GRADUATE SCHOOL OF CHINESE ACADEMY OF AGRICULTURAL SCIENCES
中国农业科学院研究生院

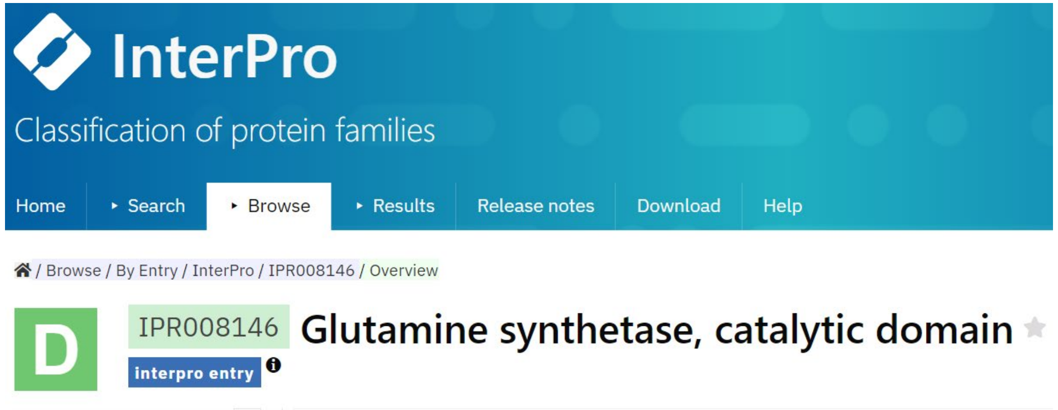
牛筋草GS基因抗性分析于结构预测

Eleusine indica glutamine synthetase

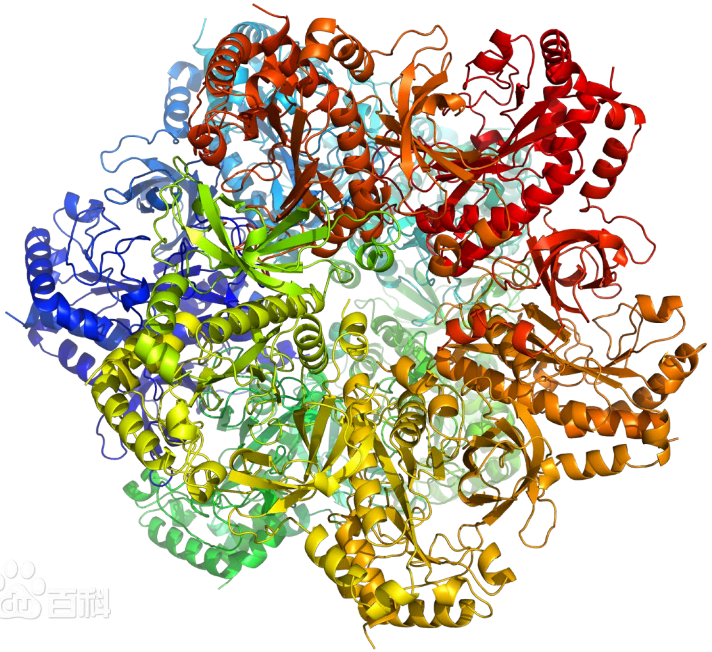
汇报人：李浩彤

研究背景

谷氨酰胺合成酶（Glutamine synthetase）是一种在植物中广泛存在的酶，参与氮代谢过程。具体来说，谷氨酰胺合成酶催化谷氨酸和氨合成谷氨酰胺，这是植物体内氮同化的关键步骤之一。



IPR008146 Glutamine synthetase, catalytic domain



谷氨酰胺合成酶可分为三大类：

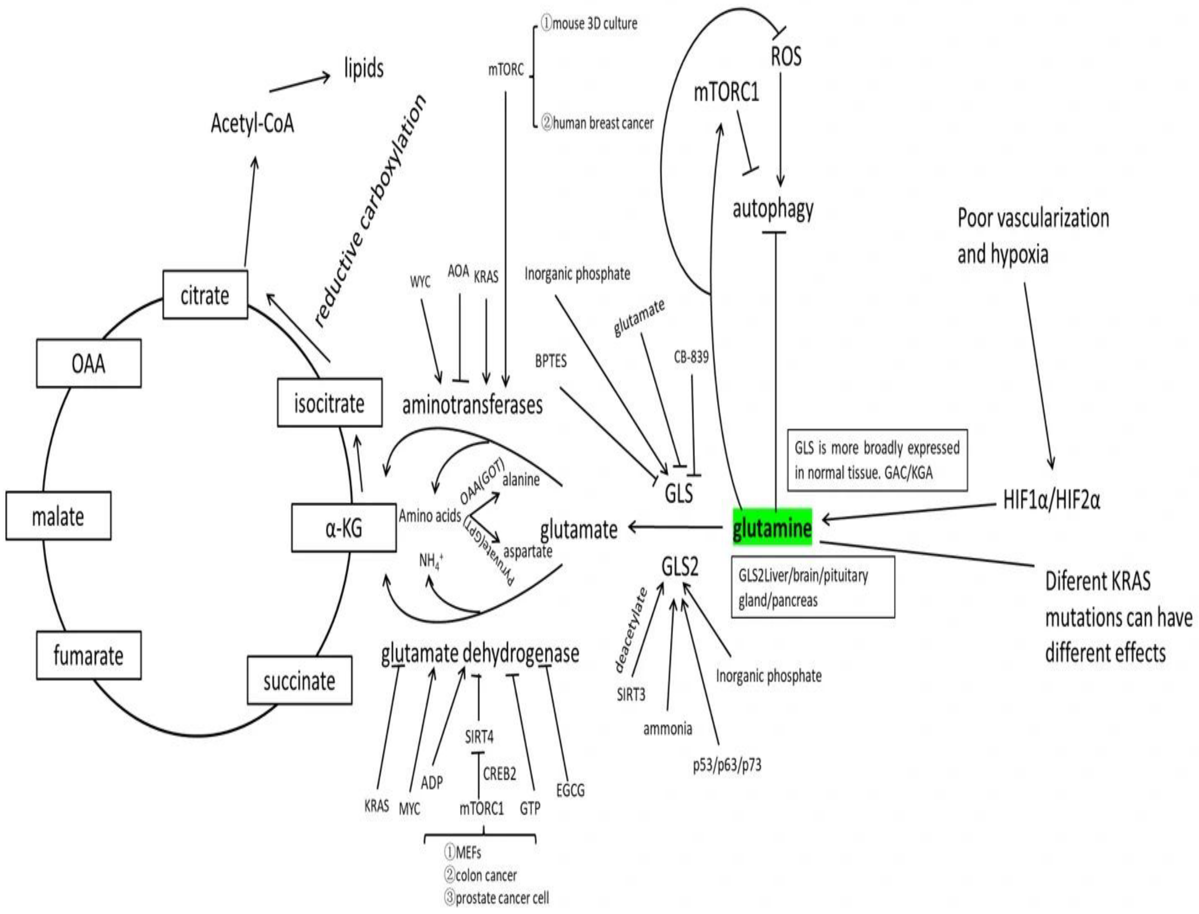
GSI：分布于原核生物。

GSII：主要分布于真核生物和少量细菌，如根瘤菌和放线菌。

GSIII：只在少量的细菌中有发现，如脆弱类杆菌和溶纤维丁酸弧菌。

研究背景

谷氨酰胺代谢



谷氨酰胺合成酶的生理功能

1. **氮同化**：将氨转化为谷氨酰胺，为植物提供氮源。

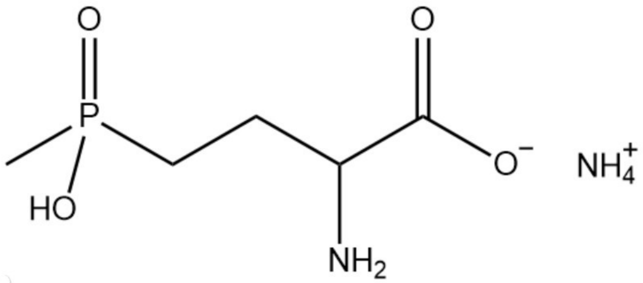
2. **调节细胞内氮平衡**：通过控制氮的吸收和利用，维持细胞的氮稳态。

3. **参与光合作用和呼吸作用**：在光合作用中提供氮源，同时在呼吸作用中参与能量代谢。

谷氨酰胺合成酶的分子机制

谷氨酰胺合成酶的分子机制包括其结构和功能。它是一个多亚基酶，通常由多个亚基组成，每个亚基包含活性位点。其活性受到多种调节因子的影响，包括ATP、谷氨酸和反馈抑制等。

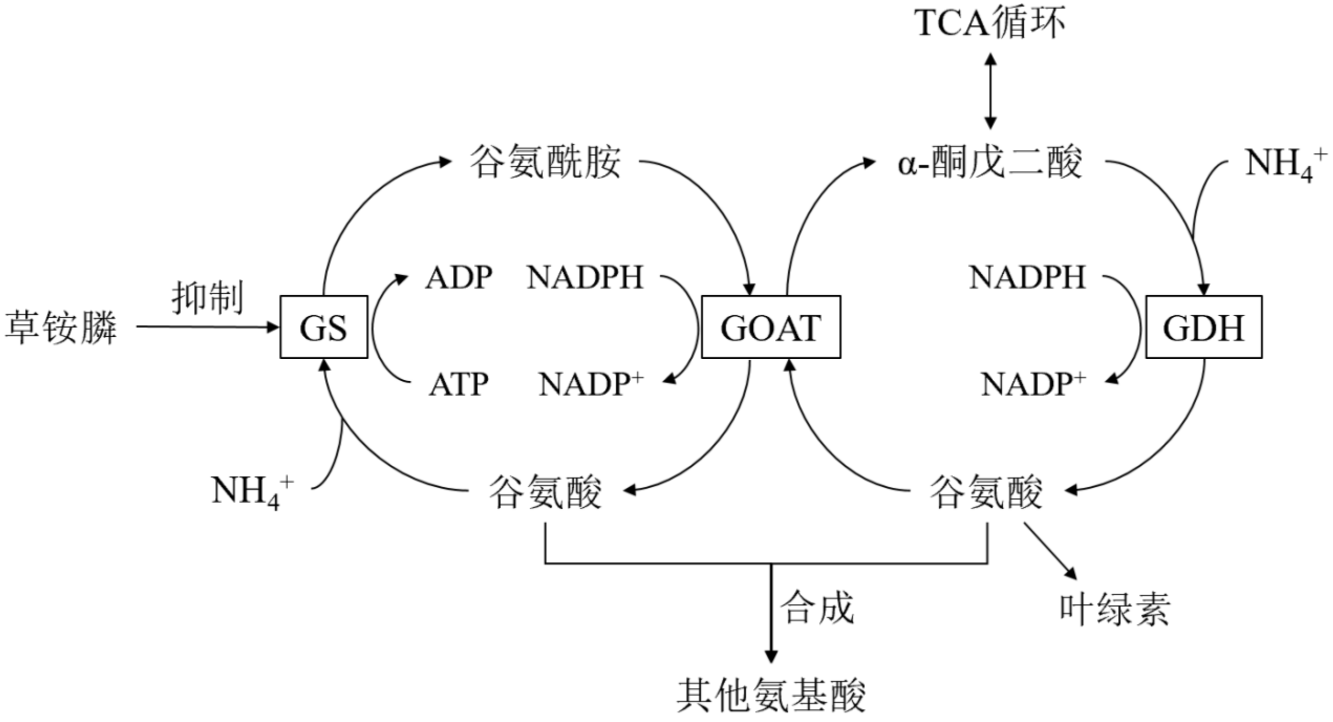
研究背景



草铵膦：

草铵膦是在20世纪80年代开发的广谱触杀型灭生性除草剂。

草铵膦属于膦酸类除草剂，能够**抑制植物氮代谢途径中的谷氨酰胺合成酶**，从而干扰植物的代谢，使植物死亡。

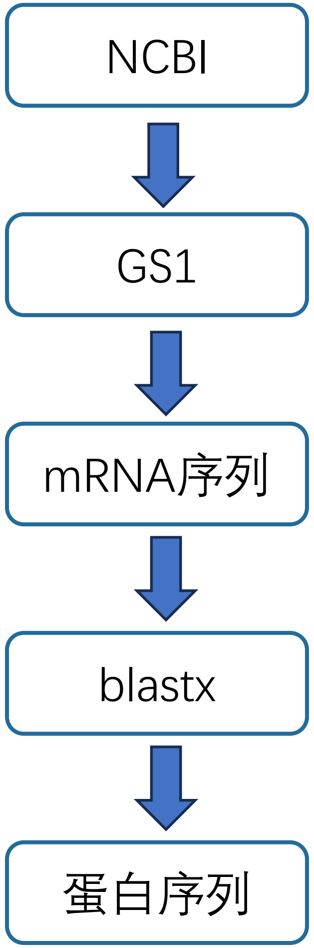


GS：谷氨酰胺合成酶；GOAT：谷氨酸合成酶；GDH：谷氨酸脱氢酶

草铵膦作用与谷氨酰胺合成酶（glutamine synthetase，GS）有关，这种酶在植物的氮代谢过程中催化谷氨酸与铵离子合成谷氨酰胺。而当草铵膦进入植物体内后，**能与ATP相结合并占据谷氨酰胺合成酶的反应位点，**从而不可逆地抑制谷氨酰胺合成酶的活性并破坏之后的一系列代谢过程。

研究内容

1、GS1-1基因与其对应的蛋白



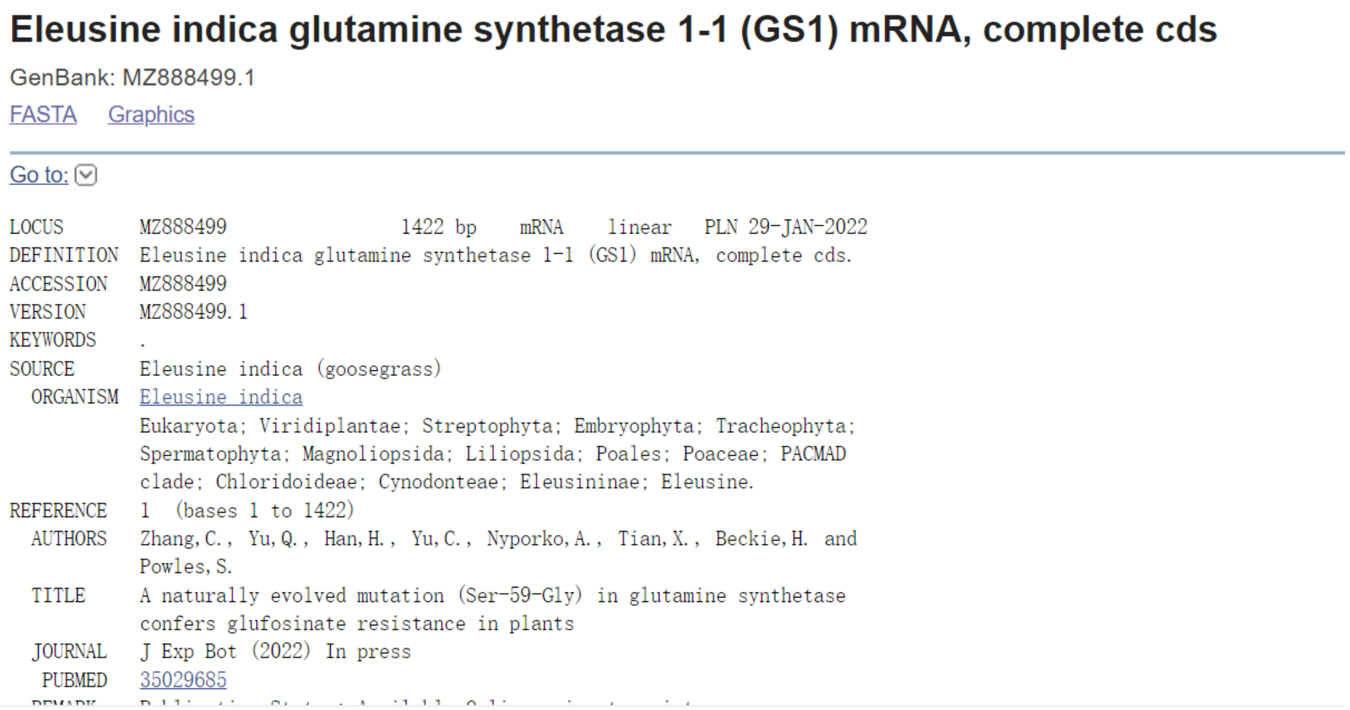
NCBI

GS1

mRNA序列

blastx

蛋白序列



Eleusine indica glutamine synthetase 1-1 (GS1) mRNA, complete cds

GenBank: MZ888499.1

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LOCUS       MZ888499                1422 bp    mRNA    linear   PLN 29-JAN-2022
DEFINITION  Eleusine indica glutamine synthetase 1-1 (GS1) mRNA, complete cds.
ACCESSION   MZ888499
VERSION     MZ888499.1
KEYWORDS    .
SOURCE      Eleusine indica (goosegrass)
  ORGANISM  Eleusine indica
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; Liliopsida; Poales; Poaceae; PACMAD
            clade; Chloridoideae; Cynodonteae; Eleusininae; Eleusine.
REFERENCE   1  (bases 1 to 1422)
  AUTHORS   Zhang,C., Yu,Q., Han,H., Yu,C., Nyporko,A., Tian,X., Beckie,H. and
            Powles,S.
  TITLE     A naturally evolved mutation (Ser-59-Gly) in glutamine synthetase
            confers glufosinate resistance in plants
  JOURNAL   J Exp Bot (2022) In press
   PUBMED   35029685
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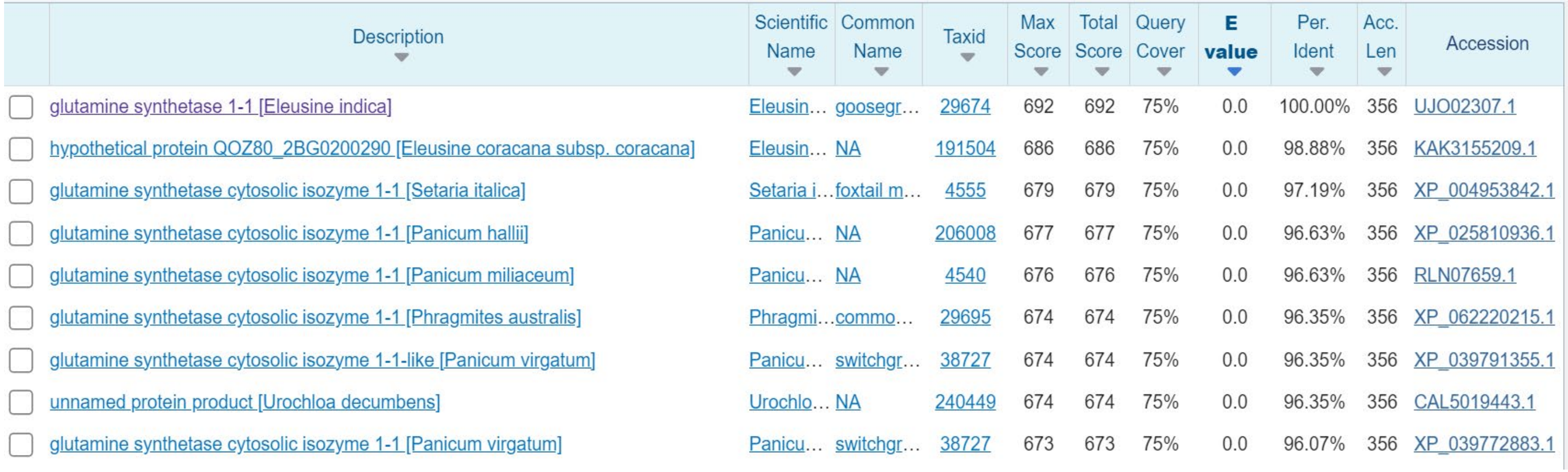

	Description	Scientific Name	Common Name	Taxid	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☐	glutamine synthetase 1-1 [Eleusine indica]	Eleusin…	goosegr…	29674	692	692	75%	0.0	100.00%	356	UJO02307.1
☐	hypothetical protein QOZ80_2BG0200290 [Eleusine coracana subsp. coracana]	Eleusin…	NA	191504	686	686	75%	0.0	98.88%	356	KAK3155209.1
☐	glutamine synthetase cytosolic isozyme 1-1 [Setaria italica]	Setaria i…	foxtail m…	4555	679	679	75%	0.0	97.19%	356	XP_004953842.1
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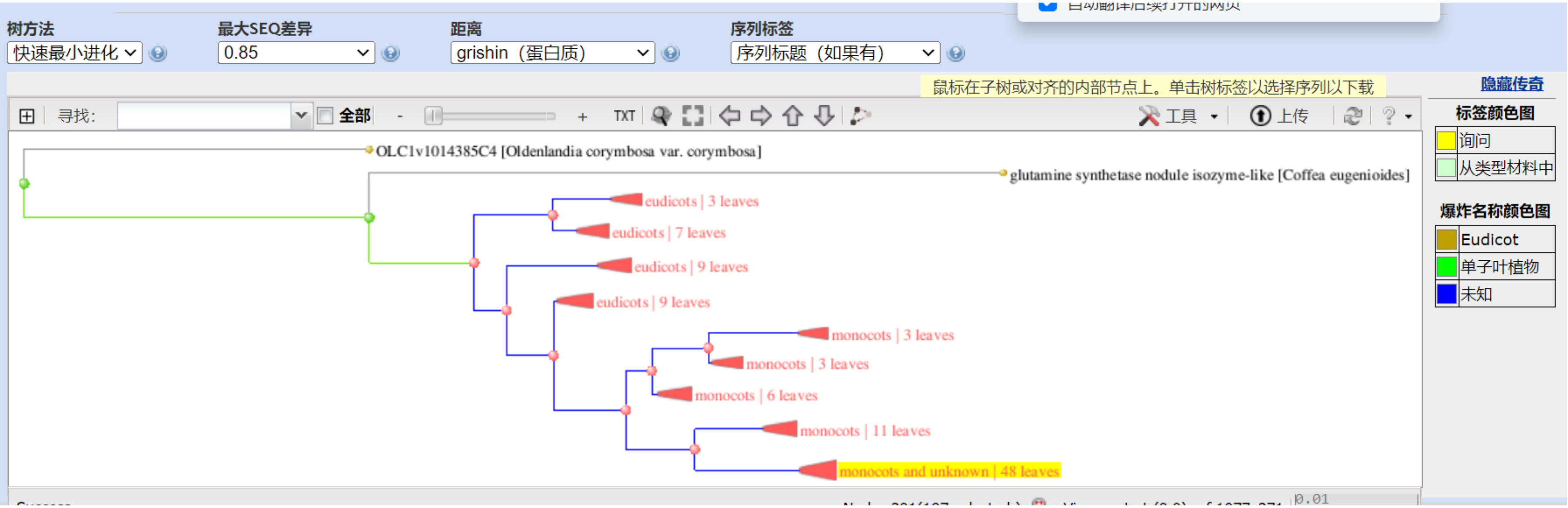
☐	glutamine synthetase cytosolic isozyme 1-1 [Panicum miliaceum]	Panicu…	NA	4540	676	676	75%	0.0	96.63%	356	RLN07659.1
☐	glutamine synthetase cytosolic isozyme 1-1 [Phragmites australis]	Phragmi…	commo…	29695	674	674	75%	0.0	96.35%	356	XP_062220215.1
☐	glutamine synthetase cytosolic isozyme 1-1-like [Panicum virgatum]	Panicu…	switchgr…	38727	674	674	75%	0.0	96.35%	356	XP_039791355.1
☐	unnamed protein product [Urochloa decumbens]	Urochlo…	NA	240449	674	674	75%	0.0	96.35%	356	CAL5019443.1
☐	glutamine synthetase cytosolic isozyme 1-1 [Panicum virgatum]	Panicu…	switchgr…	38727	673	673	75%	0.0	96.07%	356	XP_039772883.1

研究内容

通过蛋白序列利用BLAST找到其近缘种，建立发育树



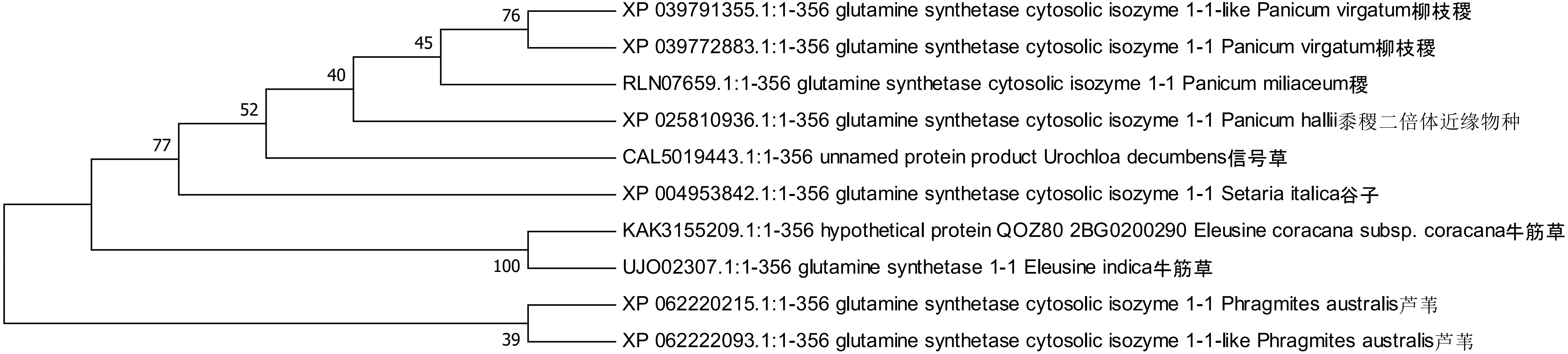
树方法：快速最小进化

最大SEQ差异：0.85

距离：grishin（蛋白质）

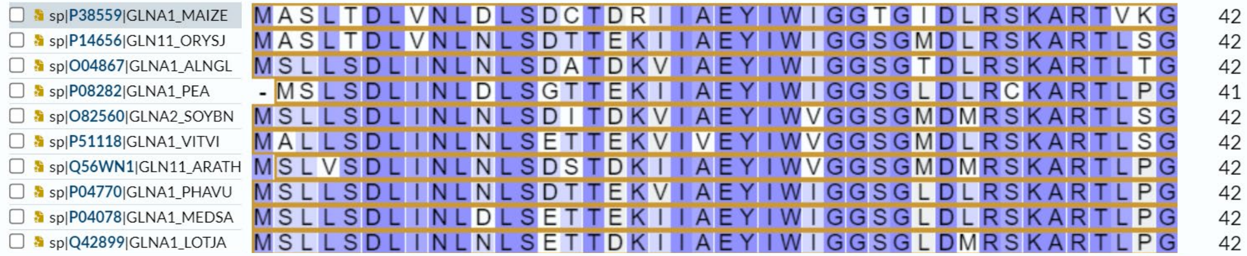
序列标签：序列标题（如果有）

鼠标在子树或对齐的内部节点上。单击树标签以选择序列以下载



研究内容

通过蛋白序列利用uniprot分析是否保守



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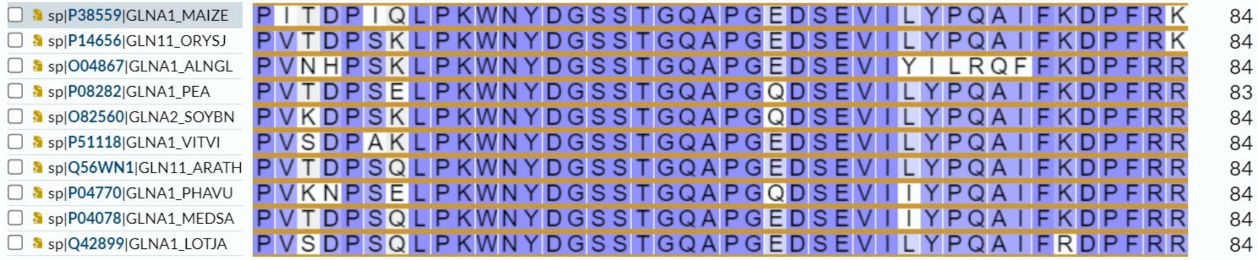

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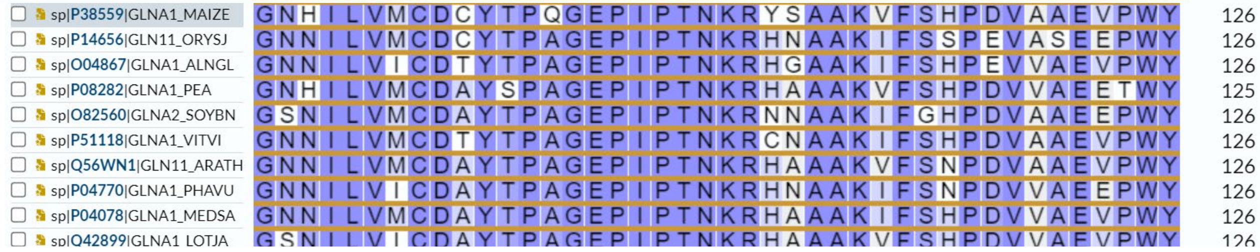

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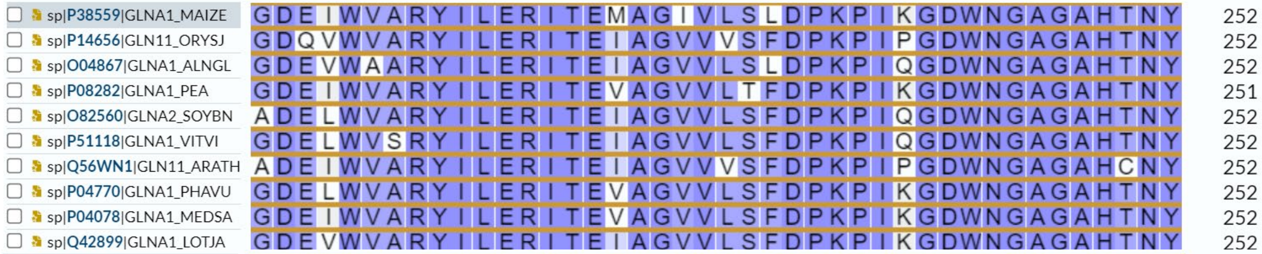

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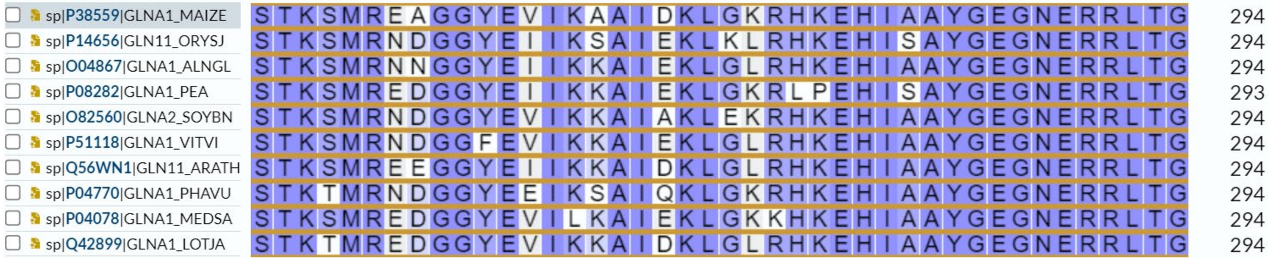

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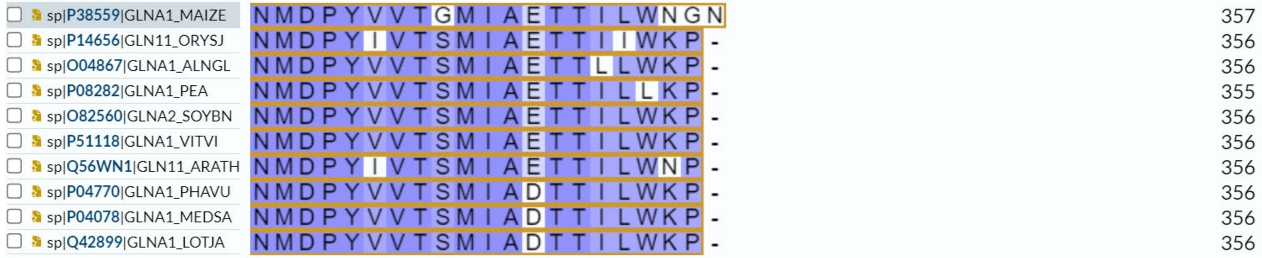

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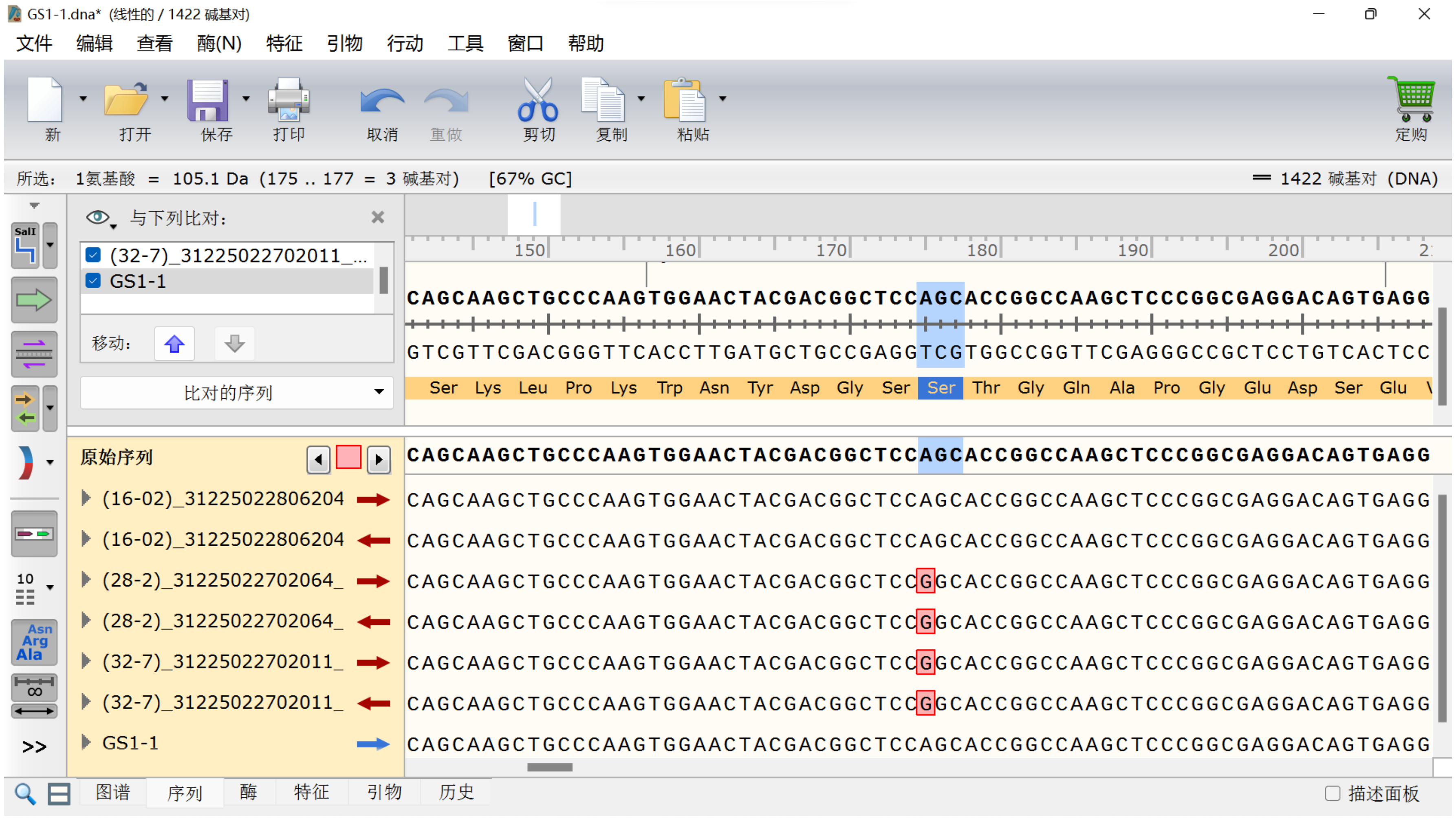
研究内容

序列对比



所选： 1氨基酸 = 105.1 Da (175 .. 177 = 3 碱基对) [67% GC]

1422 碱基对 (DNA)

☑ (32-7)_3122502702011_...
☑ GS1-1

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 Ser Lys Leu Pro Lys Trp Asn Tyr Asp Gly Ser Ser Thr Gly Gln Ala Pro Gly Glu Asp Ser Glu V

原始序列                      CAGCAAGCTGCCCAAGTGGAACTACGACGGCTCCAGCACCGGCCAAGCTCCCGGCGAGGACAGTGAGG
(16-02)_31225022806204  →   CAGCAAGCTGCCCAAGTGGAACTACGACGGCTCCAGCACCGGCCAAGCTCCCGGCGAGGACAGTGAGG
(16-02)_31225022806204  ←   CAGCAAGCTGCCCAAGTGGAACTACGACGGCTCCAGCACCGGCCAAGCTCCCGGCGAGGACAGTGAGG
(28-2)_31225022702064_  →   CAGCAAGCTGCCCAAGTGGAACTACGACGGCTCCGGCACCGGCCAAGCTCCCGGCGAGGACAGTGAGG
(28-2)_31225022702064_  ←   CAGCAAGCTGCCCAAGTGGAACTACGACGGCTCCGGCACCGGCCAAGCTCCCGGCGAGGACAGTGAGG
(32-7)_31225022702011_  →   CAGCAAGCTGCCCAAGTGGAACTACGACGGCTCCGGCACCGGCCAAGCTCCCGGCGAGGACAGTGAGG
(32-7)_31225022702011_  ←   CAGCAAGCTGCCCAAGTGGAACTACGACGGCTCCGGCACCGGCCAAGCTCCCGGCGAGGACAGTGAGG
GS1-1                   →   CAGCAAGCTGCCCAAGTGGAACTACGACGGCTCCAGCACCGGCCAAGCTCCCGGCGAGGACAGTGAGG
```

根据序列对比，其中有两个种群产生产生抗性突变。

AGC → GGC

丝氨酸突变成甘氨酸

阅读文献显示该突变造成了牛筋草对草铵膦的抗性

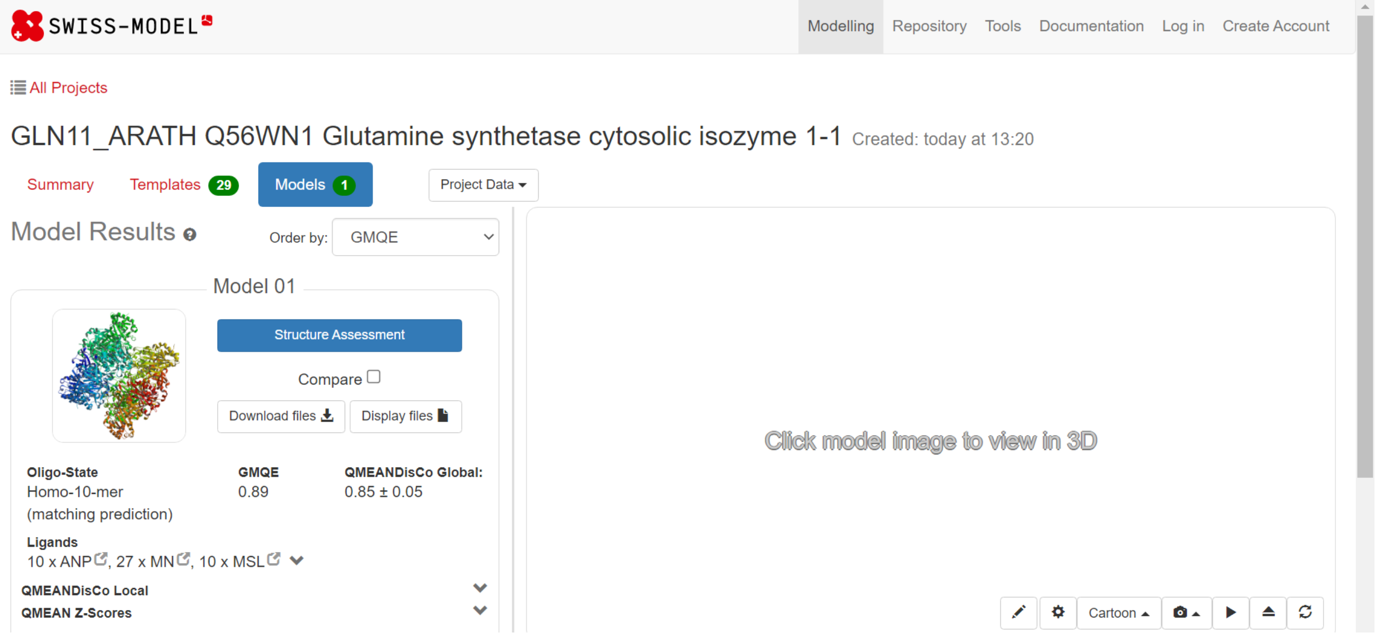
后续试验需要对此抗性进行进一步分析，究竟如何产生抗性

研究内容

寻找蛋白序列，对比序列变化。

G

>KAK3155209.1 hypothetical protein QOZ80 2BG0200290 [Eleusine coracana subsp. coracana]
MACLTDLINLDLSDSTEKIIAEYIWIGGSGMDLRSKARTLPGPVSDPSKLPKWNYDGSSTGQAPGEDSEVILYPQAIFKD
PFRKGNNILVMCDCYTPAGEPIPTNKRANAAKIFSNPEVSAEEPWYGIEQEYTLLQKDTNWPLGWPLGGFPGPQGPYYCG
VGADKSFGRDIVDSHYKACLYAGINISGINGEVMPGQWEFQVGPAVGISAGDQVWVARYILERITEIAGVVVTFDPKPIP
GDWNGAGAHSNYSTKSMRNDGGYEVIKSAIEKLKLRHREHIAAYGEGNERRLTGKHETADINTFSWGVANRGASVRVGRE
TEQNGKGYFEDRRPASNMDPYVVTSMIAETTIIWKP



GLN11_ARATH Q56WN1 Glutamine synthetase cytosolic isozyme 1-1 Created: today at 13:20

Model Results

Order by: GMQE

Model 01

Oligo-State	GMQE	QMEANDisCo Global:
Homo-10-mer (matching prediction)	0.89	0.85 ± 0.05

Ligands
10 x ANP, 27 x MN, 10 x MSL

QMEANDisCo Local

QMEAN Z-Scores

利用NBCI寻找蛋白序列

↓↓↓↓

Uniprot找到已经被注释的蛋白

↓↓↓↓

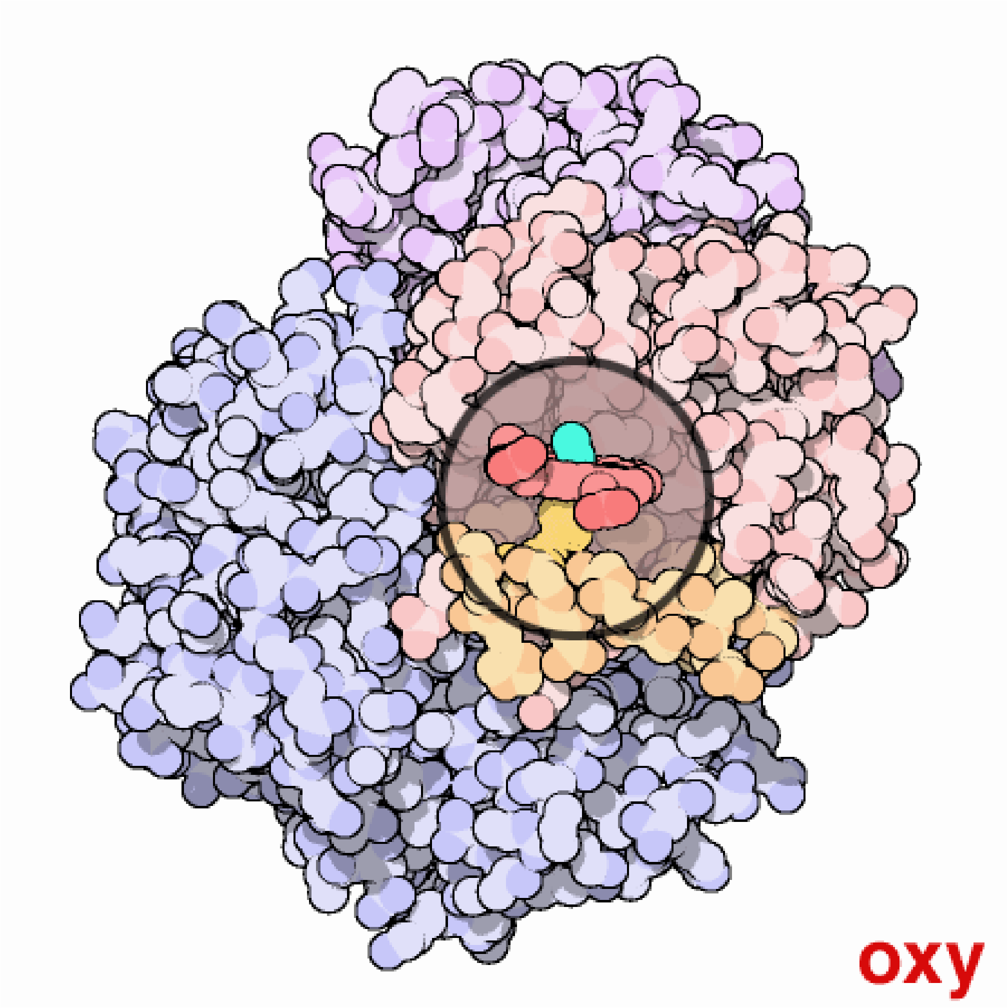
寻找三维结构

↓↓↓↓

建立模型

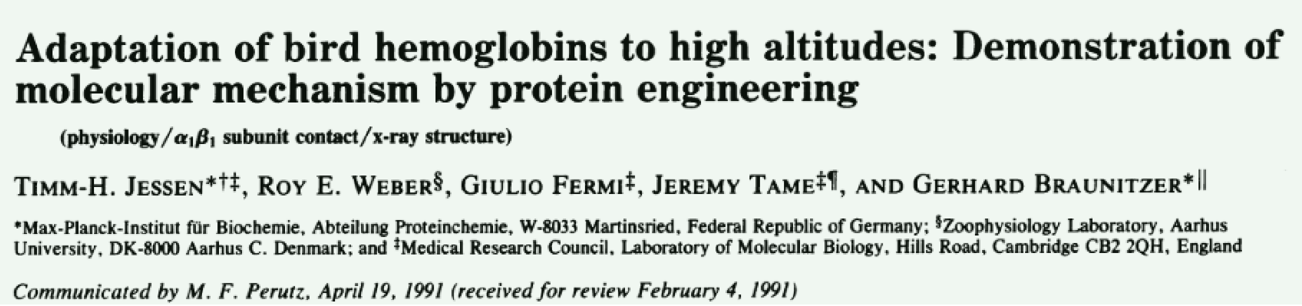
研究背景

血红蛋白对鸟类迁徙的影响？鸟类血红蛋白对高海拔的适应？



血红蛋白是一种非凡的分子机器，它使用运动和微小的结构变化来调节其作用。血红蛋白中四个血红素部位的氧结合并非同时发生。一旦第一个血红素结合氧，它就会引入相应蛋白链结构的小变化。这些变化将相邻链变成不同的形状，使它们更容易结合氧。

研究背景



Adaptation of bird hemoglobins to high altitudes: Demonstration of molecular mechanism by protein engineering

(physiology/$\alpha_1\beta_1$ subunit contact/x-ray structure)

TIMM-H. JESSEN*†‡, ROY E. WEBER§, GIULIO FERMI‡, JEREMY TAME‡¶, AND GERHARD BRAUNITZER*‖

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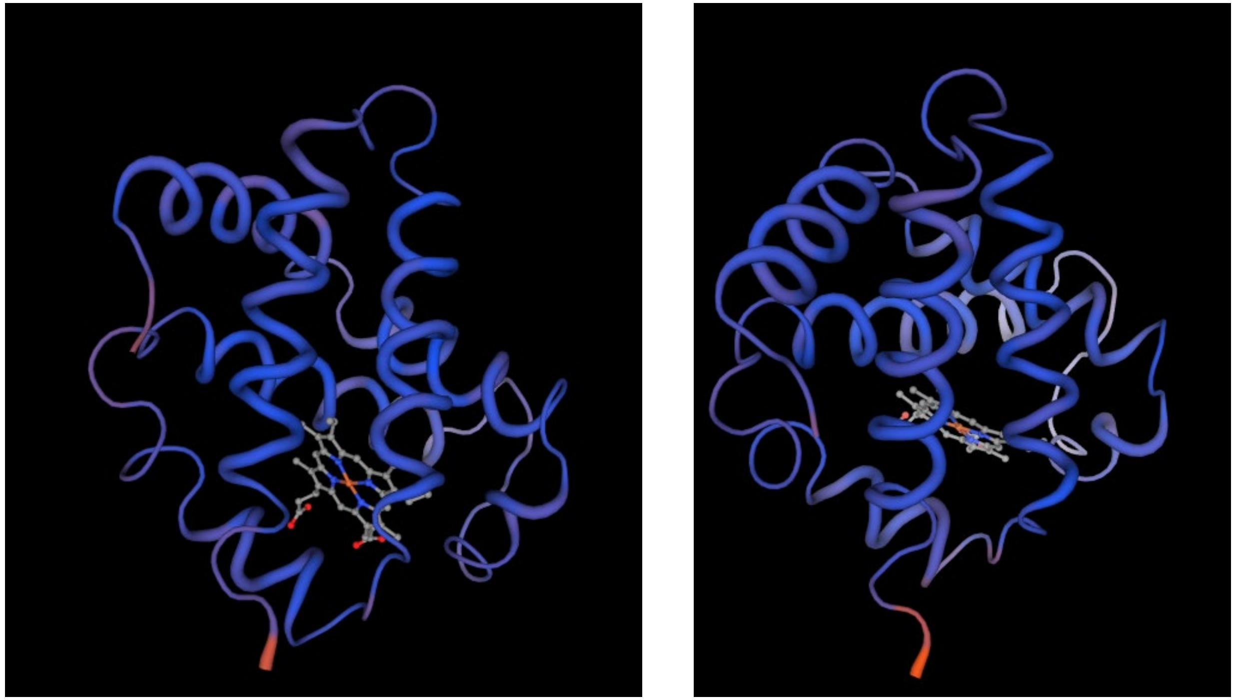
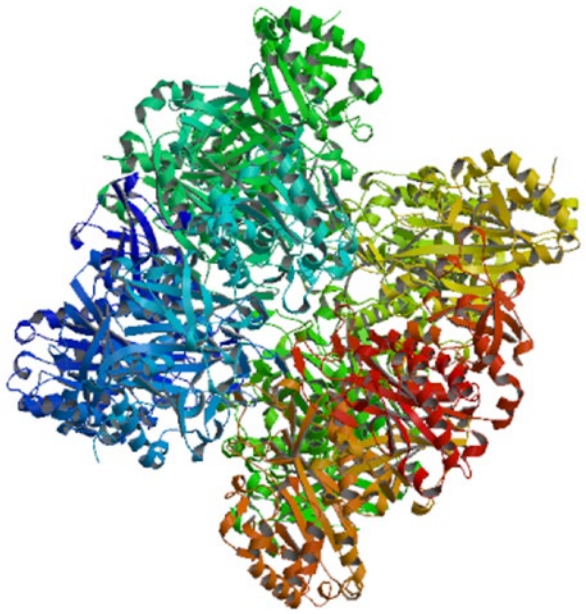
Communicated by M. F. Perutz, April 19, 1991 (received for review February 4, 1991)

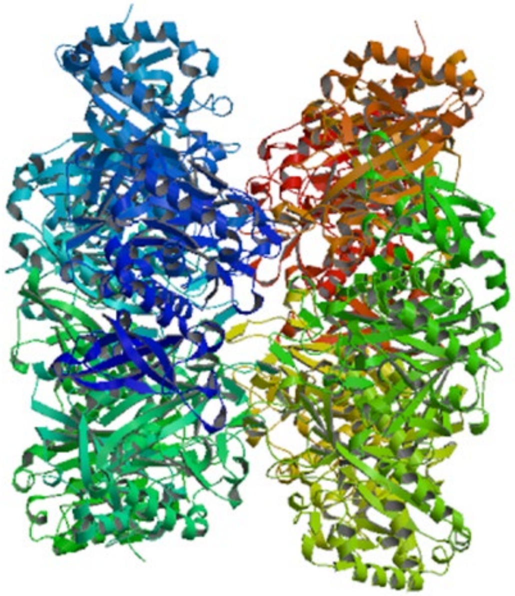


探讨了鸟类血红蛋白（Hb）如何通过分子机制适应高海拔环境。研究聚焦于两种高海拔鹅类——斑头雁（bar-headed goose）和安第斯鹅（Andean goose），它们的血红蛋白具有更高的氧亲和力，帮助它们在缺氧环境中生存和飞行。研究发现，斑头雁血红蛋白中的Pro-119α(H2)→Ala突变和安第斯鹅血红蛋白中的Leu-55β(D6)→Ser突变，均在α1β1亚基接触面留下了一个两碳的空隙，从而降低了脱氧血红蛋白（T态）的张力，提高了氧亲和力。

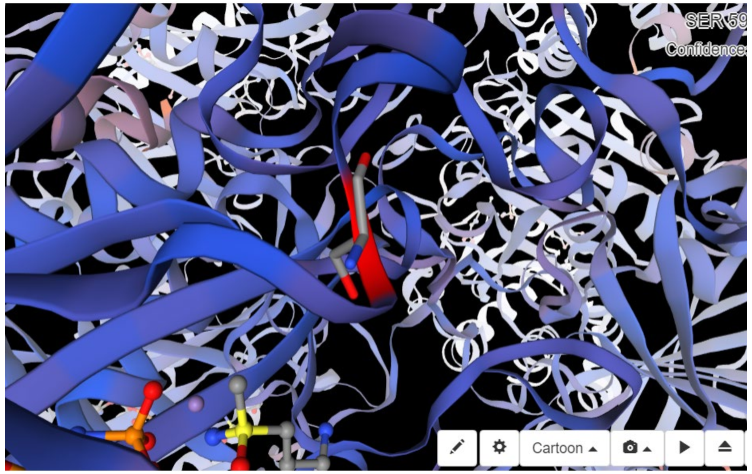
研究背景

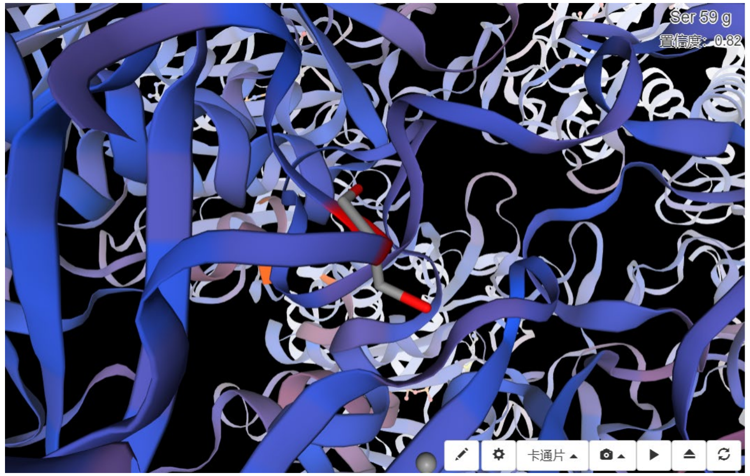


Glutamine synthetase cytosolic isozyme 1-1



突变后的模型





作为相邻亚基一侧接触界面的一部分，Ser 59不与谷氨酸或草铵膦直接相互作用，但与残基Tyr 158、Val 193、Glu 297和Asp 56（两个有利的H键）以及铵离子（不利的供体-供体相互作用）接触。**Ser 59 Gly取代诱导谷氨酸/草铵膦结合位点的结构重排，包括与Val 193和NH 4+接触的丧失**，因此，反过来，稳定了天然底物谷氨酸的结合，自由结合能降低261 kJ mol-1。因此，这允许谷氨酸在结合位点成功地与草铵膦竞争，从而赋予抗性。

研究前景

谷氨酰胺合成酶与植物抗性的关系

研究发现，谷氨酰胺合成酶的Ser59Gly突变体在glufosinate处理下表现出抗性。这种突变体在Eleusine indica等植物中自然演化而来，使得这些植物对glufosinate产生了抗性。进一步的研究表明，这种突变体在酵母中的表达也显示出对glufosinate的更高耐受性。

谷氨酰胺合成酶在农业中的应用前景

谷氨酰胺合成酶的突变体在农业上具有潜在的应用价值。通过基因工程手段，可以将这种抗性基因导入作物中，提高作物对除草剂的耐受性，从而减少除草剂的使用量，降低对环境的污染。此外，这种突变体还可以用于研究植物对除草剂的抗性机制，为农业生产提供新的策略和方法。