G04
炎症小体核心基因
NLRP3
功能与结构研究

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2022/01/16

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汇报内容

01/ 研究背景与意义

02/ NLRP3蛋白序列及功能分析

03/ NLRP3蛋白结构分析

04/ 总结与研究计划

PART ONE

1

研究背景与意义

Innate immunity

PRRs

Inflammasome

NLRP3

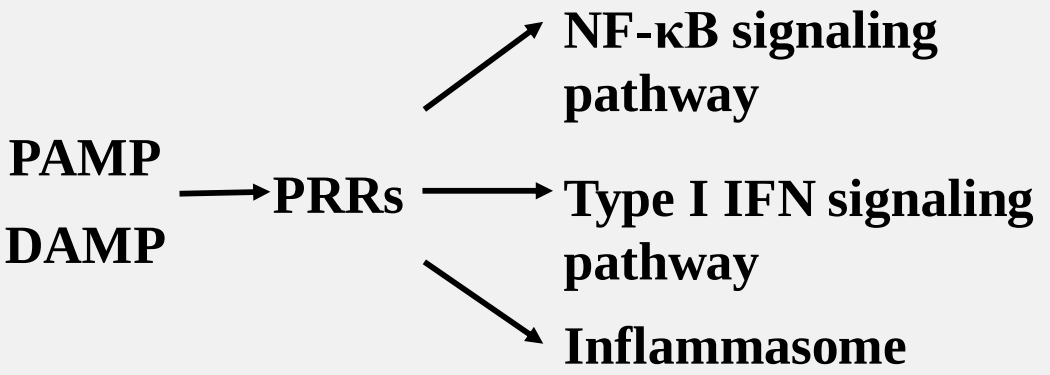
研究背景

炎症反应是天然免疫重要组成部分

Innate immunity and adaptive immunity

	Innate	Adaptive
Distribution	All multicellular organisms	Only in vertebrates
Response	General	Specific
Receptors	Primitive and broad	Highly specific (T an B cell receptors)
Kinetics	Fast (hours-days)	Slow (days-weeks)
Duration	Short (days)	Long (months/yrs)
Memory	-	+ + + +

cited from Yuluan Tang, Zhengfan Jiang's lab，Peking University.



研究背景

NLRP3是研究最广泛的炎症小体

Protein	**NACHT, LRR and PYD domains-containing protein 3**
Gene	**NLRP3**
Organism	*Homo sapiens (Human)*
Status	Reviewed - Annotation score: ●●●●● - Experimental evidence at protein level[i]

Publications[i] related to Q96P20 - NLRP3_HUMAN

Display Help video

Entry

Publications

1. **"Mutation of a new gene encoding a putative pyrin-like protein cause**
Hoffman H.M., Mueller J.L., Broide D.H., Wanderer A.A., Kolodner R.D.
Nat. Genet. 29:301-305(2001) [PubMed] [Europe PMC] [Abstract]

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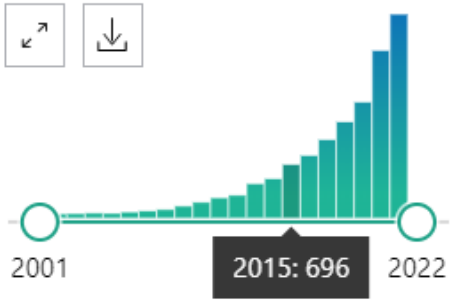
1 to 25 of 819 Show 25

:kle-Wells syndrome."

RESULTS BY YEAR



1

Cite

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NLRP3 inflammasome and its inhibitors: a review.

Shao BZ, Xu ZQ, Han BZ, Su DF, Liu C.

Front Pharmacol. 2015 Nov 5;6:262. doi: 10.3389/fphar.2015.00262. eCollection 2015.

PMID: 26594174 Free PMC article. Review.

Several **NLRP3** inflammasome inhibitors have been described, some of which show promise in the clinic. The present review will describe the structure and mechanisms of activation of the **NLRP3** inflammasome, its association with various auto-immune and auto-inflammatory ...

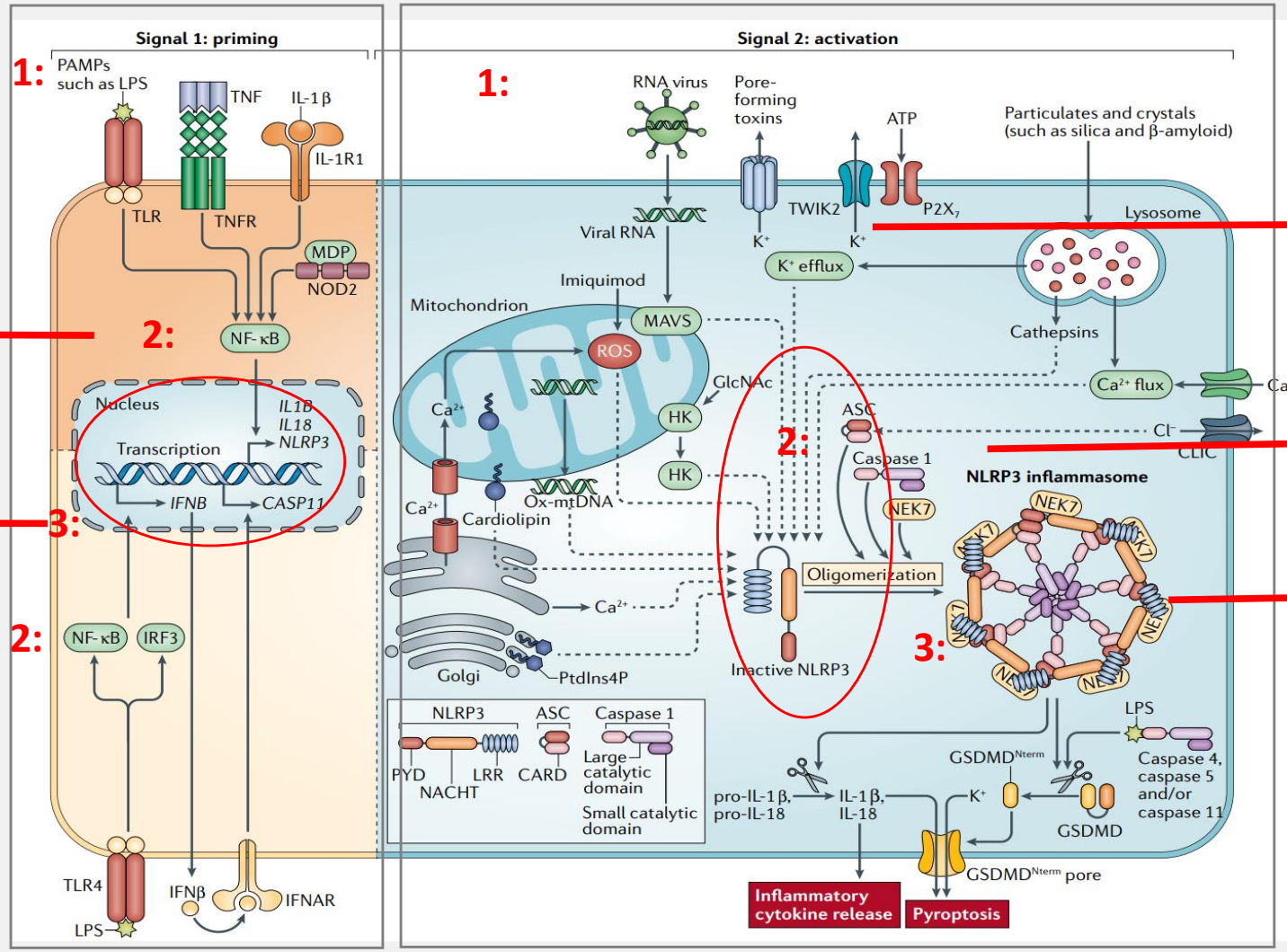
信号1促进NLRP3基因表达

信号2特异性激活NLRP3蛋白组装成炎症小体促进细胞焦亡

外界病原信号，危险信号被细胞感知

受体将信号传递到下游激活转录因子：NF-kB，IRF3

转录因子入核调控天然免疫相关基因表达：其中NLRP3基因诱导表达，NLRP3蛋白水平上升。但NLRP3处于自抑制状态。



NLRP3炎症小体激活特异性信号：胞外ATP，K+外流，RNA病毒等 多种多样

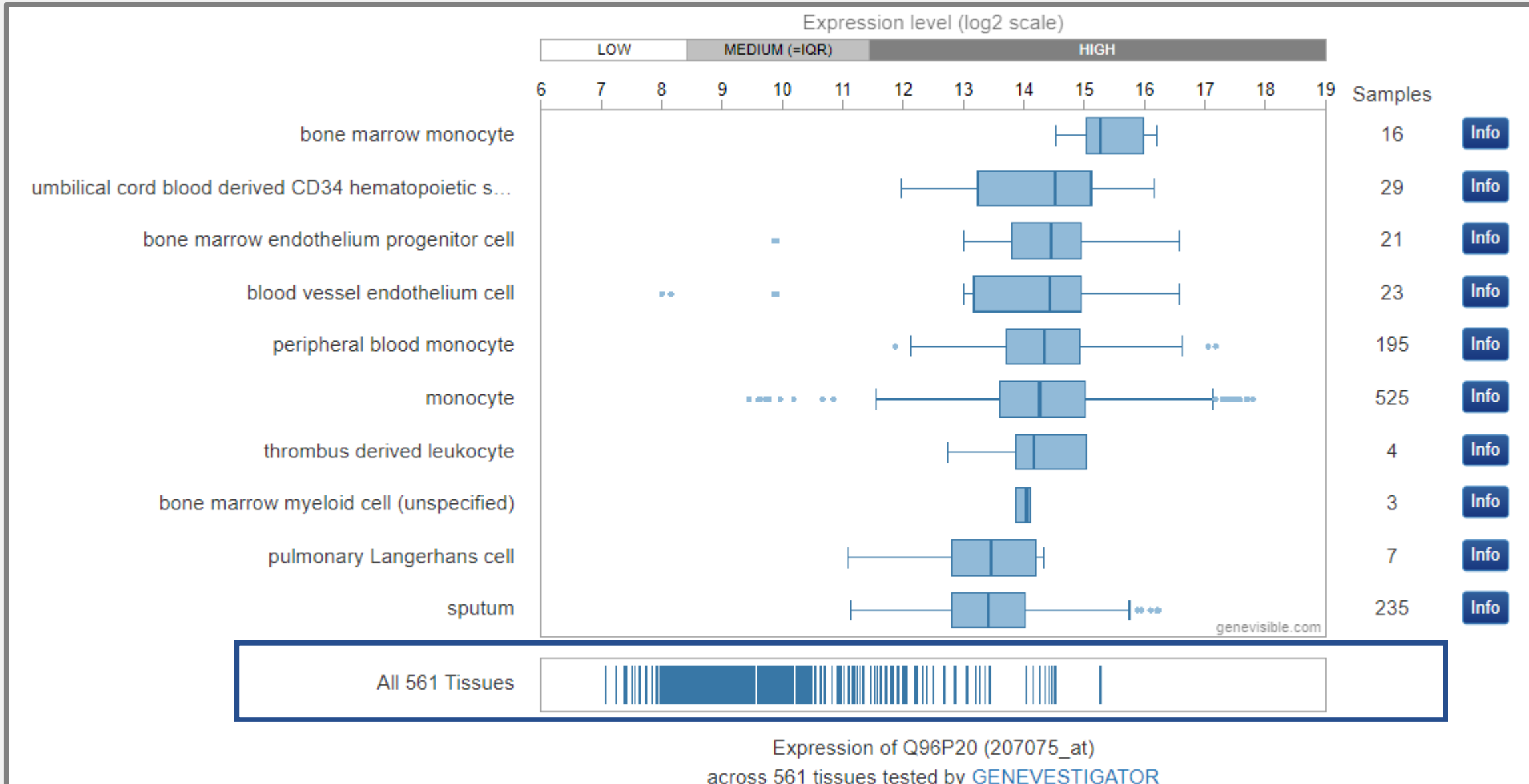
多种不同信号通过未知途径激活NLRP3蛋白

激活后的NLRP3与下游ASC，caspase1形成炎症小体超级复合物，招募并切割打孔蛋白GSDMD，导致细胞泄漏焦亡而死。

Swanson, K.V., Deng, M., and Ting, J.P. (2019). The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nature reviews Immunology 19, 477-489.

研究背景

genevisible数据库显示NLRP3在各组织表达情况

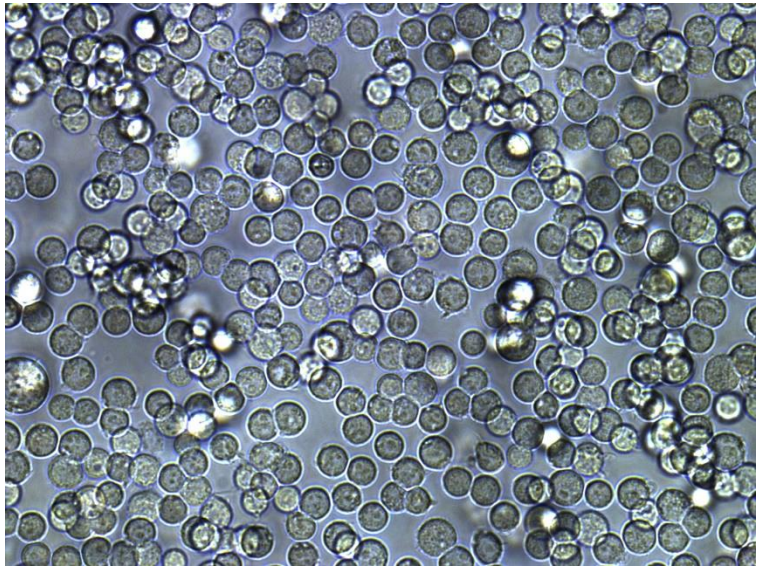


研究背景

结合NLRP3表达情况选择实验所用细胞系

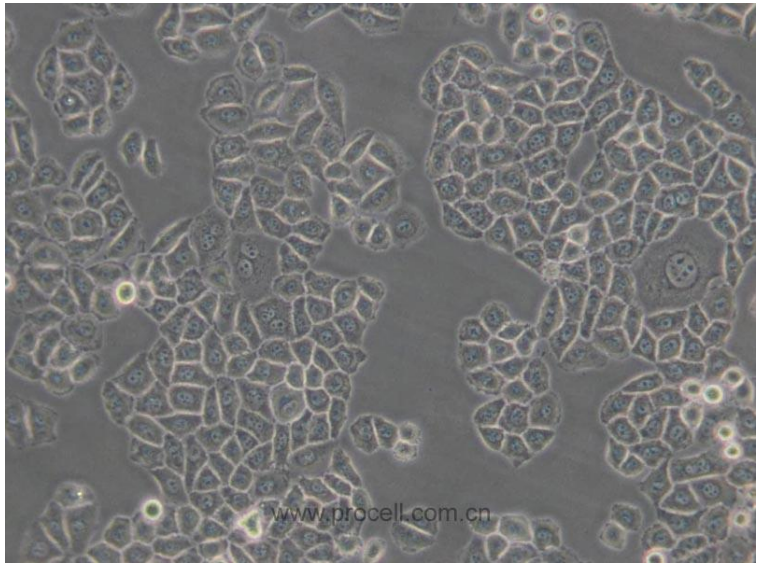
- 人单核细胞白血病 THP-1

  免疫细胞
  组成性高表达NLRP3蛋白



- 海拉细胞系 HeLa

  人宫颈癌细胞 上皮细胞
  诱导性表达NLRP3



PART
TWO

02

NLRP3蛋白
序列及功能分析

Sequence

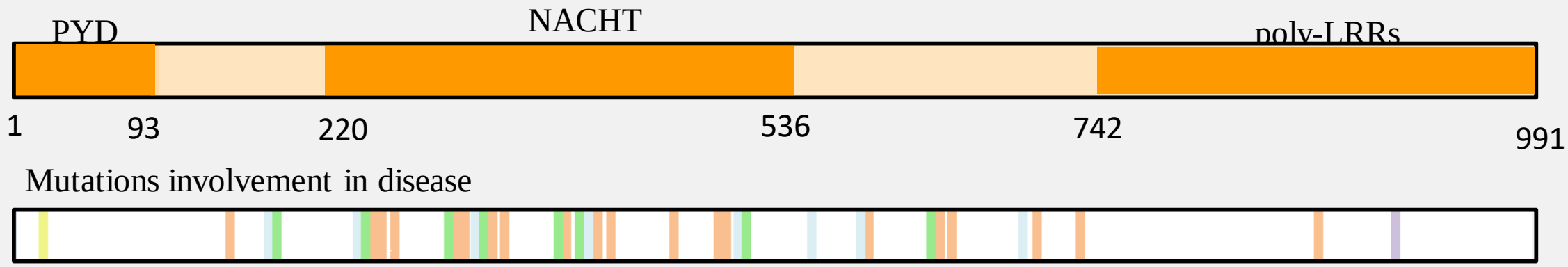
&

Function

NLRP3蛋白结构域

Domains and Repeats

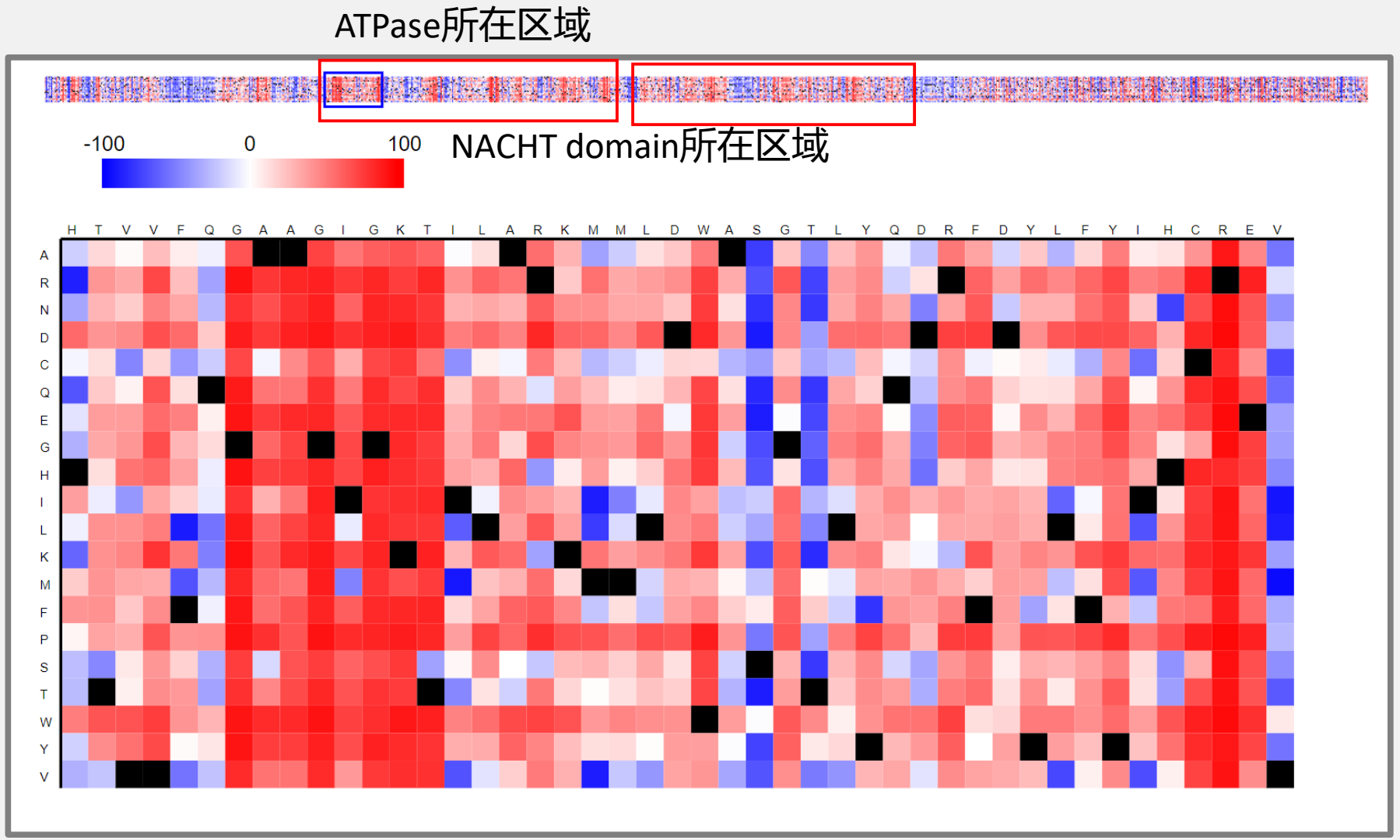
Feature key	Position(s)	Description	Actions	Graphical view	Length
Domain	1 – 93	Pyrin PROSITE-ProRule annotation	Add BLAST		93
Domain	220 – 536	NACHT PROSITE-ProRule annotation	Add BLAST		317
Repeat	742 – 762	LRR 1	Add BLAST		21
Repeat	771 – 792	LRR 2	Add BLAST		22
Repeat	799 – 819	LRR 3	Add BLAST		21
Repeat	828 – 849	LRR 4	Add BLAST		22
Repeat	856 – 876	LRR 5	Add BLAST		21
Repeat	885 – 906	LRR 6	Add BLAST		22
Repeat	913 – 933	LRR 7	Add BLAST		21
Repeat	942 – 963	LRR 8	Add BLAST		22
Repeat	970 – 991	LRR 9	Add BLAST		22



序列及功能分析

寻找实验所需自激活突变体

利用PredictProtein预测NLRP3突变位点敏感性



不可替代突变主要发生在具有ATP酶活的NACHT domain

Familial cold autoinflammatory syndrome 1 (FCAS1) 7 Publications

The disease is caused by variants affecting the gene represented in this entry.

Disease description: A rare autosomal dominant systemic inflammatory disease characterized by recurrent episodes of maculopapular rash associated with arthralgias, myalgias, fever and chills, swelling of the extremities, and conjunctivitis after generalized exposure to cold. Rarely, some patients may also develop late-onset renal amyloidosis.

Related information in OMIM

Feature key	Position(s)	Description	Actions	Graphical view	Length
Natural variant (VAR_013227)	200	V → M in FCAS1 and MWS. 4 Publications Corresponds to variant dbSNP:rs121908147	Ensembl, ClinVar.		1
Natural variant (VAR_014104)	262	R → W in FCAS1 and MWS; spontaneous polymerization into inflammasome speck. 3 Publications Corresponds to variant dbSNP:rs121908150	Ensembl, ClinVar.		1
Natural variant (VAR_014124)	307	L → P in FCAS1 and MWS. 2 Publications Corresponds to variant dbSNP:rs180177431	Ensembl, ClinVar.		1
Natural variant (VAR_043685)	355	L → P in FCAS1. 1 Publication Corresponds to variant dbSNP:rs28937896	Ensembl, ClinVar.		1
Natural variant (VAR_013229)	441	A → V in FCAS1. 1 Publication Corresponds to variant dbSNP:rs121908146	Ensembl, ClinVar.		1
Natural variant (VAR_043689)	490	R → K in FCAS1. 1 Publication Corresponds to variant dbSNP:rs145268073	Ensembl, ClinVar.		1
Natural variant (VAR_031853)	525	F → C in FCAS1. 1 Publication Corresponds to variant dbSNP:rs180177478	Ensembl.		1
Natural variant (VAR_013230)	629	E → G in FCAS1. 1 Publication Corresponds to variant dbSNP:rs121908148	Ensembl.		1

家族性寒冷自身炎症综合征

家族性寒冷自身炎症综合征(简称FCAS)，又称家族性寒冷性荨麻疹，是一种罕见的、遗传性炎症性疾病，其特征是暴露于寒冷环境下引发的间歇性皮疹、发热、关节疼痛等全身炎症体征/症状。

Muckle-Wells syndrome (MWS) 5 Publications

The disease is caused by variants affecting the gene represented in this entry.

Disease description: A hereditary periodic fever syndrome characterized by fever, chronic recurrent urticaria, arthralgias, progressive sensorineural deafness, and reactive renal amyloidosis. The disease may be severe if generalized reactive amyloidosis occurs.

Related information in OMIM

Feature key	Position(s)	Description	Actions	Graphical view	Length
Natural variant[i] (VAR_013227)	200	V → M in FCAS1 and MWS. 4 Publications Corresponds to variant dbSNP:rs121908147	Ensembl, ClinVar.		1
Natural variant[i] (VAR_014104)	262	R → W in FCAS1 and MWS; spontaneous polymerization into inflammasome speck. 3 Publications Corresponds to variant dbSNP:rs121908150	Ensembl, ClinVar.		1
Natural variant[i] (VAR_014105)	305	D → N in CINCA and MWS; spontaneous polymerization into inflammasome speck. 6 Publications Corresponds to variant dbSNP:rs121908153	Ensembl, ClinVar.		1
Natural variant[i] (VAR_014124)	307	L → P in FCAS1 and MWS. 2 Publications Corresponds to variant dbSNP:rs180177431	Ensembl, ClinVar.		1
Natural variant[i] (VAR_014366)	350	T → M in MWS and CINCA; spontaneous polymerization into inflammasome speck. 4 Publications Corresponds to variant dbSNP:rs151344629	Ensembl, ClinVar.		1
Natural variant[i] (VAR_013228)	354	A → V in MWS. 1 Publication Corresponds to variant dbSNP:rs121908149	Ensembl.		1
Natural variant[i] (VAR_014369)	441	A → T in MWS. 1 Publication Corresponds to variant dbSNP:rs180177430	Ensembl.		1
Natural variant[i] (VAR_014107)	571	G → R in MWS. 1 Publication Corresponds to variant dbSNP:rs121908151	Ensembl, ClinVar.		1

Muckle-Wells综合征

Muckle-Wells综合征(MWS)是一种由CIAS1/NLRP3基因突变引起的冷凝蛋白相关周期综合征(CAPS)。 这些综合征的特征是发热、皮疹和关节疼痛。 患有MWS的人经常有发作性发烧、发冷和关节疼痛。

Chronic infantile neurologic cutaneous and articular syndrome (CINCA) 7 Publications

The disease is caused by variants affecting the gene represented in this entry.

Disease description: Rare congenital inflammatory disorder characterized by a triad of neonatal onset of cutaneous symptoms, chronic meningitis, and joint manifestations with recurrent fever and inflammation.

Related information in OMIM

Feature key	Position(s)	Description	Actions	Graphical view	Length
Natural variant[i] (VAR_043679)	174	I → T in CINCA. 1 Publication Corresponds to variant dbSNP:rs180177449	Ensembl.		1
Natural variant[i] (VAR_043680)	262	R → L in CINCA. 1 Publication Corresponds to variant dbSNP:rs180177442	Ensembl, ClinVar.		1
Natural variant[i] (VAR_043681)	262	R → P in CINCA. 1 Publication Corresponds to variant dbSNP:rs180177442	Ensembl, ClinVar.		1
Natural variant[i] (VAR_043682)	266	L → H in CINCA. 1 Publication Corresponds to variant dbSNP:rs180177436	Ensembl.		1
Natural variant[i] (VAR_043683)	305	D → G in CINCA. 1 Publication Corresponds to variant dbSNP:rs180177447	Ensembl.		1
Natural variant[i] (VAR_014105)	305	D → N in CINCA and MWS; spontaneous polymerization into inflammasome speck. 6 Publications Corresponds to variant dbSNP:rs121908153	Ensembl, ClinVar.		1
Natural variant[i] (VAR_043684)	308	Q → K in CINCA. 1 Publication Corresponds to variant dbSNP:rs180177432	Ensembl.		1
Natural variant[i] (VAR_014106)	311	F → S in CINCA. 2 Publications Corresponds to variant dbSNP:rs121908154	Ensembl.		1

慢性婴幼儿神经系统皮肤和关节综合征

慢性婴幼儿神经系统皮肤和关节综合征(CINCA)：一种罕见的先天性炎症性疾病，以新生儿皮肤症状、慢性脑膜炎和反复发热和炎症的联合表现为特征。

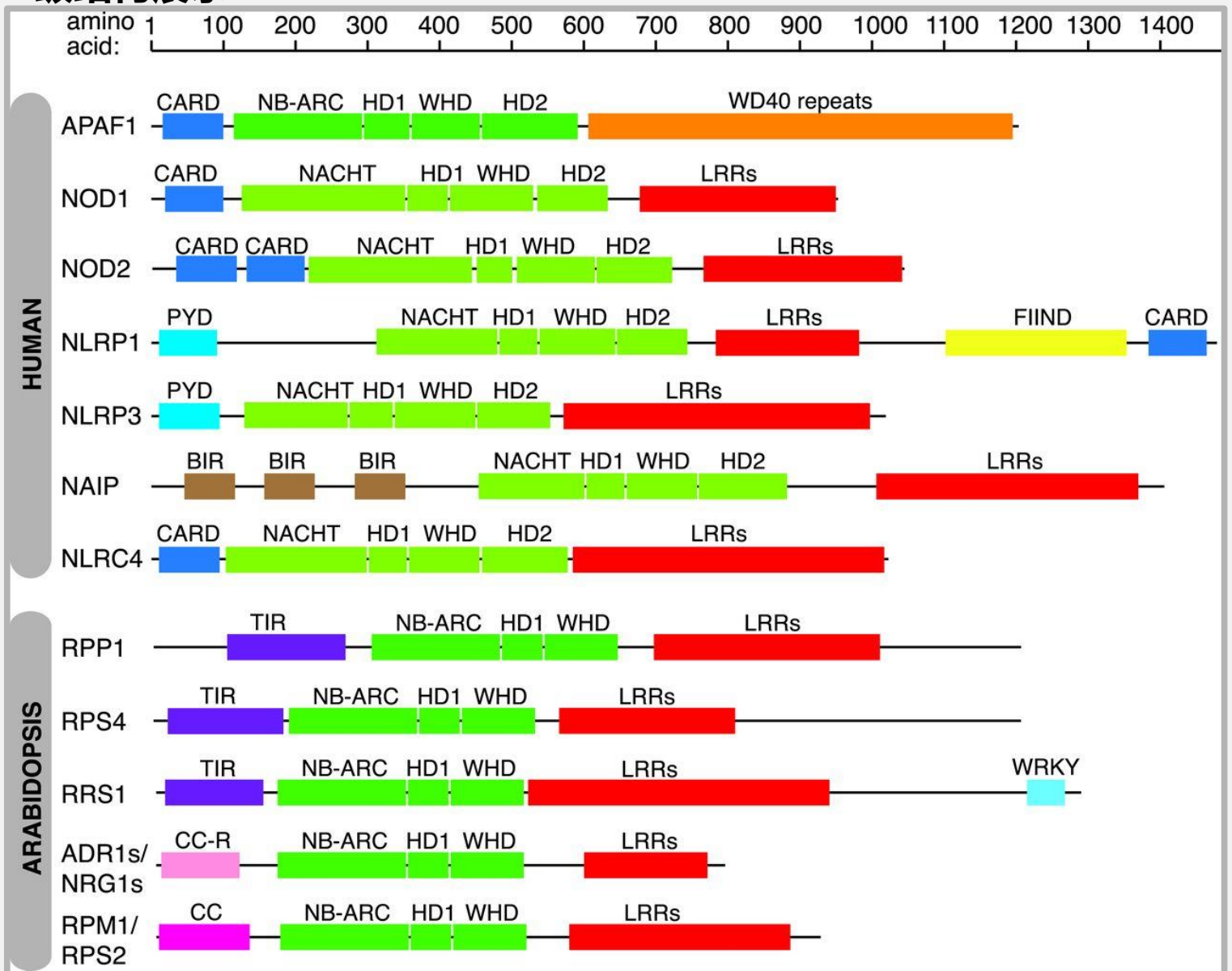
- 疾病突变位点都是预测中不可替代位点，并且90%以上集中于NACHT domain，这强调了NATCHT domain对NLRP3功能的重要作用
- 我们选择了R262W,D305N,T350M三个突变体进行实验，发现它们都是自激活突变

Position(s)	DescriptionActions	pathology
200	V→M	FCAS1
262	R→W	FCAS1
307	L→P	FCAS1
355	L→P	FCAS1
441	A→V	FCAS1
490	R→K	FCAS1
525	F→C	FCAS1
629	E→G	FCAS1
200	V→M	MWS
262	R→W	MWS
305	D→N	MWS
307	L→P	MWS
350	T→M	MWS
354	A→V	MWS
441	A→T	MWS
571	G→R	MWS

Position(s)	DescriptionActions	pathology
174	I→T	CINCA
262	R→L	CINCA
262	R→P	CINCA
266	L→H	CINCA
305	D→G	CINCA
305	D→N	CINCA
308	Q→K	CINCA
311	F→S	CINCA
350	T→M	CINCA
356	E→D	CINCA
360	H→R	CINCA
407	T→P	CINCA
438	T→I	CINCA
438	T→N	CINCA
525	F→L	CINCA
572	Y→C	CINCA
575	F→S	CINCA
634	L→F	CINCA
664	M→T	CINCA
861	Y→C	CINCA
21	D→H	KEFH

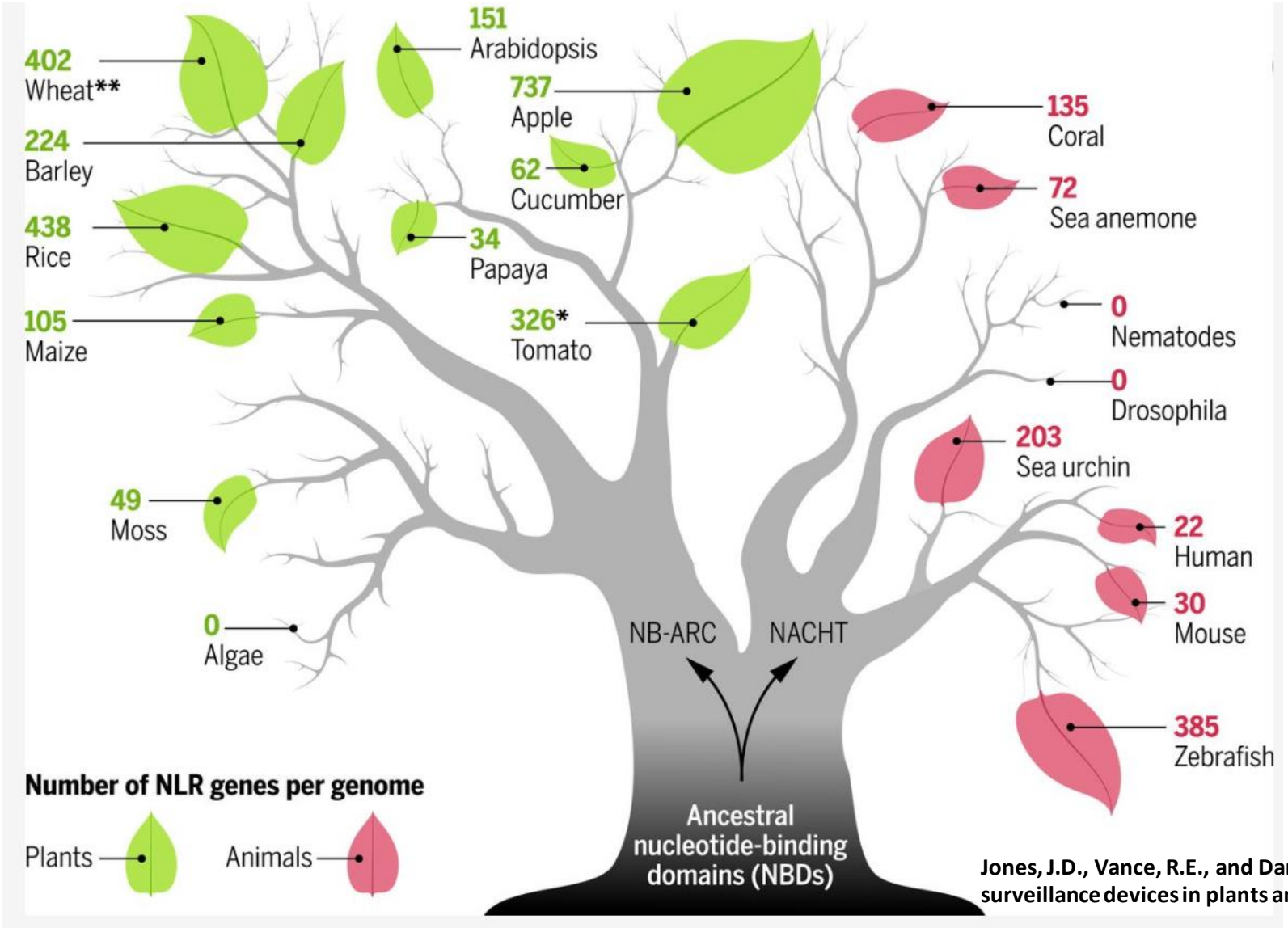
序列及功能分析

NLRP3同家族蛋白二级结构展示



序列及功能分析

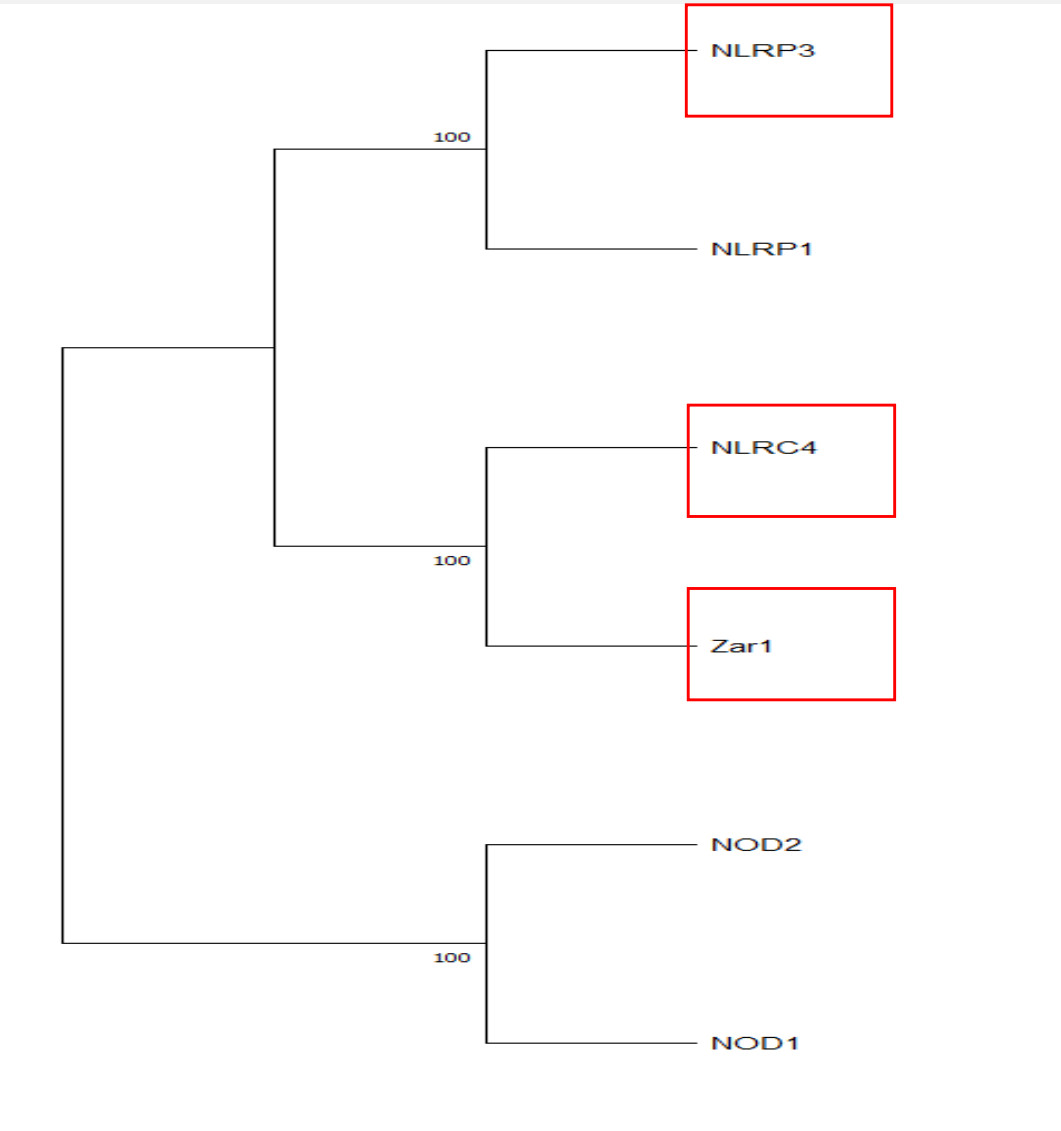
NACHT Domain在物种间进化情况



Jones, J.D., Vance, R.E., and Dangl, J.L. (2016). Intracellular innate immune surveillance devices in plants and animals. Science 354, aaf6395.

序列及功能分析

研究较为清楚NACHT-domain家族蛋白进化树



PART
THREE

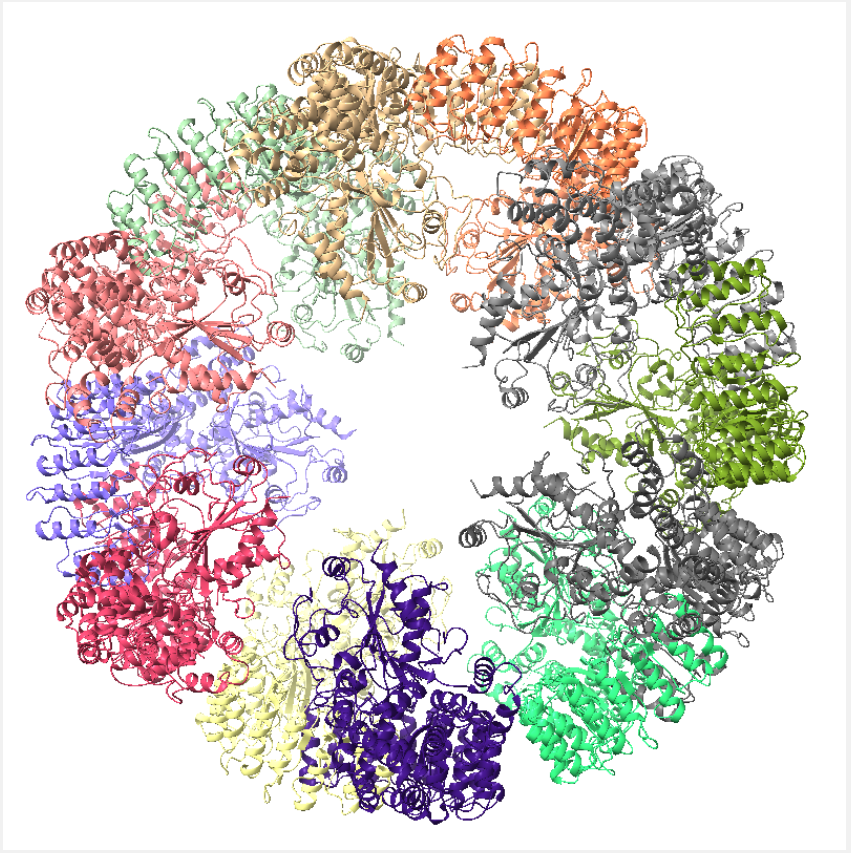
NLRP3蛋白结构分析

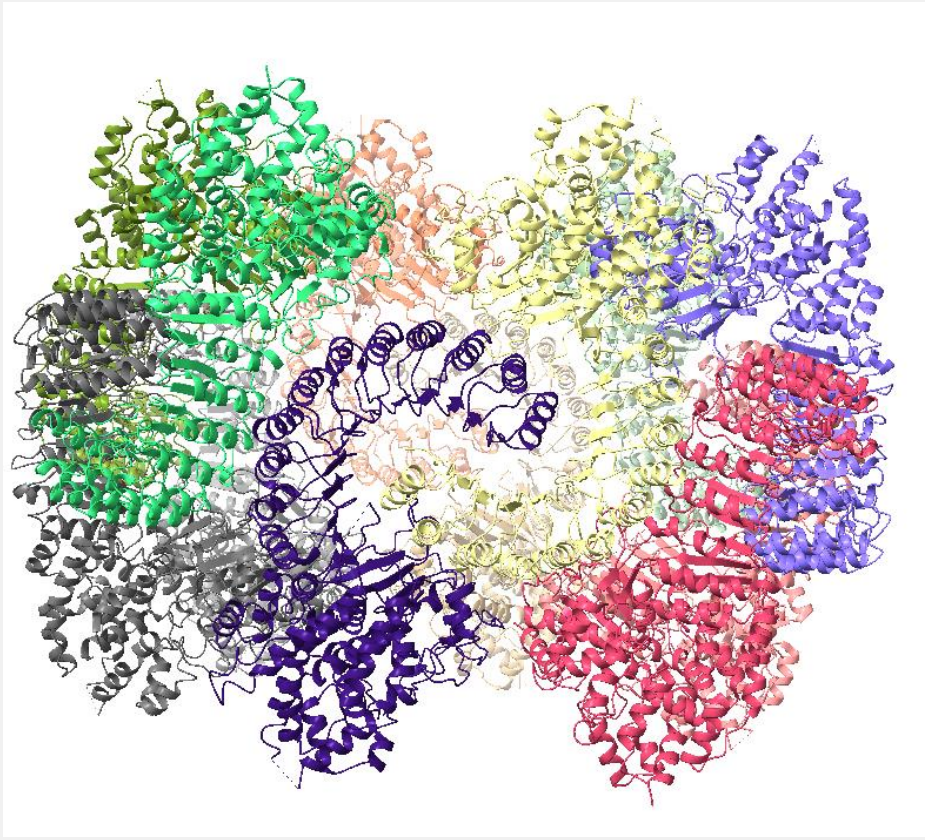
Structure

Analysis

结构分析

人类NLRP3蛋白自抑制结构　　PDB：7lfh

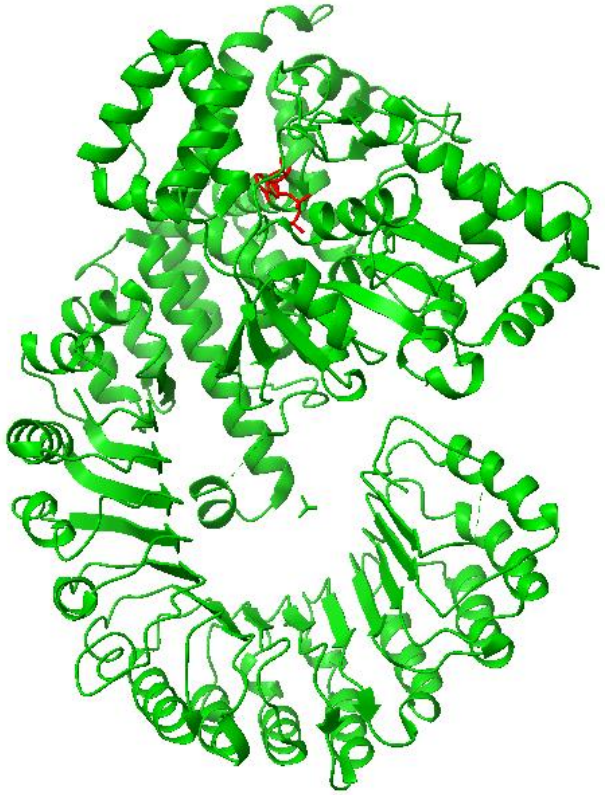




结构分析

人类NLRP3蛋白自抑制结构与小鼠NLRC4蛋白自抑制结构对比

NLRP3 inactive

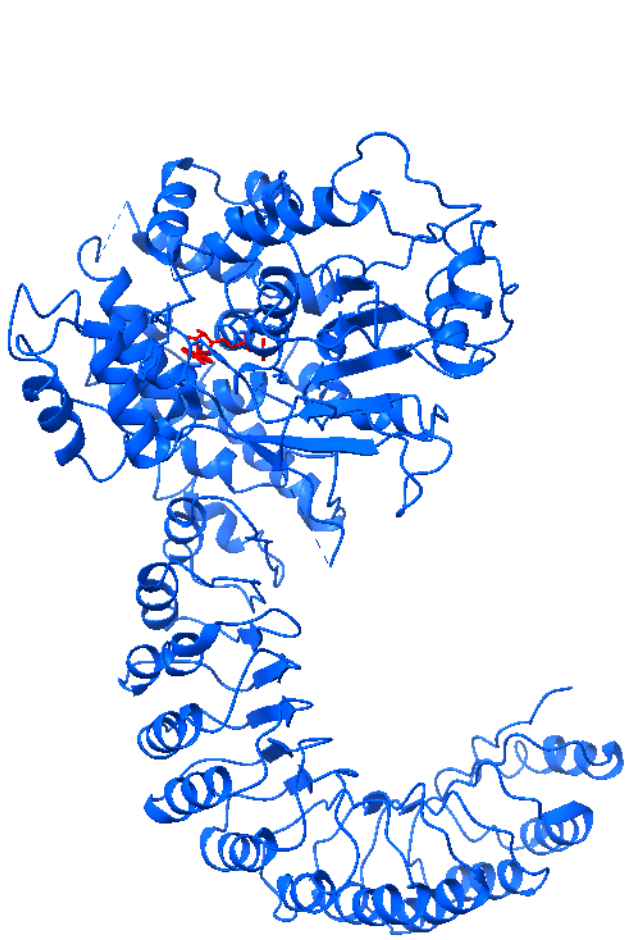


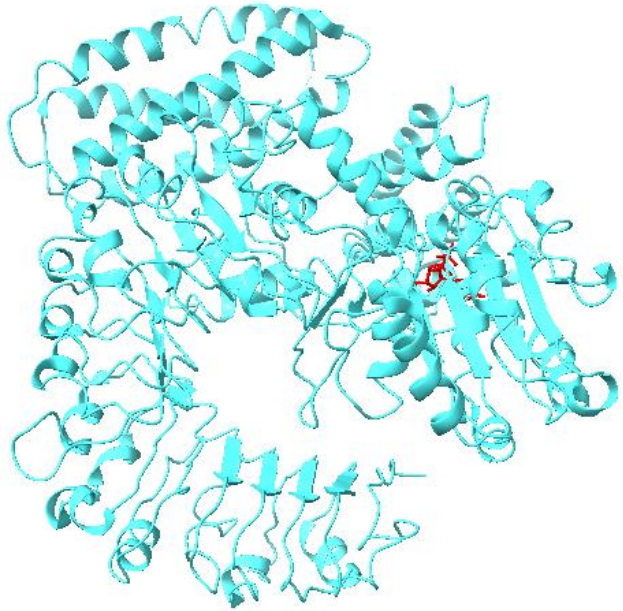
NLRC4 inactive

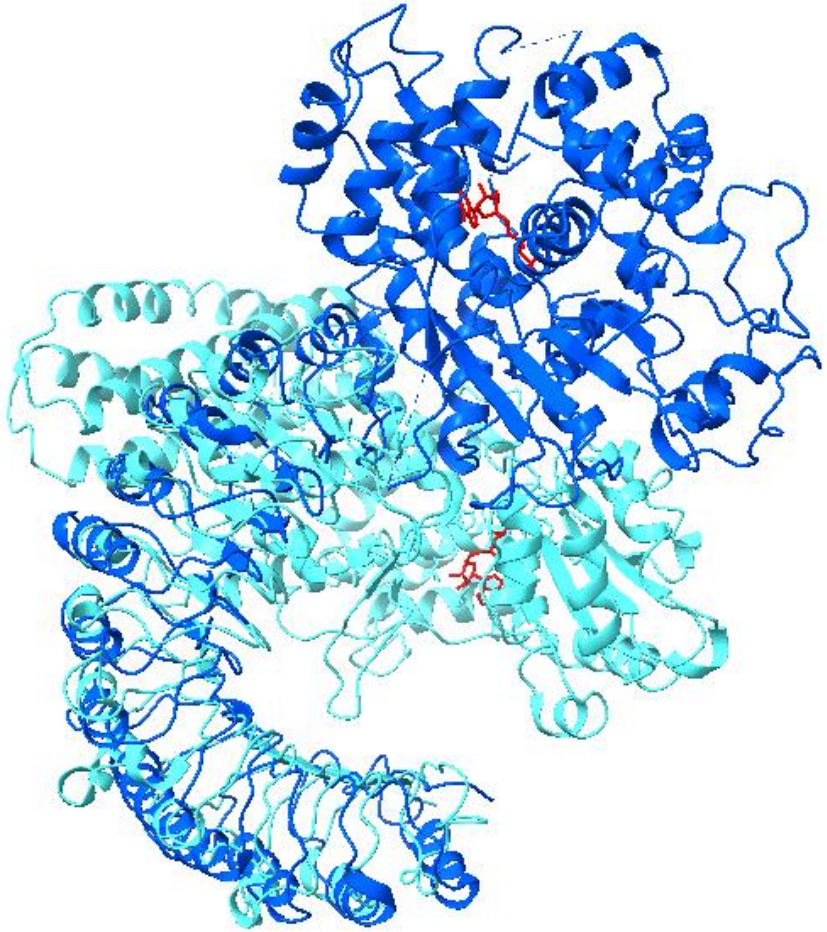
PDB：4kxf

结构分析

人类NLRP3蛋白自抑制结构与拟南芥ZAR1蛋白自抑制结构对比



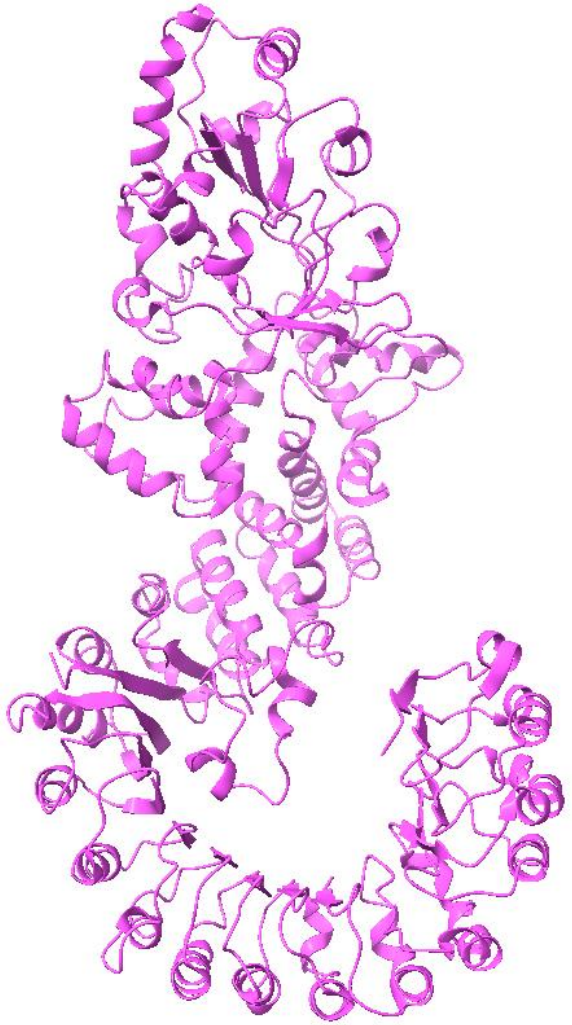




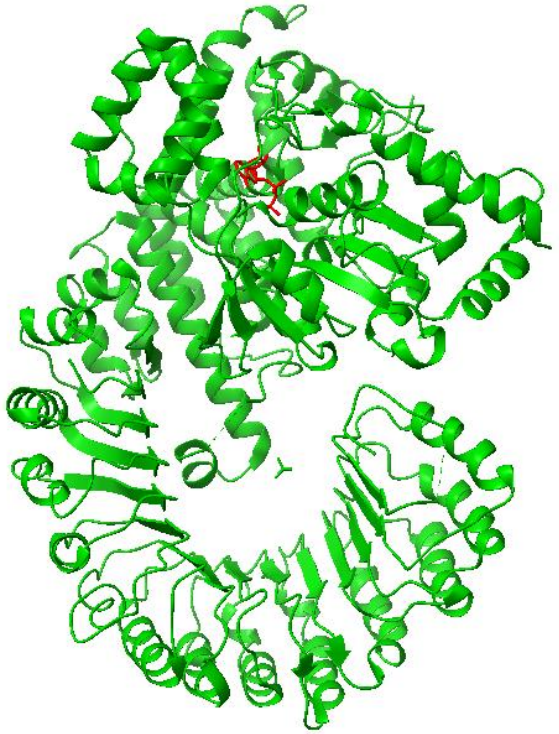
NLPR3 autoinhibition structure ZAR1 autoinhibition structure

结构分析

人类NLRP3蛋白自抑制结构与鼠NLRC4蛋白激活结构对比



NLRC4 active

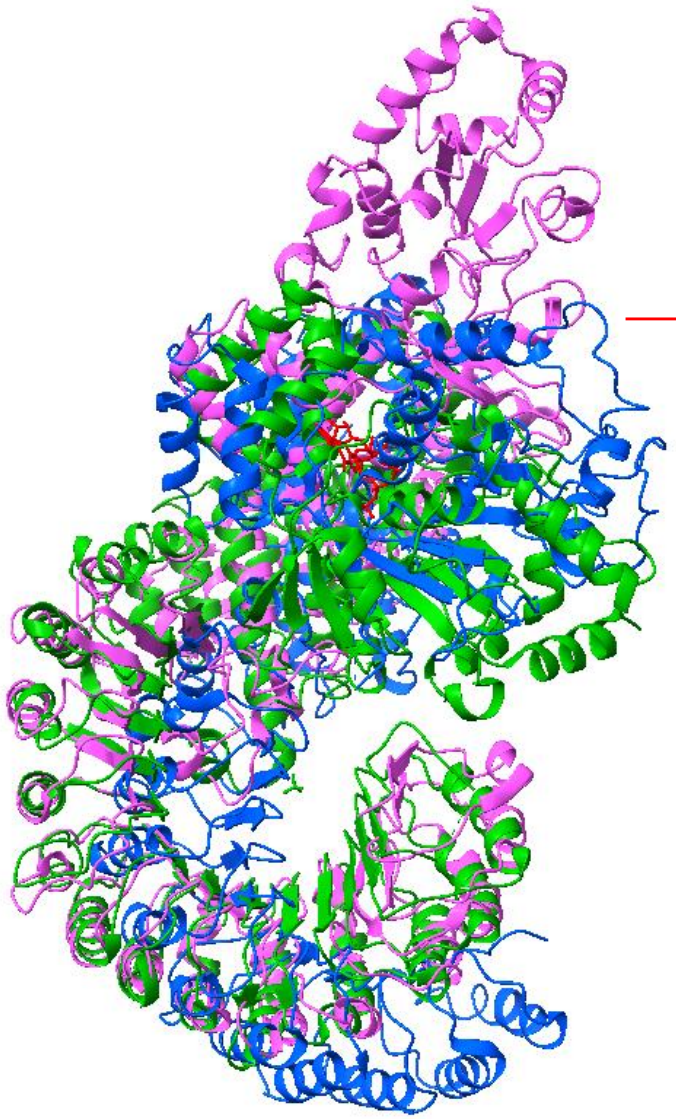


NLRC4 inactive

NLRP3 inactive

结构分析

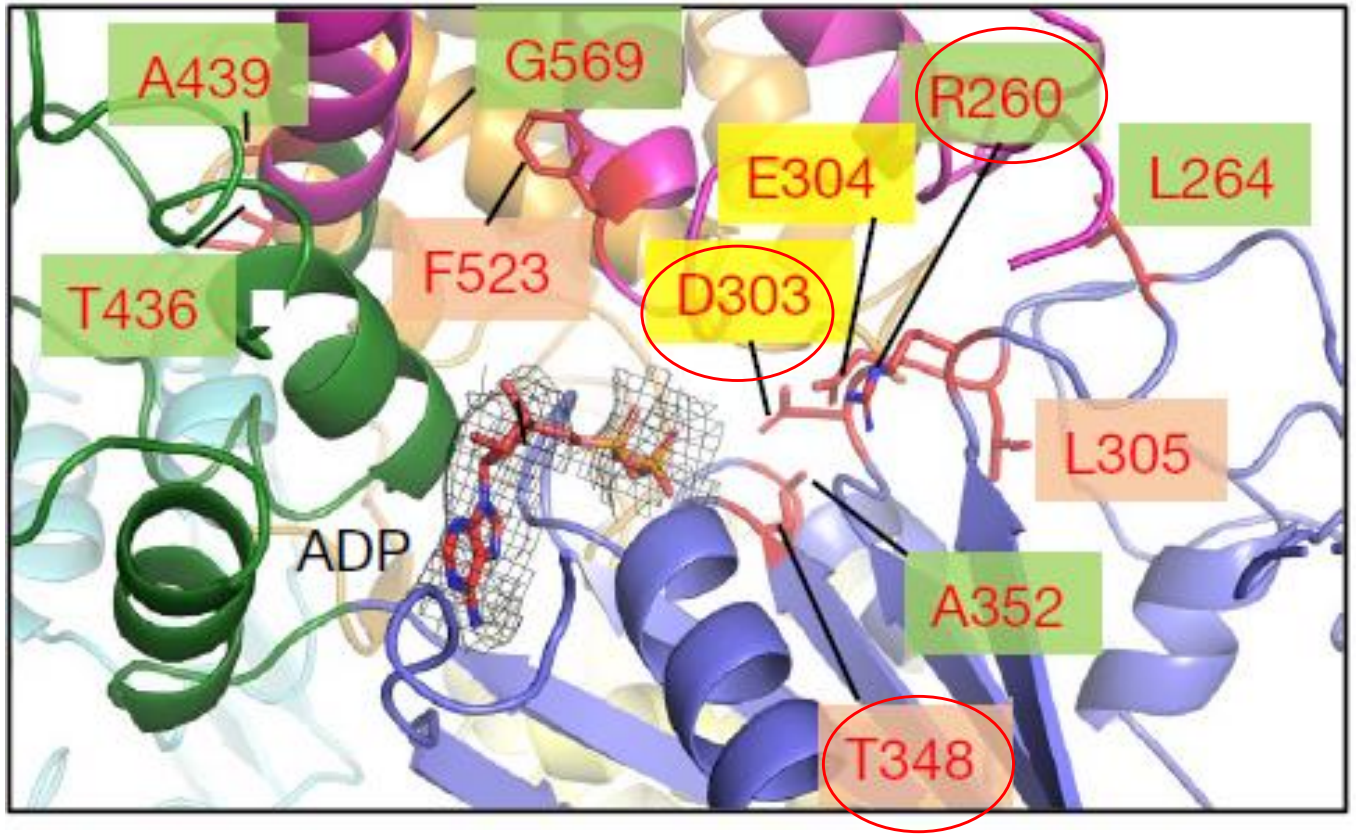
人类NLRP3蛋白自抑制结构与鼠NLRC4蛋白激活结构对比



- 可明显看到NACHT domain向外翻转约90°,有利于ADP的释放。

结构分析

NLRP3致病突变结构定位



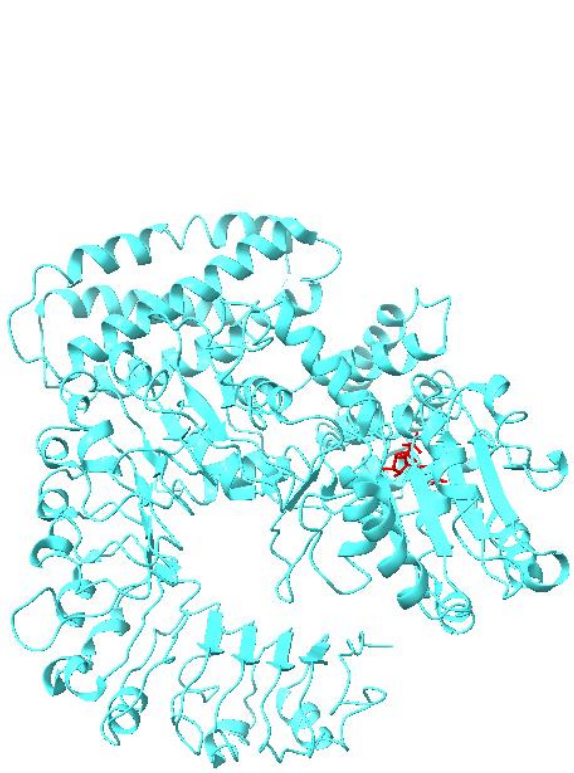
- 不可替代突变及致病突变主要存在与ADP/ATP结合区域
- 是否结合ADP抑制活性，结合ATP/水解导致激活？

自激活突变是否不再能水解ATP导致一直处于激活构象？

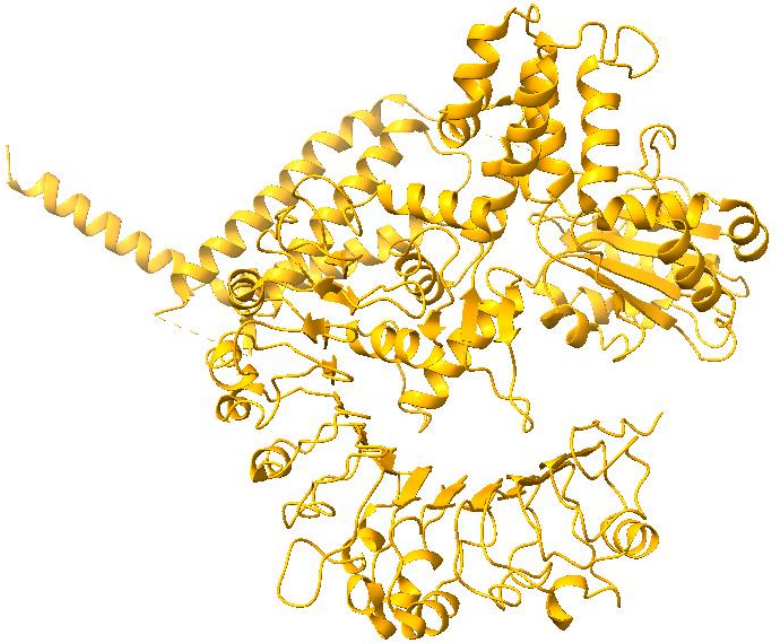
Wang, Jizong et al. “Reconstitution and structure of a plant NLR resistosome conferring immunity.” *Science (New York, N.Y.)* vol. 364,6435 (2019): eaav5870. doi:10.1126/science.aav5870

结构分析

人类NLRP3蛋白自抑制结构与拟南芥ZAR1蛋白激活结构对比

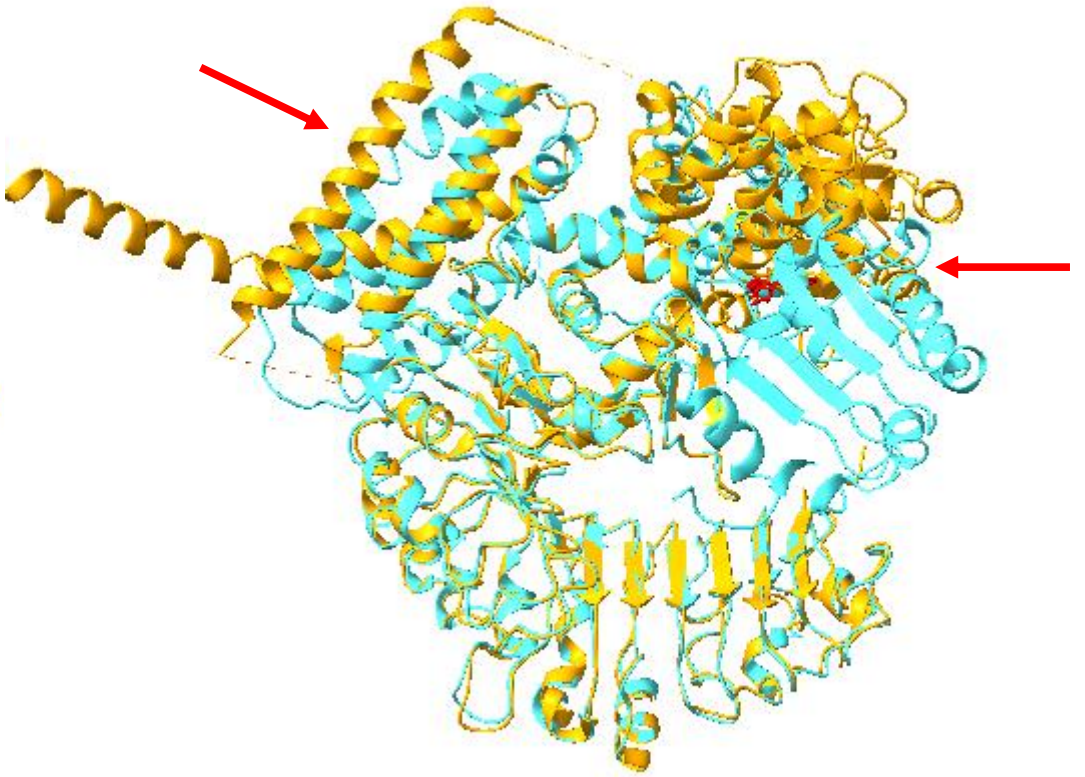


ZAR1 autoinhibition structure



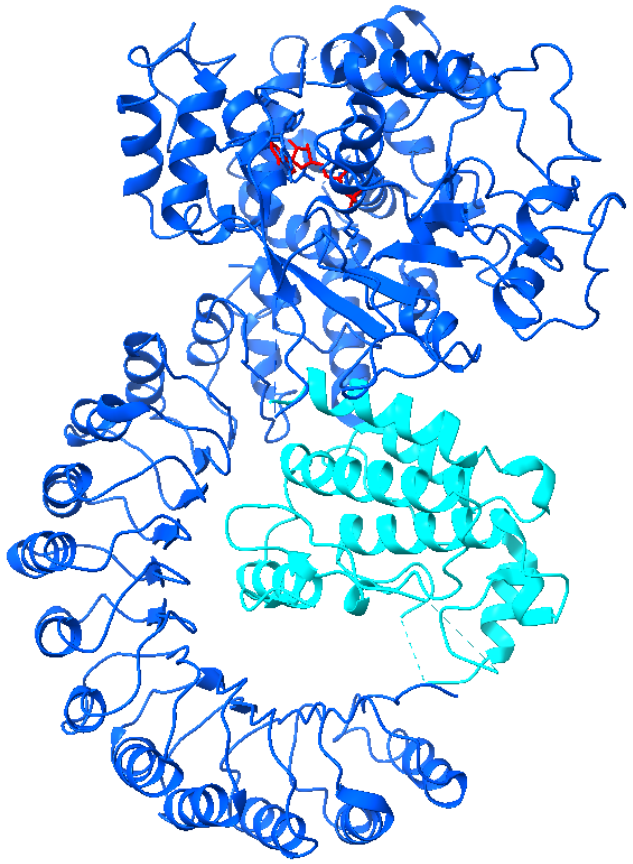
ZAR1 active structure （RKS1+PBL2）

PDB：4j5t

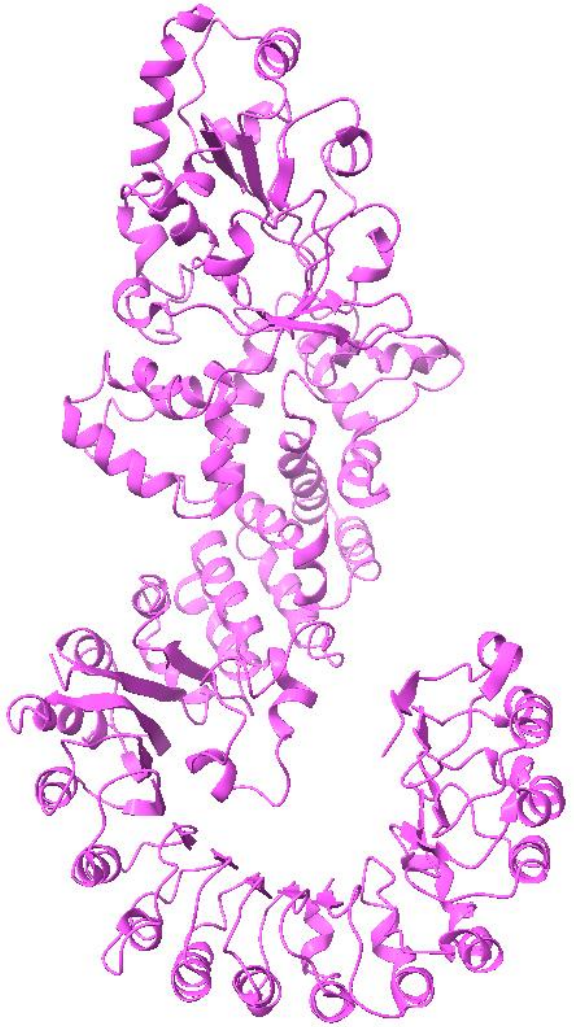


结构分析

人类NLRP3蛋白与NEK7结合后结构



NLRP3 + NEK7



NLRC4 active （NAIP）

- NACHT家族蛋白激活需要辅助蛋白导致结构变化
- 是否可以根据NLRC4,ZAR1辅助蛋白同源寻找NLRP3激活蛋白？——×
- NEK7不是NLRP3激活的辅助蛋白
- 寻找NLRP3激活所需辅助蛋白

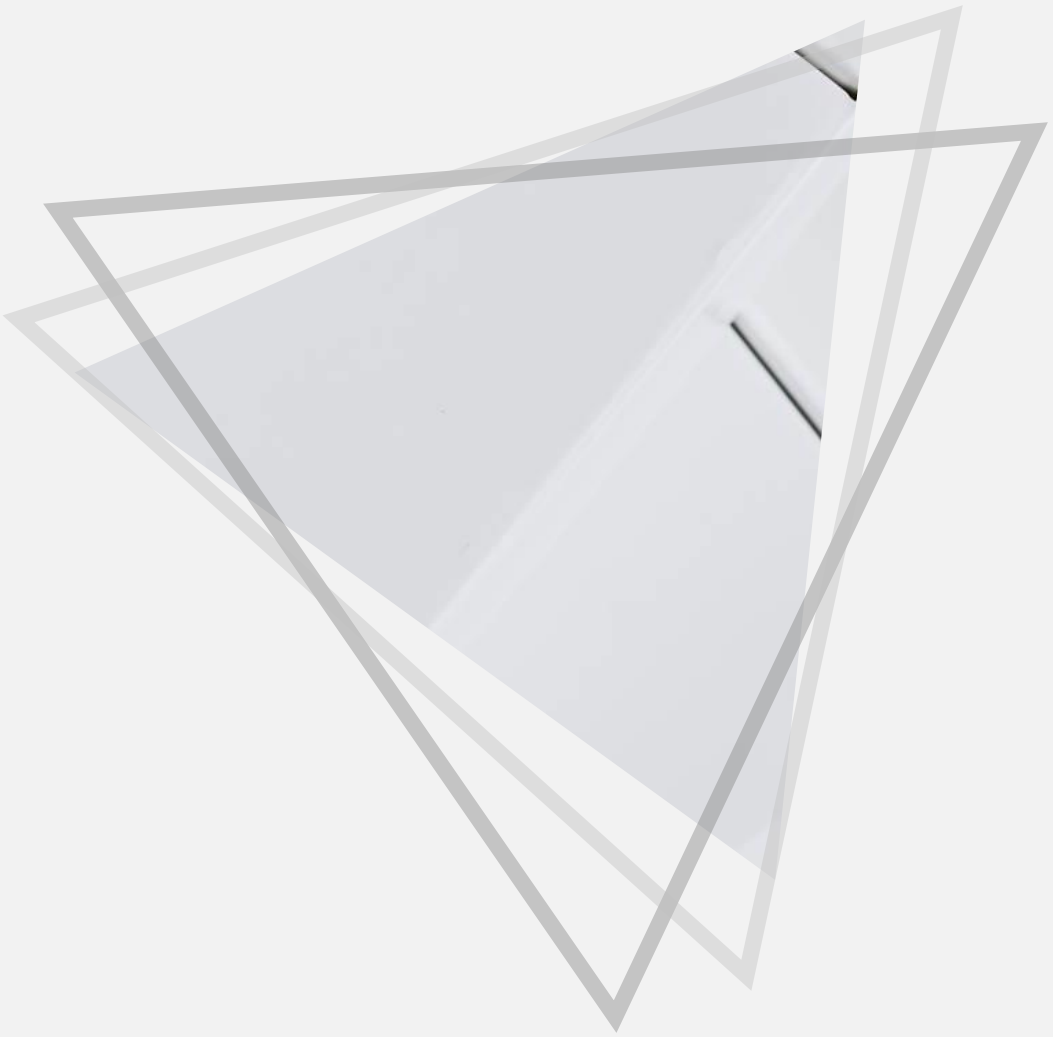
PART
Four

总结与研究计划

Summary

&

Future Plan



总结

- 选择THP1,Hela作为实现不同目的实验细胞
- 选择262，305，350位突变产生的自激活突变体
- NLRP3功能与NACNT domain尤其是ATP与ADP结合状态的改变密切相关
- NLRP3激活可能需要与辅助蛋白相互作用发生结构改变

研究计划

- 纯化NLRP3和自激活突变，检测ATPase活性
- 利用基因或生化手段寻找NLRP3激活关键蛋白

Thanks