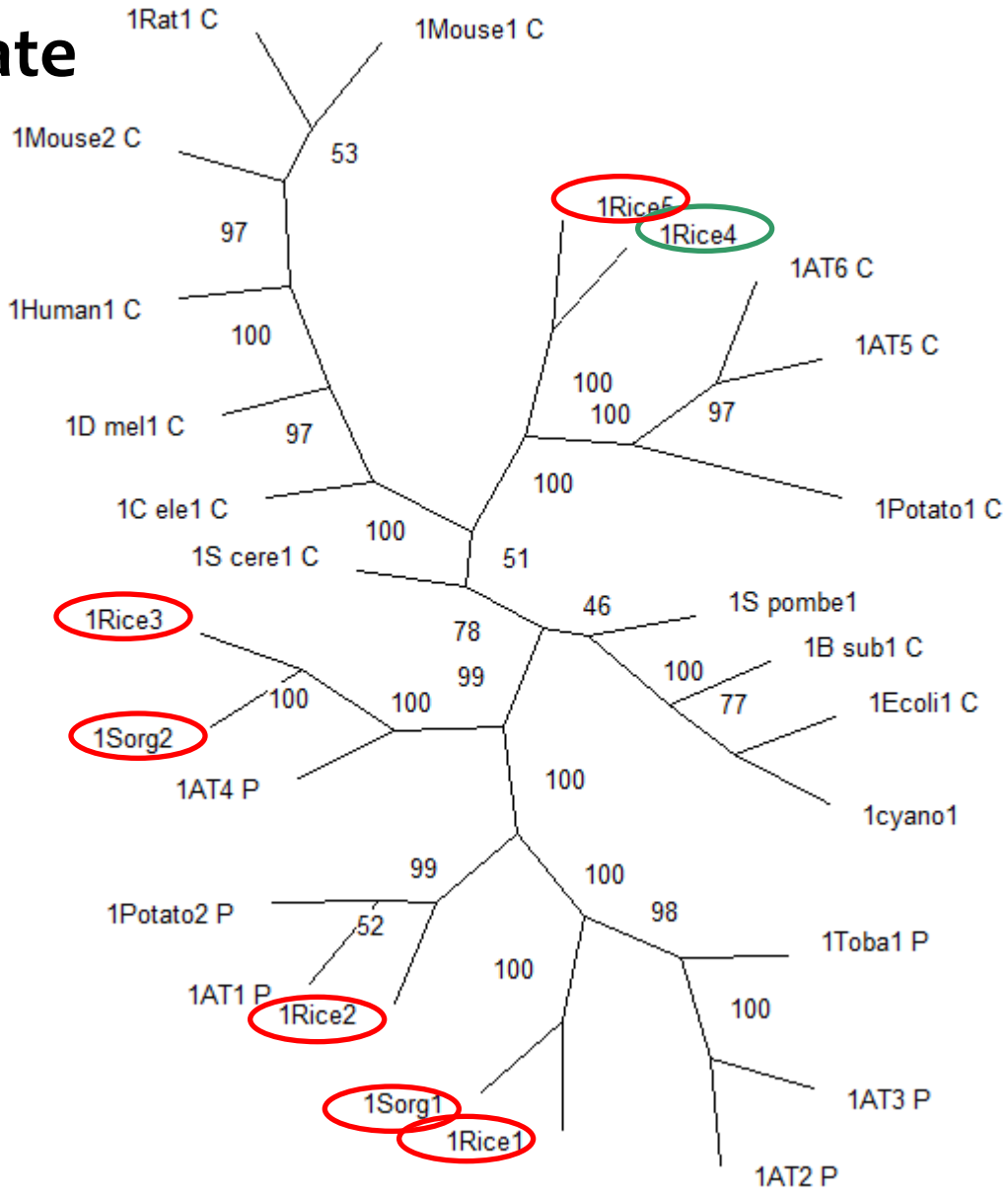
Technical University of Denmark

```
### targetp v1.1 prediction results ###################################
Number of query sequences:  1
Cleavage site predictions not included.
Using PLANT networks.
```

Name	Len	cTP	mTP	SP	other	Loc	RC
Sequence	599	0.926	0.303	0.002	0.022	C	2
cutoff		0.730	0.860	0.430	0.840		

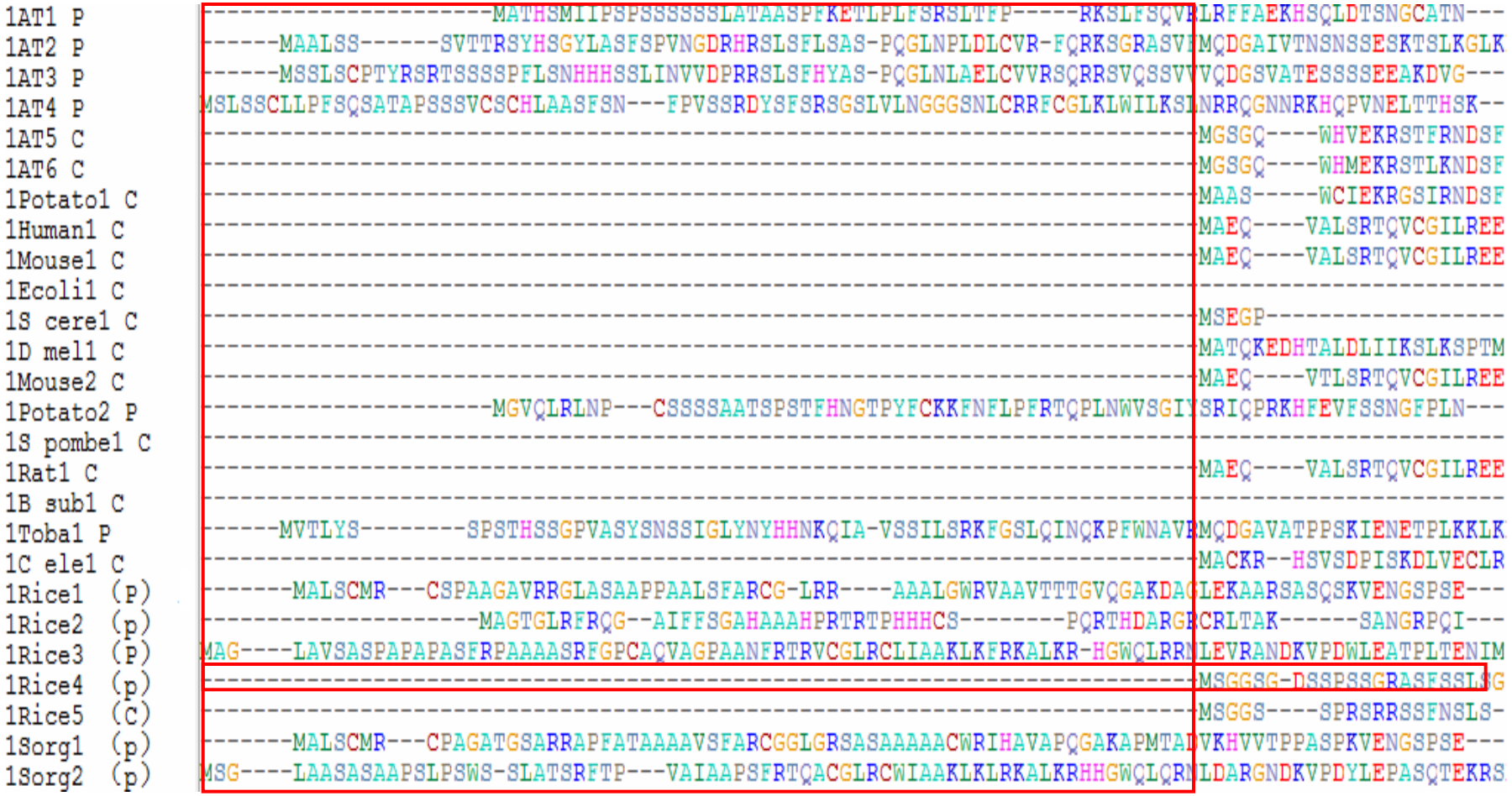
Explain the output. Go back.

Glucose-6-phosphate 1-dehydrogenase

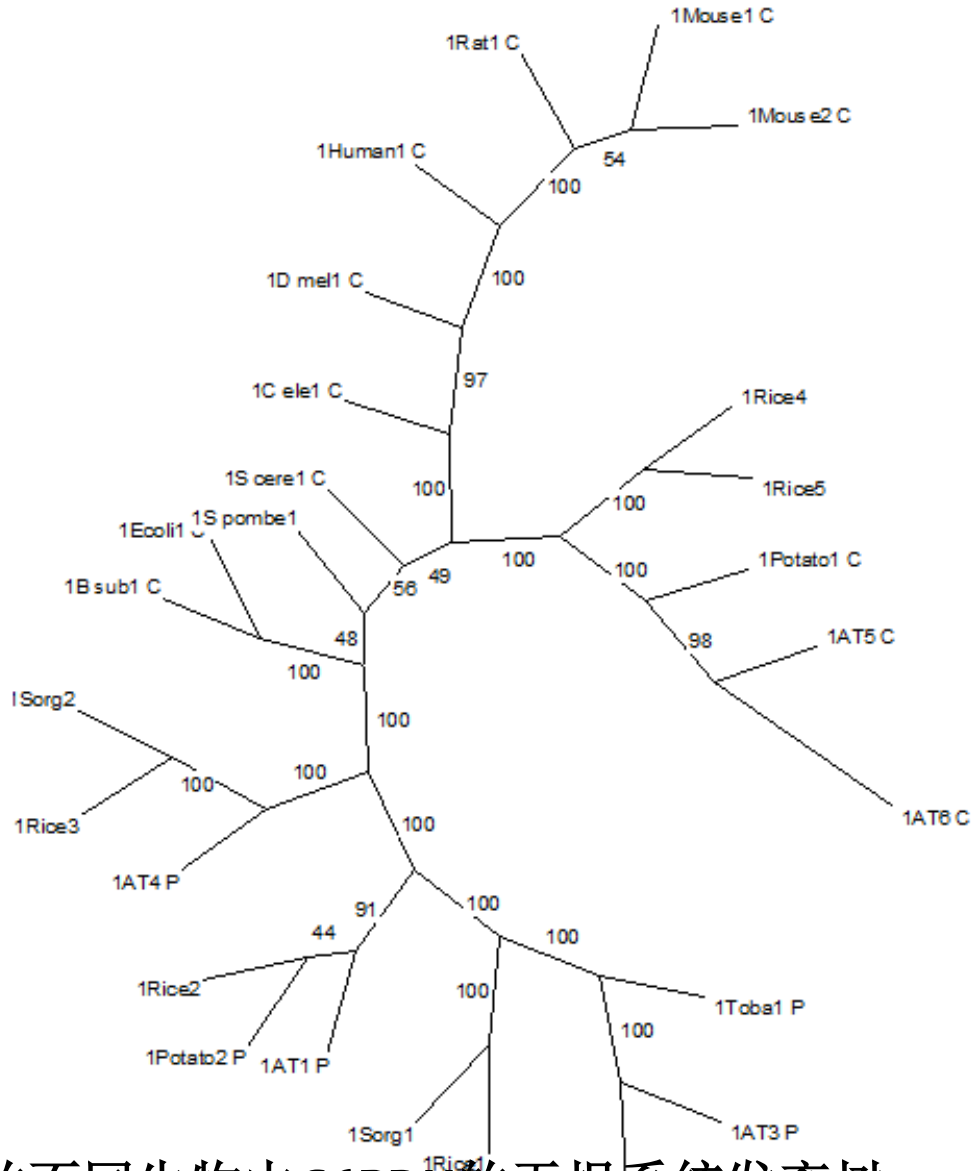


利用Phylip软件构建的不同生物中G6PDH的无根系统发育树

Mutiple alignment of G6PDH from different species



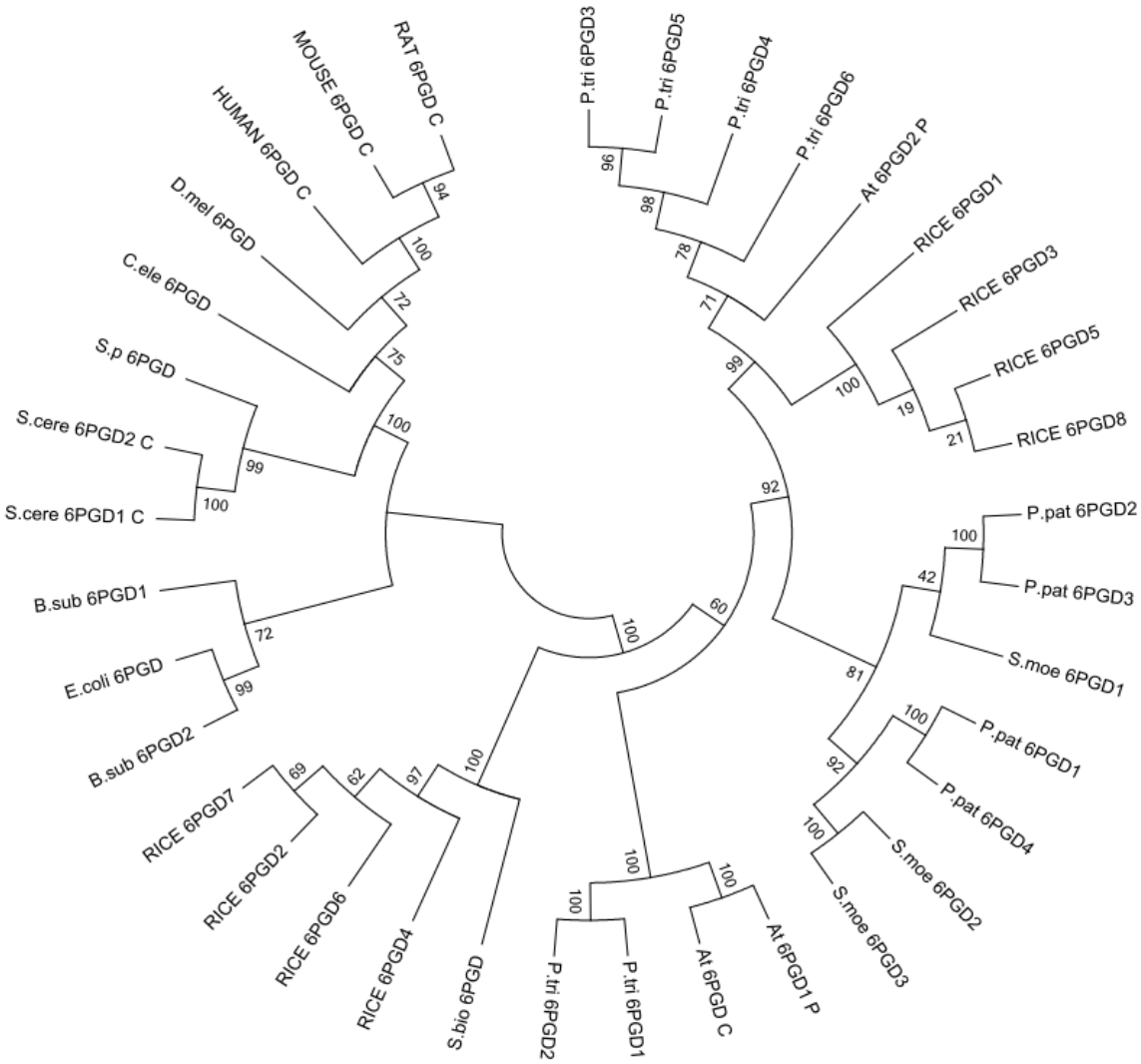
截掉信号肽后建树



利用Phylip软件构建的不同生物中G6PDH的无根系统发育树

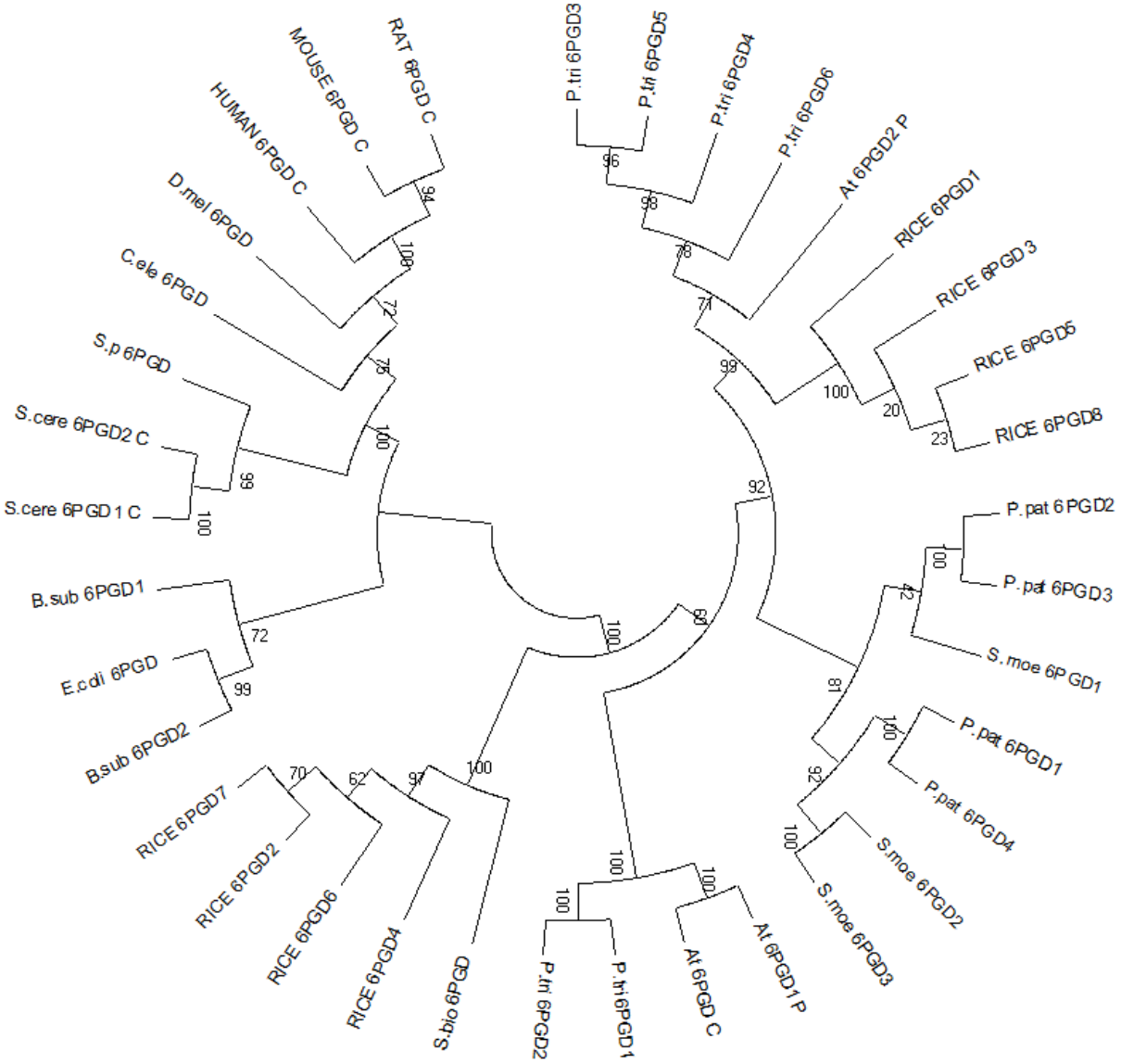
（截掉N段部分序列）

6-Phosphogluconolactonase



利用MEGA软件构建的不同生物中6GPL的无根系统发育树

6-Phosphogluconate dehydrogenase



利用MEGA软件构建的不同生物中6GPL的无根系统发育树

Summary

- 我们感兴趣的葡萄糖-6-磷酸脱氢酶，在真核和原核生物中都非常保守，我们通过NCBI和uniprot等数据库在各种生物中都找到了该步酶的同源基因。

- 通过PDB找到了人的该步酶的结构，我们利用Pdbviewer软件中的Swiss-model工具进行拟南芥G6PDH蛋白3-D结构模拟，发现该步酶在拟南芥和人的该基因在结构上也非常保守。

Summary

- 通过对找到的同源基因用mega和phylip进行建树，建树结果表明植物质体中的基因和细胞质中该基因差异比较大，也许和内共生学说相关。

- 通过比对发现在质体中的该酶在N端非常保守，怀疑这段是信号肽，同时也许由于这段保守的序列使质体中的该酶聚在了一枝，于是通过截掉这段信号肽从新建树，结果和原来一样，进一步证明了质体中的该酶在进化过程中发生了适应性的变化。

Reference

- The oxidative pentose phosphate pathway: structure and organisation. Nicholas J Kruger and Antje von Schaewen. Current Opinion in Plant Biology, 2003, 6: 236–246;
- Characterization of Arabidopsis 6-Phosphogluconolactonase T-DNA Insertion Mutants Reveals an Essential Role for the Oxidative Section of the Plastidic Pentose Phosphate Pathway in Plant Growth and Development. Yuqing Xiong, Christopher DeFraia, Donna Williams, Xudong Zhang and Zhonglin Mou, Plant Cell Physiol.2009, 50(7): 1277–1291;
- 基础生物化学 赵武玲主编

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- 感谢康菊清师姐、孙康泰师兄

Thanks for your attention !

Merry Christmas!