



水稻中依赖于ATP的解旋酶OsHRQ1蛋白 结构和功能探究

Structure and Function of ATP-dependent Helicase **OsHRQ1** Protein in Rice

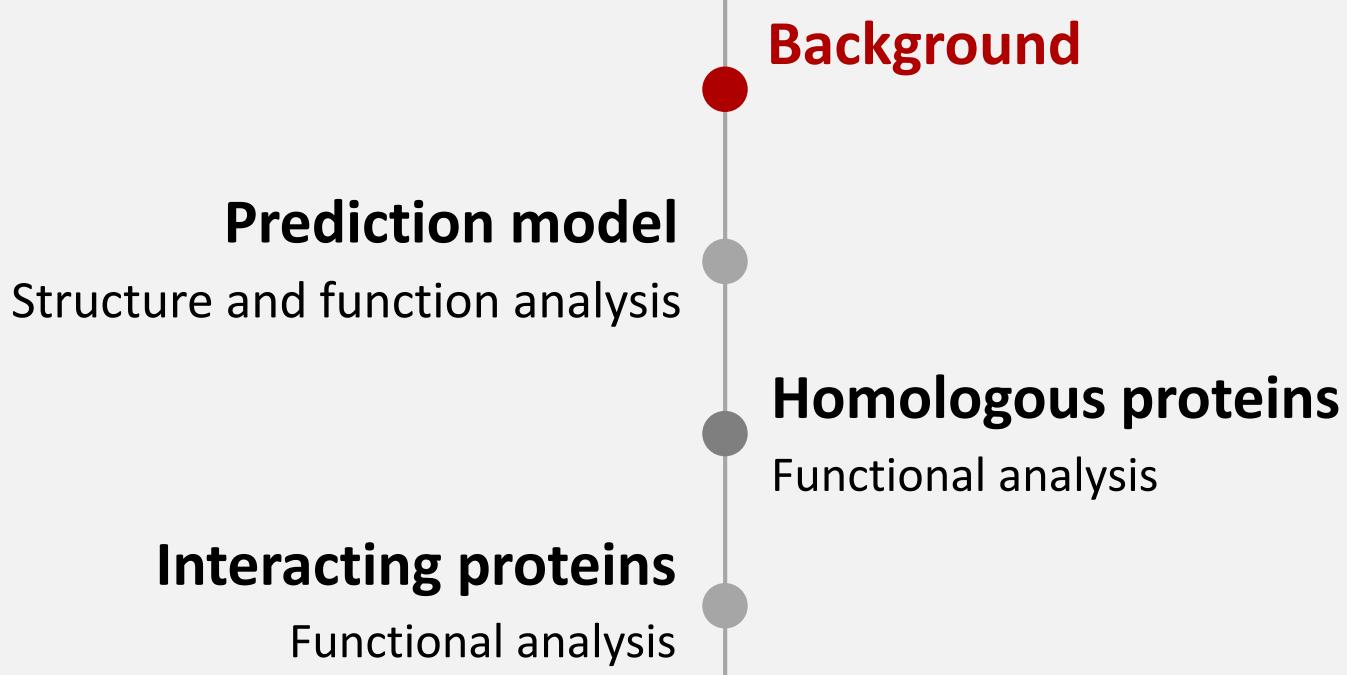
G05_06

2020.01.04

CONTENTS

- 
- Background**
 - Prediction model**
Structure and function analysis
 - Homologous proteins**
Functional analysis
 - Interacting proteins**
Functional analysis

CONTENTS



BACKGROUND

Why focus on OsHRQ1 ?

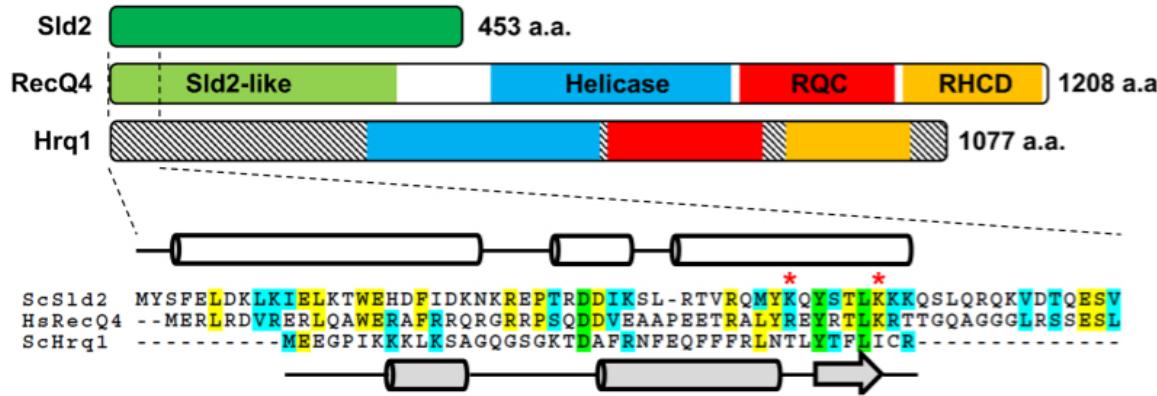
■ OsHRQ1作为RecQ家族的直系同源蛋白

RecQ4：生物体内遗传物质稳定性的重要维持者

-- 功能：DNA双链解旋、DNA重组、DNA复制、DNA修复...

-- 疾病：与三种遗传性综合征（Othmund-Thomson, Baller-Gerold, RAPADILINO）

-- 机制：尚不清楚

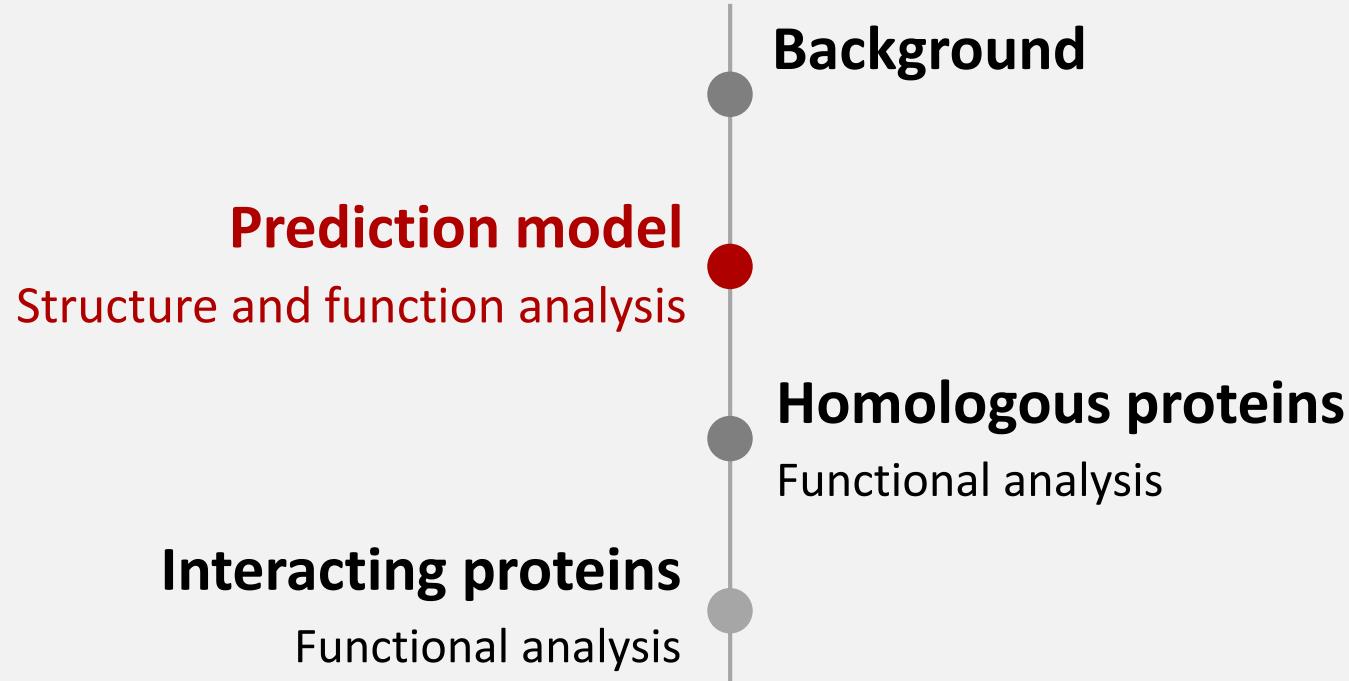


Cody M Rogers. et al. *Nucleic Acids Res.* 2017

■ HRQ1在拟南芥中行使DNA链间交联修复（Interstrand cross-link repair）有关功能

-- 范可尼贫血症（The Fanconi anemia pathway）信号通路

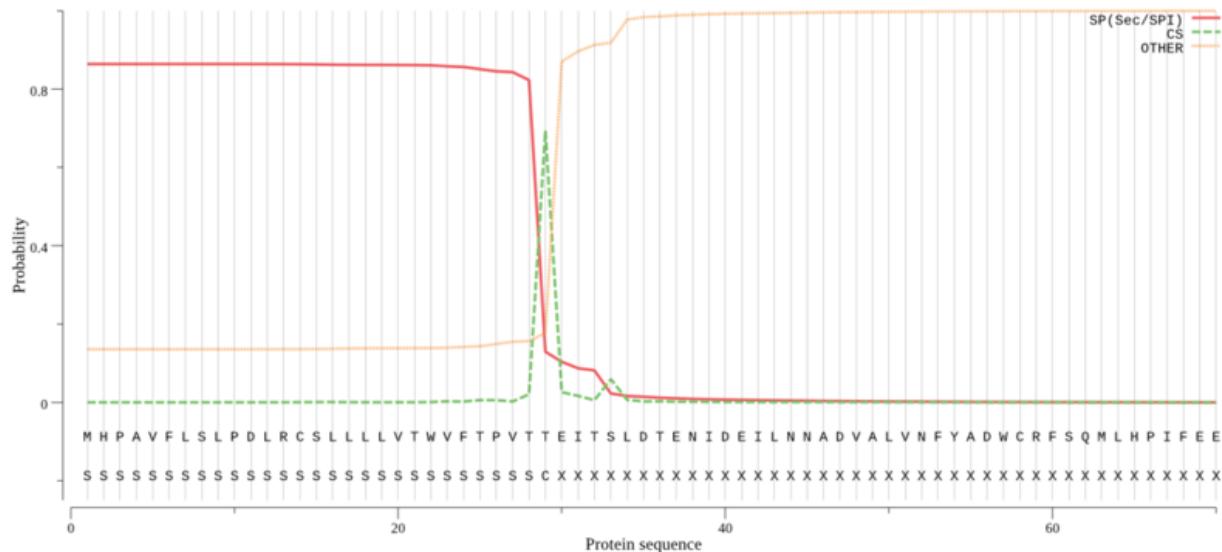
CONTENTS



Prediction model

核定位序列 (Nuclear localization sequence, NLS) 预测

cNLS Mapper Result



SignalP-5.0 prediction

NLS: 1-29 AA

Predicted NLSS in query sequence	
MAQPRVIRVRAHGRSTTVTIAASATVSDLRTL RSSFAPALVSPDFHLF	50
LKGTLIADAKVGNLPVGPGESISFIPVN AKSAPPHPSSSAPN PWRKRK	100
FSWHDGGGEDIYAKKPTNP APPRPLSCHGTQPLDPTQMVEHRLQGLGKAG	150
QITHVEEIPGREATFAELPGHLS SSMRDALRSIGVTKL YAHQAQAVQSAV	200
SGEHVVVSTSTSSGKSLCYNIPVLESISQSSAPCALYIFPTKALAQDQLK	250
TLLEMKP AFRSDFDVSIYDGDTAMKDRTIRNTARLLITNPDMHLHMSILP	300
CHAQFKRVLFNLYIVIDEAHSYKGAFGCHAAL IRLRKRICSYVYGSNP	350
TFIFCTATLANPREHVMELANLDRVVLDNDTSPCGSKNFLLWNPPQLA	400
KAEDRRPNPVLEVSYLFAEMVQHGLRAIA FCKTRKMCEQVLMQTRQILKE	450
TAEEVNSICVYRGGYVASD RRKIEADLF GGILRGVAATNALELGIDVGH	500
IDATLHLGFPGSMSAFWQQAGRSGRRAKQSIAVYYVAFEGALDQYFMRSPH	550
KLFGKPIEH CQVDSQRKVLEQHLACAASEYPLRQEHD ESYFGFSMNSVL	600
MTLKDKGCLMN NPSGGDGSVWKYIGPDKKPSHSVSIRAI EH HRYKVIDRR	650
SNRVLEEIEESKAFFQVYDGAVYMHQGVSYLV DKL DLTSRIAYCKVFDLN	700
YYTKVQDYTEISFIGGDVDEHPASECKPDIRRTAQANDCRVTTK WVGFD	750
RILKSNNQKSDSINLDHLPY SFETQAVWVQIPVSVRTT MEQMEYQLCGG	800
VHAASHALLSIIPLHMMCSGSDLG TQCAEPQENSETADRILL YDKHPGGI	850
GLASQAKLLFGELLVAALELVSSCSCTNSDGCPNCIQSFACSDYNRDLK	900
EASIFLLKGVIQYEKLYFEAIDGCYQS	927

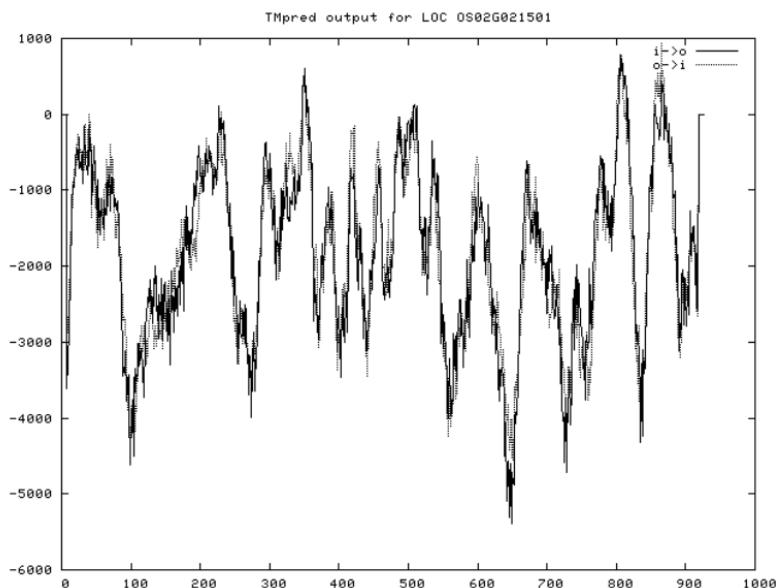
Predicted monopartite NLS		
Pos.	Sequence	Score
95	PWRKRKFSWH	10
334	ILRRRLKRICSYVY	8

NLS Mapper prediction

NLS存在且有两段

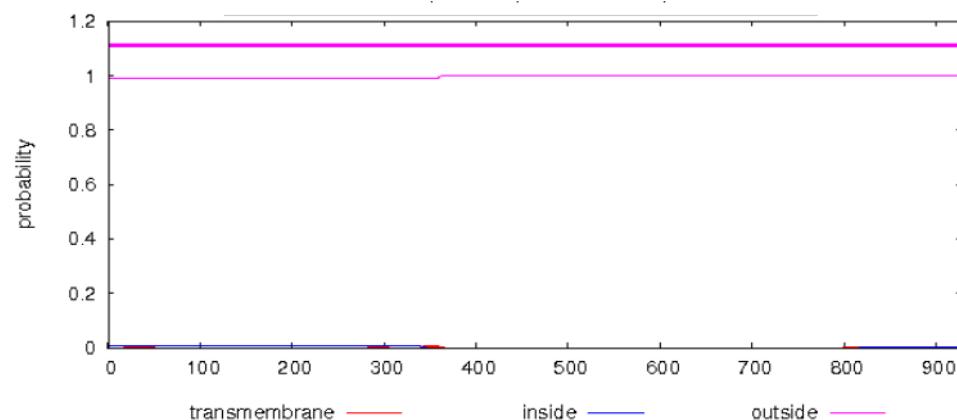
Prediction model

核定位序列 (Nuclear localization sequence, NLS) 预测

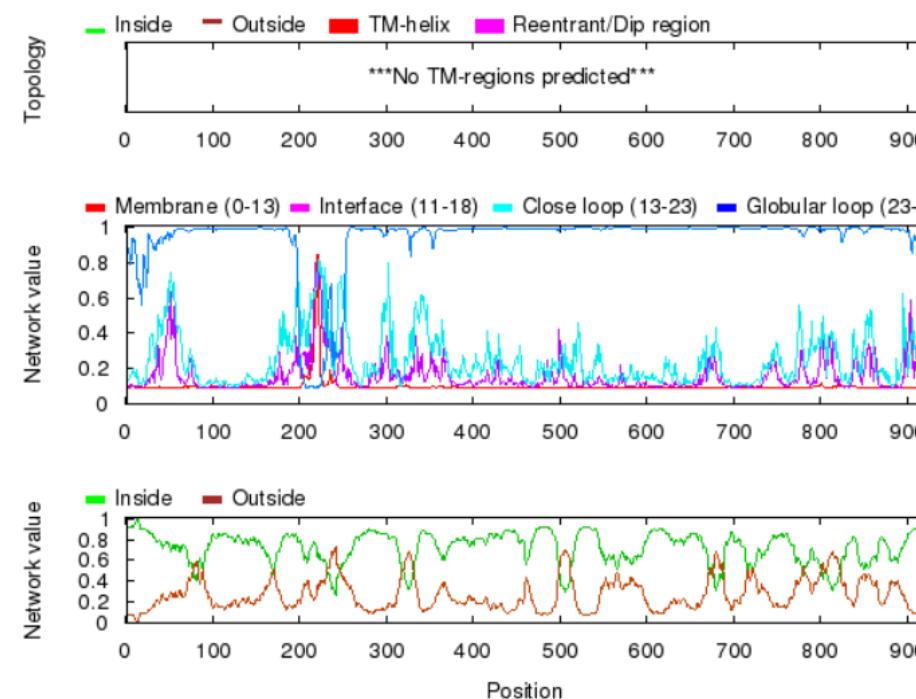


TMpred prediction

跨膜结构域得分<1000



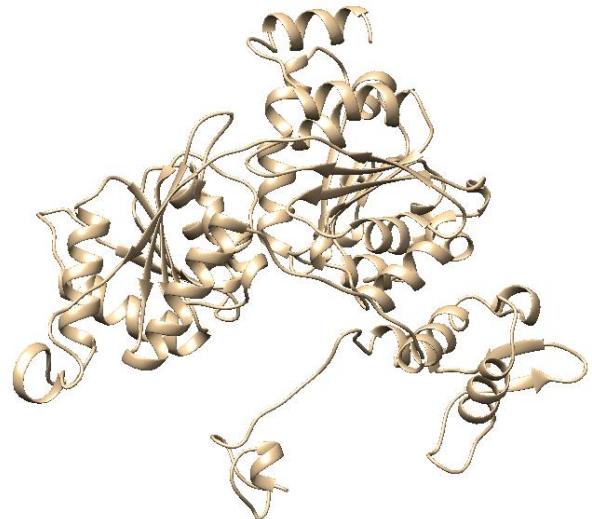
TMHMM prediction
无跨膜结构域



OCTOPUS topology
无跨膜结构域

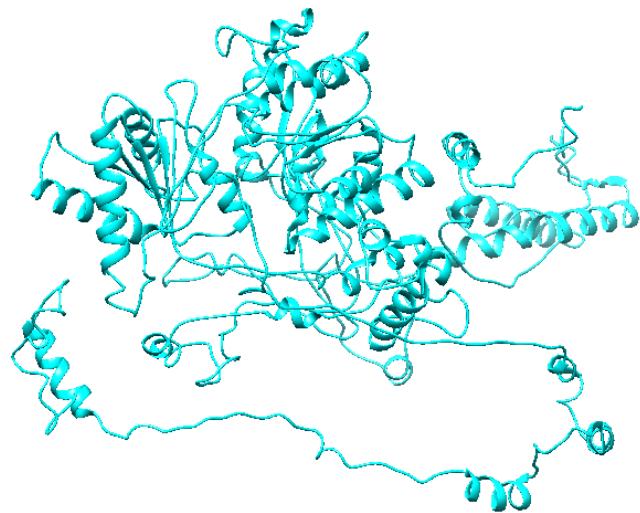
Prediction model

结构预测

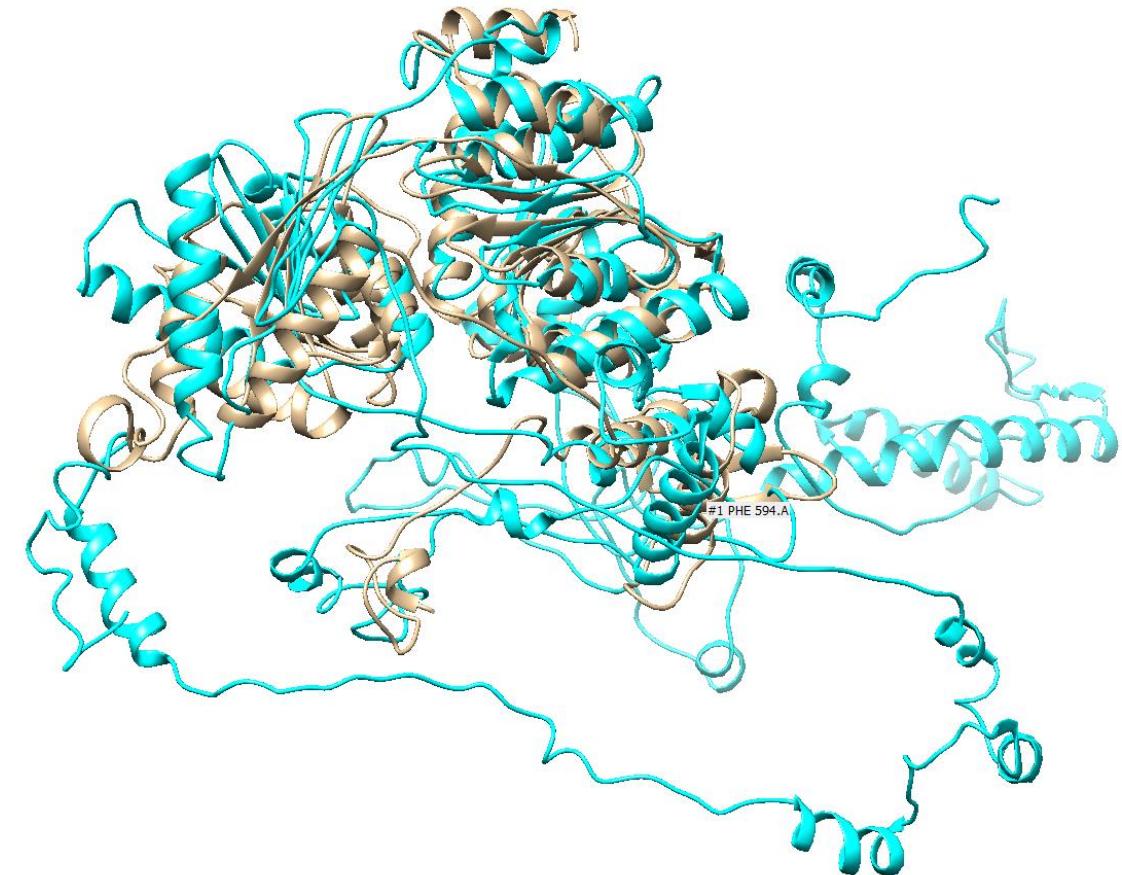


Phyre2

172-664 AA



I-TASSER



两种预测模型的结果Magic拟合

Prediction model

结构预测



模板（黄色）：与ssDNA结合的
耻垢分枝杆菌解旋酶LHR的结构
模板PDB ID： 5v9x

红框：模板的无规则卷曲序列 (loop)
与DNA单链相结合

蓝色：I-TASSER预测模型

Prediction model

GO 功能预测

分子功能

结合ATP

解旋酶活性

结合DNA

生物过程

rRNA代谢通路

非编码RNA加工

核糖体合成通路

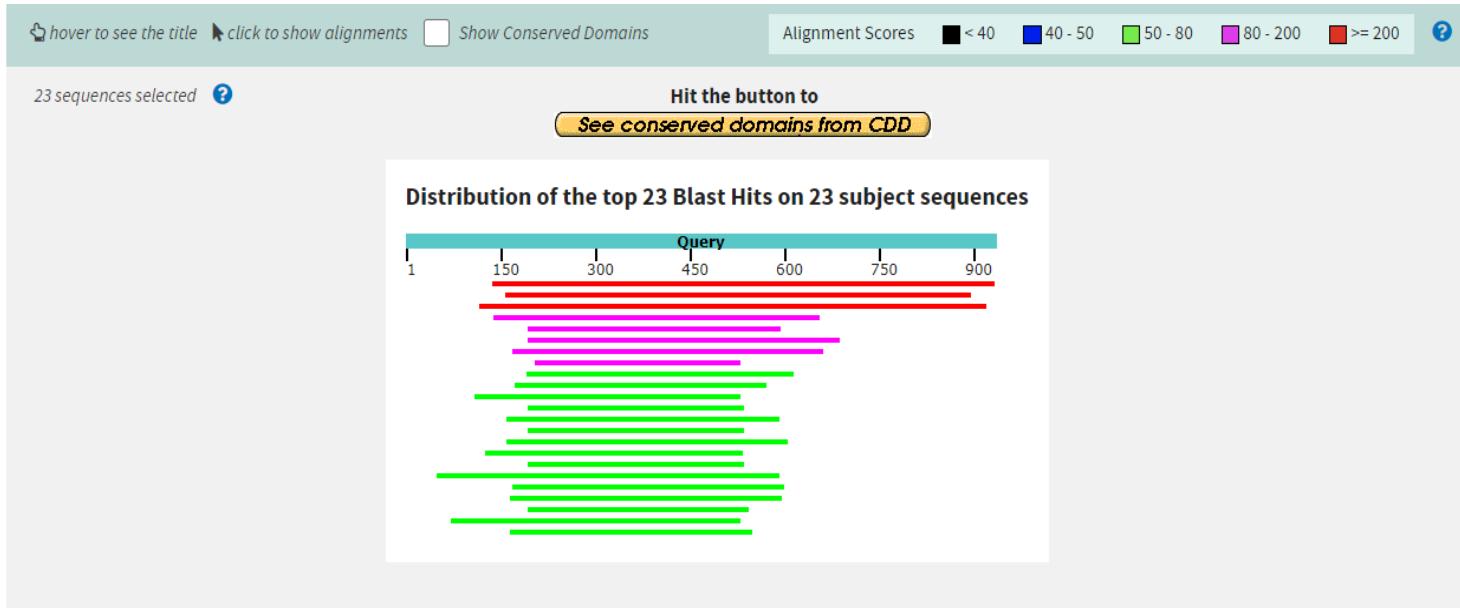
DNA修复

CONTENTS

- 
- Background**
 - Prediction model**
Structure and function analysis
 - Homologous proteins**
Functional analysis
 - Interacting proteins**
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Homologous proteins

使用BLAST

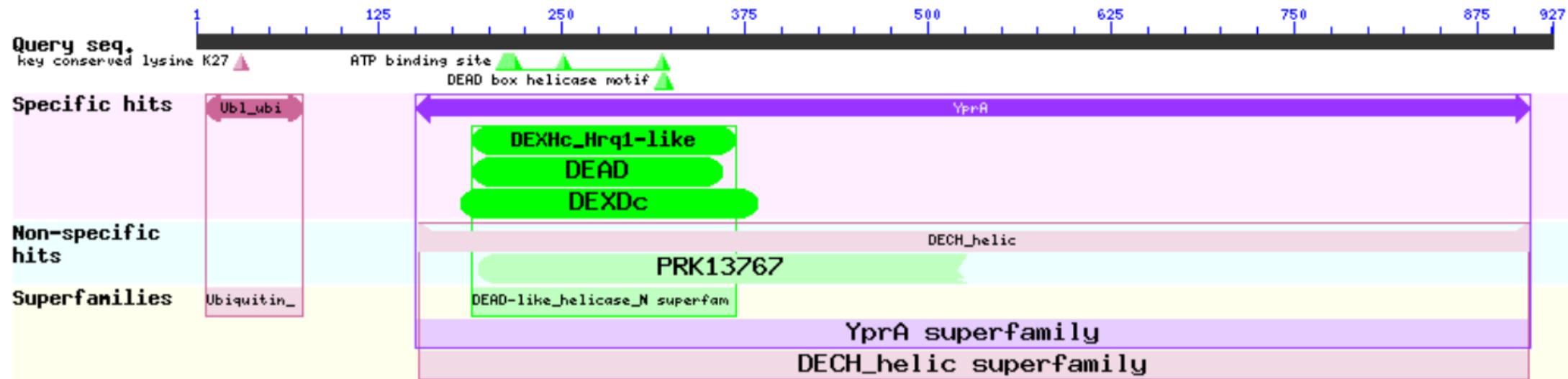


BsYprA
(枯草芽孢杆菌)
ScHRQ1
(酿酒酵母)
SpHRQ1
(裂殖酵母)

<input checked="" type="checkbox"/>	RecName: Full=Uncharacterized ATP-dependent helicase YprA [Bacillus subtilis subsp. subtilis str. 168]	504	504	78%	3e-151	36.29%	P50830.1
<input checked="" type="checkbox"/>	RecName: Full=ATP-dependent helicase HRQ1; AltName: Full=Homologous to recQ protein 1 [Saccharomyces cerevisiae S288C]	508	508	83%	1e-148	34.34%	Q05549.1
<input checked="" type="checkbox"/>	RecName: Full=ATP-dependent helicase hrq1; AltName: Full=Homologous to recQ protein 1 [Schizosaccharomyces pombe 972h-]	488	488	78%	1e-141	34.80%	O13983.4
<input checked="" type="checkbox"/>	RecName: Full=Uncharacterized ATP-dependent helicase MJ1574 [Methanocaldococcus jannaschii DSM 2661]	180	180	46%	1e-44	29.89%	Q58969.1
<input checked="" type="checkbox"/>	RecName: Full=Uncharacterized ATP-dependent helicase MTH_1802 [Methanothermobacter thermautotrophicus str. Delta H]	91.4	91.4	42%	3e-17	26.71%	O27830.1

Homologous proteins

保守结构域



List of domain hits

	Name	Accession	Description	Interval	E-value
[+]	DECH_helic	TIGR03817	helicase/secretion neighborhood putative DEAH-box helicase; A conserved gene neighborhood ...	152-910	0e+00
[+]	YprA	COG1205	ATP-dependent helicase YprA, contains C-terminal metal-binding DUF1998 domain [Replication, ...	150-911	0e+00
[+]	DEXHc_Hrq1-like	cd17923	DEAH-box helicase domain of Hrq1 and similar proteins; Yeast Hrq1, similar to RecQ4, plays a ...	188-369	5.71e-87
[+]	DEAD	pfam00270	DEAD/DEAH box helicase; Members of this family include the DEAD and DEAH box helicases. ...	189-360	8.50e-31
[+]	DEXDc	smart00487	DEAD-like helicases superfamily;	180-384	4.17e-26
[+]	PRK13767	PRK13767	ATP-dependent helicase; Provisional	192-526	1.26e-19
[+]	Ubl_ubiquitin_like	cd17039	ubiquitin-like (Ubl) domain found in ubiquitin and ubiquitin-like Ubl proteins; Ubiquitin-like ...	7-73	2.52e-03

Homologous proteins

功能预测

Q05549 HRQ1 YEAST
O13983 HRQ1 SCHPO
XP_015625093.1
P50830 YPRA_BACSU

300	NSLHQGENVIIT TSTSSGKSLIYQLAAIDLKPESTFMYIFPTKALAQDQKRAFKVIL	359
321	NHLWNGFHIVISTSTSSGKSLIYQIPILQLSLLDENQSTAFFVFPTKSLAQDQKKSLLIDIL	380
197	QSAVSGEHVVVSTSTSSGKSLCYNIPVLESISQSSAPCALYIFPTKALAQDQLKTLLEMK	256
64	QYVQKGESIVTV TPTASGKILCYNLPVLQSIAQDETNRKALYLFPTKALAQDQKSELNEII	123
	:	:
	:. . * : : * : * : * : * : : . : : * : * : * : * : :	

Q05549 HRQ1 YEAST
O13983 HRQ1 SCHPO
XP_015625093.1
P50830 YPRA_BACSU

360	SKIPELKNAVVDVTYDGDT EPEERAYIRKNARVIFTNPMDIHTSILPNHANWRHFLYHLKL	419
381	SYMP TLKNIRVDTFDGDTPLESRESIIRSANIIFTNPMDLHQTLPNANRWWYFFKNLKL	440
257	PAFR--SDFDVSIVYDGDTAMKDRTRIRNTARLLITNPMDLHMSILPCHAQFKRVLFNLK	314
124	DEMG -- IDIKSFTYDGDTSPAIRQKV RKAGHIVITNPMDLHSAILPHHTKVVSLFENLK	181
	:	:
	: * : * : * : * : * : . : : * : * : * : * : * : * : * : * : * : * :	

Q05549 HRQ1 YEAST
O13983 HRQ1 SCHPO
XP_015625093.1
P50830 YPRA_BACSU

420	VV VDELHIYKGGLFGSHVALVMRRRLRLCHCFYENSGLQFISCATLKSPVQHMKDMFGIN	479
441	FV LDEAHVYNGIFGVHVAFVLRMMRIAE-YFGNSQYRFVSCSATIEDPLQHMKKIFGV	499
315	IVIDEAHSYKGAFGCHAALIIRRKLKRICSYY-GSNFTFICATLANPREHVMELANLD	373
182	IVI DELHTYRGVFGSHVANVIRRKICRF-Y-GSDPVFICTSATIANPKELGEQLTG-K	238
	: * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	

Q05549 HRQ1 YEAST
O13983 HRQ1 SCHPO
XP_015625093.1
P50830 YPRA_BACSU

480	EVTL I HEDGSPPTGAKHLVWNPPILPQHE--RKRENFIRESAKILVQLLNNVRTIAFCY	537
500	NIKL I NYTSSPSGSKKFVMWNPPVDPKHPDDGKKS AISE KSKLLIKFAEKRVRTIVFCR	559
374	RVLVDNDTSPCGSKNPLLWNPLQLAKAE-DRRPNPVLEVSILYFAEMVQHGLRAIAFCK	432
239	PMRLV DDNGAPSGRKHVFVYNNPIVNKPLN-IRR-SATA EVNE LAKEFLNKVKQTIVFAR	296
	: * : . : * : * : * : * : * : * : . : * : * : * : * : * : * : * : * :	

Q05549 HRQ1 YEAST
O13983 HRQ1 SCHPO
XP_015625093.1
P50830 YPRA_BACSU

538	VRRV CELLMKEVRNIFIETGREDLVTEVMSYRGGGYASDRRKIEREMFHGNLKAVISTNA	597
560	VRKTC ESLMLRIVRQELKTKQGDLLSKIQSYRAGTYVQERRKIESEMFNGKLYGIATNA	619
433	TRKMCEQVLMQTRQILKETA-AELVNSICVYRGGGYVASDRRKIEADLFGGILRGAATNA	491
297	SRVR VEIIILSHIQELVK KEI --GTKSIRGYRGGLPKERREIERGLREGDILGVVSTNA	353
	* : * :	

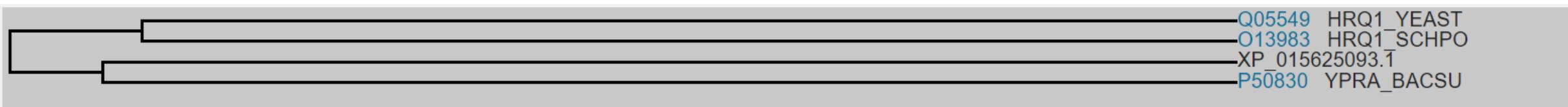
Q05549 HRQ1 YEAST
O13983 HRQ1 SCHPO
XP_015625093.1
P50830 YPRA_BACSU

598	LELGIDIGGLDAVLMCGFPLSMANFHQQSGRAGRNNDSLTVVASDSDPVQHYVAH PES	657
620	LELGIDIGSLDAVITIGFPY SLNSLRQQFGRAGRNNKSSLAVYIVETFPVDFQFYLKHPIL	679
492	LELGIDVGHIDATLHLGFPGSMASFWQQAGRSRRAKQSIAVYVAFEGALDQYFMRSPHK	551
354	LELGVDIGQLQVCMTGYPGSV ASAQQAGRAGRHHGESLIIMVANSTPIDQYIVRHPEY	413
	****: * :	

Q05549 HRQ1 YEAST
O13983 HRQ1 SCHPO
XP_015625093.1
P50830 YPRA_BACSU

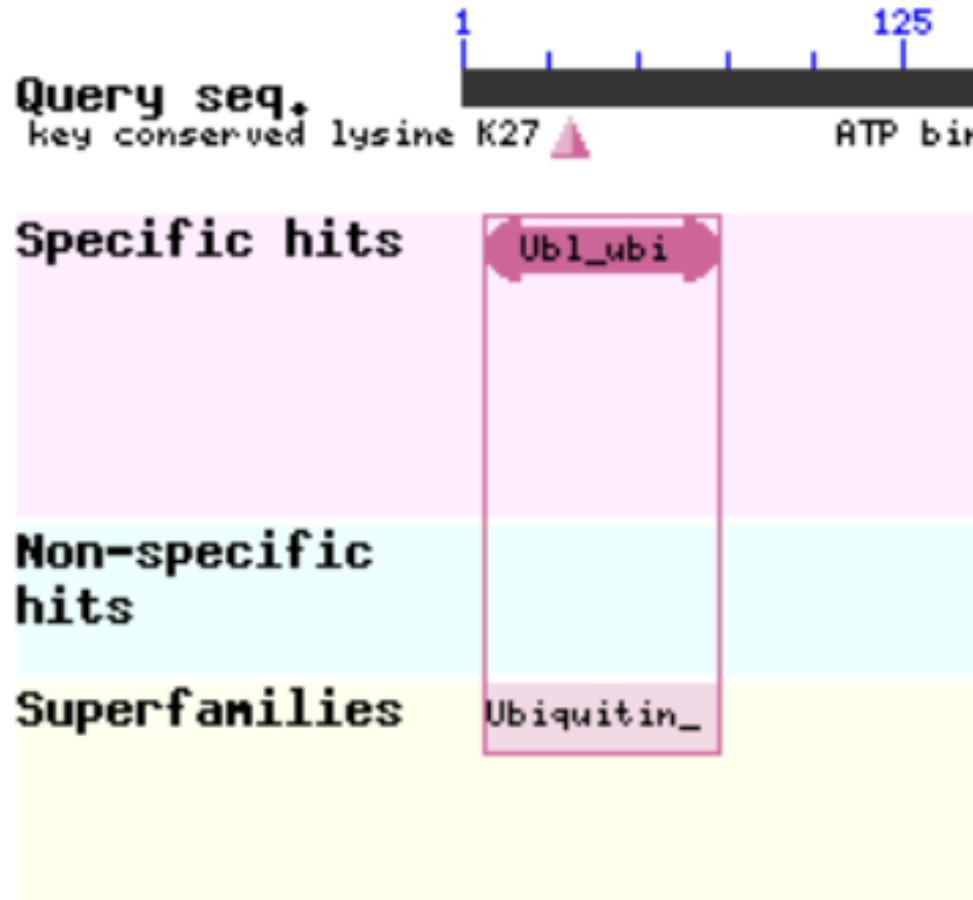
658	LLEVNNFESYQDLVLD FNIIILEGHIQCAAFLPEPINFERDKQYFTESHLR-----KI	710
680	IHTQP ----NAELTLLDTNEVLLASHI QCAAYBLPFINR SDKFFG-NQIQ-----DI	727
552	LFGKP---IEHCQVDSQNPKVLEQHLACAASEYPLRQEHD ESYFGFSMNSV LMTLKDKG	607
414	FFNRS ----PESARINPENL I LVVDHLKCAAYELPFRADEEF--GAMEVSDILEYLQEEA	467
	: : : * : * : * : * : * : * :	

Tree



Homologous proteins

独特的类泛素结构域



■ 类泛素结构域

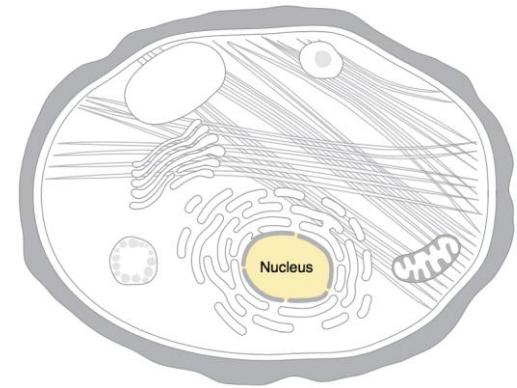
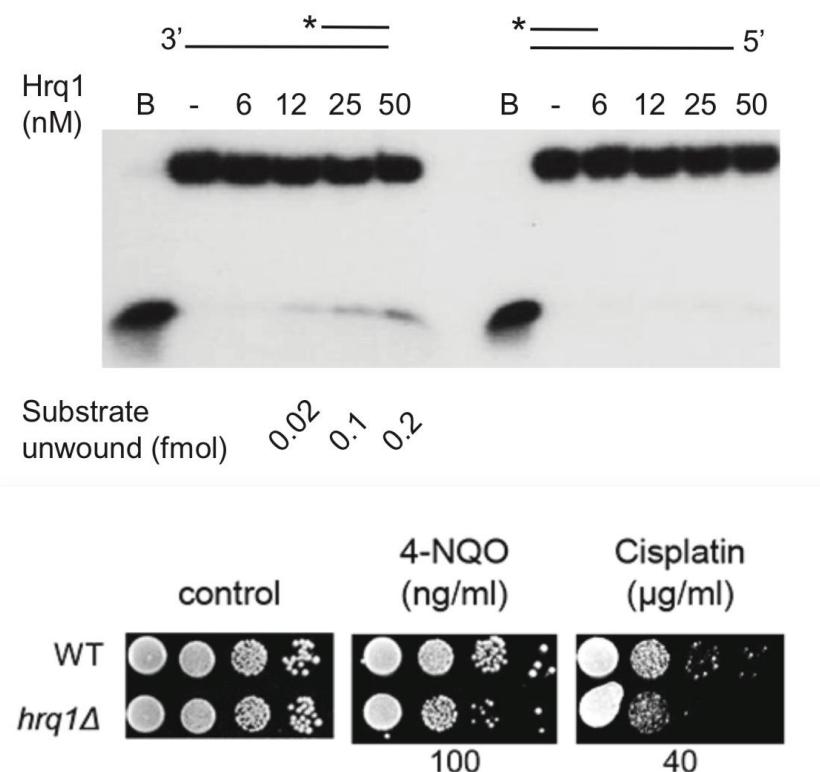
- 共价修饰目的蛋白
 - 介导蛋白质间的相互作用

K27

- 此类蛋白中较为保守的一个位点
 - 涉及介导蛋自质间相互作用的机理

Homologous proteins ATP-dependent helicase **ScHRQ1**

- 酿酒酵母 *Saccharomyces cerevisiae* (strain ATCC 204508 / S288c)
- 注释信息完善
- 与OsHRQ1有35%的一致性



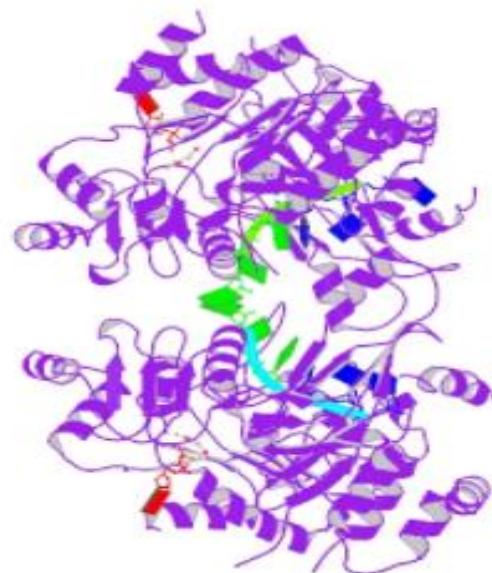
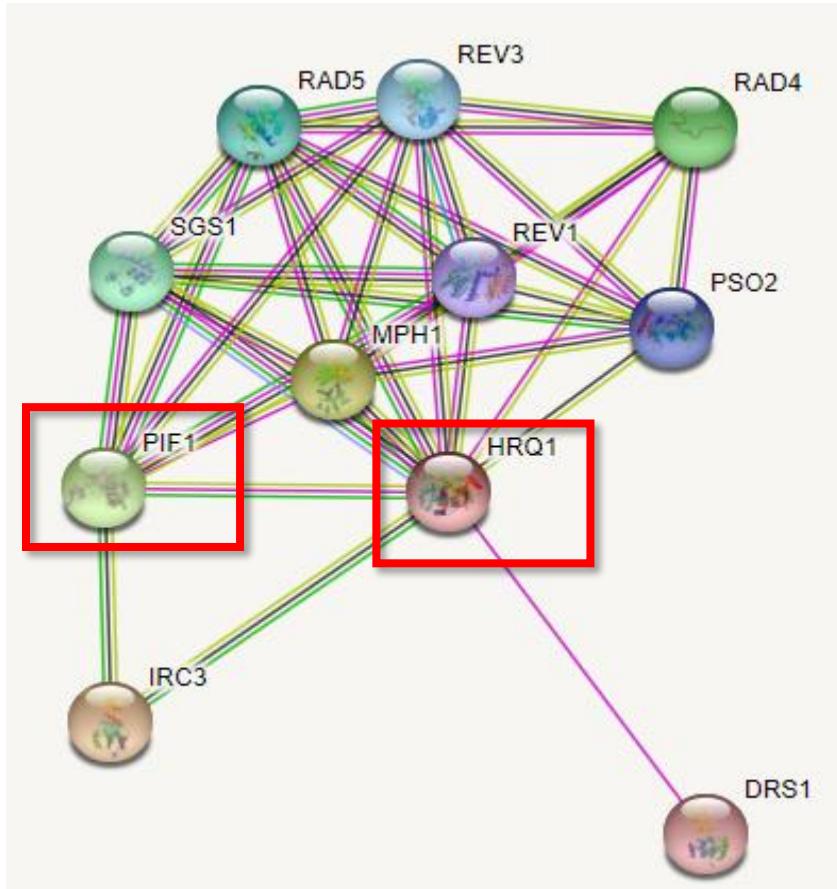
- DNA复制过程中3'-5' 解旋酶活性
- 维持基因组稳定性
 - ✓ 核酸切除修复(nucleotide excision repair, NER)
 - ✓ DNA 链间交联修复
- 影响端粒长度的稳定

CONTENTS

- 
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Interacting proteins

ScHRQ1与ScPIF1互作



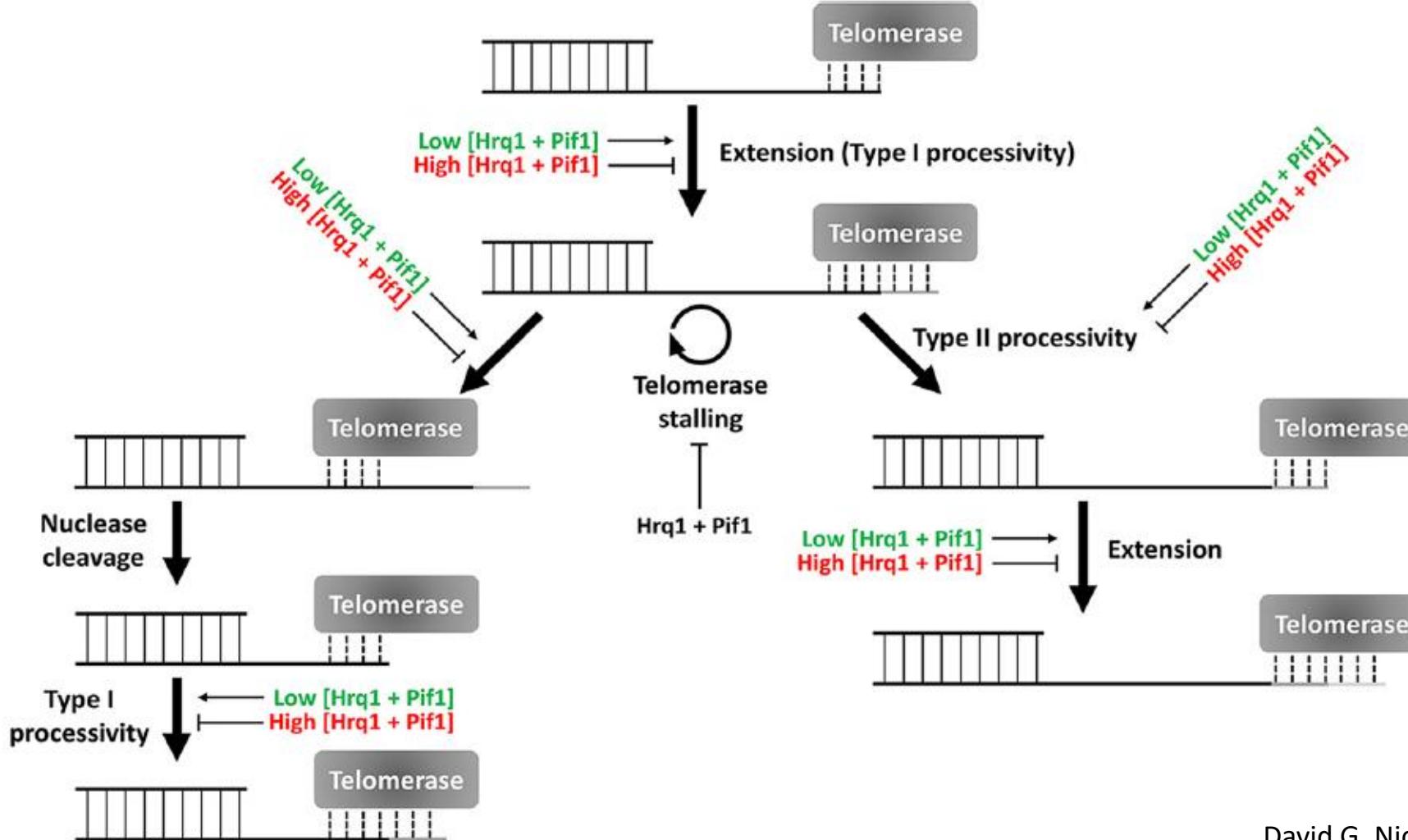
名称	ATP-dependent DNA helicase PIF1
登录号	P07271
长度	859 AA
亚细胞定位	细胞核 & 线粒体
功能	端粒酶抑制剂； 参与DNA修复和重组

ScPIF1 结构
PDB ID: 5O6B

Interacting proteins

ScHRQ1与ScPIF1互作

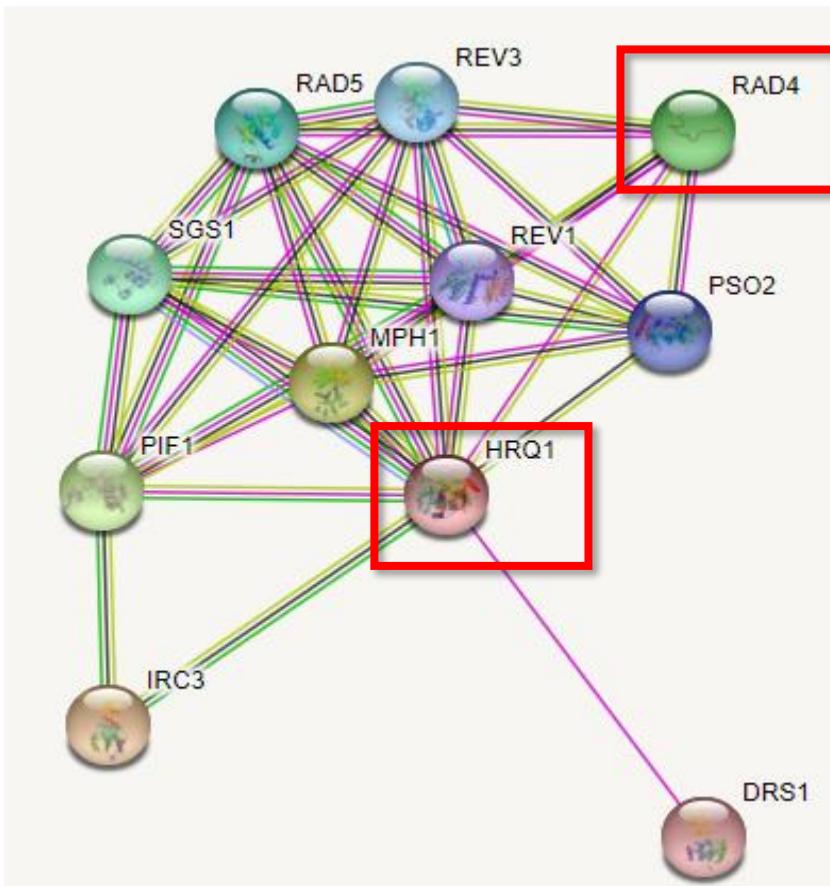
- HRQ1与PIF1的协同作用确保了酿酒酵母细胞内端粒长度的稳态。



Interacting proteins

ScHRQ1与ScRAD4互作

- RAD4 在HRQ1上位， HRQ1与RAD4相互作用共同完成DNA损伤修复过程。



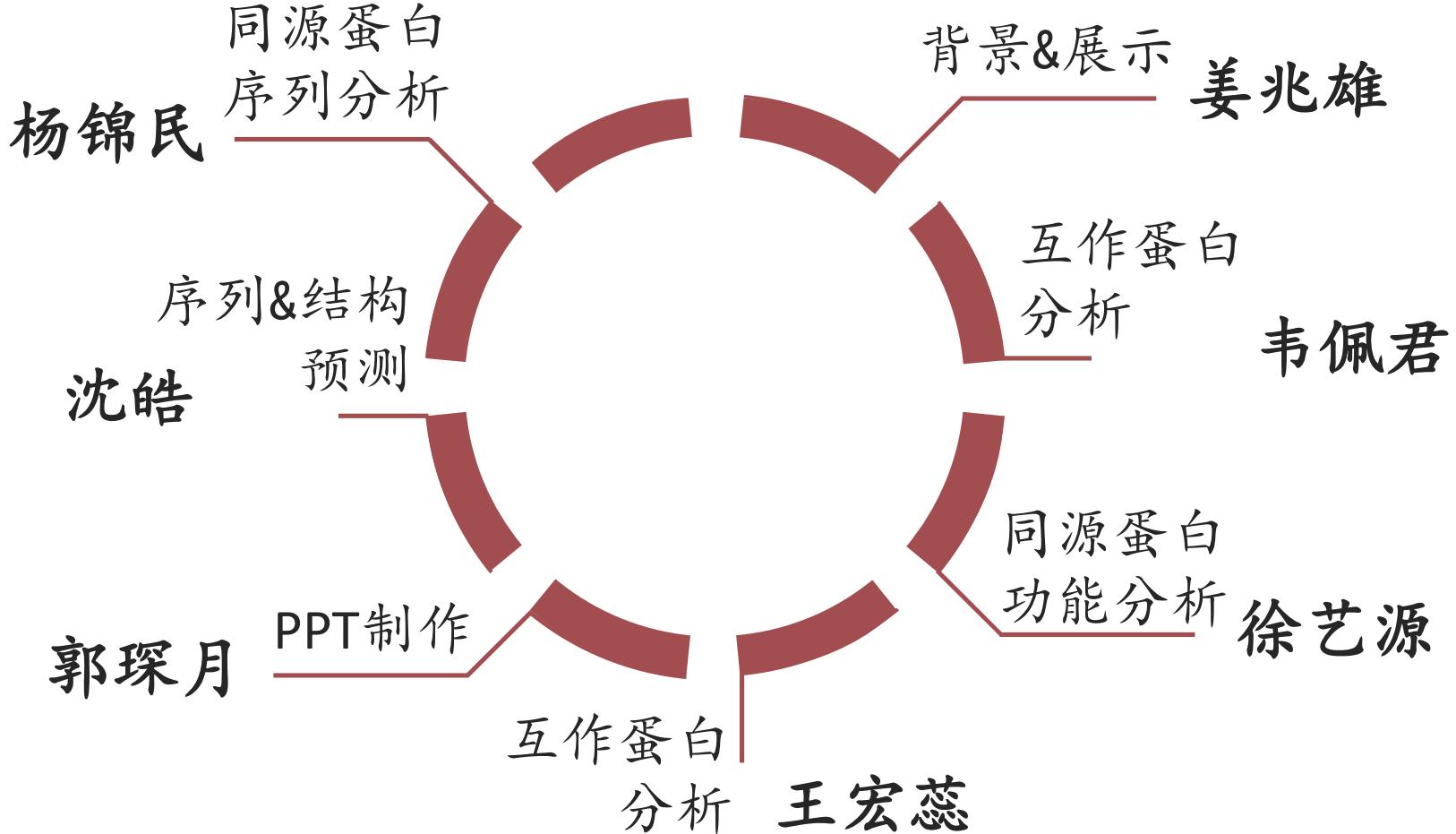
名称	DNA repair protein RAD4
登录号	P14736
长度	754 AA
亚细胞定位	细胞核 & 细胞质
功能	DNA损伤的核苷酸切除修复

ScRAD4-RAD23结构
PDB ID: 2QSF

References

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7. Kwon S.H., Choi D.H., Lee R., Bae S.H. *Saccharomyces cerevisiae* Hrq1 requires a long 3'-tailed DNA substrate for helicase activity. *Biochem. Biophys. Res. Commun.* 427:623-628(2012)
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9. David G. Nickens et al. The *Saccharomyces cerevisiae* Hrq1 and Pif1 DNA helicases synergistically modulate telomerase activity in vitro. *J. Biol. Chem.* 2018
10. Do-Hee Choi et al. Hrq1 Facilitates Nucleotide Excision Repair of DNA Damage Induced by 4-Nitroquinoline-1-Oxide and Cisplatin in *Saccharomyces cerevisiae*. *Journal of Microbiology*. 2014

Group Members



Thanks!

BACKGROUND

范可尼贫血症信号通路

The Fanconi anemia pathway (FA)

- 修复受损的DNA，尤其是链间交联（ICL）。
- DNA ICL被FANCM和相关蛋白直接识别，这些蛋白募集了FA核心复合物。
- FA核心复合物是单泛素化的FANCD2和FANCI。单泛素化的FANCD2 / FANCI成为活性形式，并与一系列DNA修复蛋白相互作用，促进下游修复途径。
- 范可尼贫血是由至少13个FA基因之一的突变引起的，其特征是先天性生长异常、骨髓衰竭和癌症易感性等。

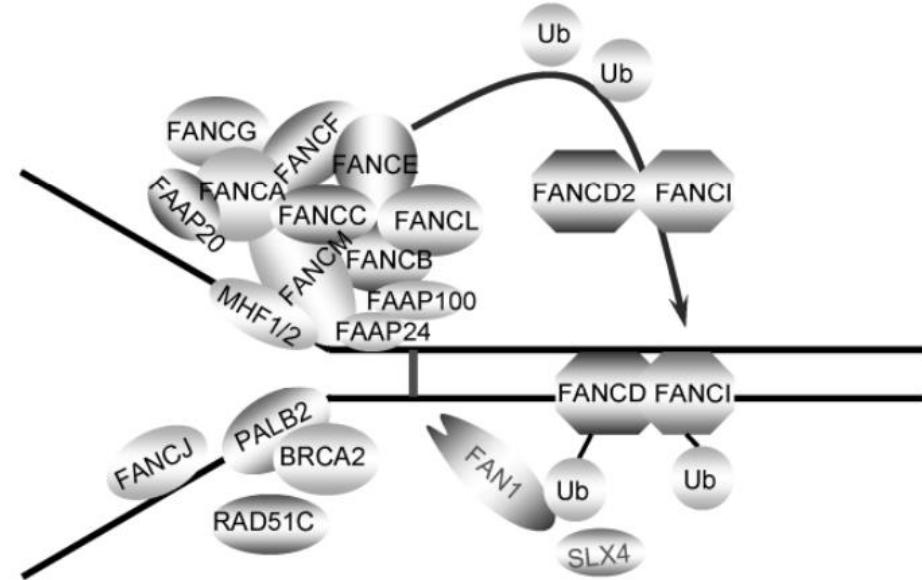
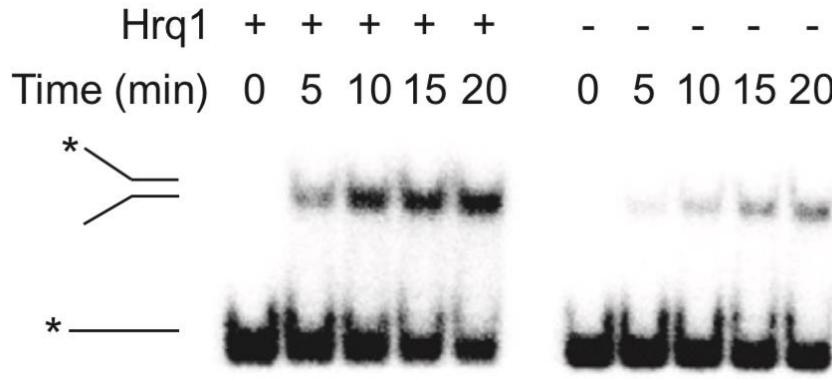


图1 范可尼贫血症信号通路作用模式图

Homologous proteins

ATP-dependent helicase **ScHRQ1**



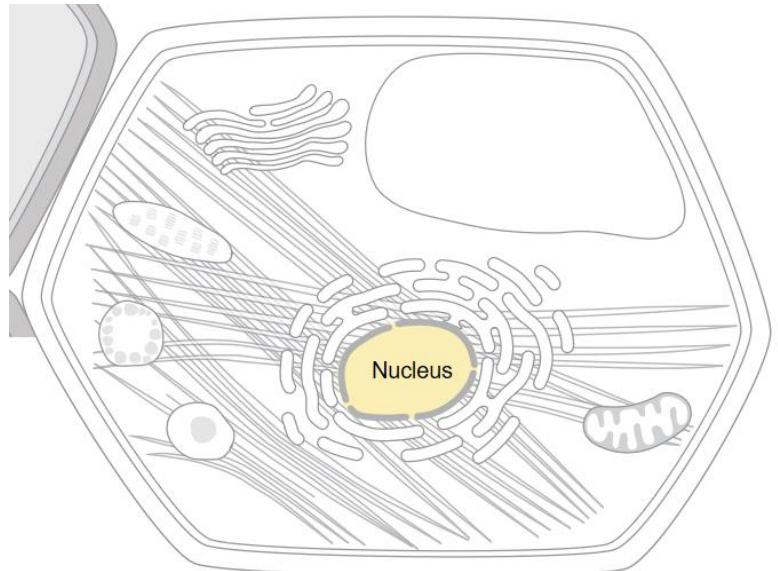
Helicase & DNA strand annealing activities

Affects telomere length by a non-catalytic mechanism.

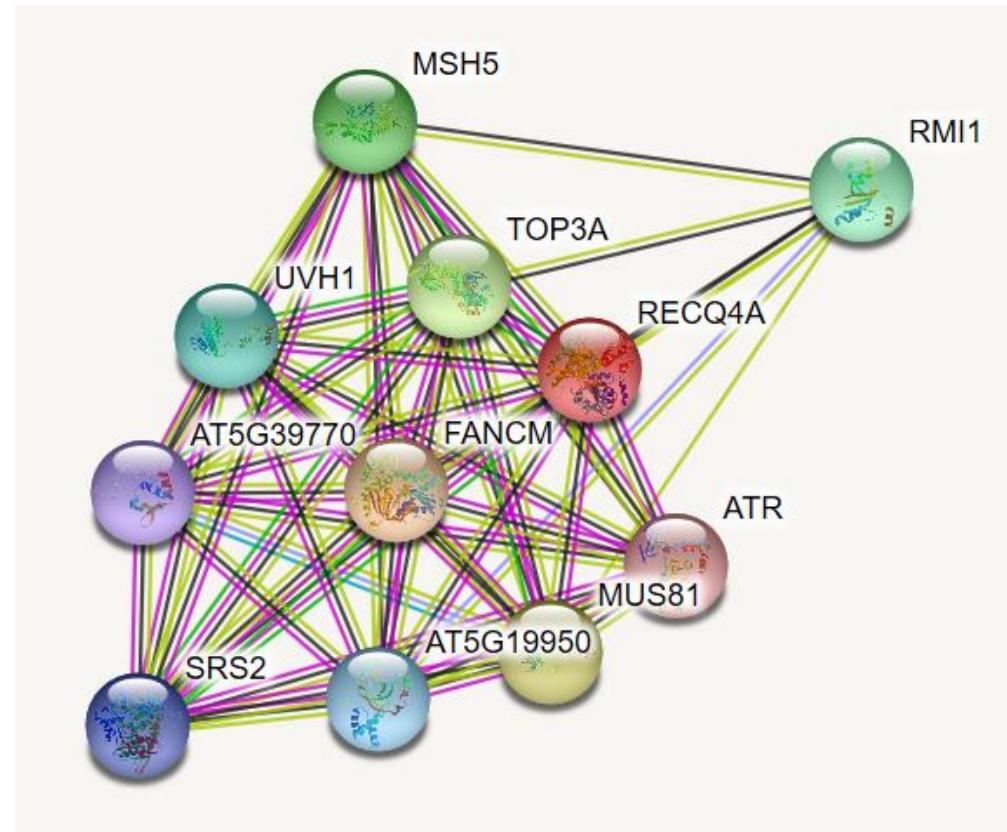
- *HRQ1* suppressed telomere addition (TA) to DSBs, an activity it shares with Pif1
- probably through inhibiting telomerase by competing with it for ssDNA binding
- also important for telomerase-independent telomere maintenance.

Homologous proteins

in *Arabidopsis thaliana*



AtRECQ4A



<https://string-db.org/network/3702.AT1G10930.1>

Homologous proteins in *Arabidopsis thaliana*

AtHRQ1

- 参与DNA的交联修复
- 与范科尼贫血症相关的核酸酶FAN1和复制后修复的ATP酶RAD5A有共同的途径

