

The structure prediction of SAPK9 and its function in ABA-signaling pathway leading to gene expression in *Oryza sativa*

CAAS First Class

Group One

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The structure prediction of SAPK9 and its function in ABA-signaling pathway leading to gene expression in *Oryza sativa*

- Background
- SAPK9 Primary Structure Analysis
- SAPK9 Structure Prediction
- SAPK9 Function Analysis

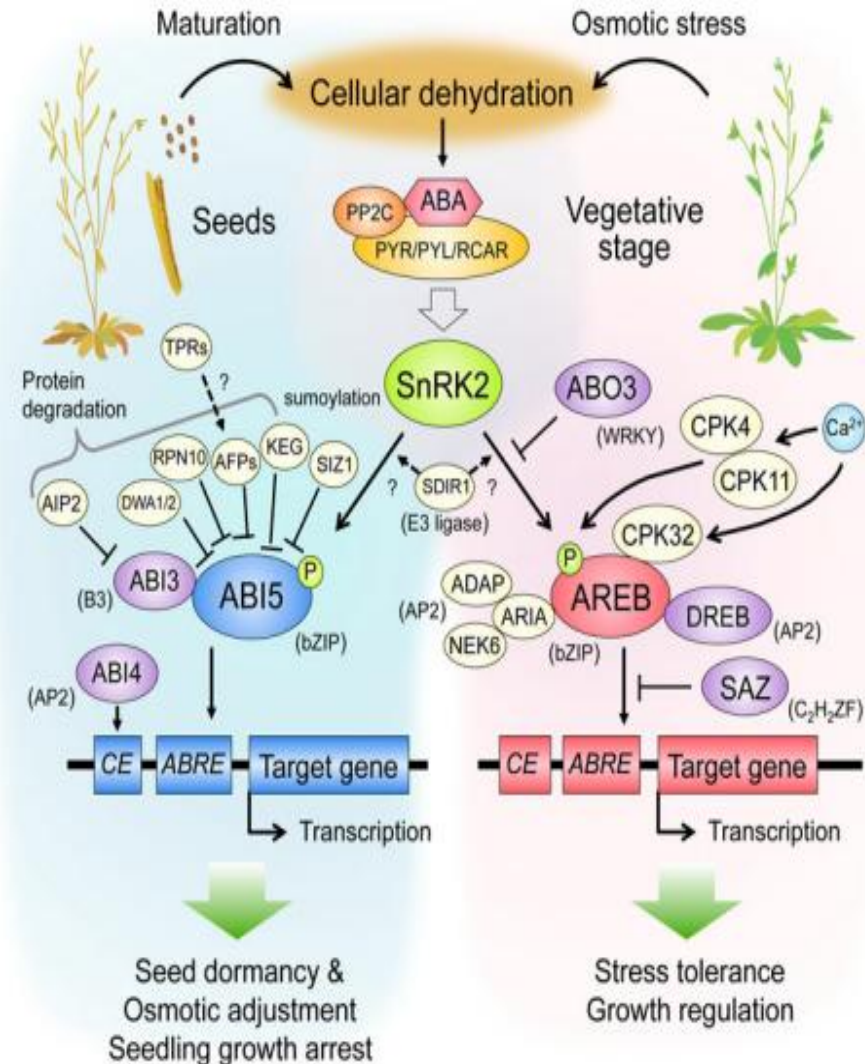
Function of ABA

1. developmental processes
2. adaptive stress responses to environmental stimuli
3. Cellular dehydration during the seed maturation
4. control many dehydration responsive genes in vegetative growth stages .

(Yasunari Fujita et al.,2011)



While a large number of signaling factors have been identified that are involved in stomata regulation by ABA, relatively less is known about the ABA-signaling pathway that leads to **gene expression**. (Yuhko Kobayashi et al., 2005)



SnRK2 family members

- plant-specific serine/threonine kinases
- in plant response to abiotic stresses
abscisic acid (ABA)-dependent plant development.
- SnRK2s have been classed into three groups:
 - group 1 comprises kinases **not** activated by ABA
 - group 2 comprises kinases not activated or activated **very weakly** by ABA
 - group 3 comprises kinases **strongly** activated by ABA.

(Anna Kulik et al., 2011)

SnRK2 family in Rice

- all members of the rice SnRK2 family, designated SAPK1–10, are activated in response to hyperosmotic stress via phosphorylation by an unidentified upstream protein kinase, and that SAPK8, **SAPK9** and SAPK10 are also activated by ABA (Kobayashi et al., 2004).

Our goal

- SAPK9 3D Structure
- SAPK9 function in ABA-signaling pathway leading to gene expression

SAPK9 Primary Structure Analysis

- The CDS OF SAPK9 GENE BANK [JF733767.1](#)

>gi|332806313|gb|JF733767.1| [Oryza sativa Japonica Group cultivar 9804](#) serine/threonine-protein kinase (SAPK9) mRNA, complete cds

ATGGAGAGGGCGGCGGGCCGCTGGGGATGGAGATGCCGATAATGCACGACGGTGACAGGTACGAGC
TGGTGAAGGAGATCGGGTTCGGGGAACCTTCGGCGTCGCCCGCCTCATGCGCAACCGCGCCTCCGGCGACCT
CGTCGCCGTCAAGTACATCGACCGCGGGCGAGAAGATTGACGAGAACGTGCAGAGGGAGATCATCAACCAC
AGGTCGCTGCGCCACCCCAACATCATCCGATTCAAGGAGGTTATTCTGACGCCGACGCATCTCGCGATCG
TCATGGAGTACGCCTCCGGCGGGCGAGCTCTTCGAGCGCATCTGCAGCGCCGGCCGCTTCAGCGAGGACGA
GGCTCGTTTCTTCTCCAGCAGCTGATATCTGGAGTTAGCTACTGCCATTCCATGCAAGTATGCCATCGT
GACTTAAAGCTGGAGAACACTCTGCTAGATGGAAGTACTGCTCCTCGTTGAAGATATGTGACTTTGGTT
ACTCGAAGTCATCGGTTCTTCATTCACAACCAAAATCAACAGTTGGAACTCCAGCTTATATTGCTCCAGA
AGTTTTGCTCAAGAAAGAATACGATGGAAAGATTGCCGATGTTTGGTCATGCGGTGTGACGCTCTACGTG
ATGTTGGTTGGCGCATAACCCTTTCGAGGATCCTGAAGATCCCAAGAACTTCAGAAAGACAATTCAGAAAA
TATTGGGTGTTCACTCAATTCCAGACTATGTCCACATATCTCCGGAGTGCCGCGATCTCATTACGAG
GATTTTTGTTGGCAACCCAGCTAGTAGGATCACCATGCCTGAGATAAAGAACCACCCATGGTTCATGAAG
AACATCCCGGCTGACCTCATGGATGATGGCATGGTTAGCAATCAGTACGAGGAGCCTGACCAGCCGATGC
AGAATATGAACGAGATCATGCAGATACTGGCAGAAGCAACAATTCAGCAGCAGGCACCCAGTGGAATCAA
CCAGTTCTTGACTGACAGCCTTGACCTCGACGACGACATGGAGGATATGGACTCGGACCTTGACCTTGAC
ATTGAGAGCAGCGGAGAGATCGTATATGCCATG**TAA**

1086bp

protein

Evidence at transcript level 361aa

- >SAPK9

MERAAAGPLGMEMPIMHDGDRYELVKEIGSGNFGVARLMRNRASGDL
VAVKYIDRGEKIDENVQREIINHRSLRHPNIIRFKEVILTPTHLAIVMEYASG
GELFERICSAGRFSEDEARFFFQQLISGVSYSCHSMQVCHRDLKLENTLLDG
STAPRLKICDFGYSKSSVLHSQPKSTVGTPAYIAPEVLLKKEYDGKIADVWS
CGVTLYVMLVGAYPFEDPEDPKNFRKTIQKILGVQYSIPDYVHISPECRDLIT
RIFVGNPASRITMPEIKNHPWFMKNIPADLMDDGMVSNQYEEPQPM
QNMNEIMQILAEATIPAAGTSGINQFLTDSLDDDDMEDMDSDDLDDIE
SSGEIVYAM

Regions

<input type="checkbox"/>	Domain	22 – 278	257	Protein kinase	
<input type="checkbox"/>	Nucleotide binding	28 – 36	9	ATP <small>By similarity</small>	
<input checked="" type="checkbox"/>	Compositional bias	332 – 350	19	Asp-rich	

Sites

<input type="checkbox"/>	Active site	141	1	Proton acceptor <small>By similarity</small>	
<input type="checkbox"/>	Binding site	51	1	ATP <small>By similarity</small>	

The analysis of AA in the SAPK9

- Molecular weight = 40628.32
- Residues = 361
- Average Residue Weight = 112.544
- Charge = -16.5
- Isoelectric Point = 4.5669
- A280 Molar Extinction Coefficient = 28020
- A280 Extinction Coefficient 1mg/ml = 0.69
- Probability of expression in inclusion bodies = 0.682

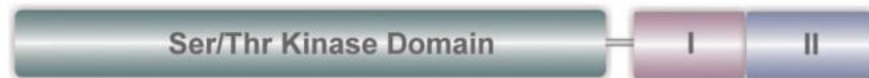
• Residue	Number	Mole%	DayhoffStat
• A = Ala	22	6.094	0.709
• B = Asx	0	0.000	0.000
• C = Cys	6	1.662	0.573
• D = Asp	29	8.033	1.461
• E = Glu	27	7.479	1.247
• F = Phe	13	3.601	1.000
• G = Gly	24	6.648	0.791
• H = His	9	2.493	1.247
• I = Ile	30	8.310	1.847
• J = ---	0	0.000	0.000
• K = Lys	16	4.432	0.672
• L = Leu	28	7.756	1.048
• M = Met	18	4.986	2.933
• N = Asn	14	3.878	0.902
• O = ---	0	0.000	0.000
• P = Pro	20	5.540	1.065
• Q = Gln	12	3.324	0.852
• R = Arg	19	5.263	1.074
• S = Ser	25	6.925	0.989
• T = Thr	13	3.601	0.590
• U = ---	0	0.000	0.000
• V = Val	21	5.817	0.881
• W = Trp	2	0.554	0.426
• X = Xaa	0	0.000	0.000
• Y = Tyr	13	3.601	1.059
• Z = Glx	0	0.000	0.000

Property	Residues	Number	Mole%
• Tiny	(A+C+G+S+T)	90	24.931
• Small	(A+B+C+D+G+N+P+S+T+V)	174	48.199
• Aliphatic	(A+I+L+V)	101	27.978
• Aromatic	(F+H+W+Y)	37	10.249
• Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	197	54.571
• Polar	(D+E+H+K+N+Q+R+S+T+Z)	164	45.429
• Charged	(B+D+E+H+K+R+Z)	100	27.701
• Basic	(H+K+R)	44	12.188
• Acidic	(B+D+E+Z)	56	15.512

Phylogenetic Analysis

- Using Blast to search the ten sequences (SAPK1-10) from the SnRKs family of *Oryza sativa* and the ten sequences (SRK2A-SRK2J) from the SnRKs family of *Arabidopsis thaliana*.
- Using MEGA5.0 to construct a neighbor-joining tree of the 20 sequences.

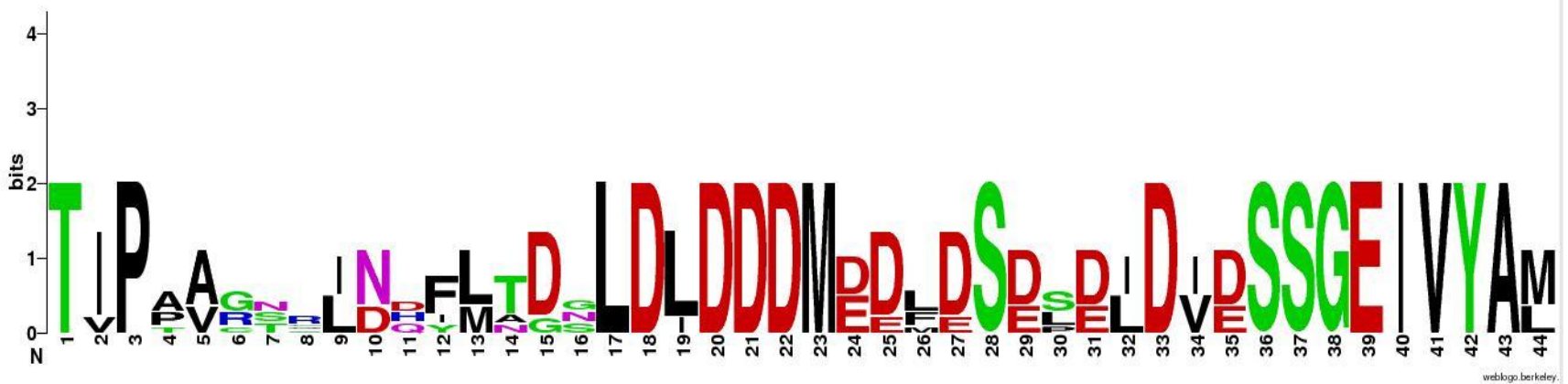
Structure of Arabidopsis thaliana SnRK2s



- Ser/Thr kinase domain —kinase catalytic
- domain, I —domain involved in ABA-independent activation in response to osmotic stress, characteristic for all SnRK2.
- Domain, II — needed for ABA-dependent activation of SnRK2s.

(Anna Kulik et al., 2011)

SAPK8-10 & SRK2E,2D,2I Domain II----Weblogo

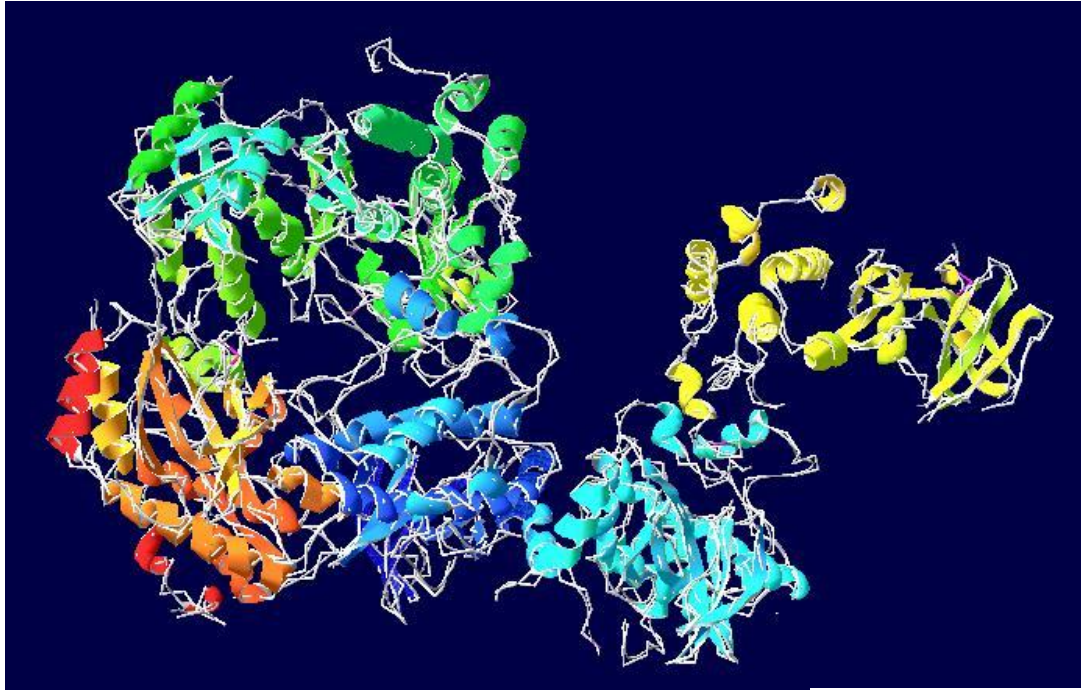


SAPK9---SRK2E Pairwise Alignment Result

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
363	1521.0	294/363 (81.0%)	331/363 (91.2%)	3/363 (0.8%)

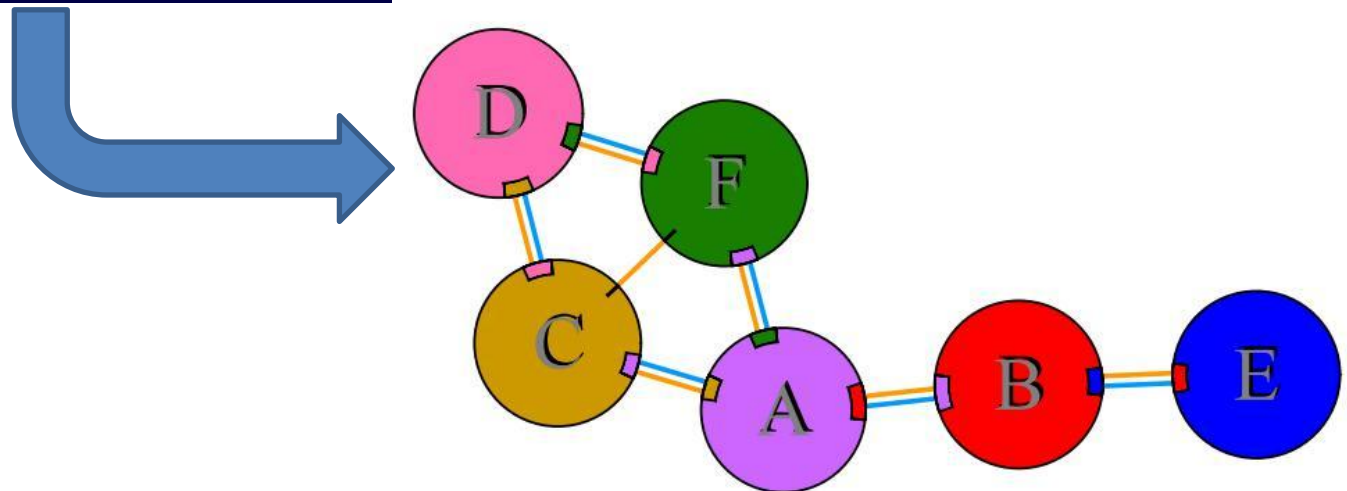
SRK2E	1	-MDRPAVSGPMDLPIMHDSDRYELVKDIGSGNFGVARLMRDKQSNELVAV	49
	 :: . : : : . .:	
SAPK9	1	MERAAAGPLGMEMPIMHDGDRYELVKEIGSGNFGVARLMRNRASGDLVAV	50
SRK2E	50	KYIERGEKIDENVKREIINHRSLRHPNIVRFKEVILTPTHLAIVMEYASG	99
		: : :	
SAPK9	51	KYIDRGEKIDENVQREIINHRSLRHPNIIRFKEVILTPTHLAIVMEYASG	100
SRK2E	100	GELFERICNAGRFEDEARFFFQQLISGVSYCHAMQVCHRDLKLENTLLD	149
		:	
SAPK9	101	GELFERICSAGRFEDEARFFFQQLISGVSYCHSMQVCHRDLKLENTLLD	150
SRK2E	150	GSPAPRLKICDFGYSKSSVLHSQPKSTVGTPAYIAPEVLLKKEYDGKVAD	199
		.	
SAPK9	151	GSTAPRLKICDFGYSKSSVLHSQPKSTVGTPAYIAPEVLLKKEYDGKIAD	200
SRK2E	200	VWSCGVTLYVMLVGAYPFEDPEEPKNFRKTIHRILNVQYAIIPDYVHISPE	249
SAPK9	201	VWSCGVTLYVMLVGAYPFEDPEDPKNFRKTIQKILGVQYSIPDYVHISPE	250
SRK2E	250	CRHLISRIFVADPAKRISIPAIRNHEWFLKNLPADLMNDNTMTTQFDESD	299
		. : .:. . : : . : : : ...:. : .	
SAPK9	251	CRDLITRIFVGNPASRITMPEIKNHPWFMKNIPADLMDDGMVSNQYEEPD	300
SRK2E	300	QPGQSIIEIMQIIAEATVPPAGTQNLNHLYLTGSLDIDDDMEEDLESDDLDD	349
		. : . : : . .:. : . :	
SAPK9	301	QPMQNMNEIMQILAEATIPAAGTSGINQFLTDSLDDDDM-EDMDSL-D	348
SRK2E	350	LDIDSSGEIVYAM	362
		:	
SAPK9	349	LDIESSGEIVYAM	361

Goal 1: SAPK9 Structure Prediction

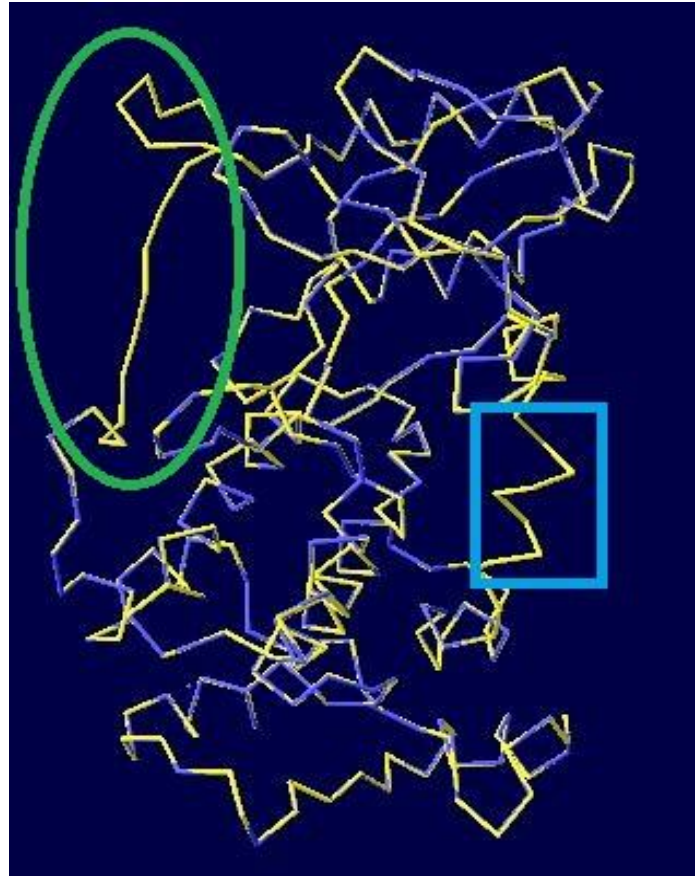


SRK2E: PDB
ID **3udb**

6 chains: A B D F 275aa
C E 261aa

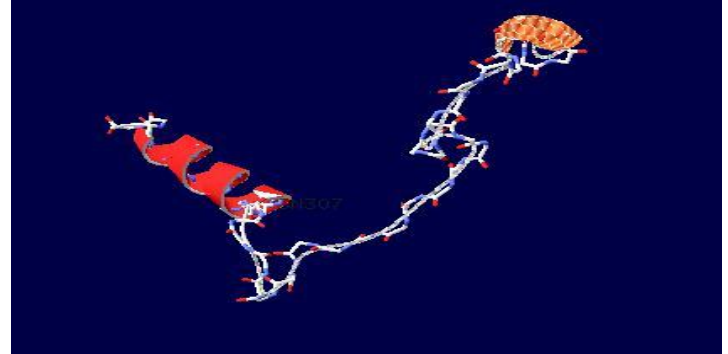
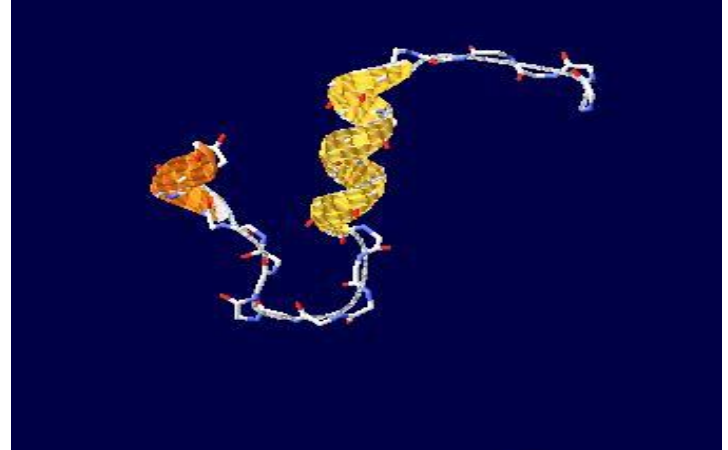
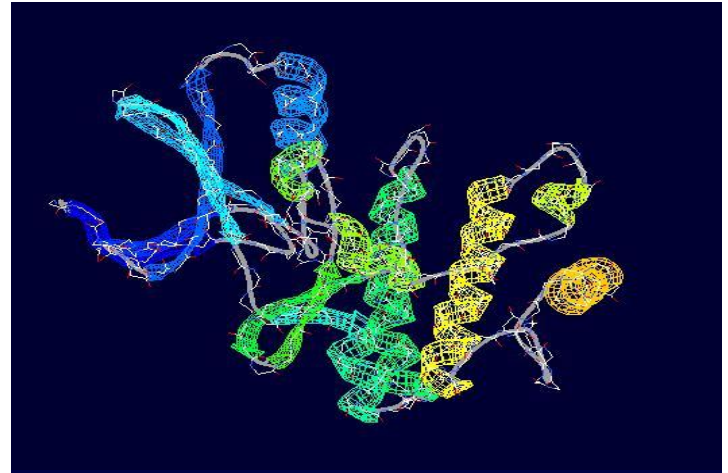
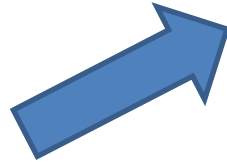
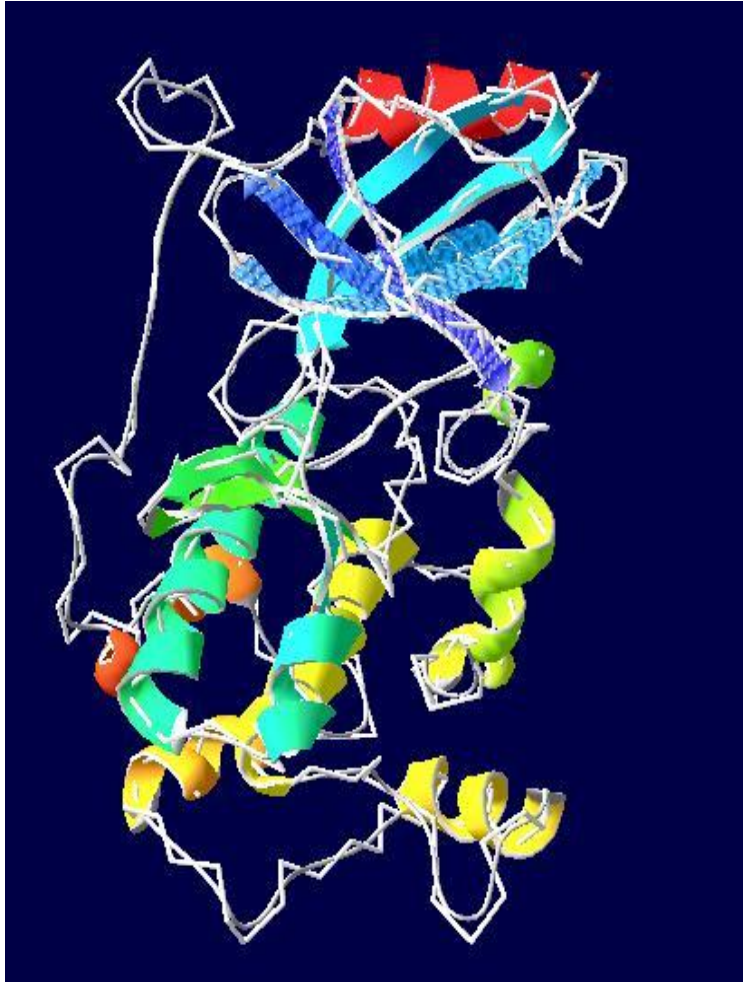


SAPK9&3udb.a----Interactive Magic Fit

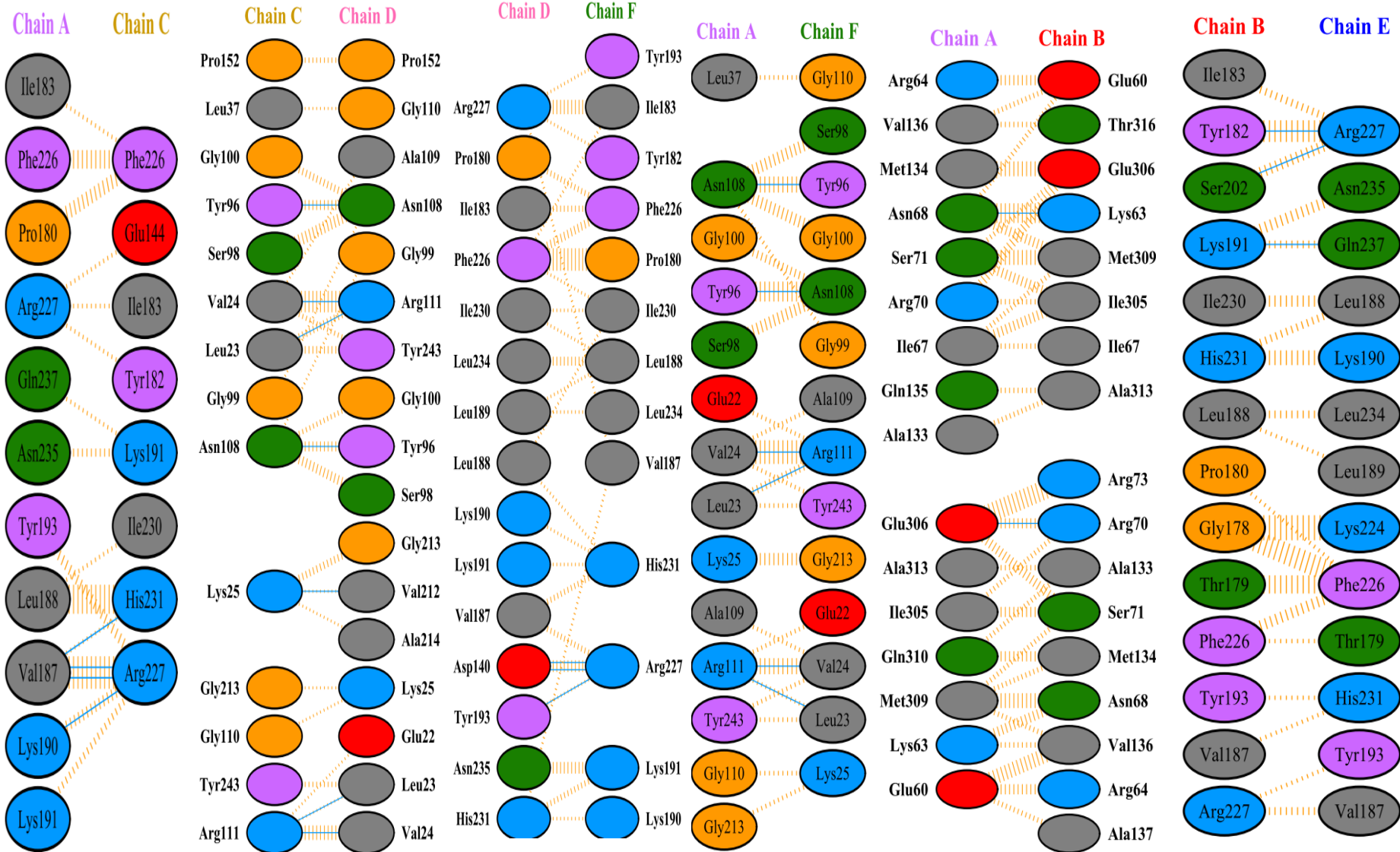
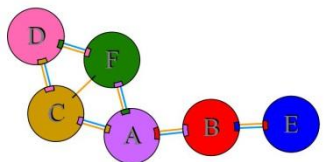


RMS=0.08

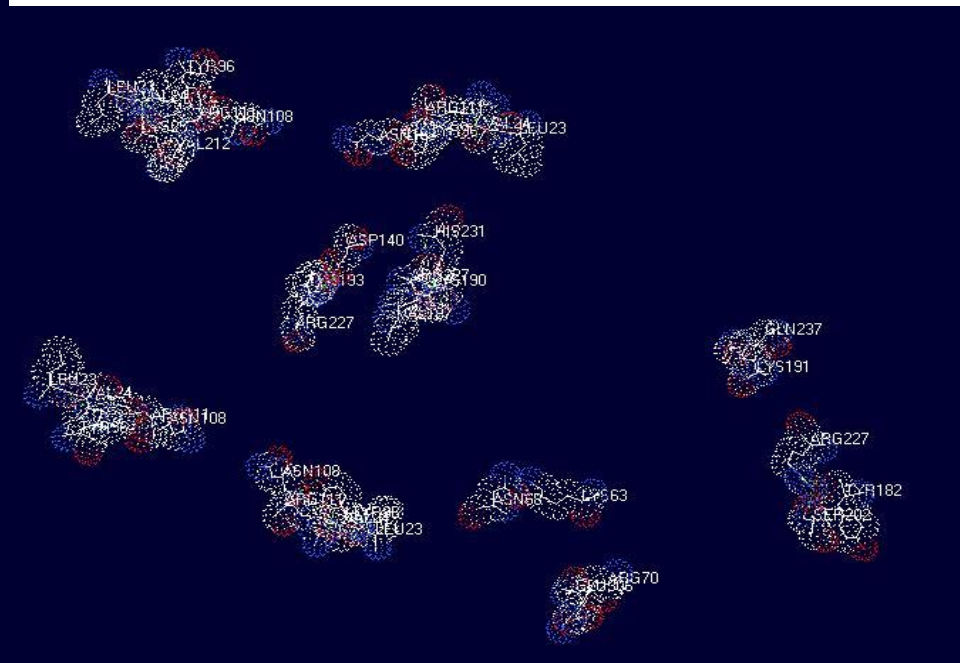
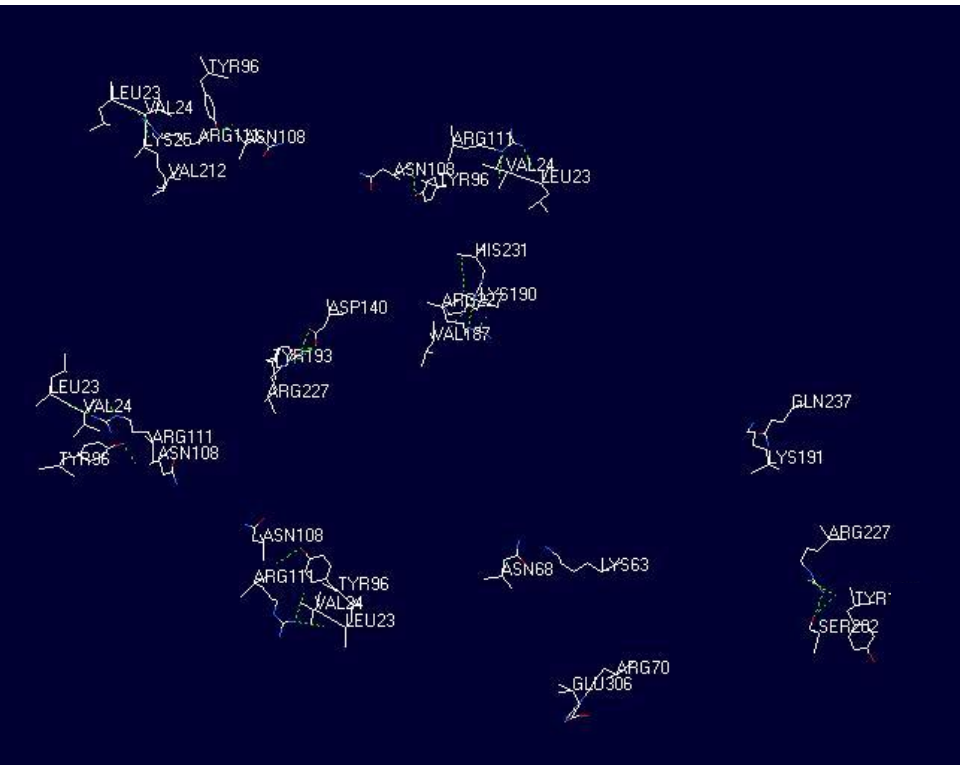
SAPK9 Domains in 3D Structure



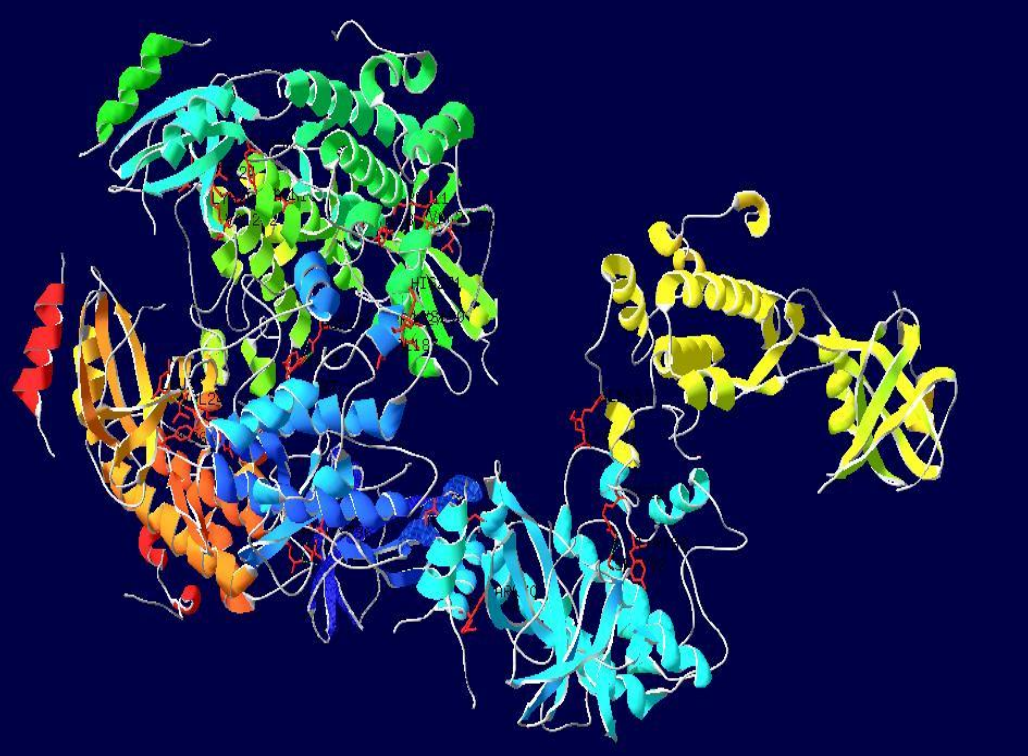
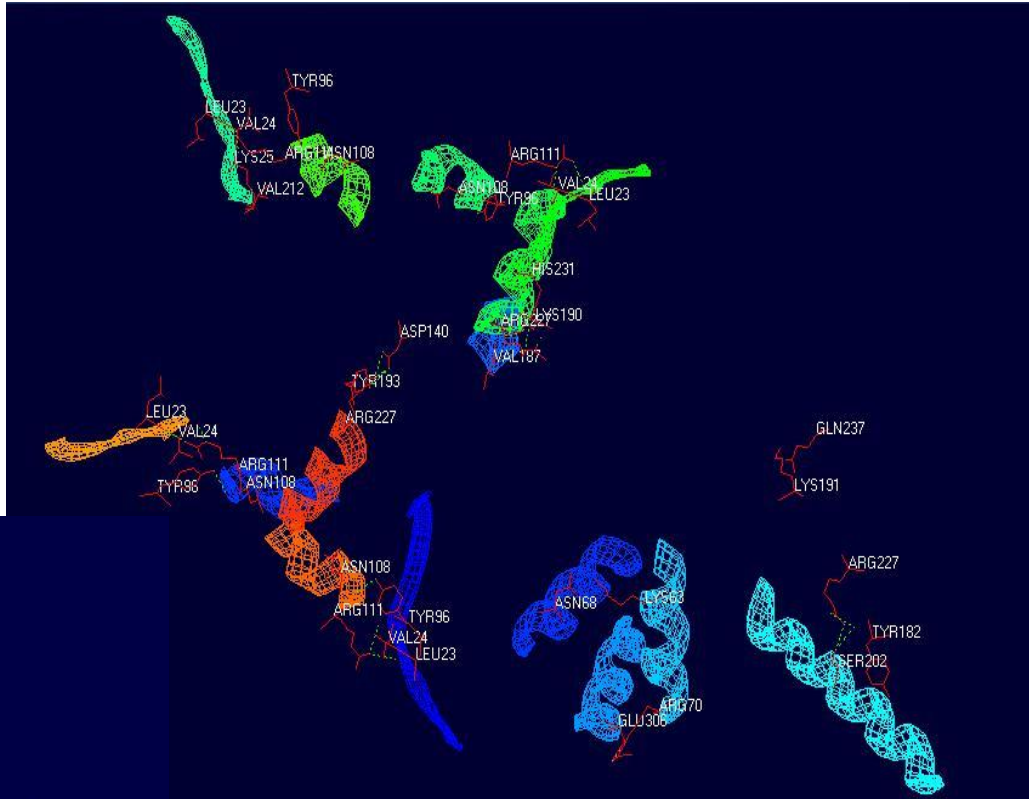
SRK2E: Interaction between chains



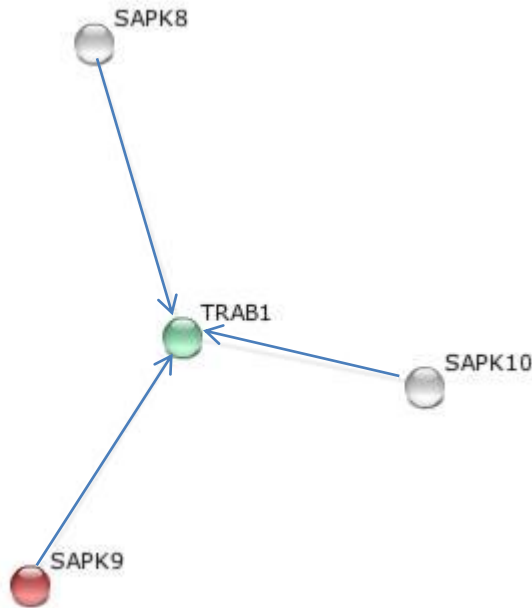
SRK2E: Interaction between chains



SRK2E: Interaction between chains



Goal 2 : SAPK9 function in ABA-signaling pathway leading to gene expression




TRAB1 [Close](#)

Actions

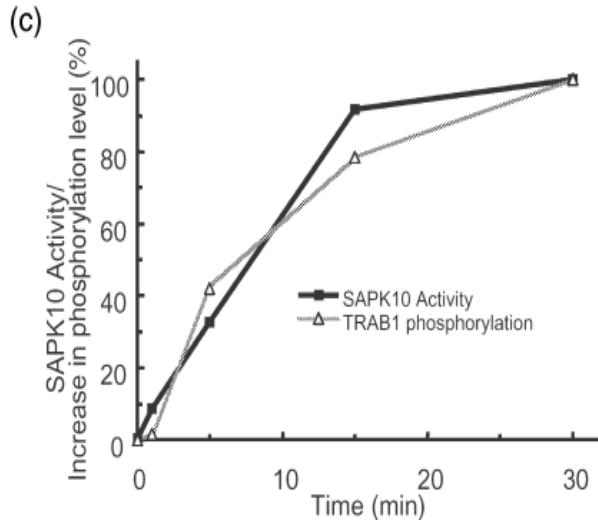
- [re-center network on this node](#)
- [add this node to input nodes](#)

Information

Os08g0472000 ; Transcription activator that mediates abscisic acid (ABA) signaling. Binds specifically to the ABA-responsive element (ABRE) of the EMP1 and RAB16A gene promoters

Identifier: BGIOSIBCE028180 

SAPK10 directly phosphorylates TRAB1



SAPK10 activities (closed squares) and TRAB1 phosphorylation levels (open triangles) were quantified using the radioactive and luminescence images obtained in the experiments. SAPK10 activities were normalized by immunoblot signals. Changes in the levels of Ser102-phosphorylation in TRAB1 were expressed as the increase in S-band intensity/total band intensity at each time point relative to the 0-min value.

(Yuhko Kobayashi et al., 2005)

TRAB1 aa Sequence

46

- >TRAB1
- MNMDELLRSIWTAESQAMASASAAAAAAEGGLQRQGS LTP**RTLS**VKTVDEVW
 RDLEREASPGAAAAD**GGGGGG**EQQQPRRQPTLGEMTLEEF LVRAGVVRENTAAA
 AAMVAAAAAPPVAPRSIPAVNNSSIFFGNYGGVND**AAAAA**GAMGFSPVGIGDP
 TMGNRLMSGVAGIGGGAITVAPVDTSVGQMDSAGKGDGDLSSPMAPVPYPFEG
 VIRGRRSGGNVEKVVE**RRQRRMIKNRESAARSARK**QAYTME**LEAEVQKLKEQNM**
ELQKKQEEIMEMQKNFFPEMQKNQVLEAVNNPYGQKKRCLRRTLTPW

<input type="checkbox"/> Chain	1 – 318	318	bZIP transcription factor TRAB1		PRO_0000377394
Regions					
<input type="checkbox"/> Domain	258 – 272	15	Leucine-zipper		
<input type="checkbox"/> DNA binding	232 – 251	20	Basic motif		
<input type="checkbox"/> Compositional bias	70 – 75	6	Poly-Gly		
<input type="checkbox"/> Compositional bias	144 – 151	8	Poly-Ala		

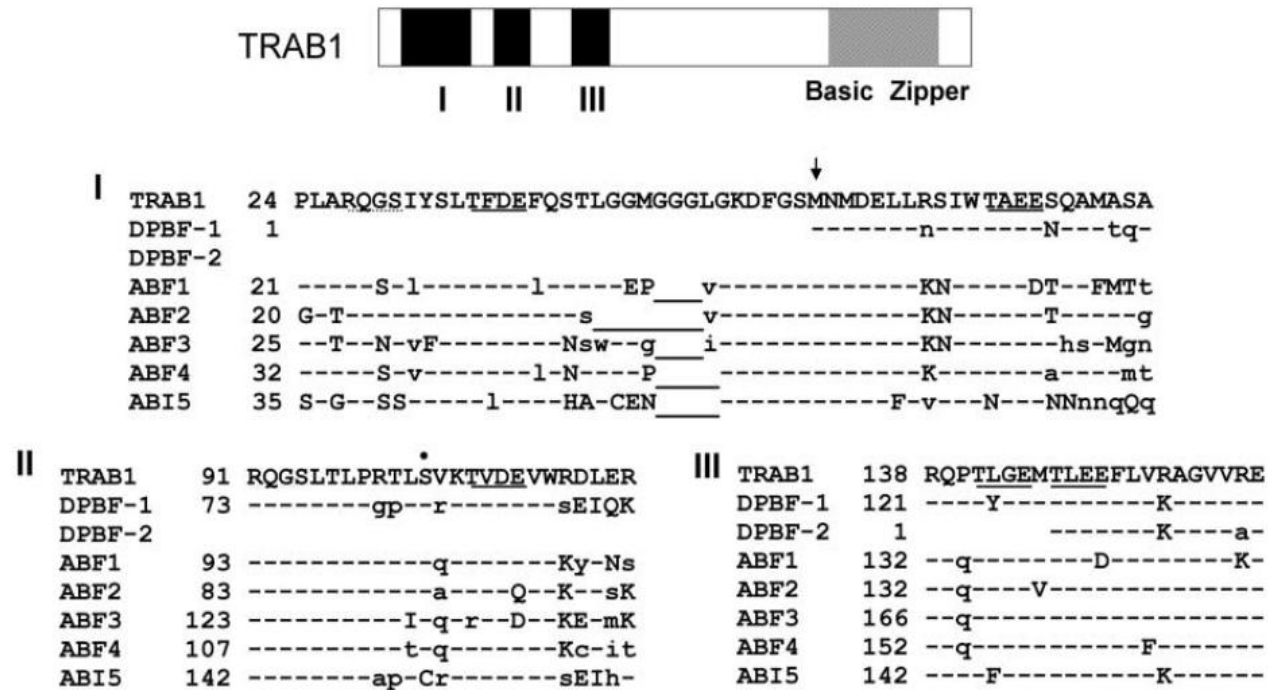
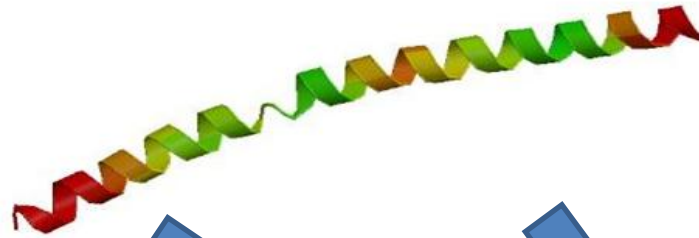


Figure 1. The Ser residue in the protein kinase C phosphorylation signature is indicated by a dot. The arrow points to the Met residue formerly predicted to be the N terminus from the sequence of the TRAB1 cDNA clone. The amino acids of TRAB1 are numbered based on the putative full-length sequence, which includes the N-terminal 56 amino acids predicted from the genome sequence obtained from the Syngenta draft sequence database .

(Yasuaki Kagaya et al., 2002)

The 3D structure prediction of Trab1

Length : ARG233—MET283 51AA

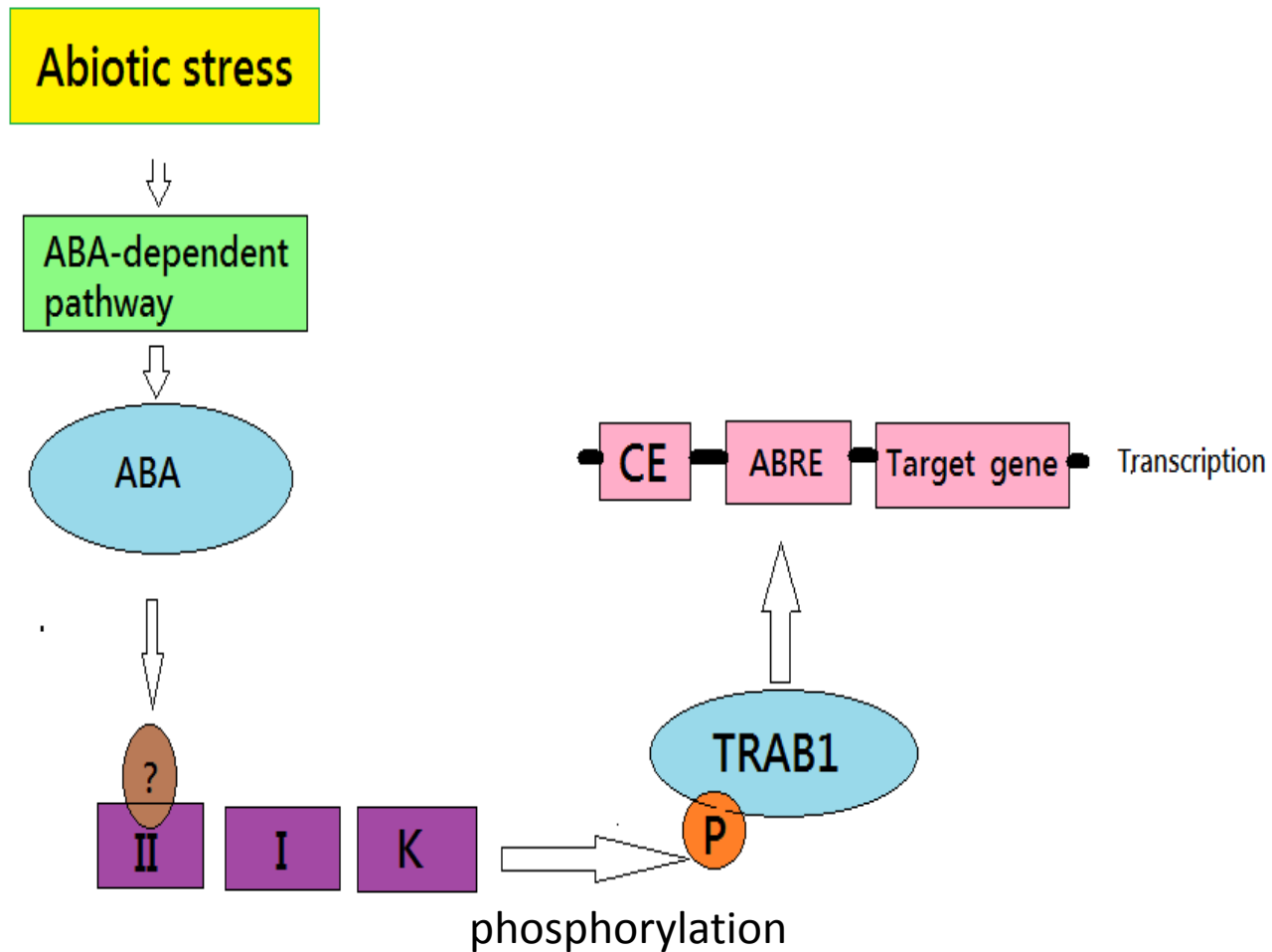


Leucine Zipper(258-272)

DNA binding(232-251)



Signaling pathway predication



致谢

感谢罗静初老师的精心指导！
感谢小组成员的共同努力！
感谢实验室的黄立钰师兄和
张帆同学提供的建议！



Reference

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