

Protein Prediction and Structure Analysis of WRKY Transcription Factors Involved in Response to Cold in *Cucumis Sativus*

12组：陈璐 张晓孟 孟姗姗 张吉祥

Background



Protein Sequence Alignment and Phylogeny

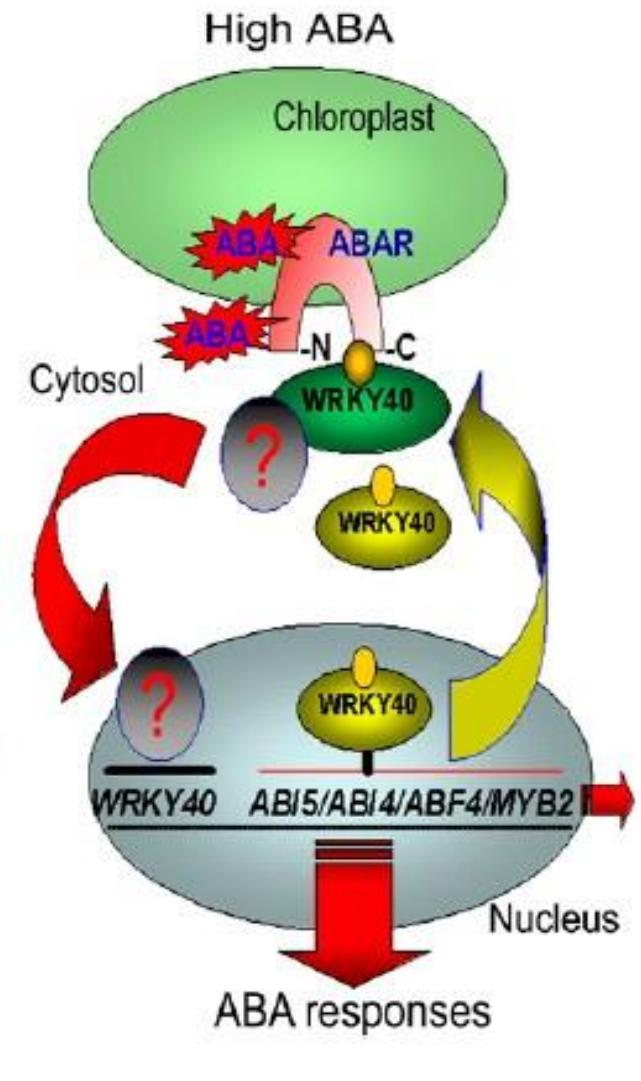
Protein modeling



Future Plan

Transcription factor

- sequence-specific DNA binding
- activate or repress transcription of downstream target genes.



Yi Shang et al. ,2010

WRKY Transcription factor

Group1

AtWRKY20C	SEVDILDDGYRWRKYGQKVVRGNPNPRSYKKCT---AHGCPVRKHVERAS---	HDPKAVITTYEGKHDV
CsWRKY17C	SEVDILDDGYRWRKYGQKVVRGNPNPRSYKKCT---NVGCPVRKHVERAS---	HDPKAVITTYEGKHNHDV
CsWRKY2C	SDIDILDDGYRWRKYGQKVVKGNPNPRSYKKCT---NP GCPVRKHVERAS---	HDL RAVITTYEGKHNHDV
CsWRKY15	SEVDILDDGYRWRKYGQKVVKGNPNPRSYKKCT---NP GCTVRKHVERAS---	HDL KSVITTYEGKHNHDV
CsWRKY8C	TEVDILEDGYRWRKYGQKVVKGNPNPRSYKKCT---SAGCLVRKHVERAS---	HDL KCVITTYEGKHNHEVP
CsWRKY37C	SEVDLLDDGYRWRKYGQKVVKGNPNPRSYKKCT---SAGCNVRKHVERSS---	TDSKAVVTTYEKGHNHDV
CsWRKY39	SNVDKLDGYWWRKYGQKVVKGNPNPRSYKKCT---YP GCGVRKHIERAS---	HDFRAVVTTYEKGHNHDIP
CsWRKY23C	SEIDILPDGYRWRKYGQKVVKGNPNPRSYKKCT---SL GCPVRKHIERAA---	NDMRAVITTYEGKHNHEVP
CsWRKY49C	SEDIVNDGYRWRKYGQKFVKGNPNPRSYRCS---	SPGCPVKKHVERAS---
CsWRKY4C	GDVGISGDGYRWRKYGQKMWKGNPHPRNYYRCT---	SAGCPVRKHIESAV---
CsWRKY24C	TGIEISGKGVRWRKYGQKVVKGNLYPRSYYRCT---	ENPNAVIITYKGVHDHDT
		EDPDSFITTYEKGHNHG

Group3

AtWRKY54	VEAKSSEDRYAWRKYGQKEILNTTPRSYFRCTHKPTQQCKATKQVQKQD-Q-DSE-MFQITYIGYHTCTAN	
CsWRKY34	RTSRTTEDNYGWRKYGQKAIHNTTYPRSYYRCTHKFDQGCQATKQVQRMEGD-DSEIMYNITYISDHTCRP	
CsWRKY22	ESCDLYDDGHAWRKYGQKTILNAKYPRNYYRCTHKYDQTCQATKQVQRLQ---	DNPPKFRTTYGNHTCSNF
CsWRKY20	AIEGSLDDGFAWRKYGQKGILGAKHPRGYYRCTHRNLQGCLATKQVQRSD---	DDPTIFEITYRGKHSQS
CsWRKY50	AVEGPGCDGFSWRKYGQKDILGSKFPRTSYFRCSHRFTQGCLATKQVQKSD---	NDPTIYEVTYKGRHTCNKA
CsWRKY31	GFE GPHEDGYSWRKYGQKDILGATYPRSYYRCTFRNTQNCWAVKQVQRSD---	EDPSVFEITYRGKHTCSQG
CsWRKY35	NTELPPDDGFTWRKYGQKEILGSRFPRGYFRCTHQKLYHCPAKKHVQLD---	DDPHTFEVTYRGEHTCHMS

Jian Ling et al. 2011

WRKY Transcription factor

- contain at least one highly conserved signature domain of about 60 amino acid residues, which includes the conserved WRKYGQK sequence followed by a zinc finger motif.

Eulgem T et al. , 2000

WRKY Transcription factor- PubMed

Results: 8

PubMed

[Genome-wide analysis of WRKY gene family in Cucumis sativus.](#)

1. Ling J, Jiang W, Zhang Y, Yu H, Mao Z, Gu X, Huang S, Xie B.
BMC Genomics. 2011 Sep 28;12:471.

PMID: 21955985 [PubMed - indexed for MEDLINE] [Free PMC Article](#)

[Related citations](#)

[Capsicum annuum WRKYb transcription factor that binds to the CaPR-10 promoter functions as a positive regulator in innate immunity](#)

2. [upon TMV infection.](#)

Lim JH, Park CJ, Huh SU, Choi LM, Lee GJ, Kim YJ, Paek KH.

Biochem Biophys Res Commun. 2011 Aug 5;411(3):613-9. Epub 2011 Jul 13.

PMID: 21771584 [PubMed - indexed for MEDLINE]

[Related citations](#)

[Expression and functional analysis of two genes encoding transcription factors, VpWRKY1 and VpWRKY2, isolated from Chinese wild](#)

3. [Vitis pseudoreticulata.](#)

Li H, Xu Y, Xiao Y, Zhu Z, Xie X, Zhao H, Wang Y.

Planta. 2010 Nov;232(6):1325-37. Epub 2010 Sep 2.

PMID: 20811906 [PubMed - indexed for MEDLINE]

[Related citations](#)

[Male gametophyte-specific WRKY34 transcription factor mediates cold sensitivity of mature pollen in Arabidopsis.](#)

4. Zou C, Jiang W, Yu D.

J Exp Bot. 2010 Sep;61(14):3901-14. Epub 2010 Jul 19.

PMID: 20643804 [PubMed - indexed for MEDLINE] [Free PMC Article](#)

[Related citations](#)

WRKY Transcription factor- UniProt

Uniprot

Entry	Entry name	Status	Protein names	Gene names	Organism	Length
022921	WRK25_ARATH	★ Probable WRKY transcription factor 25	WRKY25 At2g30250 T9D9.6	Arabidopsis thaliana (Mouse-ear cress)	393	
Q8S8P5	WRK33_ARATH	★ Probable WRKY transcription factor 33	WRKY33 At2g38470 T19C21.4	Arabidopsis thaliana (Mouse-ear cress)	519	
065590	WRK34_ARATH	★ Probable WRKY transcription factor 34	WRKY34 At4g26440 M3E9.130	Arabidopsis thaliana (Mouse-ear cress)	568	



WRKY Transcription factor- UniProt

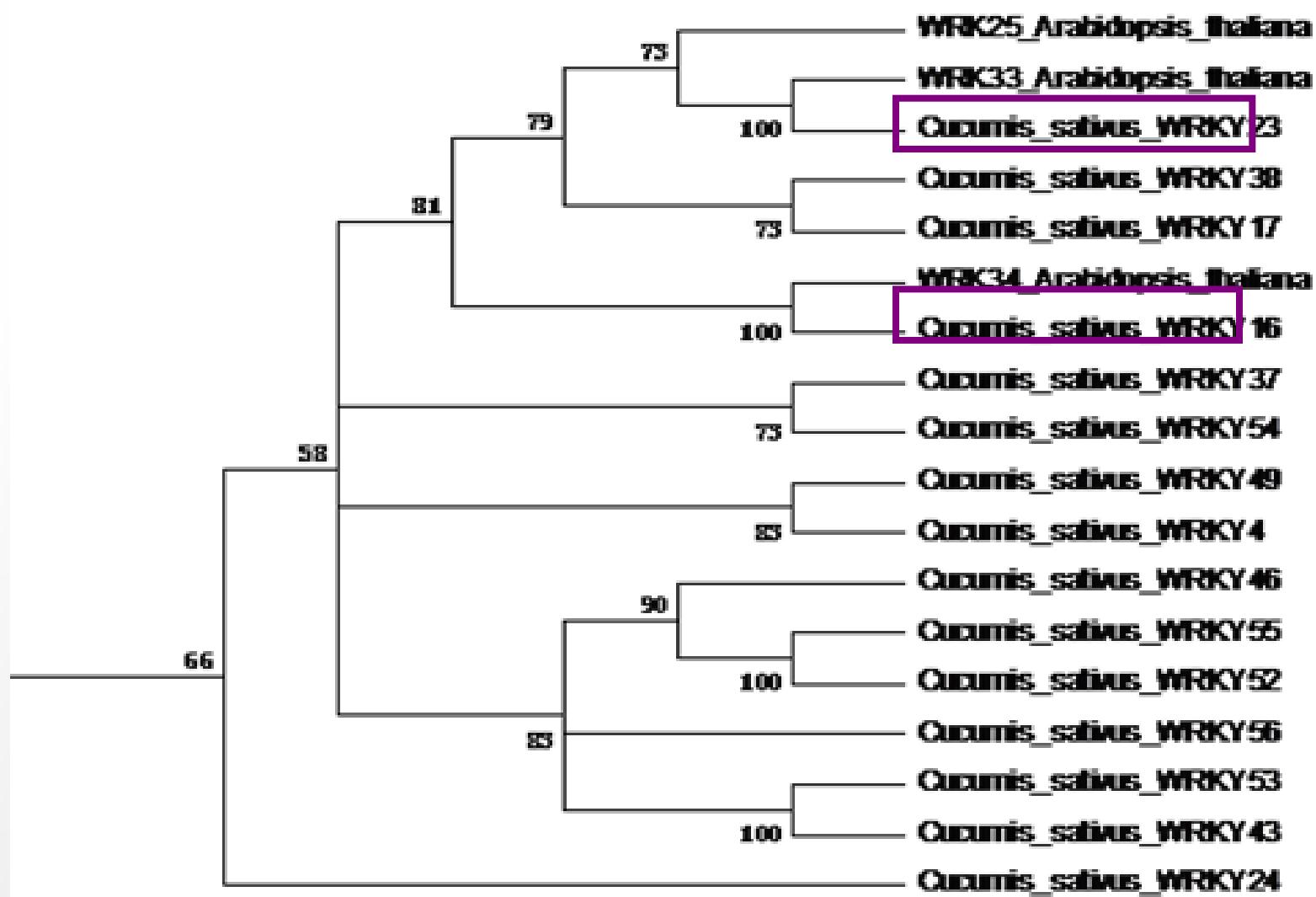
- › Restrict term "wrky" to gene name (33), protein name (34)
- › Expand search to "Cucumis sativus (Cucumber) [3659]" to include lower taxonomic ranks

Uniprot

Entry	Entry name	Status	Protein names	Gene names	Organism	Length
E7CEW1	E7CEW1_CUCSA	★	WRKY protein	WRKY9 WRKY10	Cucumis sativus (Cucumber)	348
E7CEV9	E7CEV9_CUCSA	★	WRKY protein	WRKY5	Cucumis sativus (Cucumber)	282
E7CEX6	E7CEX6_CUCSA	★	WRKY protein	WRKY33	Cucumis sativus (Cucumber)	383
E7CEY5	E7CEY5_CUCSA	★	WRKY protein	WRKY51	Cucumis sativus (Cucumber)	352
E7CEX3	E7CEX3_CUCSA	★	WRKY protein	WRKY25	Cucumis sativus (Cucumber)	350
E7CEW4	E7CEW4_CUCSA	★	WRKY protein	WRKY14	Cucumis sativus (Cucumber)	293
E7CEW7	E7CEW7_CUCSA	★	WRKY protein	WRKY18	Cucumis sativus (Cucumber)	336
E7CEY3	E7CEY3_CUCSA	★	WRKY protein	WRKY49	Cucumis sativus (Cucumber)	433
E7CEX0	E7CEX0_CUCSA	★	WRKY protein	WRKY22	Cucumis sativus (Cucumber)	280

Species/Abbrv	*	**	***	****	*****	*	**	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
1. WRK25 Arabi	G	F	P	W	R	K	Y	G	Q	K	V	V	G	N	T	N	P	R	S	Y	Y	
2. WRK33 Arabi	G	Y	P	W	R	K	Y	G	Q	K	V	V	G	N	P	N	P	R	S	Y	Y	
3. WRK34 Arabi	G	Y	P	W	R	K	Y	G	Q	K	V	V	G	N	P	N	P	R	S	Y	Y	
4. Cucumis sat	D	Y	S	W	R	K	Y	G	Q	K	P	I	K	G	S	P	H	P	R	G	Y	Y
5. Cucumis sat	E	Y	S	W	R	K	Y	G	Q	K	P	I	K	G	S	P	Y	P	R	G	Y	Y
6. Cucumis sat	G	Y	P	W	R	K	Y	G	Q	K	V	V	G	N	P	N	P	R	S	Y	Y	Y
7. Cucumis sat	I	W	A	W	R	K	Y	G	Q	K	P	I	K	G	S	P	Y	P	R	G	Y	Y
8. Cucumis sat	G	C	Q	W	R	K	Y	G	Q	K	I	A	G	N	P	C	P	R	A	Y	Y	Y
9. Cucumis sat	G	Y	Q	W	R	K	Y	G	Q	K	V	T	R	D	N	P	C	P	R	A	Y	Y
10. Cucumis sa	S	W	G	W	R	K	Y	G	Q	K	P	I	K	G	S	P	Y	P	R	S	Y	Y
11. Cucumis sa	G	Y	Q	W	R	K	Y	G	Q	K	V	T	R	D	N	P	S	P	R	A	Y	Y
12. Cucumis sa	G	Y	W	W	R	K	Y	G	Q	K	V	V	G	N	P	N	P	R	S	Y	Y	Y
13. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	A	V	K	N	S	A	Y	P	R	S	Y	Y
14. Cucumis sa	S	W	A	W	R	K	Y	G	Q	K	P	I	K	G	S	P	Y	P	R	G	Y	Y
15. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	V	V	K	N	T	Q	H	P	R	S	Y	Y
16. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	A	V	K	N	N	K	F	P	R	S	Y	Y
17. Cucumis sa	G	V	P	W	R	K	Y	G	Q	K	V	V	K	N	L	Y	P	R	S	Y	Y	Y
18. Cucumis sa	S	W	A	W	R	K	Y	G	Q	K	P	I	K	G	S	P	Y	P	R	S	Y	Y
19. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	A	V	K	N	S	P	F	P	R	S	Y	Y
20. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	V	V	K	N	T	L	H	P	R	S	Y	Y
21. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	F	V	K	G	N	P	N	P	R	S	Y	Y
22. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	M	V	K	G	N	P	H	P	R	N	Y	Y
23. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	V	V	K	G	N	P	N	P	R	S	Y	Y
24. Cucumis sa	G	H	A	W	R	K	Y	G	Q	K	T	I	L	N	A	K	Y	P	R	N	Y	Y
25. Cucumis sa	D	Y	S	W	R	K	Y	G	Q	K	P	I	K	G	S	P	H	P	R	G	Y	Y
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27. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	V	V	K	G	N	P	N	P	R	S	Y	Y
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29. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	K	S	V	K	N	S	P	N	P	R	S	Y
30. Cucumis sa	E	Y	S	W	R	K	Y	G	Q	K	P	I	K	G	S	P	Y	P	R	G	Y	Y
31. Cucumis sa	-	Y	G	W	R	K	Y	G	Q	K	A	I	H	N	T	T	P	R	S	Y	Y	Y
32. Cucumis sa	G	Y	Q	W	R	K	Y	G	Q	K	V	T	R	D	N	P	S	P	R	A	Y	Y
33. Cucumis sa	G	F	S	W	R	K	Y	G	Q	K	O	I	L	G	S	K	F	P	R	S	Y	Y
34. Cucumis sa	D	Y	S	W	R	K	Y	G	Q	K	P	I	K	G	S	P	H	P	R	G	Y	Y
35. Cucumis sa	G	Y	P	W	R	K	Y	G	Q	K	A	V	K	N	S	P	H	P	R	S	Y	Y

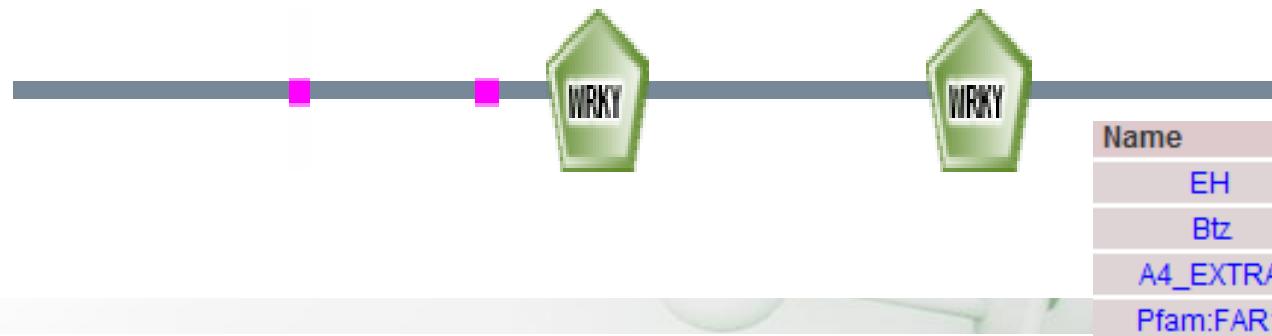
WRKY Transcription Factor Phylogeny



WRKY16 Domains-Smart

Domains within the query sequence of 720 residues

1 100 200



Confidently predicted domains, repeats, motifs and features:

Name	Begin	End	E-value
low complexity	157	169	-
low complexity	263	276	-
WRKY	303	361	1.00e-34
WRKY	518	577	6.83e-37

Name	Begin	End	E-value	Reason
EH	46	118	1.71e+03	threshold
Btz	281	437	1.34e+05	threshold
A4_EXTRA	285	432	5.32e+02	threshold
Pfam:FAR1	301	361	8.80e+01	overlap
Pfam:WRKY	303	361	8.00e-30	overlap
internal repeat 1	304	375	3.97e-18	overlap
Pfam:Ogr_Delta	311	348	8.90e+00	overlap
CTD	384	536	1.59e+05	threshold
Glyco_25	389	490	2.15e+03	threshold
SPT2	422	490	8.30e+04	threshold
Pfam:FAR1	506	579	1.00e+00	overlap
Pfam:WRKY	518	577	3.70e-30	overlap
GYR	519	536	2.35e+03	threshold
internal repeat 1	519	591	3.97e-18	overlap
Pfam:Ogr_Delta	530	555	1.10e+01	overlap
ZnF_C2H2	542	560	3.60e+02	threshold

Domains within the query sequence of 452 residues

1 100 200

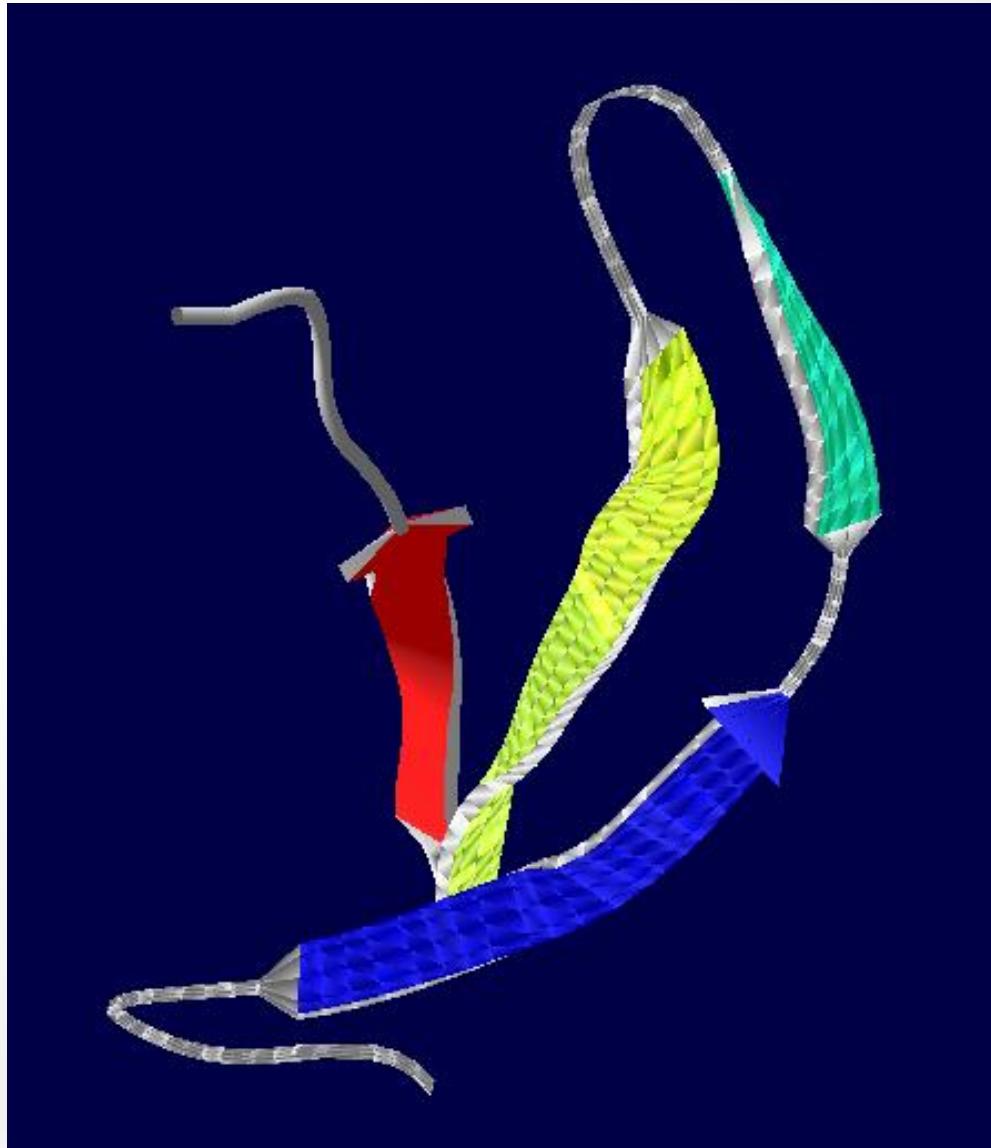


Confidently predicted domains, repeats, motifs and features:

Name	Begin	End	E-value
low complexity	4	31	-
low complexity	86	108	-
WRKY	145	203	5.30e-34
low complexity	263	274	-
WRKY	294	353	1.68e-36

Name	Begin	End	E-value	Reason
Pfam:FAR1	77	203	3.40e+02	overlap
PhnA_Zn_Ribbon	100	147	1.00e+05	threshold
CG-1	107	199	2.28e+04	threshold
Pfam:CG-1	112	164	2.70e-03	overlap
Pfam:DNA_pol3_tau_4	117	166	3.20e+00	overlap
Pfam:WRKY	145	203	8.00e-31	overlap
internal repeat 1	147	217	7.74e-24	overlap
SEP	203	278	2.19e+03	threshold
Pfam:DNA_pol3_tau_4	203	270	1.70e+01	overlap
Pfam:DNA_pol3_tau_4	276	314	5.40e+02	overlap
Pfam:FAR1	286	355	4.00e-01	overlap
Pfam:WRKY	294	353	7.10e-31	overlap
Pfam:CG-1	294	309	7.00e+02	overlap
internal repeat 1	296	365	7.74e-24	overlap
CBF	308	366	3.32e+03	threshold

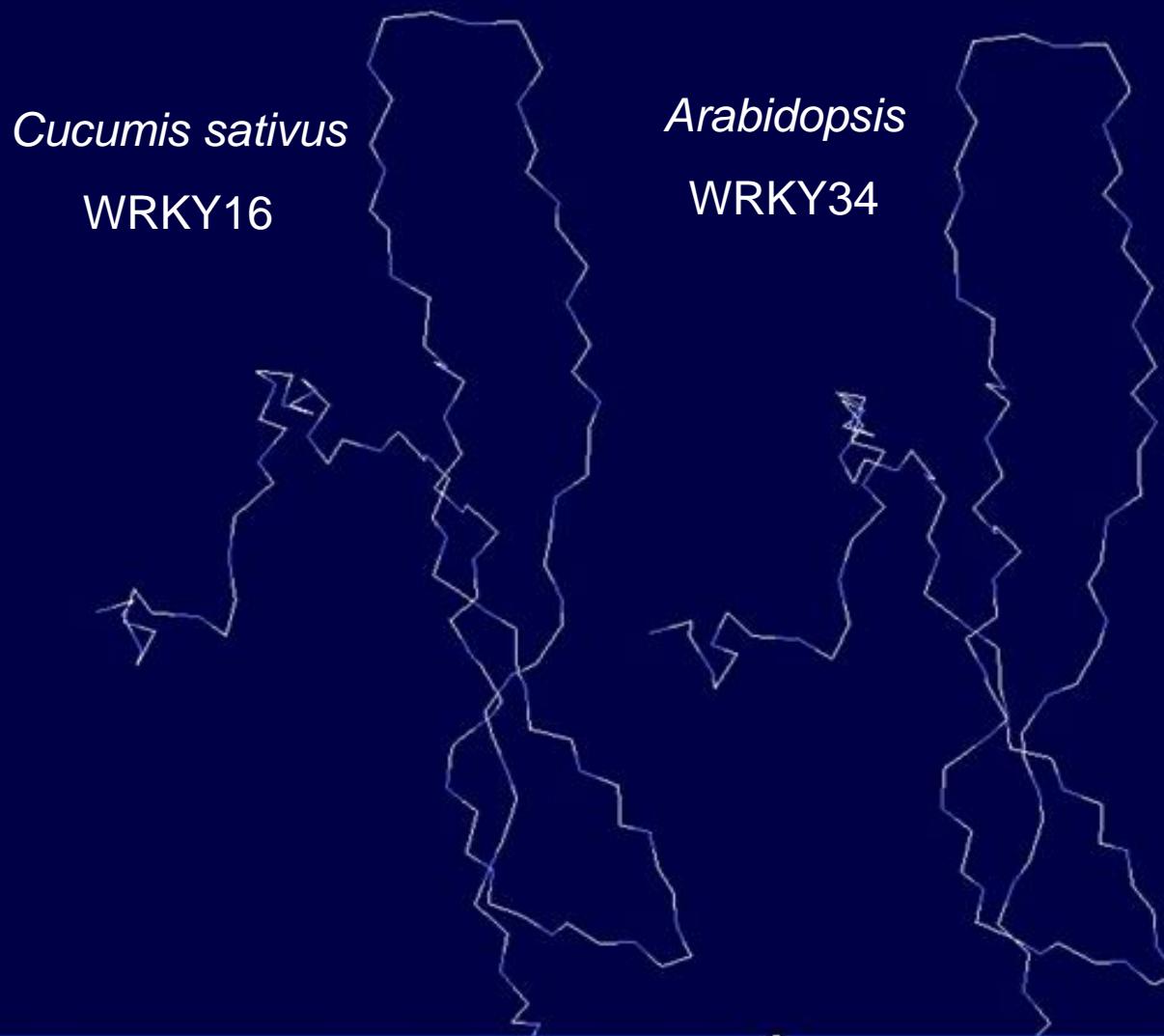
WRKY16- 3D Structure-Swiss Model



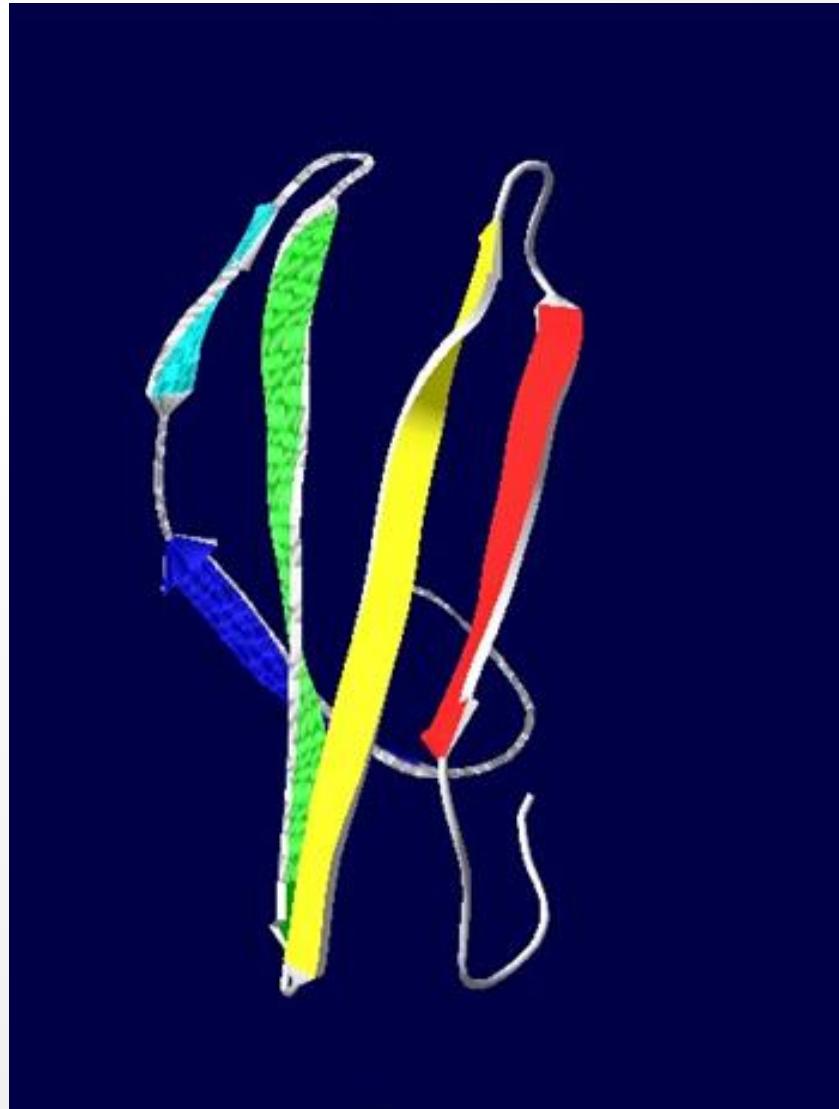
黄瓜WRKY16-
3D结构图



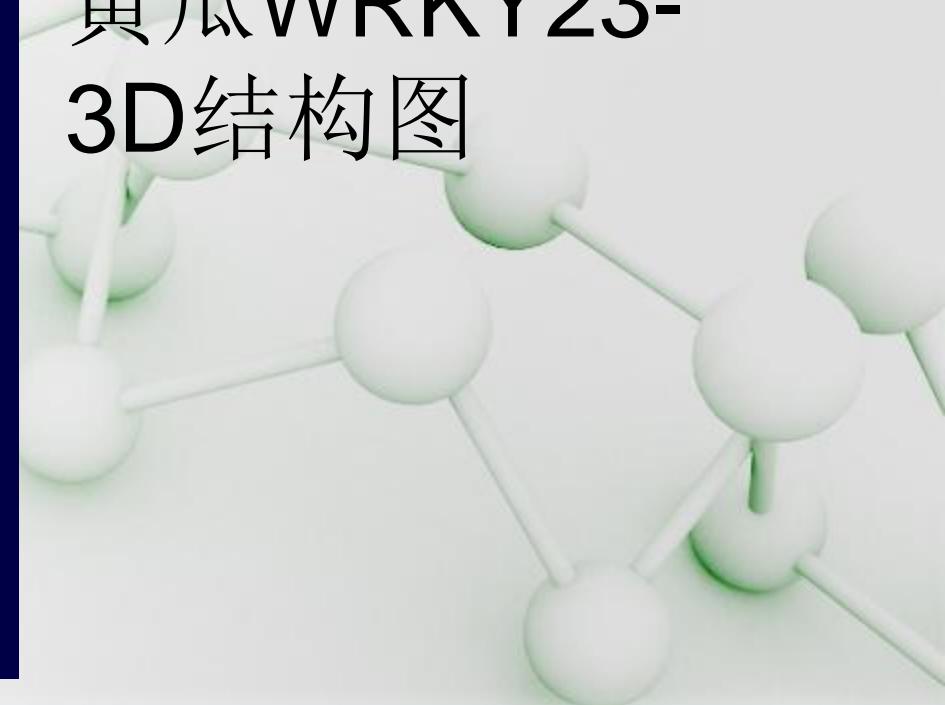
WRKY Transcription factor-SPDBV



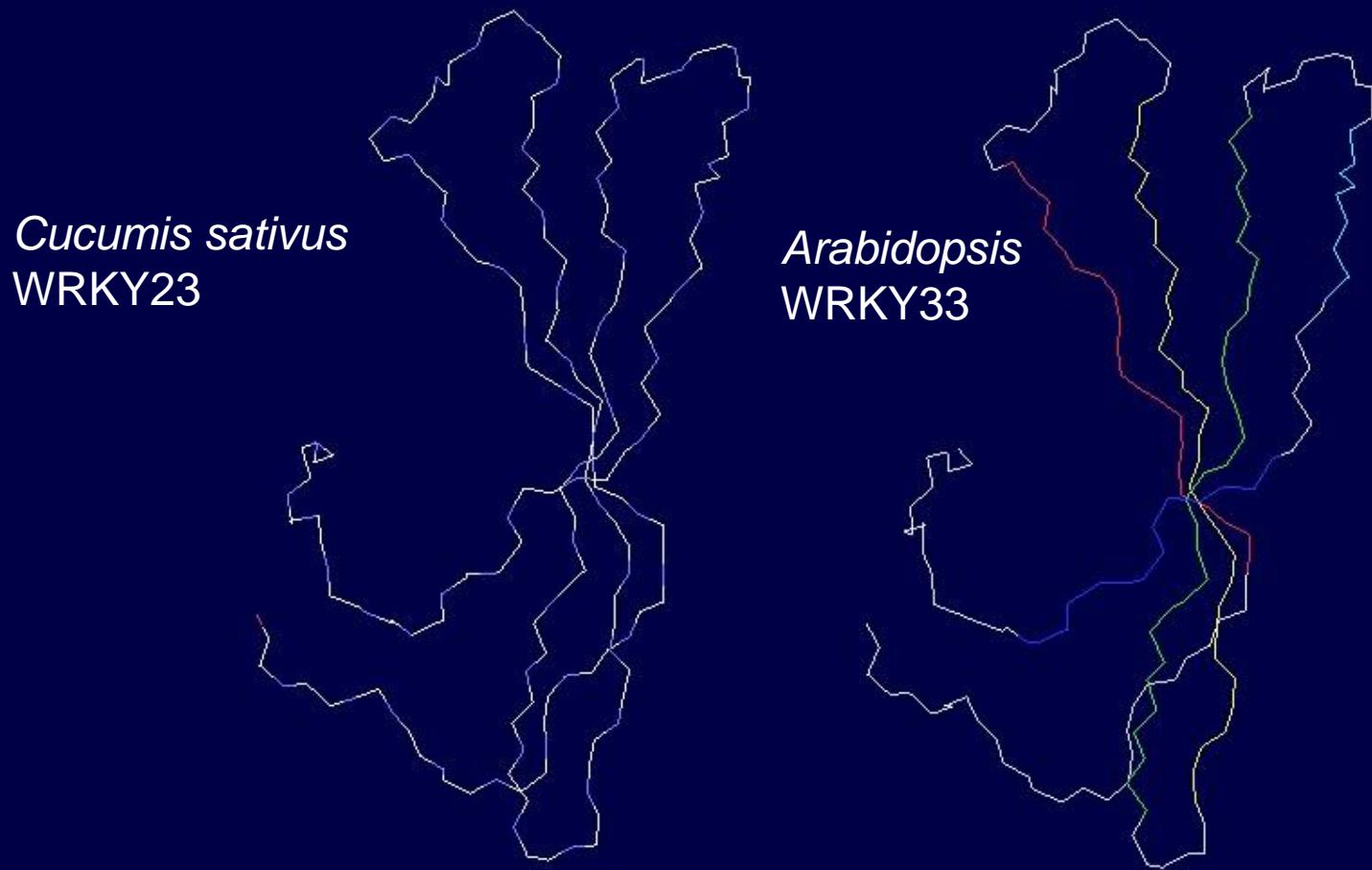
WRKY23- 3D Structure-Swiss Model



黄瓜WRKY23-
3D结构图



WRKY Transcription factor-SPDBV



- 1、Do the WRKY 16 and WRKY 23 have NLS(Nuclear Localization Signal)?
 - 2、Do the WRKY 16 and WRKY 23 response to cold?
 - 3、Which downstream gene do they regulate?
-

Acknowledgements

- Pro. Jiang, Pro. Luo
- Ying Zhang
- Lu Chen, Jixiang Zhang, Shanshan Meng

References

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- Jian Ling, Weijie Jiang*, Ying Zhang et al. Genome-wide analysis of WRKY gene family in *Cucumis sativus*. *BMC Genomics* 2011, 12:471.
- Yi Shang, Lu Yan, Zhi-Qiang Liu, et al. The Mg-Chelatase H Subunit of *Arabidopsis* Antagonizes a Group of WRKY Transcription Repressors to Relieve ABA-Responsive Genes of Inhibition. *Plant Cell* 2010, Vol. 22: 1909–1935.
- Changsong Zou, Wenbo Jiang and Diqu Yu. Male gametophyte-specific WRKY34 transcription factor mediates cold sensitivity of mature pollen in *Arabidopsis*. *Journal of Experimental Botany* 2010, Vol. 61, No. 14: 3901–3914.

Thanks

thank you

