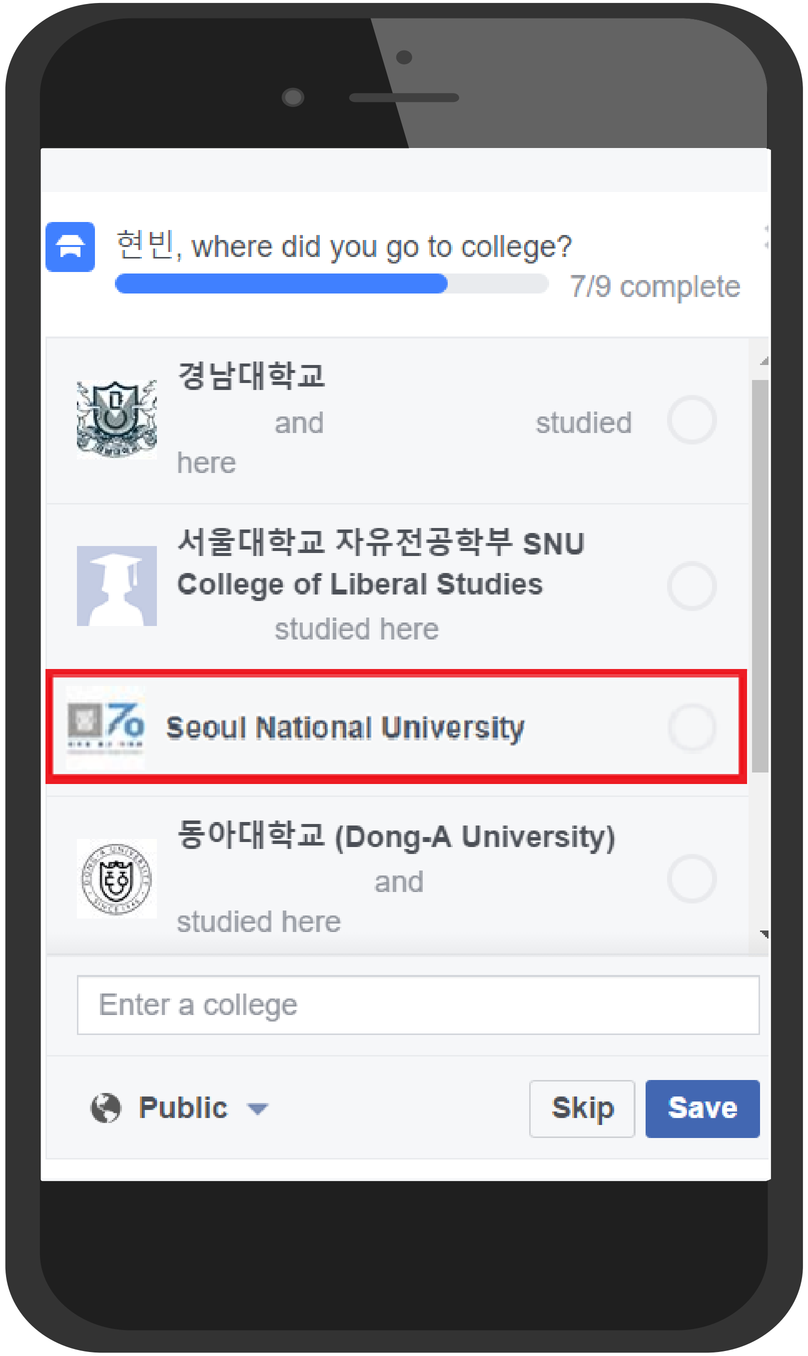
MagNet: the integrated gene network of the rice blast fungus *Magnaporthe oryzae*

Hyunbin Kim
Seoul National University

How Facebook knows you?

How can Facebook predict the college which I graduated from?



현빈, where did you go to college?

7/9 complete

경남대학교

and studied here ○

서울대학교 자유전공학부 SNU College of Liberal Studies

studied here ○

Seoul National University ○

동아대학교 (Dong-A University)

and studied here ○

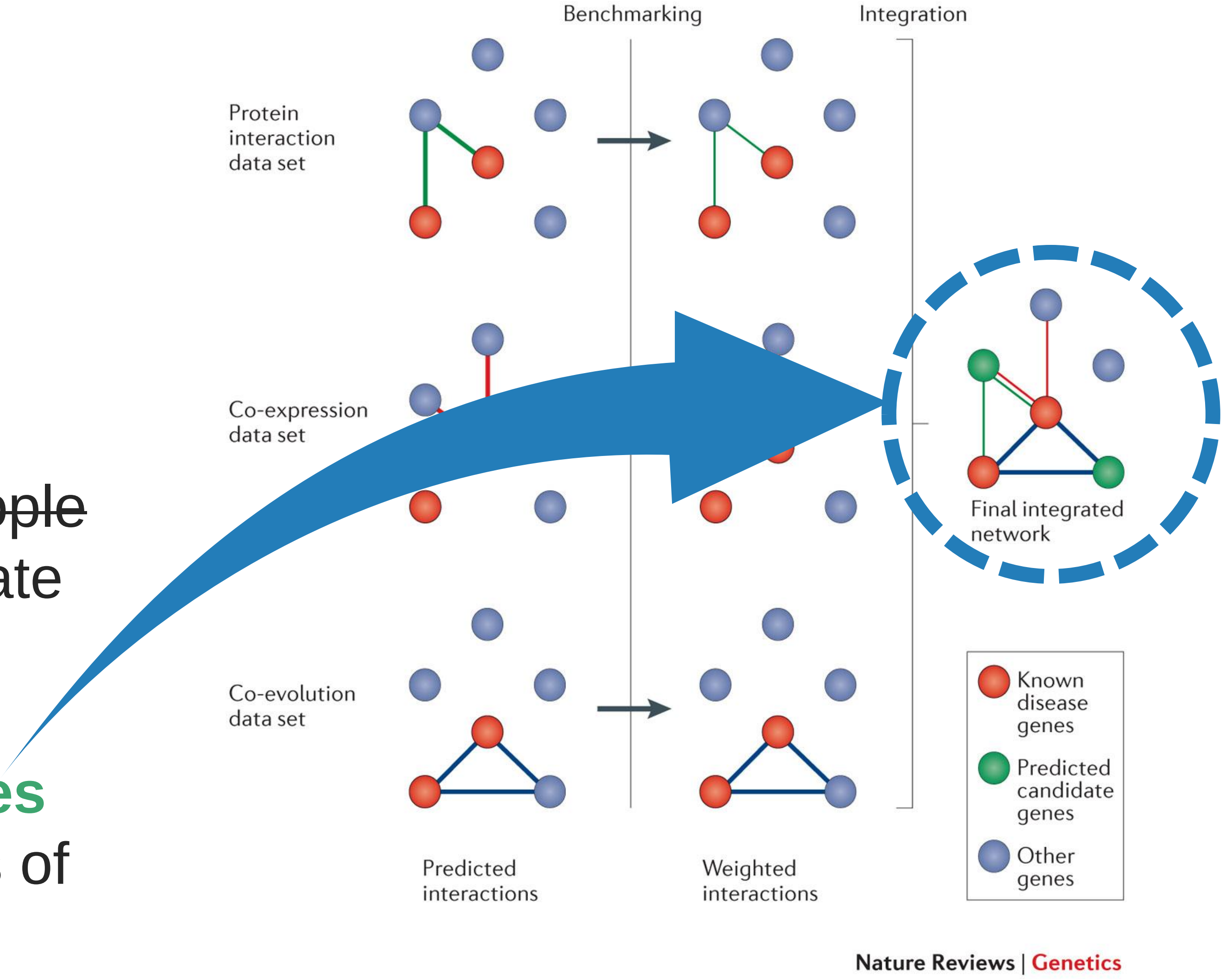
Enter a college

Biological network

Guilt-by association

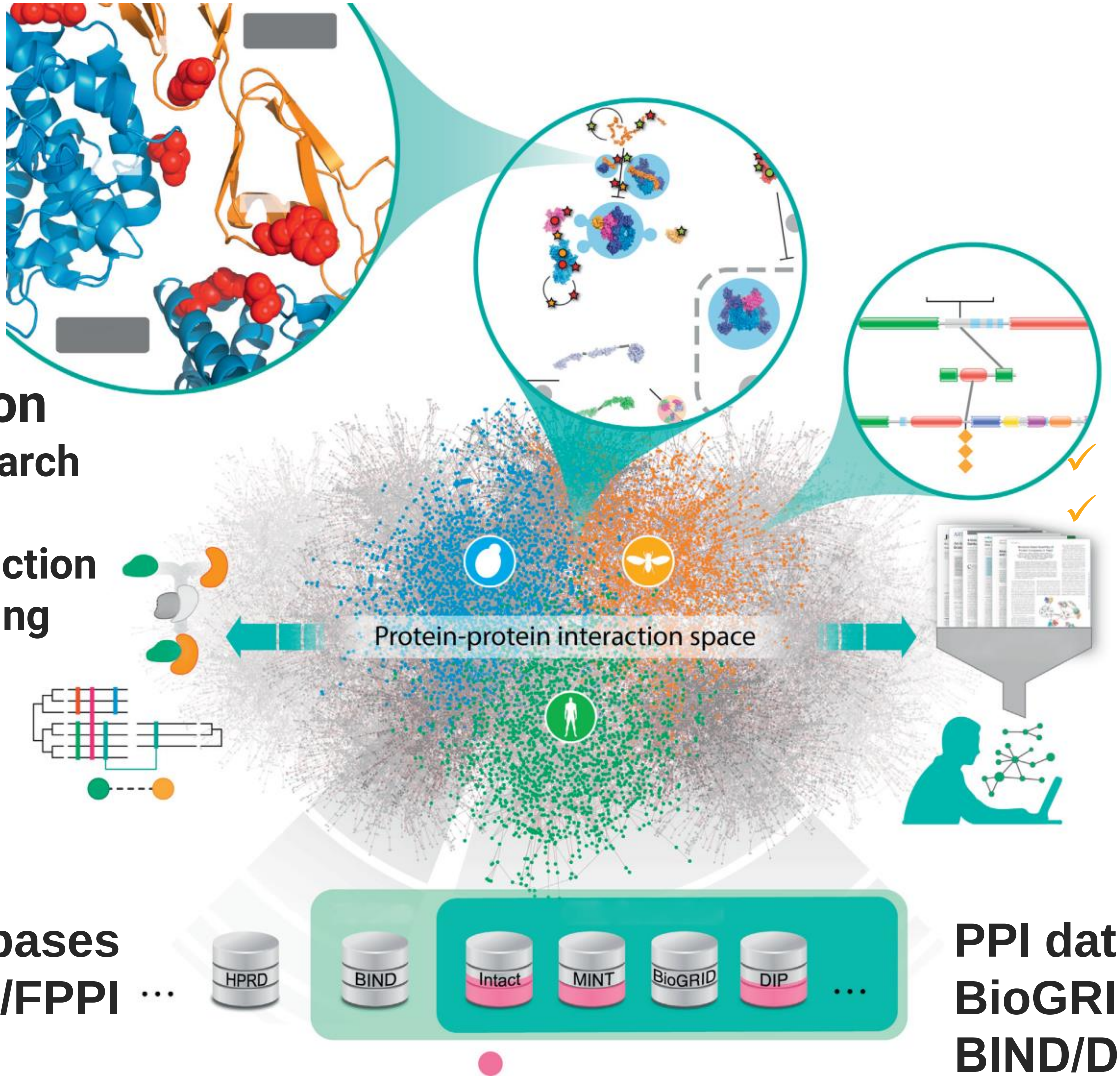
The attribution of ~~guilt~~ **function** to individuals because of the ~~people~~ **proteins** with which they associate

You can **predict candidate genes** for a disease with gene networks of **known disease genes**



Lehner, B. 2013. *Nature Reviews Genetics*

Protein-Protein Interaction (PPI)



Computational Prediction

- ✓ Homology-based Interolog search
- ✓ Domain-Domain interaction
- ✓ Machine-learning based prediction
- ✓ Structure-based protein docking

Experiments

- ✓ Yeast two-hybrid screening
- ✓ Affinity purification coupled to MS

Predicted PPI databases
STRING/MPID/FPPI …

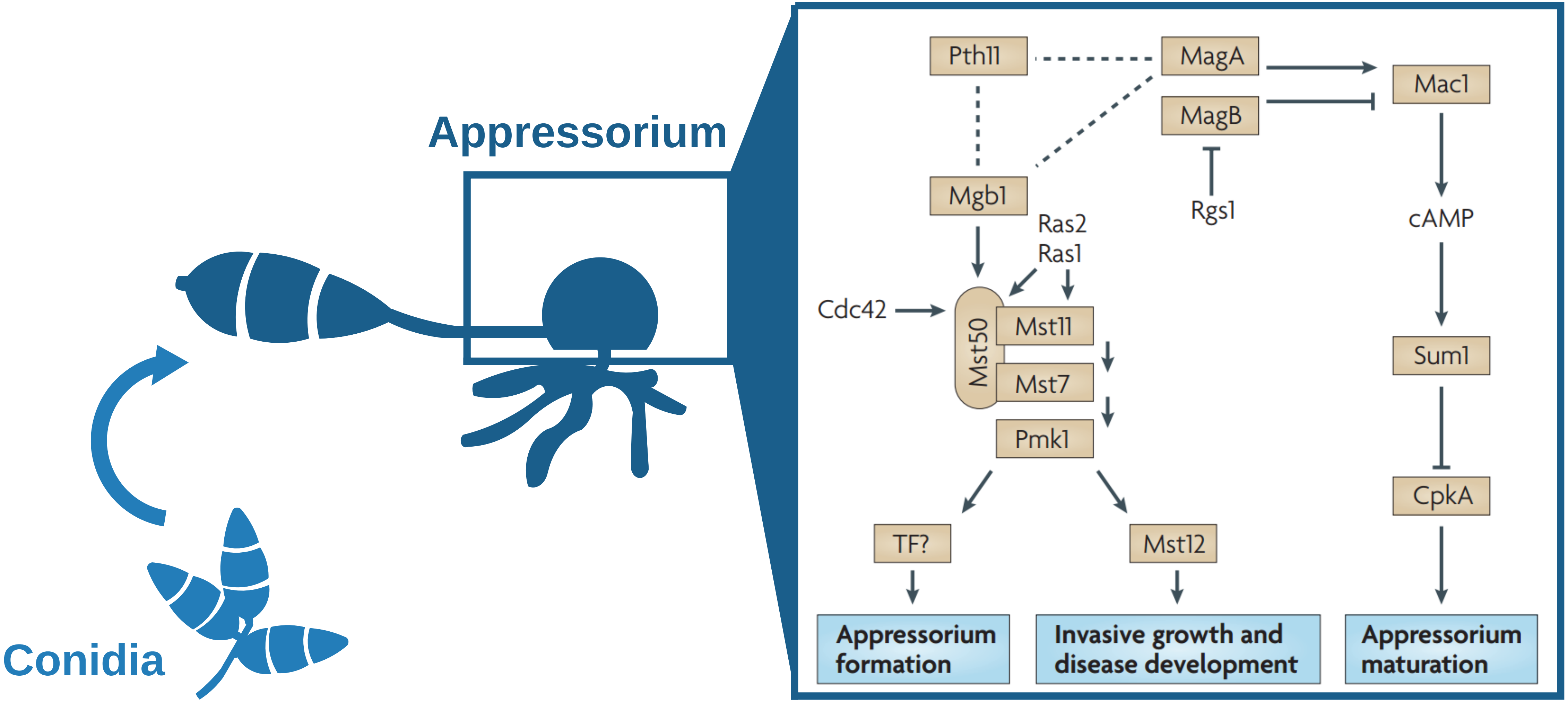
PPI databases
BioGRID/Intact/MINT/
BIND/DIP …

Mosca, R., 2013, *Current opinion in structural biology*

Magnaporthe oryzae

The rice blast caused by *Magnaporthe oryzae* occurs worldwide and is one of the most important plant diseases. Several rice blast epidemics result in great yield losses of the expected crop.

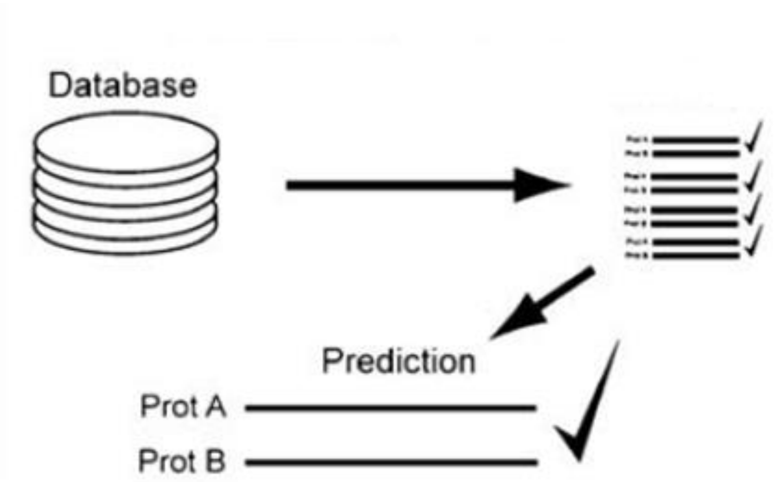
Magnaporthe oryzae



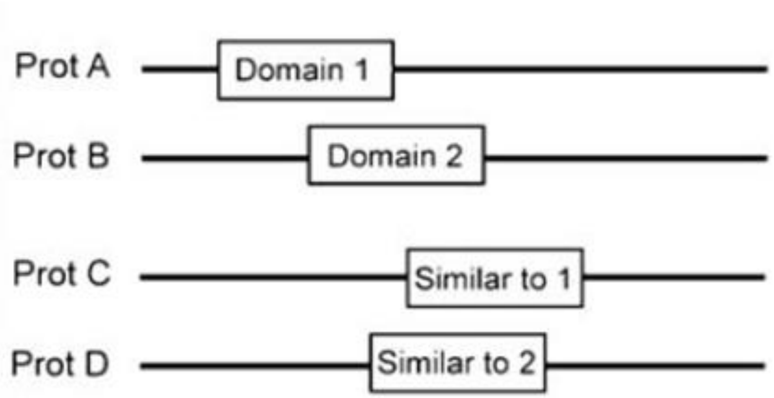
Wilson RA, 2009, *Nature Reviews Microbiology*

Construction method of MagNet

1. Homology based “Interolog” search

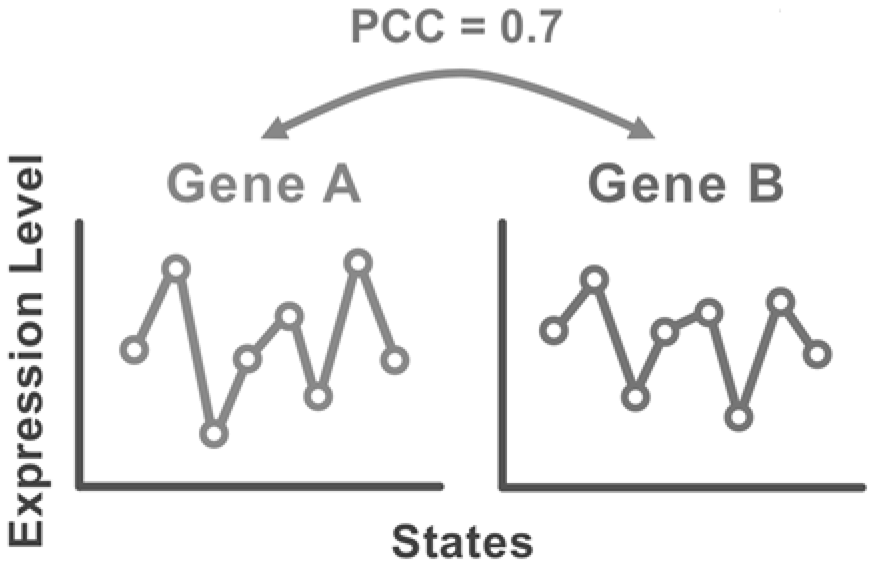


2. Domain-Domain Interaction



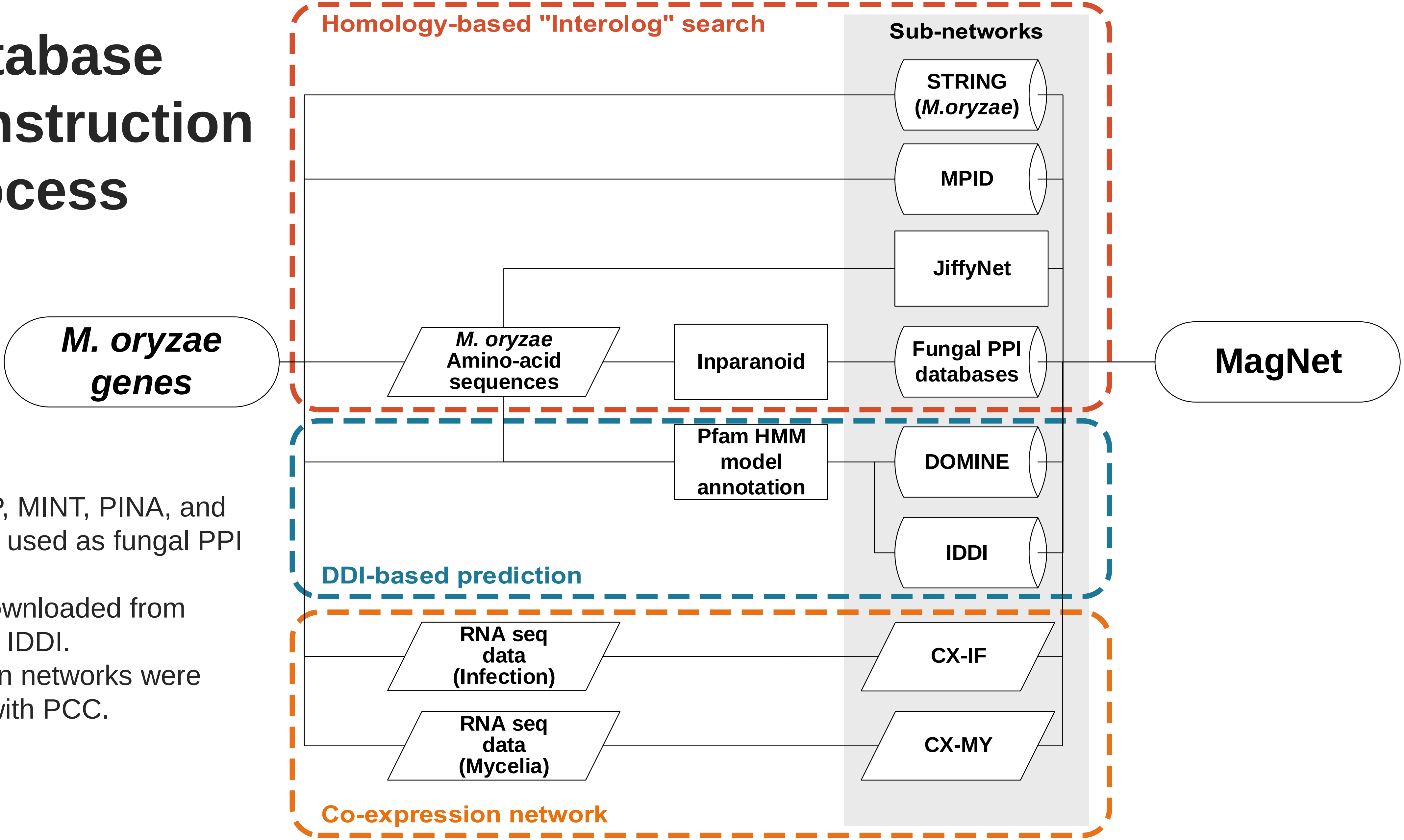
Pitre, S. 2008. *Protein–Protein Interaction*. Springer Berlin Heidelberg.

3. Co-expression network



Aoki, Y. 2016. *Plant and Cell Physiology*

Database construction process

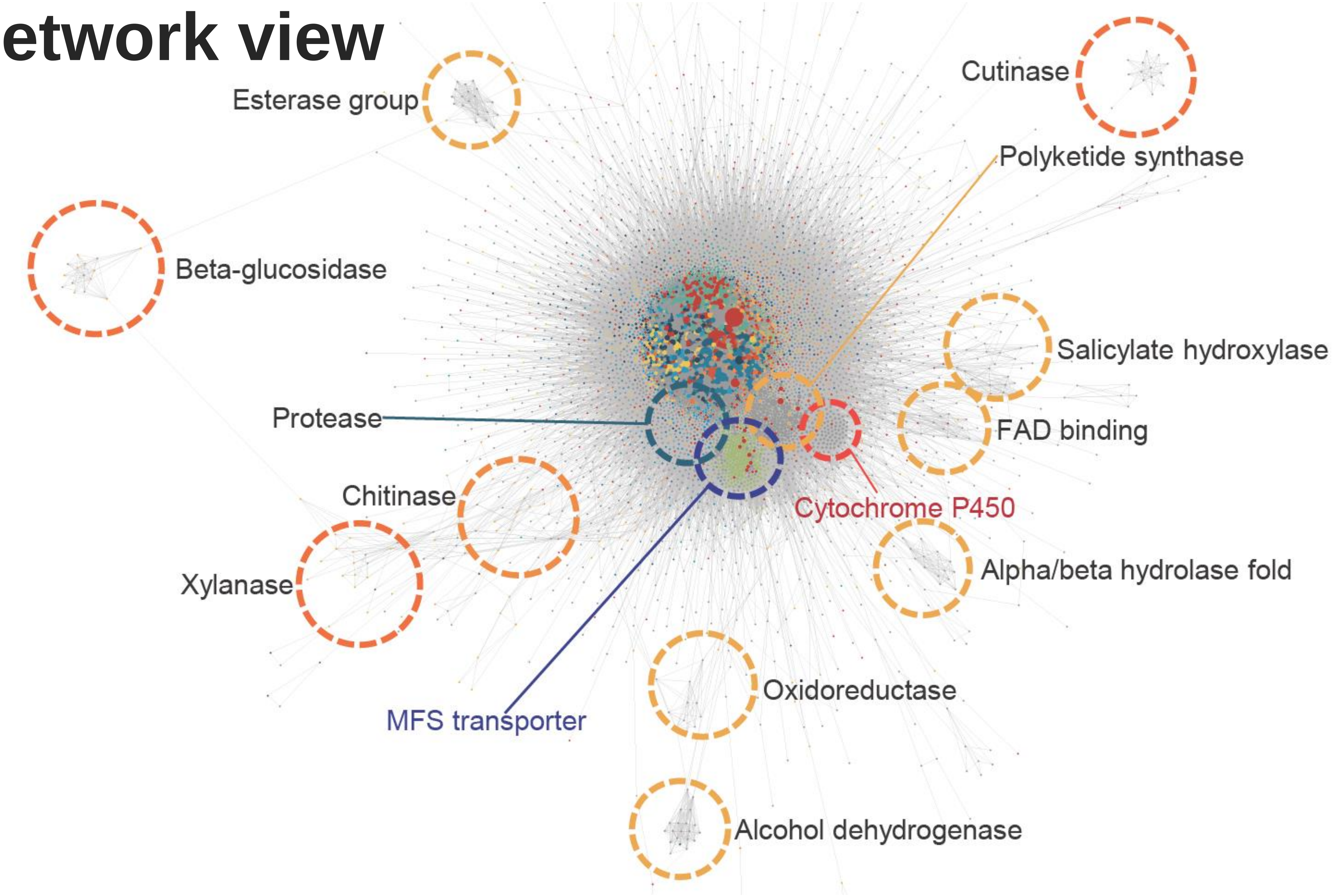


- BioGRID, DIP, MINT, PINA, and INTACT were used as fungal PPI repositories.
- DDIs were downloaded from DOMINE and IDDI.
- Co-expression networks were constructed with PCC.

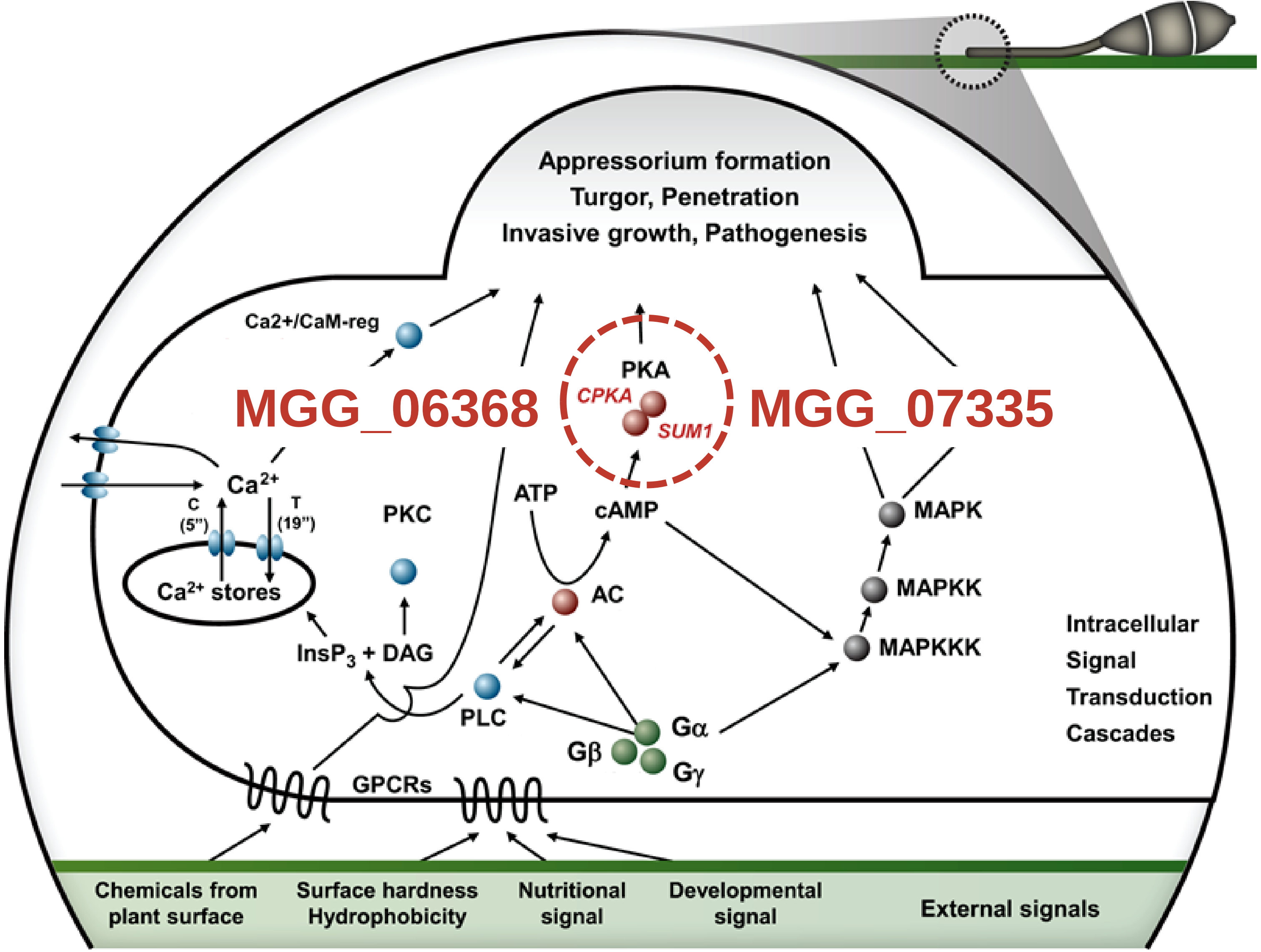
Size of MagNet and sub-networks

Network	Proteins	Interactions
STRING	6,498	1,097,966
MPID	3,017	11,674
BioGRID	3,503	166,492
JiffyNet	4,690	266,409
Vegetative stage	10,356	1,173,767
Infection stage	8,131	819,480
DOMINE	5,727	1,450,218
IDDI	6,231	4,247,425
MagNet (over 3)	6,005	217,531
MagNet	11,734	5,600,976

Network view



PKA pathway in MagNet

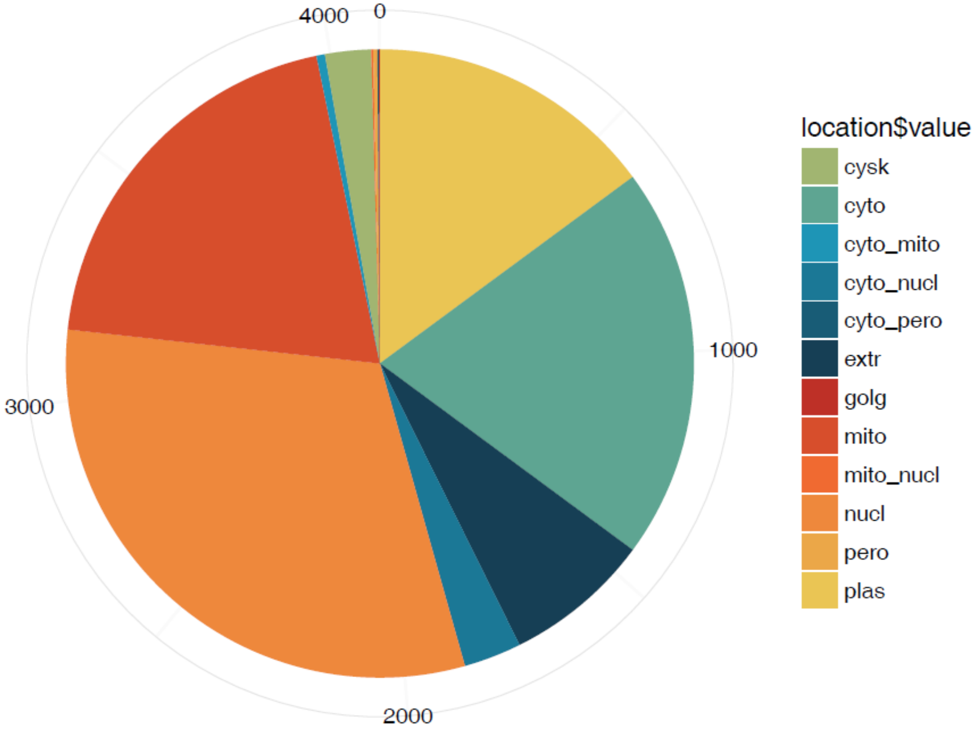


CPKA (MGG_06368)

		Interolog									Domain		Co-expression							
protein1	protein2	BIOGRID	DIP	INTACT	JIFFY	MINT	MPID	PINA	STR	STR_0.7	DOM	IDDI	CX_IF	CX_MY	CX_PROT	ATMT	BLAST	description	localization	sum
MGG_06368	MGG_07335	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	cAMP-dependent protein kinase regulatory subunit	cyto_nucl	11
MGG_06368	MGG_06399	1	0	1	1	1	0	1	1	0	1	1	0	0	0	0	1	CMGC/DYRK/YAK protein kinase	nucl	9
MGG_06368	MGG_14773	1	0	0	1	0	0	0	1	1	1	1	1	0	0	0	1	AGC/AKT protein kinase	nucl	8
MGG_06368	MGG_04714	1	0	1	0	1	0	1	1	0	1	1	1	0	0	0	0	DNA-directed RNA polymerase II subunit RPB2	nucl	8
MGG_06368	MGG_03729	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	1	CAMK/CAMKL/CHK1 protein kinase	plas	8
MGG_06368	MGG_08065	1	0	1	1	1	0	1	1	0	1	1	0	0	0	0	0	WD domain-containing protein	nucl	8
MGG_06368	MGG_00371	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	cell division control protein 25	nucl	7
MGG_06368	MGG_17442	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	hypothetical protein	cysk	7
MGG_06368	MGG_02829	0	0	0	1	0	0	0	1	0	1	1	1	0	0	0	1	CK1/CK1/CK1-D protein kinase	cyto	6
MGG_06368	MGG_03064	1	0	0	1	0	0	0	1	0	1	1	1	0	0	0	0	Rho1 guanine nucleotide exchange factor 1	nucl	6
MGG_06368	MGG_06154	1	0	0	1	0	0	0	1	0	1	1	0	1	0	0	0	Ras-like protein	nucl	6
MGG_06368	MGG_00345	1	0	0	1	0	0	0	1	0	1	1	0	0	0	0	1	AGC protein kinase	nucl	6
MGG_06368	MGG_06599	1	0	0	1	0	0	0	1	0	1	1	0	0	0	0	1	AGC/AKT protein kinase	mito	6
MGG_06368	MGG_09912	1	0	0	1	0	0	0	1	0	1	1	0	0	0	0	1	CAMK/CAMK1 protein kinase	cyto	6
MGG_06368	MGG_00101	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	ATPase NPA3	cyto	6
MGG_06368	MGG_03165	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	heat shock protein 60	mito	6
MGG_06368	MGG_03215	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	DNA-directed RNA polymerase II subunit RPB3	cyto	6
MGG_06368	MGG_04652	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	DNA-directed RNA polymerase II largest subunit	cyto	6
MGG_06368	MGG_09898	1	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	adenylate cyclase	nucl	6
MGG_06368	MGG_11491	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	transcription elongation factor SPT6	nucl	6
MGG_06368	MGG_15672	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	threonyl-tRNA synthetase	mito	6

Interaction partners of CPKA

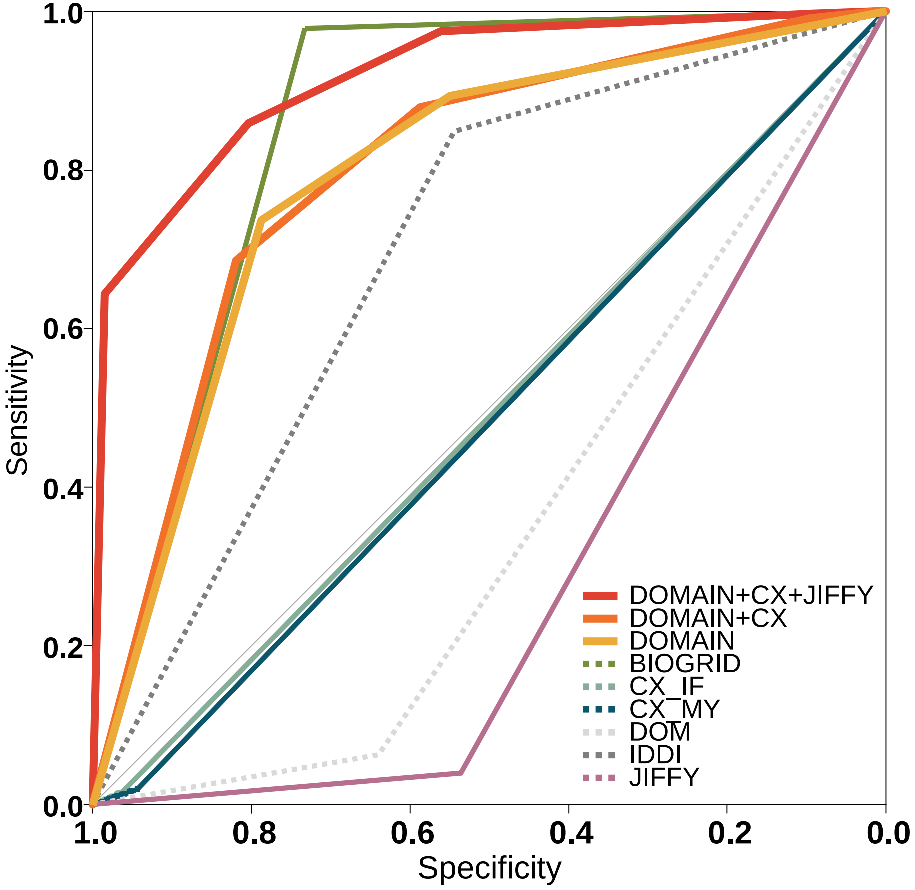
Subcellular localization of CpkA neigbors



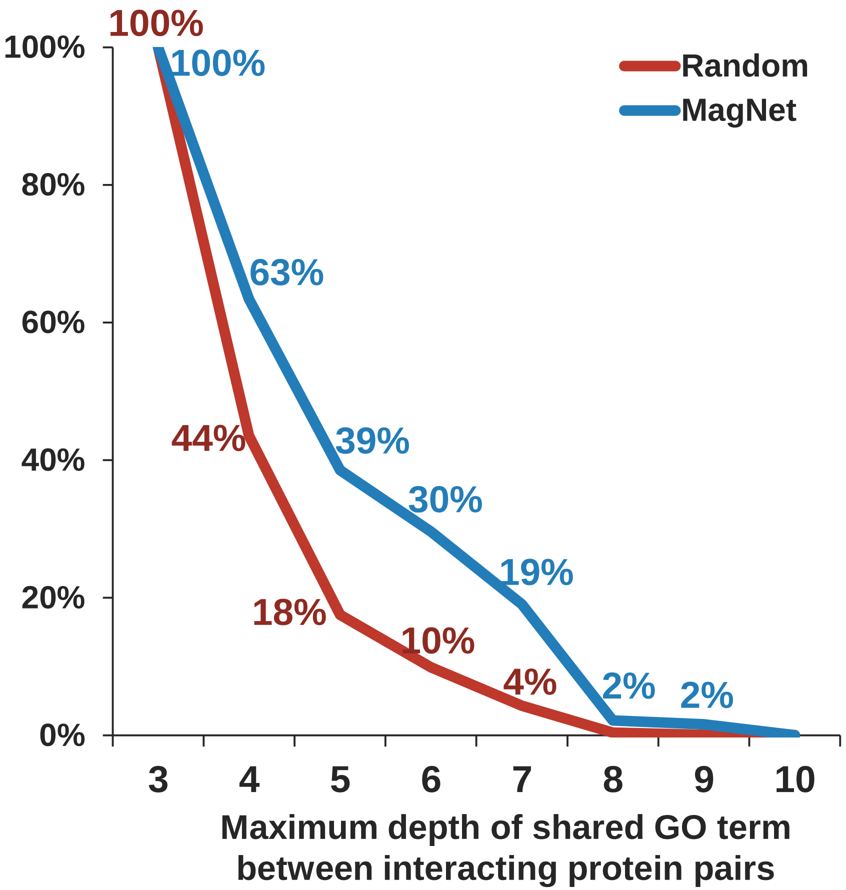
Enriched GO terms of CpkA neighbors

GO:0009987	BP	cellular process
GO:0006468	**BP**	**protein phosphorylation**
GO:0031326	BP	regulation of cellular biosynthetic process
GO:0051171	BP	regulation of nitrogen compound metabolic process
GO:0019219	BP	regulation of nucleobase-containing compound metabolic process
GO:0006200	BP	obsolete ATP catabolic process
GO:0006184	BP	obsolete GTP catabolic process
GO:0051169	BP	nuclear transport
GO:0007018	BP	microtubule-based movement
GO:1901363	MF	heterocyclic compound binding
GO:0017111	MF	nucleoside-triphosphatase activity
GO:0004672	**MF**	**protein kinase activity**
GO:0004497	MF	monooxygenase activity
GO:0051192	MF	prosthetic group binding
GO:0016705	MF	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen
GO:0016620	MF	oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor
GO:0015662	MF	ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism
GO:0016877	MF	ligase activity, forming carbon-sulfur bonds

Validation with computational methods



ROC curve of MagNet subnetworks tested with MPID & STRING intersection



Validation of the predicted PPI network based on GO terms

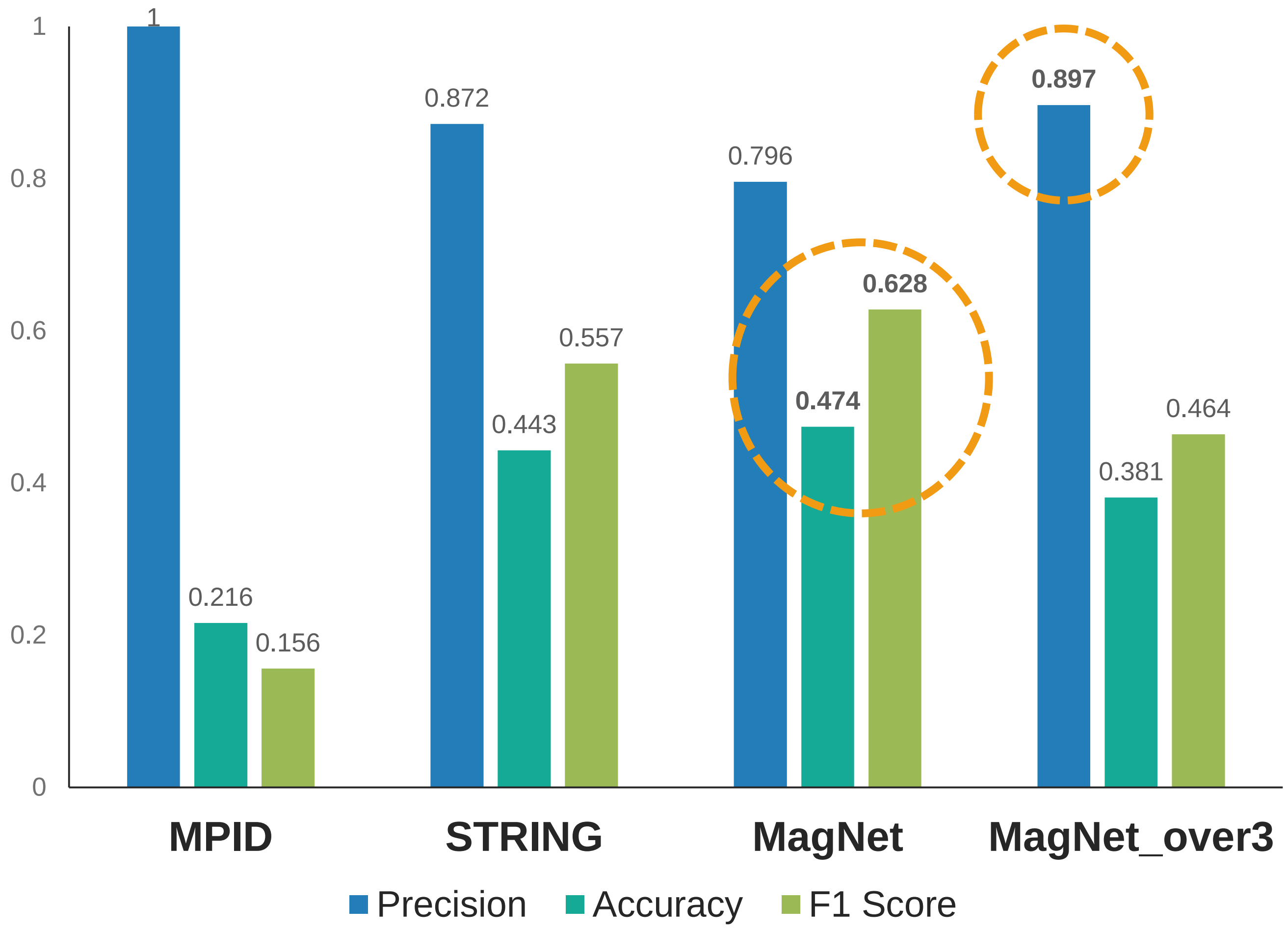
Experimental evidences

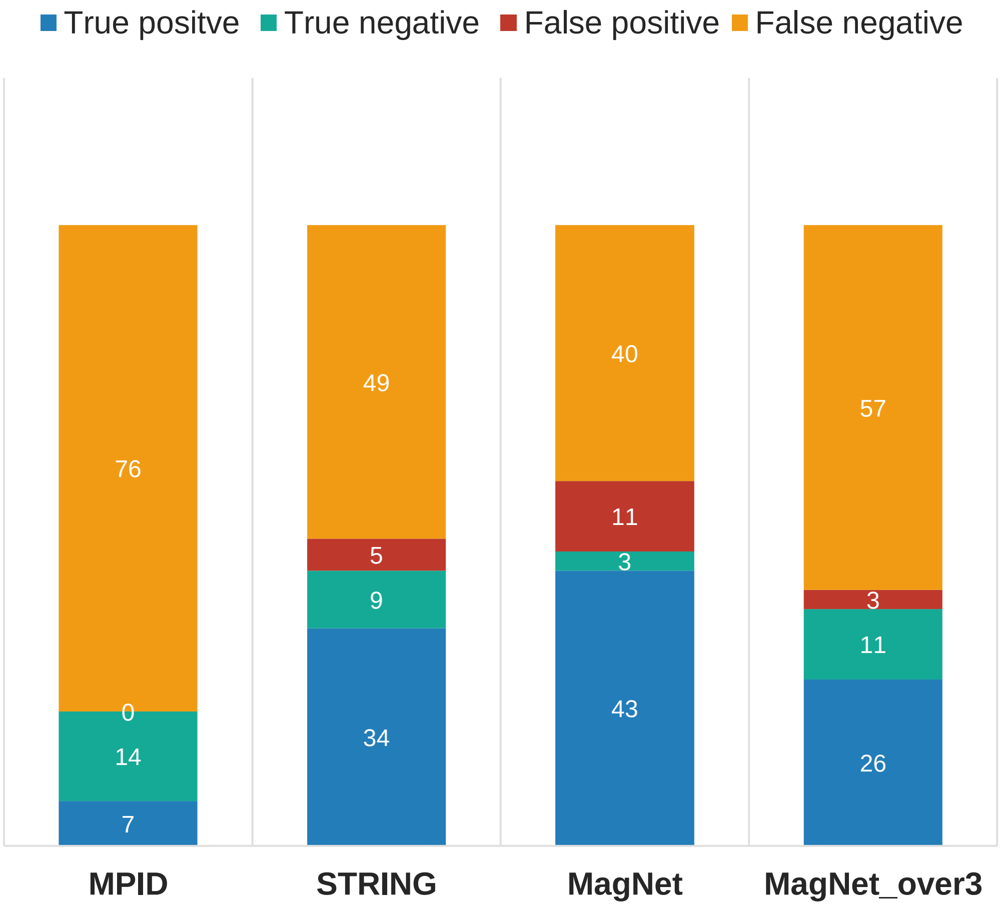
Locus 1	Locus 2	Result	Experiment	STRING	MPID	MagNet	Gene name 1	Gene name 2	Reference
MGG_07312	MGG_07173	+	Y2H	-	+	+	MoSln1p	MoYpd1p	(Jacob *et al*., 2015)
MGG_02897	MGG_07173	+	Y2H	+	-	+	MoSsk1p	MoYpd1p	(Jacob *et al*., 2015)
MGG_11174	MGG_07173	+	Y2H	+	-	+	MoHik1p	MoYpd1p	(Jacob *et al*., 2015)
MGG_11174	MGG_07312	+	Y2H	+	-	+	MoHik1p	MoSln1p	(Jacob *et al*., 2015)
MGG_01822	MGG_00501	+	Y2H	+	-	+	MoOsm1	MoMsn2	(Zhang *et al*., 2014)
MGG_11141	MGG_12868	+	Y2H	-	-	+	MAP1	ECH1	(Cui *et al*., 2015)
MGG_11141	MGG_10668	+	Y2H	-	-	+	MAP1	CGT	(Cui *et al*., 2015)
MGG_11141	MGG_04042	-	Y2H	+	-	+	MAP1	LRS	(Cui *et al*., 2015)
MGG_11141	MGG_04317	+	Y2H	-	-	-	MAP1	C3HC	(Cui *et al*., 2015)
MGG_11141	MGG_00253	-	Y2H	-	-	+	MAP1	hisHF	(Cui *et al*., 2015)
MGG_11141	MGG_15393	+	Y2H	-	-	-	MAP1	C2H2	(Cui *et al*., 2015)
MGG_11141	MGG_07808	-	Y2H	-	-	-	MAP1	MFT	(Cui *et al*., 2015)

+: positive / -: negative / Y2H: yeast two-hybrid

17 references/ 97 interactions

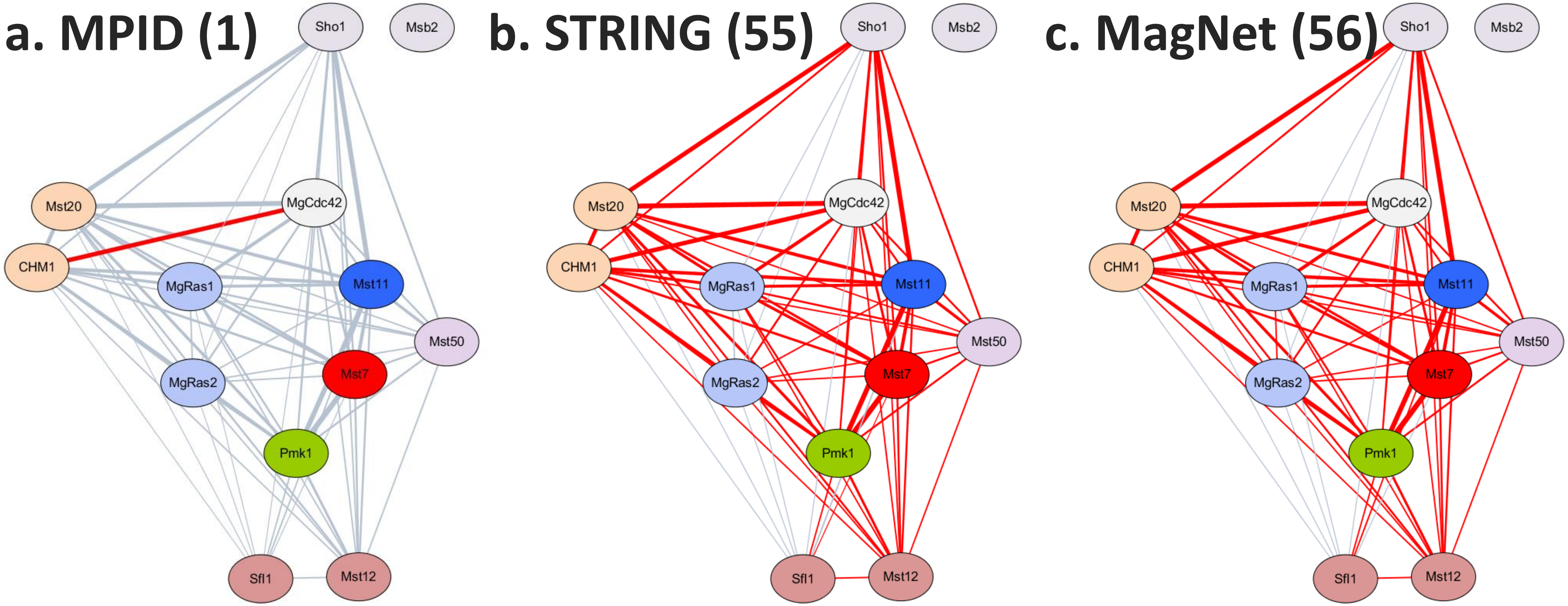
Experimental evidences





PPIs in MAPK pathway

Database (number of interactions)



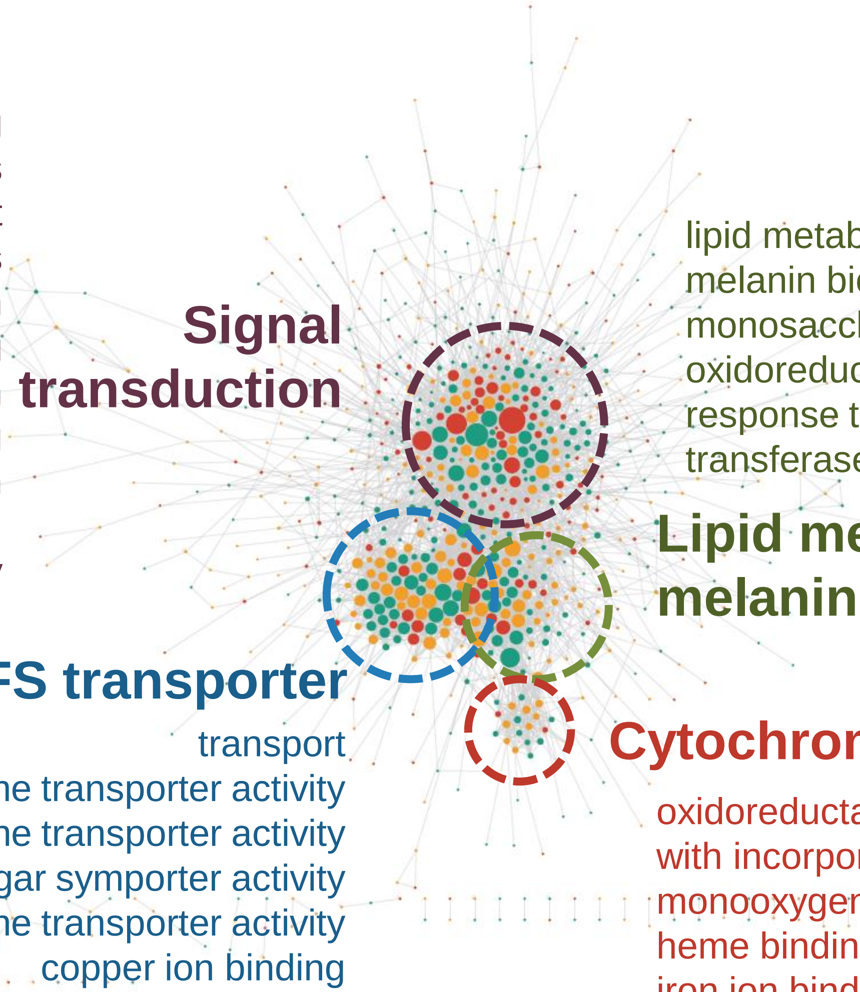
a. MPID (1)

b. STRING (55)

c. MagNet (56)

Total edges are more in STRING than high confident MagNet (over3) (STRING: 1097966, MagNet over3: 217531) but the edge coverage of two networks are similar, which means MagNet over3 may have less false positives than STRING.

Differentially expressed genes (DEGs) during appressorium development



Signal transduction

GTP binding
cofactor metabolic process
microtubule-based movement
negative regulation of cellular process
organelle organization
phospholipid binding
protein dephosphorylation
ribonucleotide binding
signal transduction
small GTPase mediated signal transduction
two-component response regulator activity

Lipid metabolic / melanin biosynthetic process

lipid metabolic process
melanin biosynthetic process
monosaccharide metabolic process
oxidoreductase activity, acting on CH-OH group of donors
response to cAMP
transferase activity, transferring acyl groups

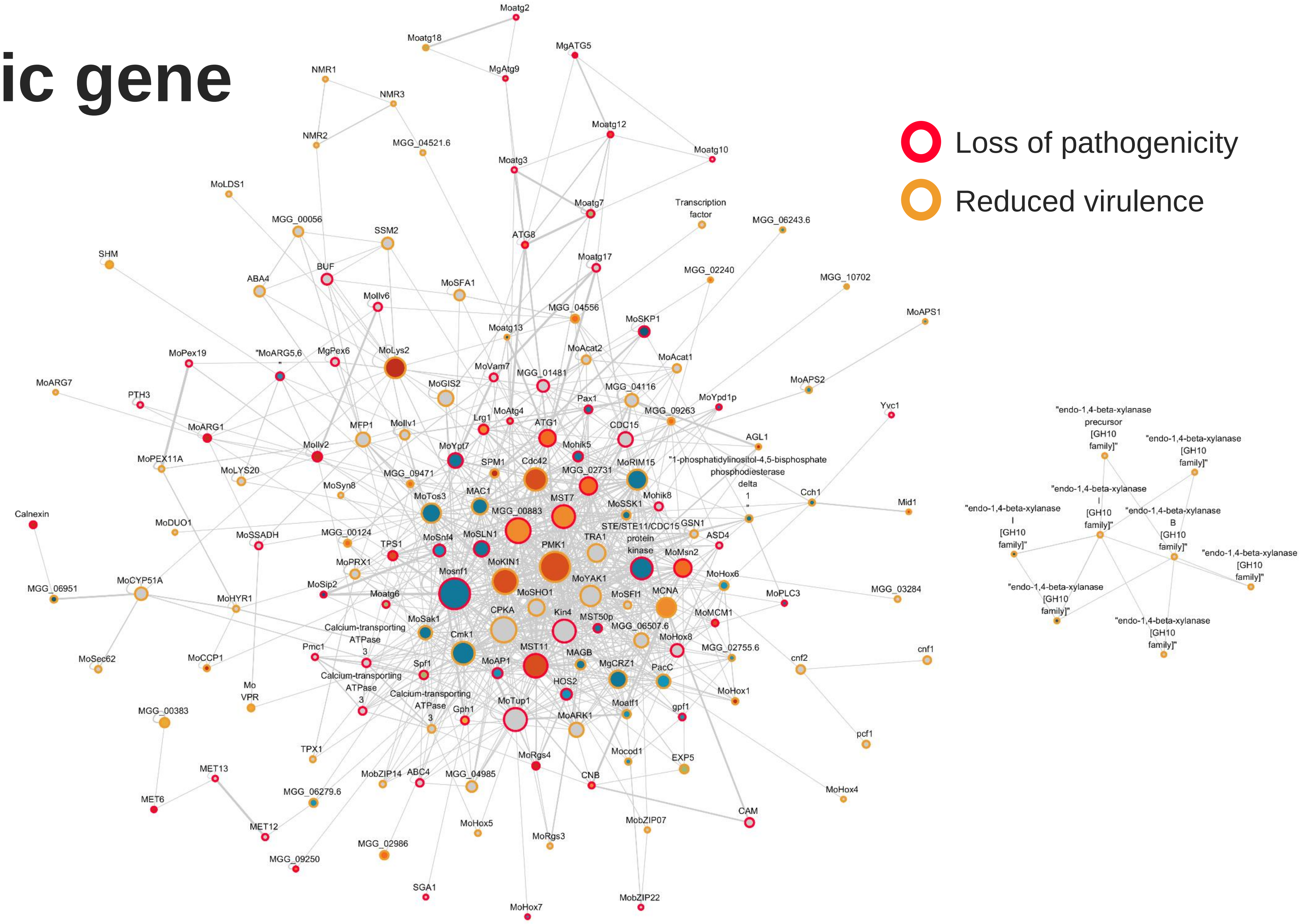
MFS transporter

transport
secondary active transmembrane transporter activity
active transmembrane transporter activity
cation:sugar symporter activity
carbohydrate transmembrane transporter activity
copper ion binding

Cytochrome P450

oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen
monooxygenase activity
heme binding
iron ion binding

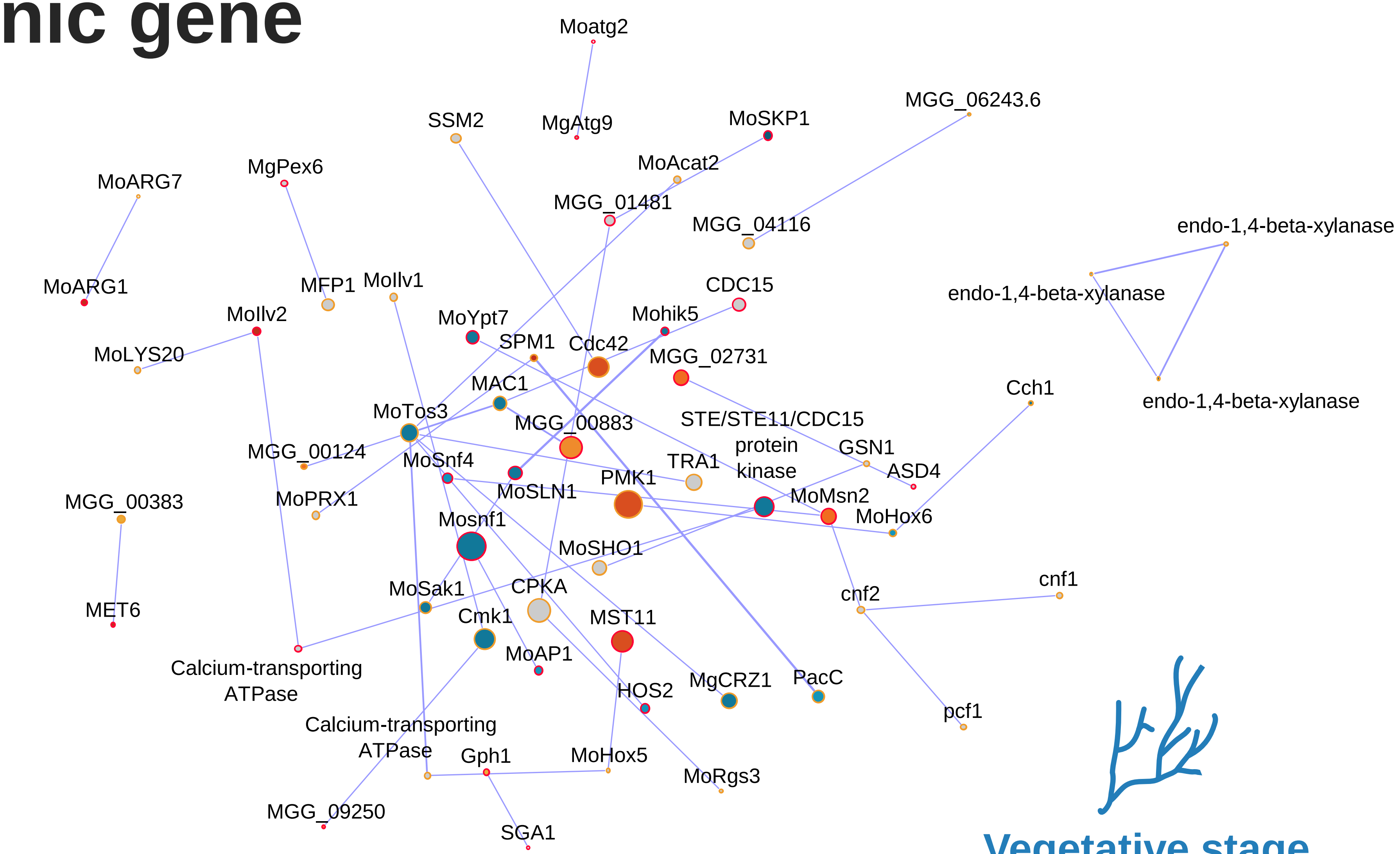
Pathogenic gene network



○ Loss of pathogenicity

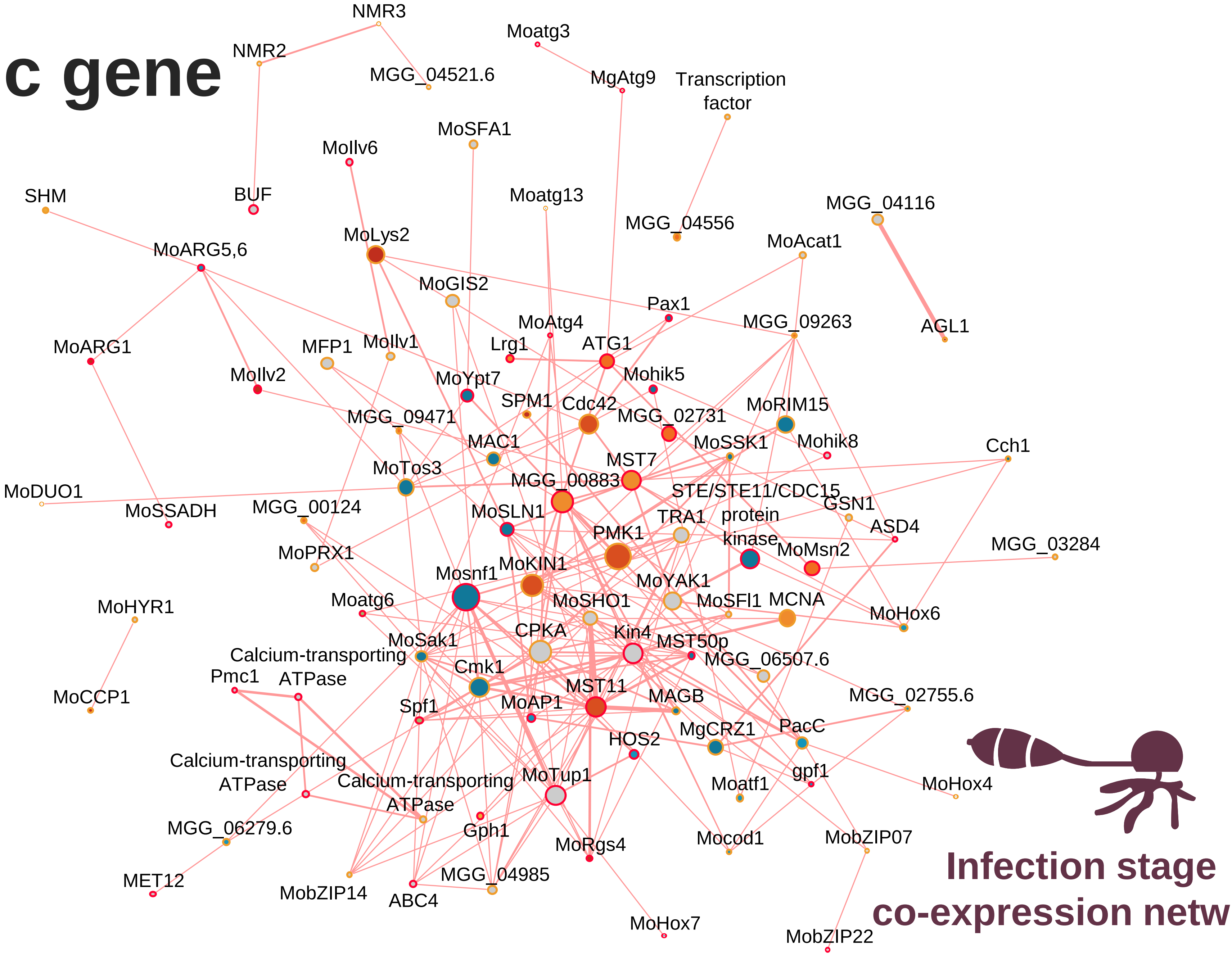
○ Reduced virulence

Pathogenic gene network



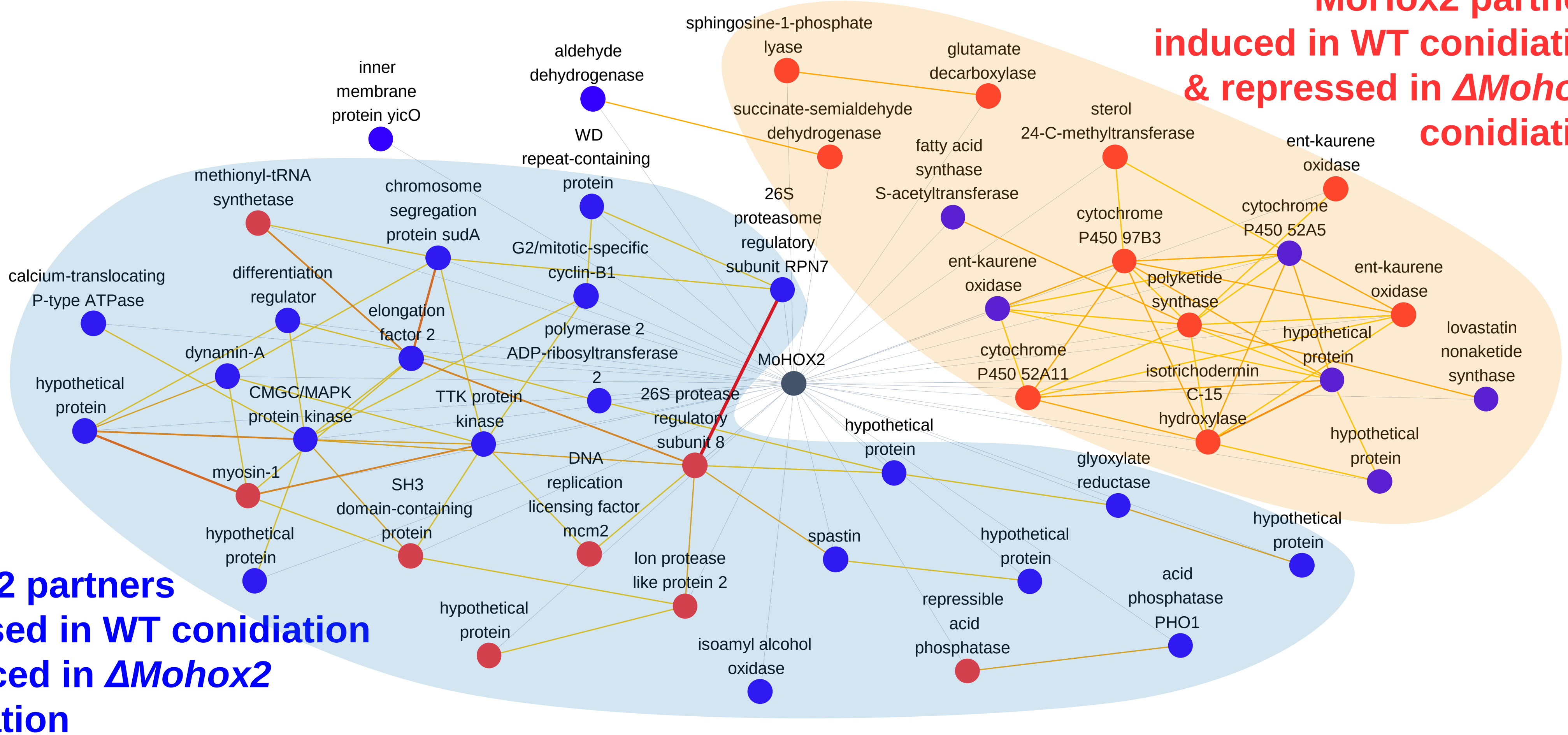
Vegetative stage co-expression network

Pathogenic gene network



Infection stage
co-expression network

DEGs + PPI: WT vs *ΔMoHOX2* (no conidia)

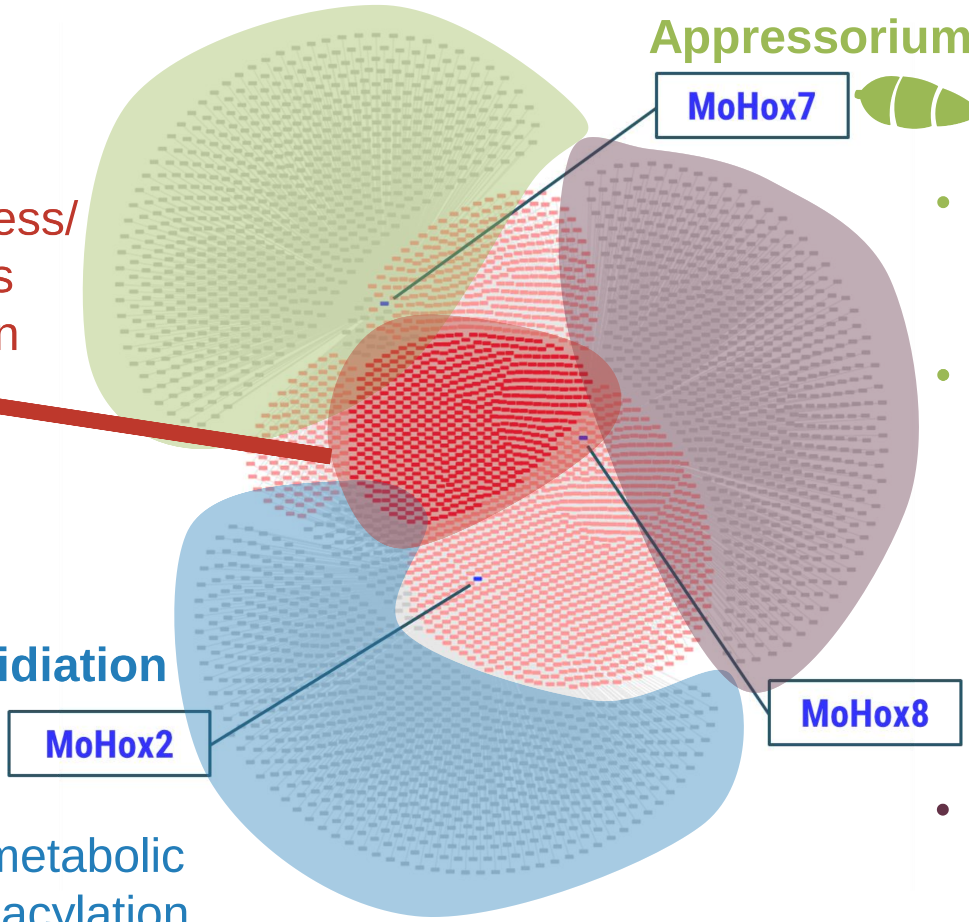


MoHox2 partners induced in WT conidiation & repressed in *ΔMohox2* conidiation

MoHox2 partners repressed in WT conidiation & induced in *ΔMohox2* conidiation

Genes repressed in WT are likely to interact with WT repressed genes (containing kinases)

Specific interaction partners of homeobox TFs



Intersection

- BP: regulation of cellular process/ nucleic acid metabolic process
- MF: nucleotide binding/ protein histidine kinase activity…

Appressorium

- BP: Carbohydrate transport / transcription from RNA polymerase II promoter
- MF: sugar hydrogen symporter activity/ peptide transporter activity/ hydrolase activity

Conidiation

- BP: Carboxylic acid metabolic process/ tRNA aminoacylation for protein translation
- MF: monooxygenase activity …

Invasive growth

- BP: macromolecule biosynthetic process/ purine ribonucleotide metabolism
- MF:zinc ion binding/ ubiquitin…

Summary

- Integrated gene network of *M. oryzae*, **MagNet**, was constructed
    - Homology-based “Interolog” search
    - Domain-Domain interaction
    - Co-expressed gene network.

- Subnetworks of pathogenic genes and DEGs were found from high-confident network

- The information of **MagNet** can be used in identifictation of putative gene functions, construction of hypothesis, and integration of biological informations.

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Prof. Yong-Hwan Lee

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1. Kyeongchae Chung
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3. Jongbum Jeon
4. Gobong Choi

Fungal Plant Pathology Lab

FPPL Seoul National University Fungal Plant Pathology Laboratory

NRF National Research Foundation of Korea

Poster presentation

PgmNr 260T

MagNet: the integrated gene network of the rice blast fungus *Magnaporthe oryzae*.

Today PM 7:30 – 8:30

THANK YOU
for
LISTENING

Statistics of MagNet subnetworks

Method	Network	Proteins	Interactions	Avg. num of neighbors	Clustering coefficient	Network centralization	Connected components	Network diameter	Network density
Homology based network	STRING	6498	1097966	168.97	0.309	0.445	71	9	0.026
	MPID	3017	11674	7.234	0.105	0.041	108	9	0.002
	BioGRID	3503	166492	61.961	0.207	0.375	4	5	0.018
	DIP	2344	10110	8.438	0.130	0.054	28	9	0.004
	MINT	2542	23868	8.094	0.127	0.045	30	10	0.003
	PINA	2849	38336	26.688	0.251	0.493	2	6	0.009
	INTACT	2825	31756	21.848	0.239	0.543	5	6	0.008
	JiffyNet	4690	266409	113.607	0.303	0.184	2	7	0.024
Co-expression network	Vegetative stage	10356	1173767	226.683	0.546	0.075	6	15	0.022
	Infection stage	8131	819480	201.569	0.539	0.071	5	11	0.025
Domain interaction network	DOMINE	5727	1450218	372.438	0.663	0.412	182	10	0.065
	IDDI	6231	4247425	991.802	0.662	0.514	51	6	0.159
High-confident	MagNet	6005	217531	71.936	0.324	0.223	57	11	0.012
Total	MagNet	11734	5600976						

Co-expression network comparison

	Vegetative stage (8 sets)	Infection stage (10 sets)
Used sets	KJ201_Control 98-06_Mycelia Normoxia Complete_medium 70-15_WT_mycelia_rep1 70-15_WT_mycelia_rep2 70-15_WT_mycelia_rep3 KJ201_methylation_mycelia	KJ201_1618hpi KJ201_2527hpi KJ201_3436hpi KJ201_4045hpi KJ201_72hpi 98-06_0h 98-06_8h 98-06_24h 98-06_48h 98-06_72h
PCC cutoff	0.903	0.846
P-value	0.001	0.001

PCC: Pearson Correlation Coefficient
(0 is no correlation, +1 is total positive correlation)

Co-expression networks

Enriched GO terms of Vegetative Stage Specific Co-expressed Proteins

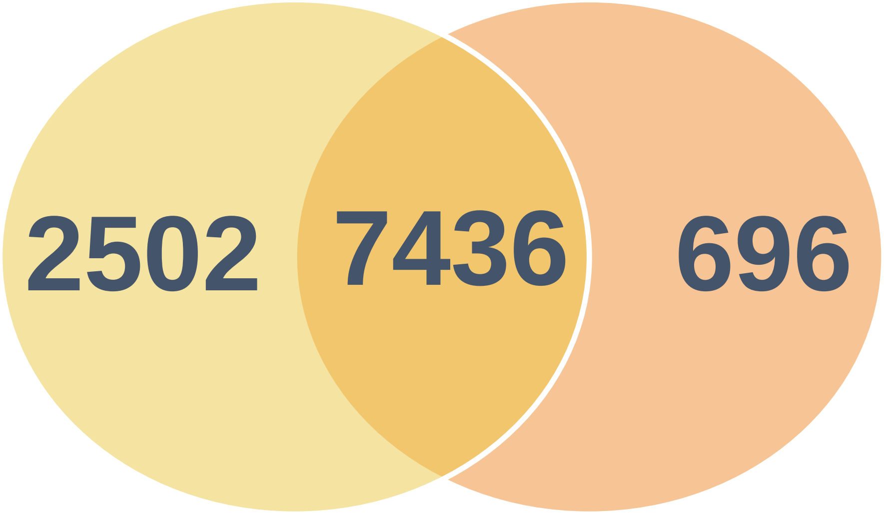
Cellular macromolecule metabolic process

Cellular protein metabolic process

Cellular component organization at cellular level

Mycelium development

Translation



Enriched GO terms of Infection Stage Specific Co-expressed Proteins

Interaction with host via substance released outside of symbiont

Response to cAMP

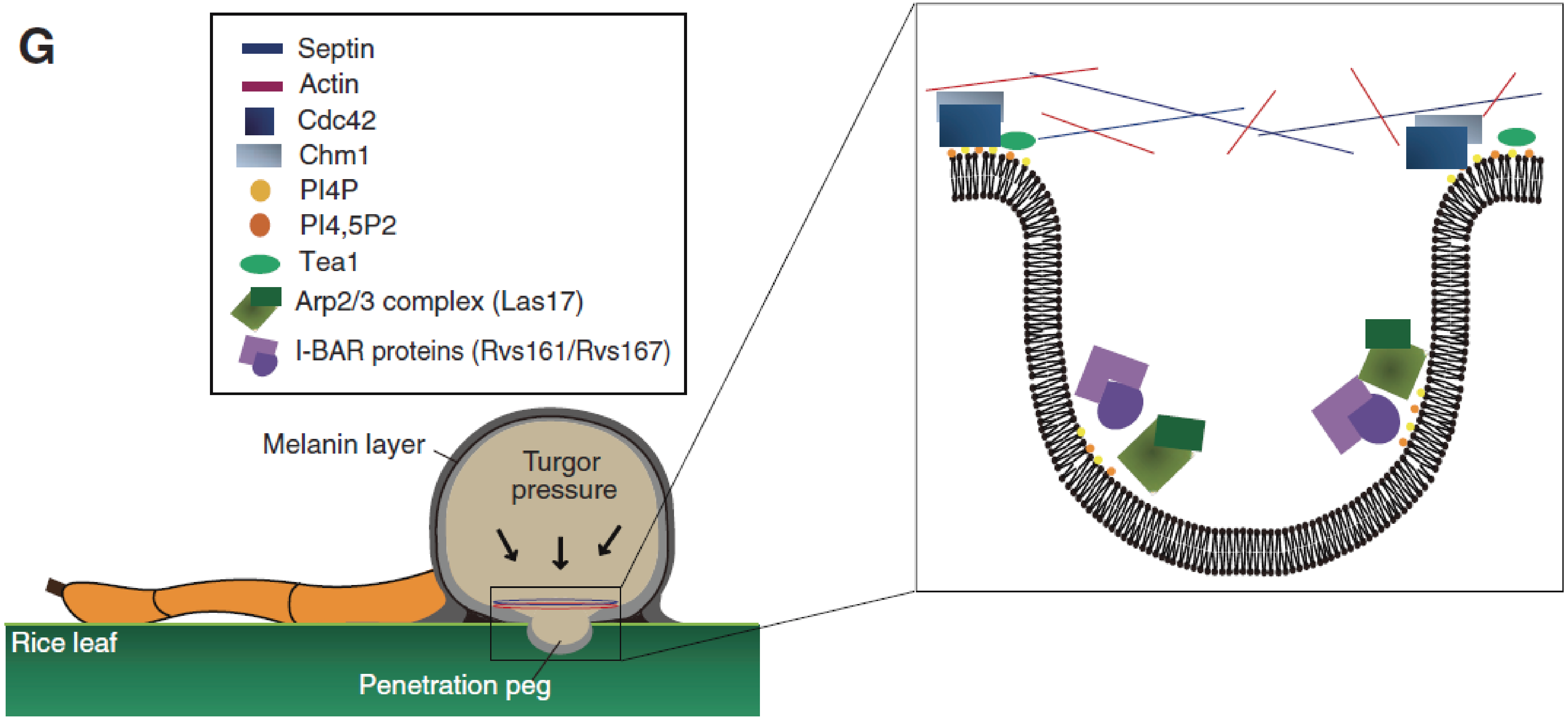
Experimental evidences

Parameter	MPID	STRING	MagNet	Integrated	MagNet_over3
True positve (No.)	7	34	40	43	26
True negative (No.)	14	9	4	3	11
False positive (No.)	0	5	10	11	3
False negative (No.)	76	49	43	40	57
Precision	1.000	0.872	0.800	0.796	0.897
Accuracy	0.216	0.443	0.454	0.474	0.381
F1 Score	0.156	0.557	0.602	0.628	0.464

MagNet only true positives & false positives

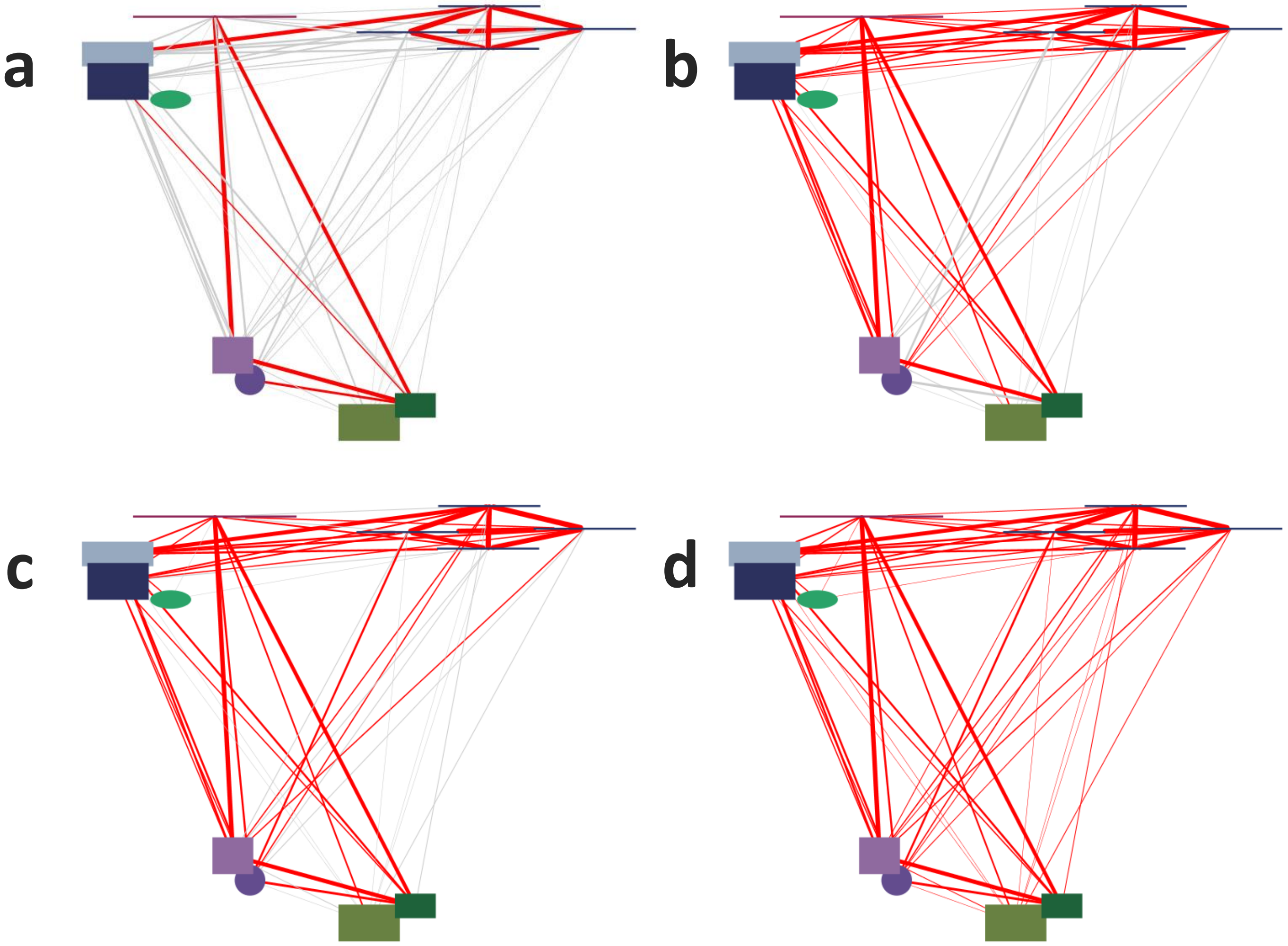
Protein 1	Protein 2	MPID	STRING	MagNet	MagNet_over3	Experiment	MPID	STRING	MagNet	MagNet_over3	MGG_BIOGRID.txt	MGG_DIP.txt	MGG_INTACT.txt	MGG_JIFFY.txt	MGG_MINT.txt	MGG_MPID.txt	MGG_PINA.txt	MGG_STR.txt	MGG_STR_0.7.txt	MGG_DOM.txt	MGG_IDDI.txt	MGG_CX_IF.txt	MGG_CX_MY.txt	MGG_CX_PROT.txt	MGG_ATMT.txt	MGG_BLAST.txt	sum	Experiment
MGG_11141	MGG_12868	0	0	1	0	1	FN	FN	TP	FN	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	Y2H
MGG_11141	MGG_10668	0	0	1	0	1	FN	FN	TP	FN	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	Y2H
MGG_11141	MGG_13781	0	0	2	0	1	FN	FN	TP	FN	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	Y2H
MGG_09565	MGG_01078	0	0	1	0	1	FN	FN	TP	FN	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Y2H
MGG_09898	MGG_05533	0	0	1	0	1	FN	FN	TP	FN	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Y2H
MGG_14008	MGG_00365	0	0	1	0	1	FN	FN	TP	FN	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Y2H
MGG_03198	MGG_00446	0	0	1	0	1	FN	FN	TP	FN	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AP
MGG_12958	MGG_13778	0	0	2	0	1	FN	FN	TP	FN	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	AP
MGG_11141	MGG_00253	0	0	2	0	0	TN	TN	FP	TN	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	Y2H
MGG_11141	MGG_05587	0	0	2	0	0	TN	TN	FP	TN	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	Y2H
MGG_11141	MGG_03136	0	0	1	0	0	TN	TN	FP	TN	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	Y2H
MGG_11141	MGG_02695	0	0	1	0	0	TN	TN	FP	TN	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Y2H
MGG_14008	MGG_01818	0	0	1	0	0	TN	TN	FP	TN	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Y2H
MGG_14008	MGG_04204	0	0	1	0	0	TN	TN	FP	TN	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Y2H

PPIs in penetration peg



Dagdas, Y. F., Yoshino, K., Dagdas, G., Ryder, L. S., Bielska, E., Steinberg, G., & Talbot, N. J. (2012). Septin-mediated plant cell invasion by the rice blast fungus, Magnaporthe oryzae. Science, 336(6088), 1590-1595.

PPIs in penetration peg

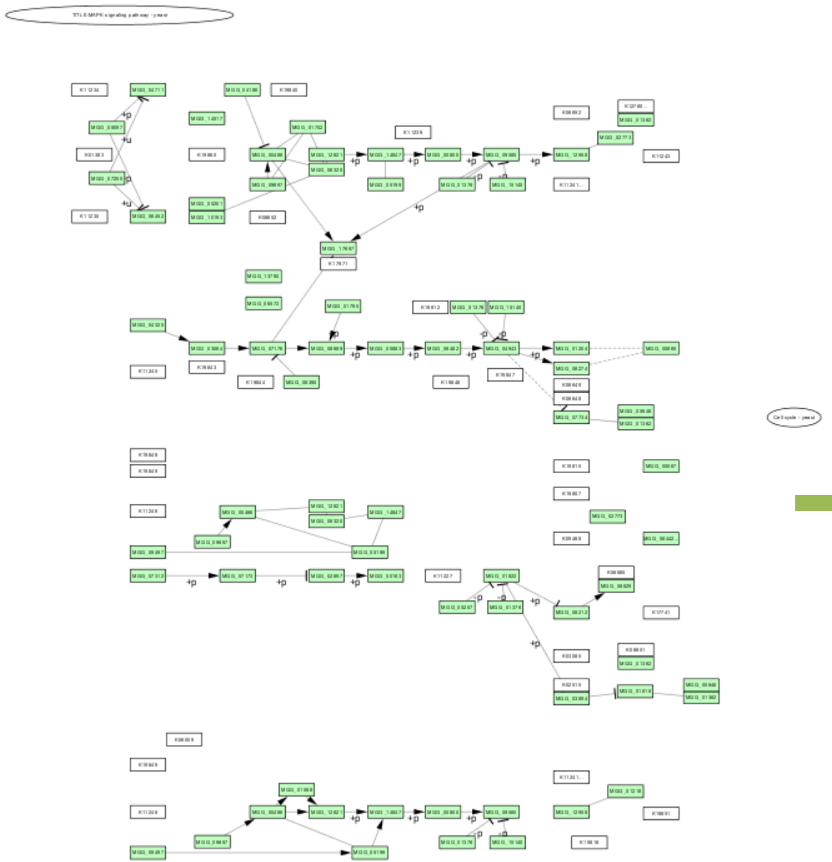


Comparison of PPI databases

Database (edge num)

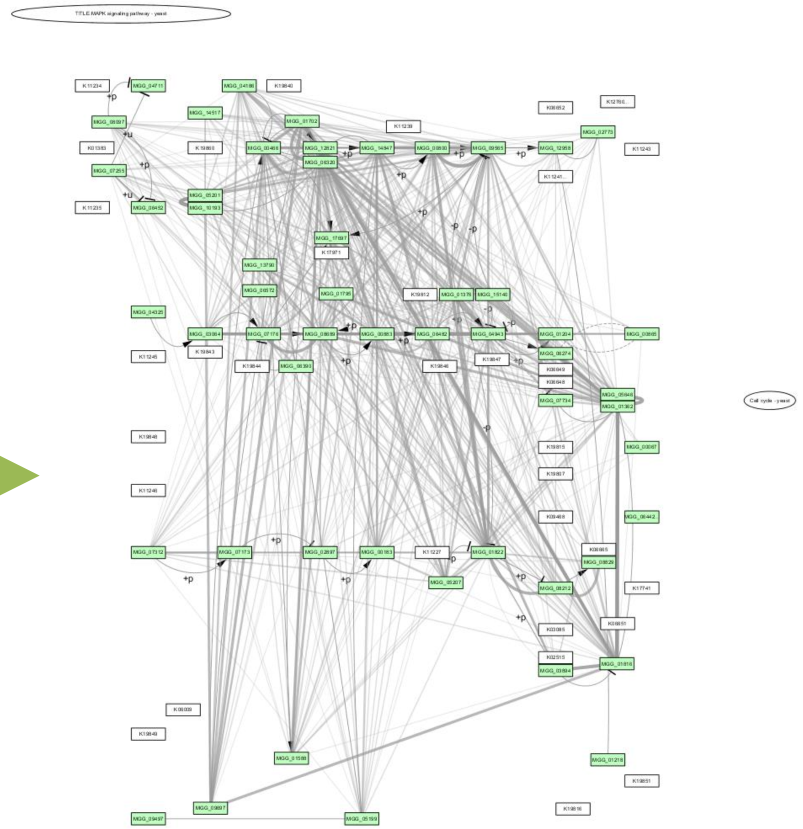
a. MPID (14)
b. STRING (40)
c. MagNet over3 (37)
d. MagNet (56)

KEGG - MAPK



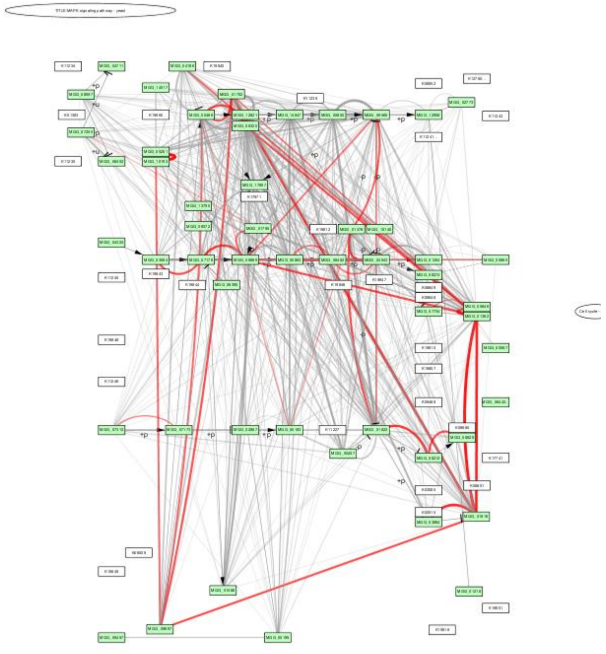
M. oryzae genes mapped on KEGG – MAPK pathway (yeast)

Cytoscape App
- KEGGParser
- LayoutMapper

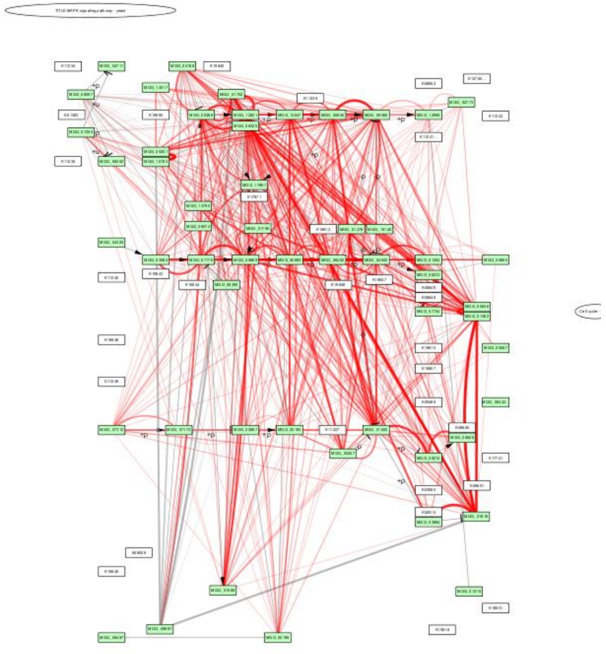


Network of MagNet was overlayed on the pathway

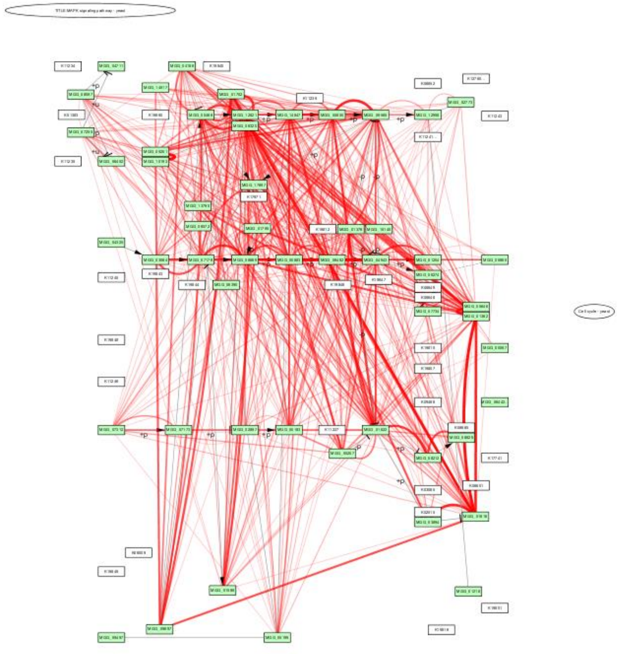
KEGG - MAPK



MPID: 36 edges



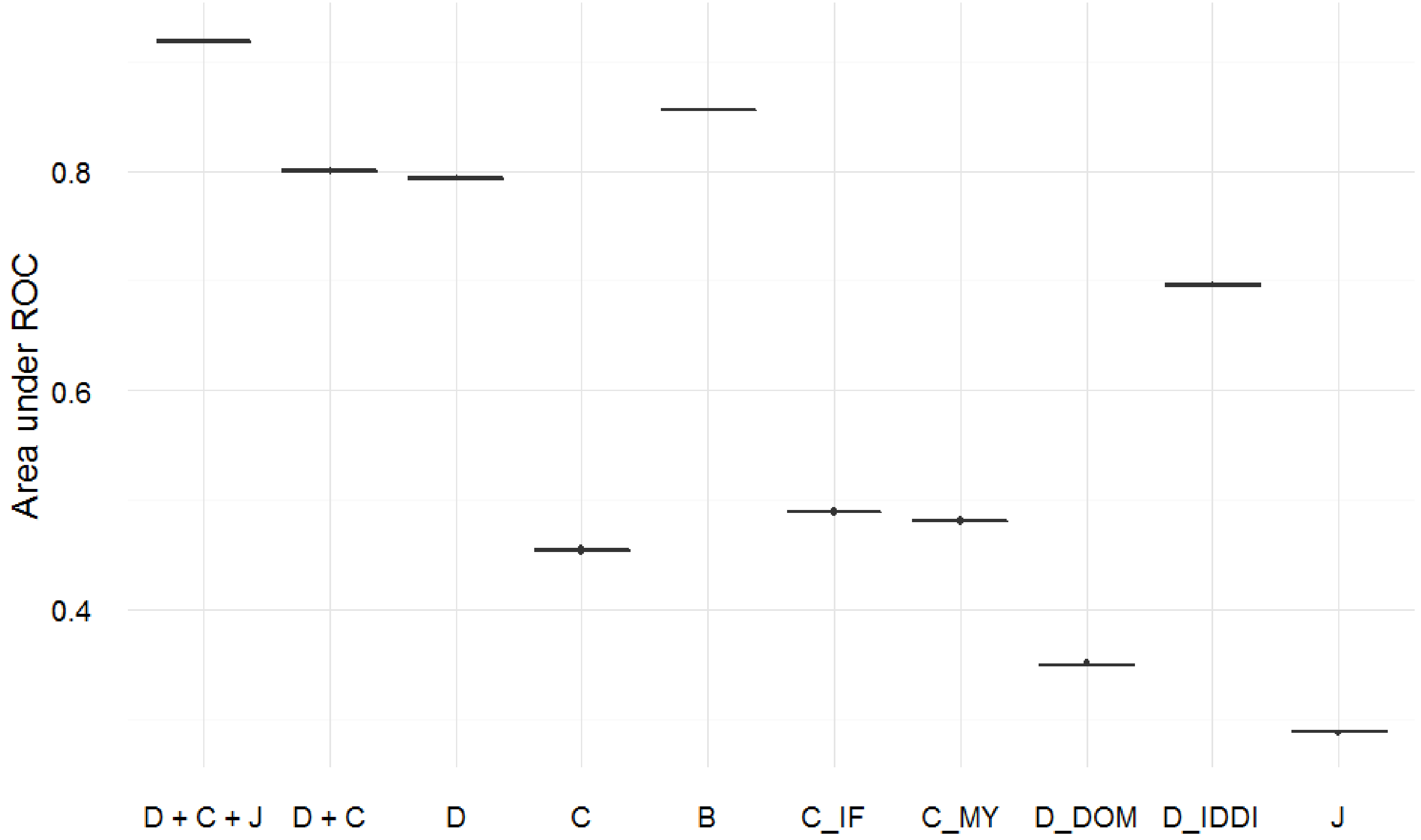
STRING: 541 edges



MagNet over3: 530 edges

MagNet total: 956 edges

Validation with computational methods



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VERI TAS LUX MEA

FPPL Seoul National University Fungal Plant Pathology Laboratory

NRF National Research Foundation of Korea