

Knr4/Smi1 Family: Conserved Fungal Chaperones of Puzzling Origin

Hélène MARTIN-YKEN

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Physiologie et Génomique Fonctionnelle des Eucaryotes
UMR-CNRS 5504 & UMR-INRA 792, Toulouse, France.
Email: helene.martin@insa-toulouse.fr

I Introduction

**Signaling pathways controlling Cell Wall Synthesis
Uncovering of ScKnr4/Smi1**

II Coordinating Cell Wall Synthesis and Cell Cycle Progression

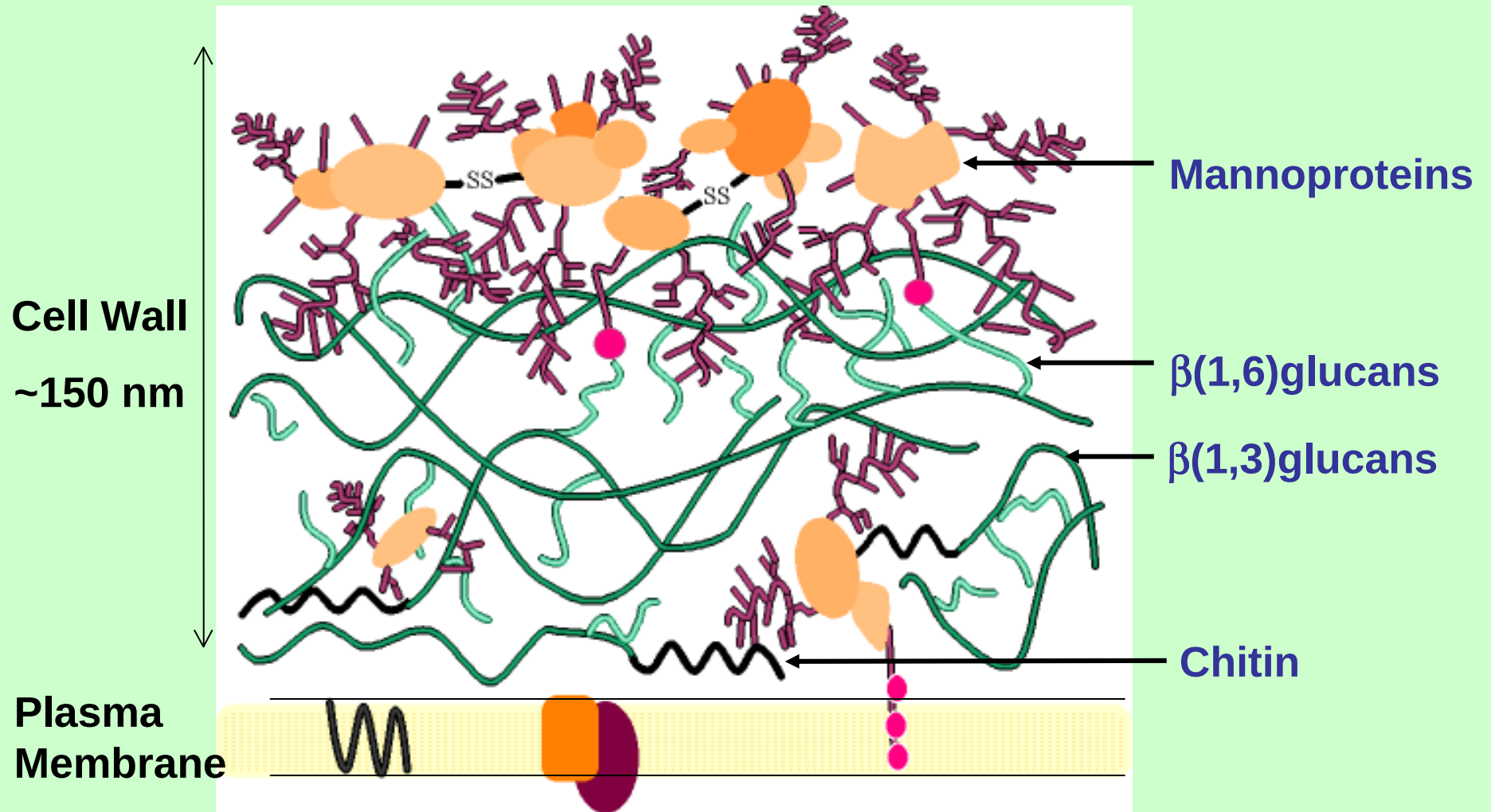
**The role of ScKnr4/Smi1 in cell signaling
So many partners !
A very peculiar structure...**

III Gene and function conserved among fungi

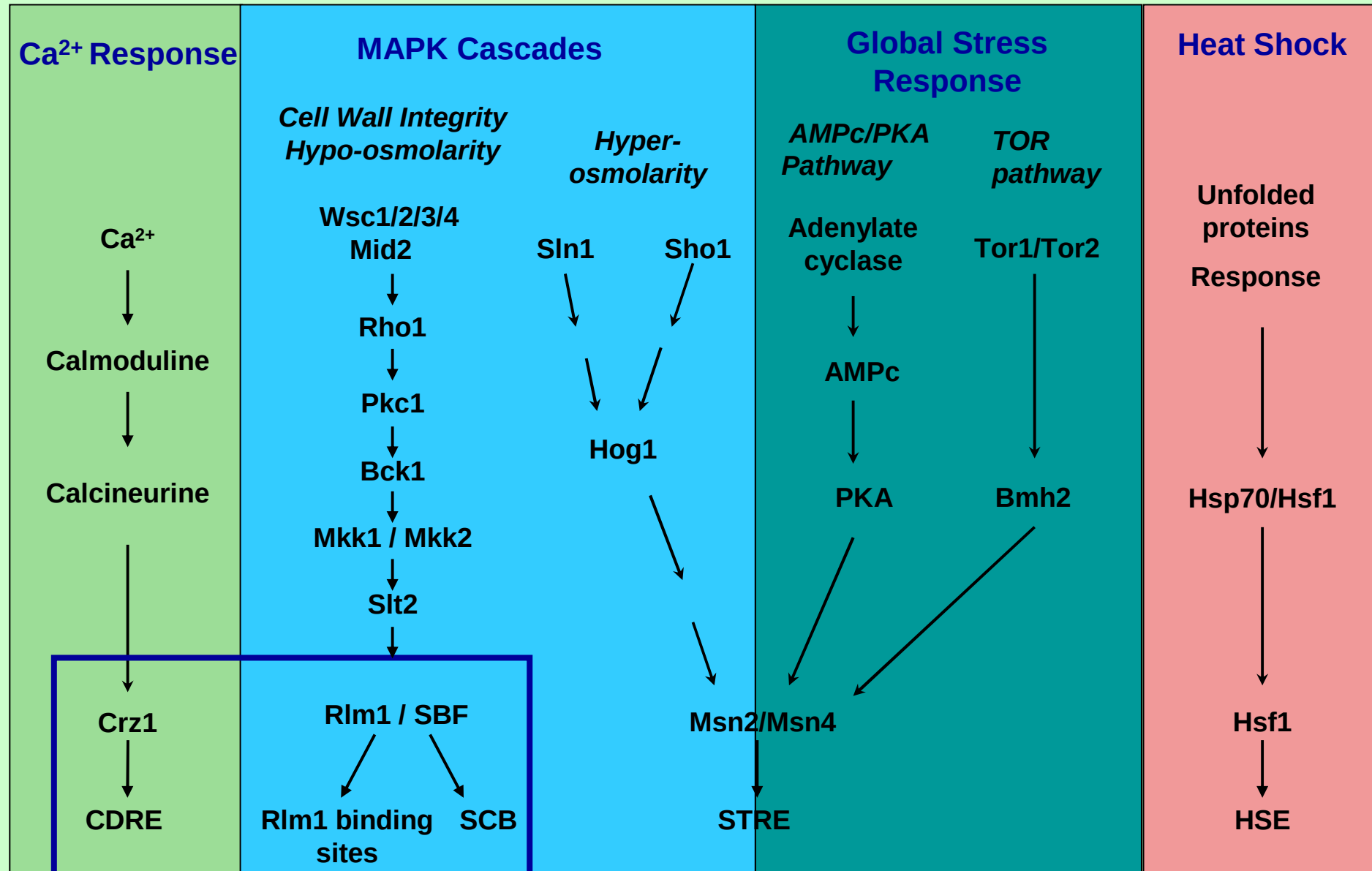
**Complementation of *S. cerevisiae* mutant
Candida albicans Smi1 and Smi1B
Neurospora crassa GS-1.**

IV Integrative Overview and Perspectives

Cell Wall Architecture in *S. cerevisiae* :



Signaling pathways involved in coping with Cell Wall Stress :



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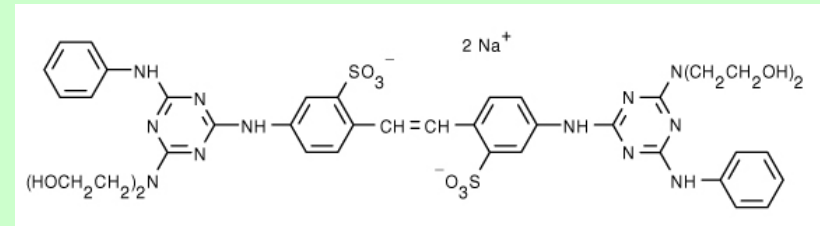
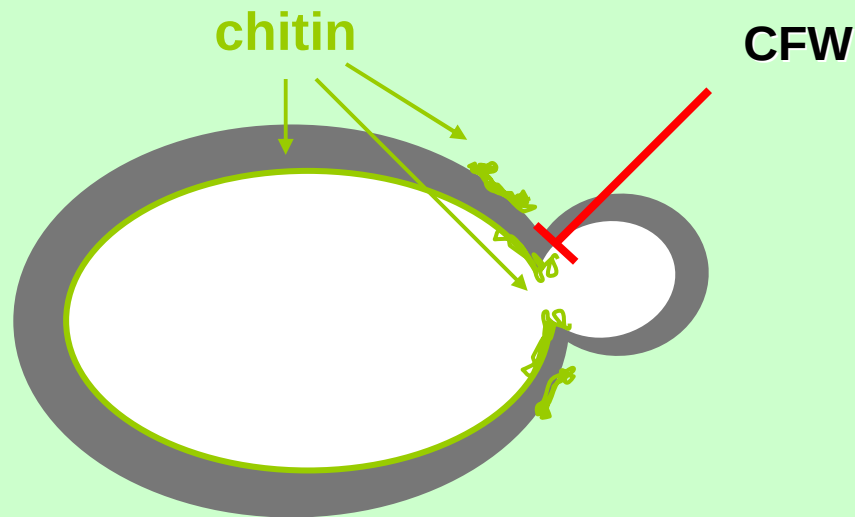
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Screen for Calcofluor White hypersensitive mutants



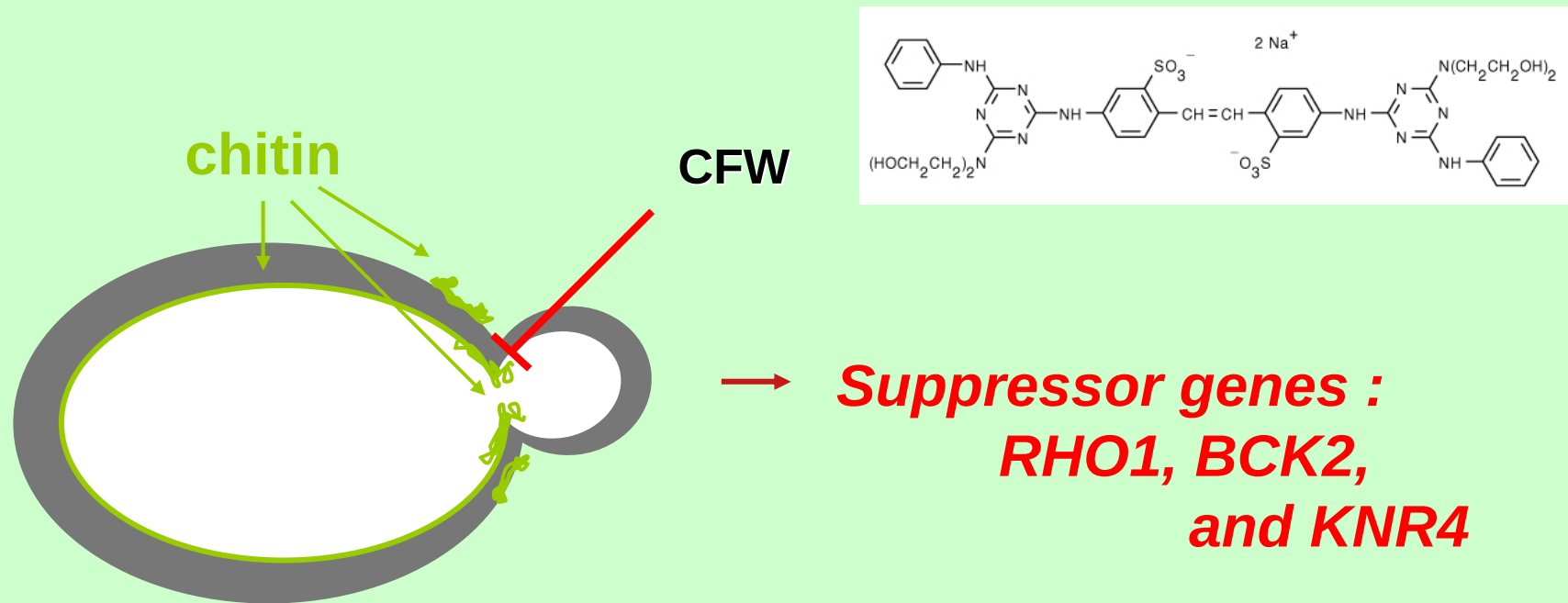
Calcofluor White

- **63 cell wall mutants hypersensitive to calcofluor white.**
- ***cwh39, cwh43-1, 2...***

(Ram et al., 1994, Yeast. (8):1019-30.)

Uncovering *KNR4* gene in the lab :

Complementation of Calcofluor White hypersensitive mutants



KNR4, a suppressor of *S. cerevisiae* *cwh* mutants, is involved in the **transcriptional control of chitin synthase genes.**

(Martin et al., 1999, Microbiology. 145, 249-258.)

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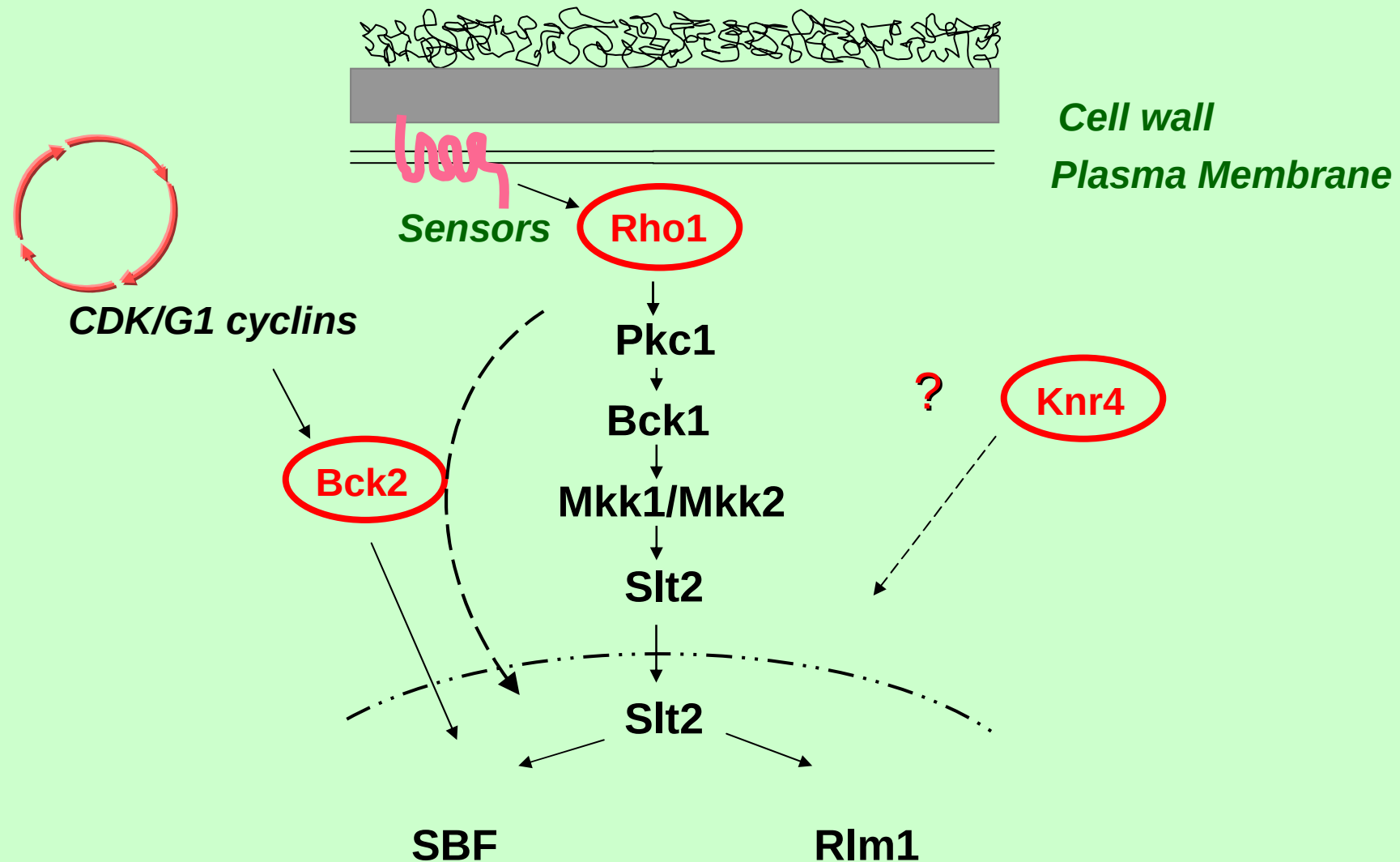
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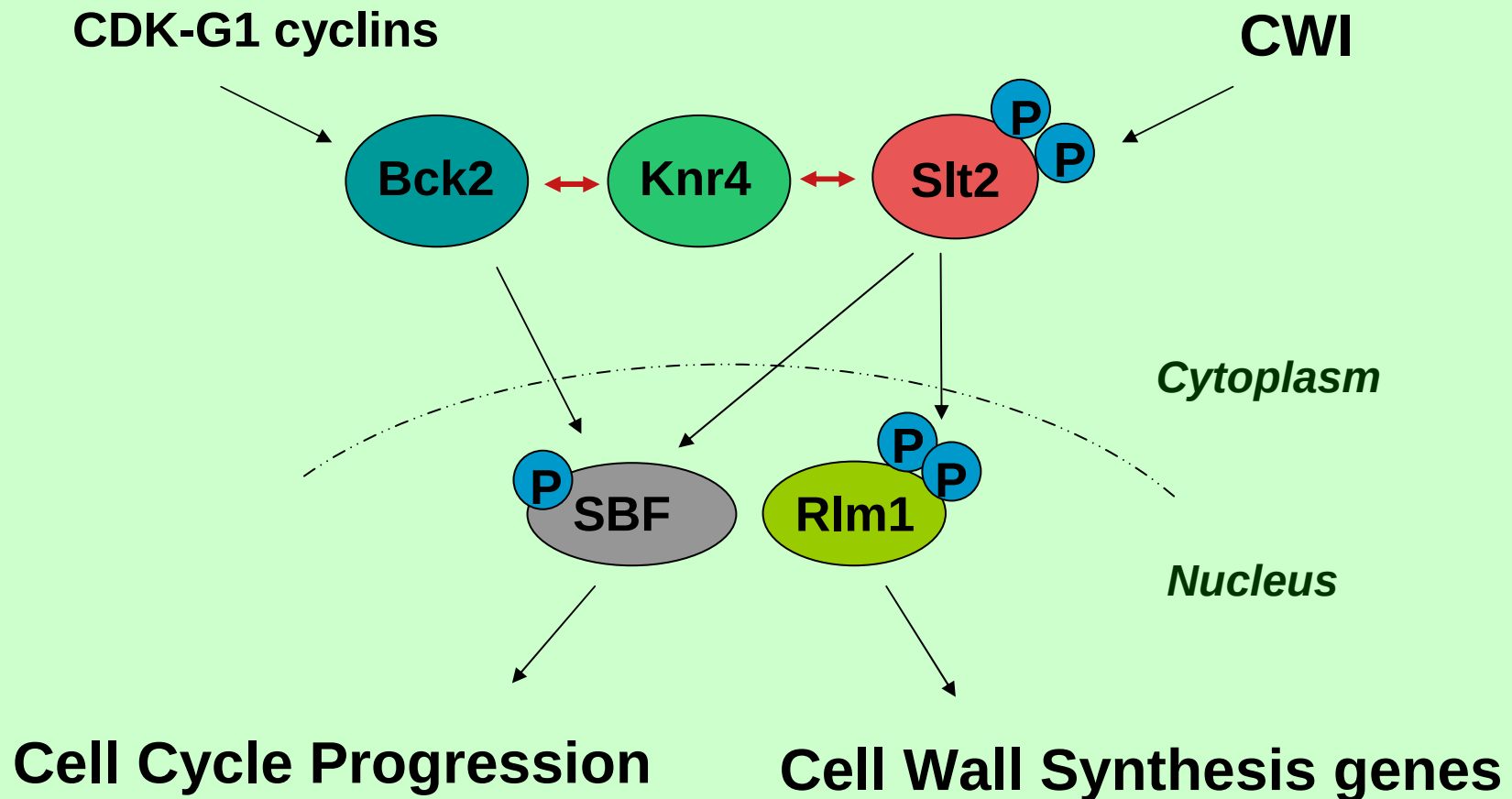
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Position of the suppressor genes isolated in cell signaling pathways :



G1/S transition, Bud emergence, cell wall synthesis genes

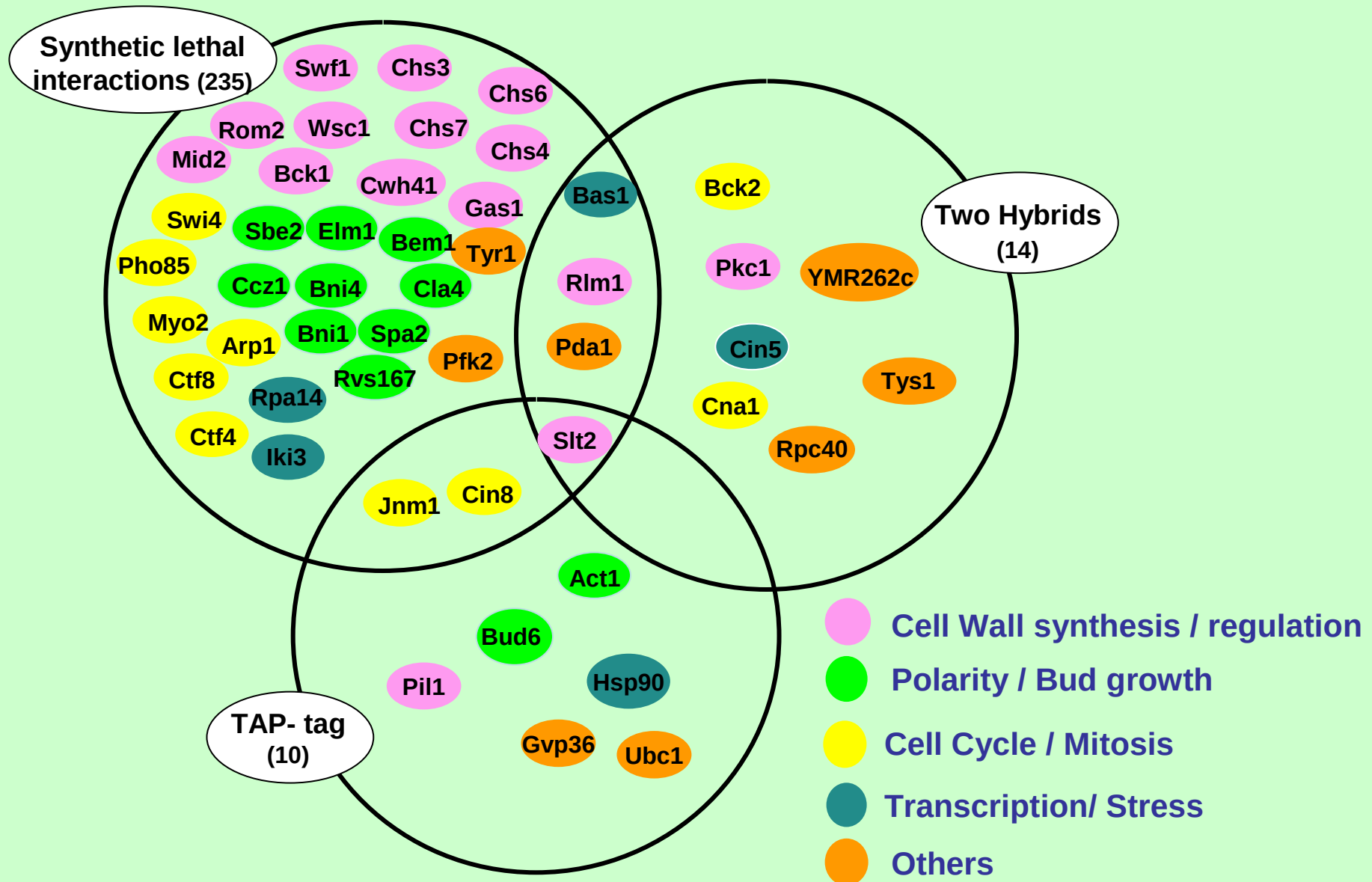
Knr4 affects transcriptional Control of Cell Cycle and Cell Wall Synthesis genes



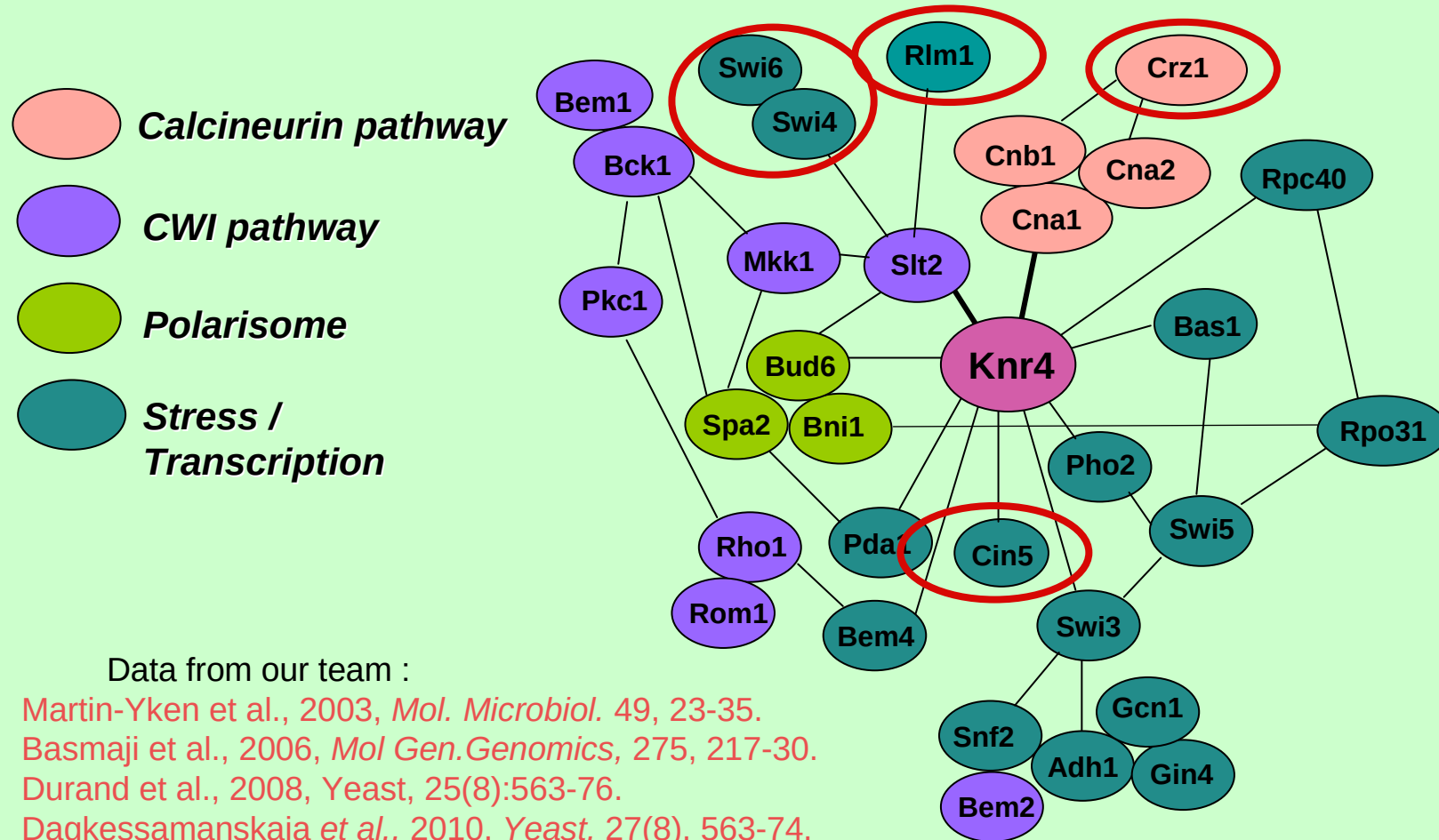
Martin-Yken H., et al., 2002, *Current Genetics*, 41: 323-332.

Martin-Yken H., et al., 2003, *Molecular Microbiology*. 49(1), 23-35.

Knr4 partners identified by complementary methods



Knr4 Functional Interactions Network :



Data from our team :

Martin-Yken et al., 2003, *Mol. Microbiol.* 49, 23-35.

Basmaji et al., 2006, *Mol Gen.Genomics*, 275, 217-30.

Durand et al., 2008, *Yeast*, 25(8):563-76.

Dagkessamanskaia et al., 2010, *Yeast*, 27(8), 563-74.

And global studies :

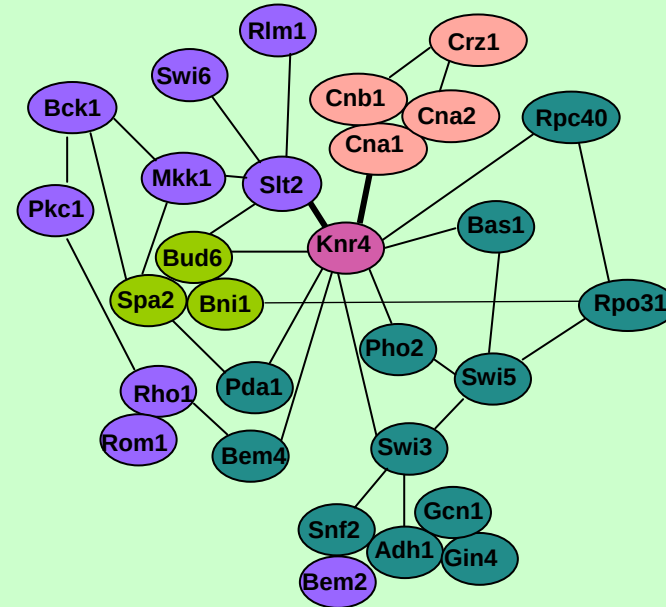
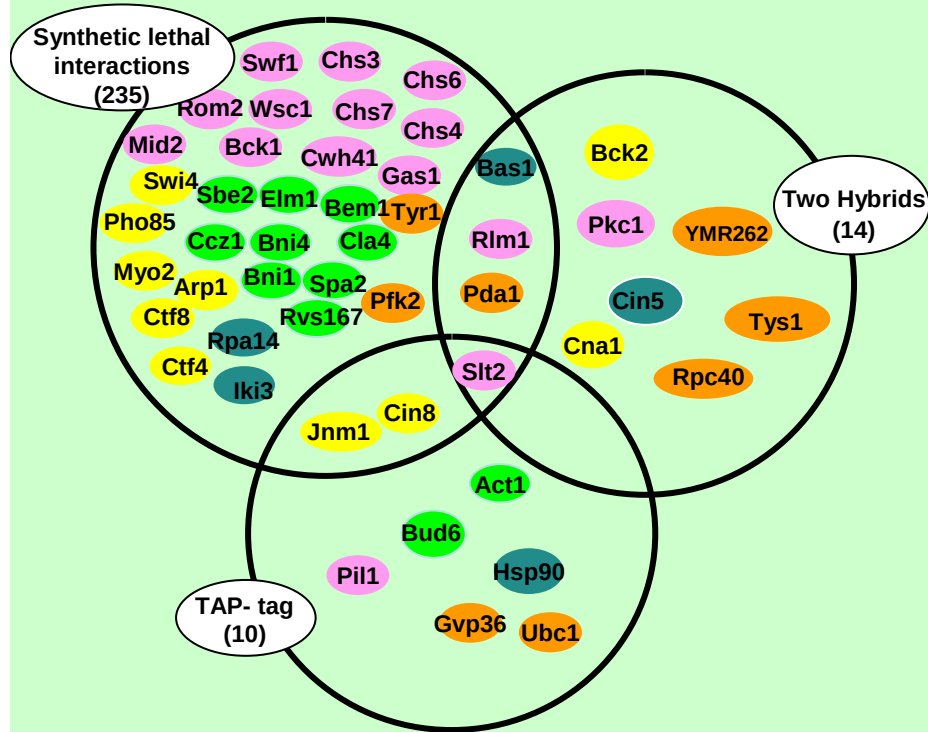
Ito T. et al., 2001 *PNAS U S A.*; 98, 4277-8.

Goehring AS. et al., 2003 *Mol Biol Cell.* 14, 1501-16.

Tong, A. H., et al., 2001, *Science* 294, 2364-8.

Uetz, P. et al., 2000. *Nature* 403, 623-627.

Questions raised by the multiplicity of Knr4 interactions :

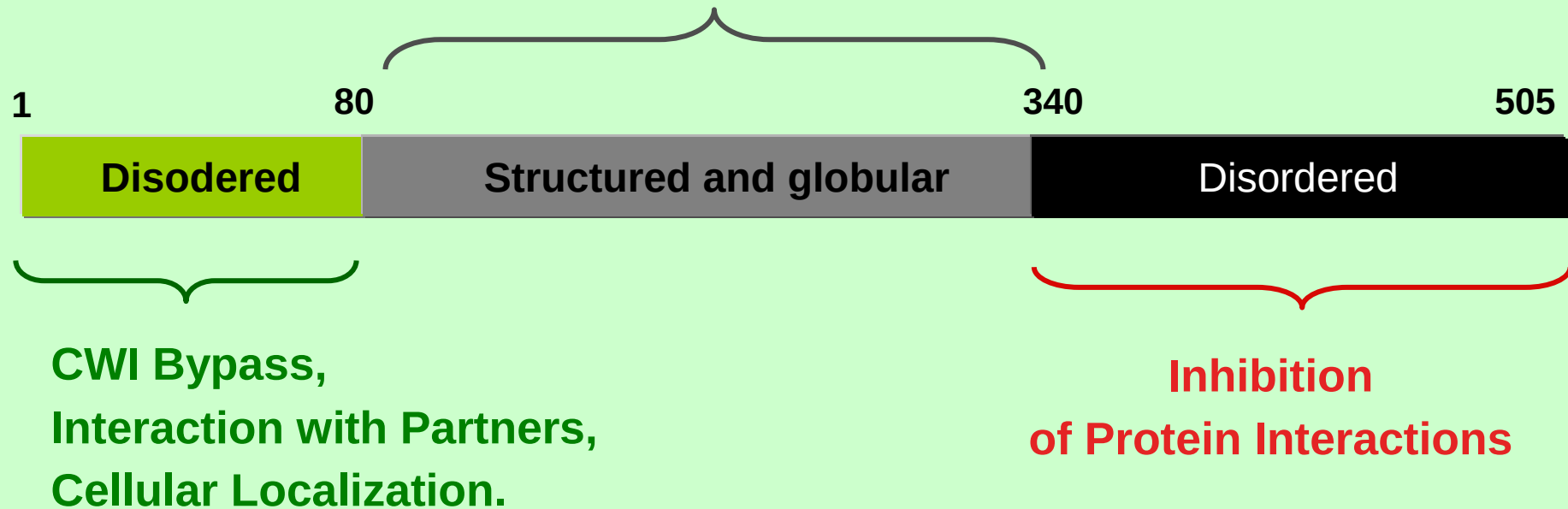


→ **Genetic : why so many Synthetic Lethals ?**

→ **Physical : is there a structural basis to this ability ?**

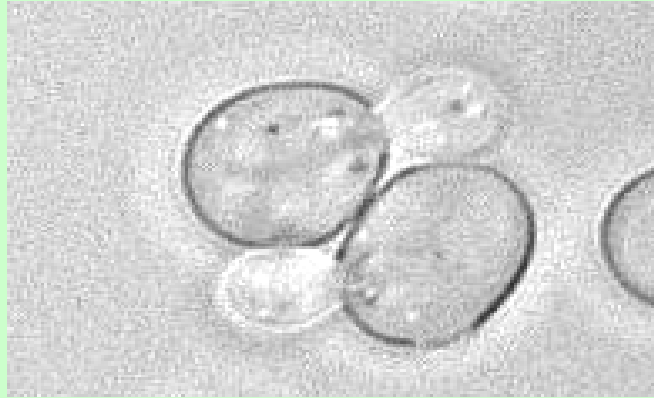
Different functions of the distinct Knr4 domains :

Complements the *knr4* Δ mutant phenotypes,
and almost all the synthetic lethal interactions...



Dagkessamanskaia A., 2010. Protein Science, 19(7):1376-85.

Cellular Localization of Knr4 in Budding Haploid and Diploid cells



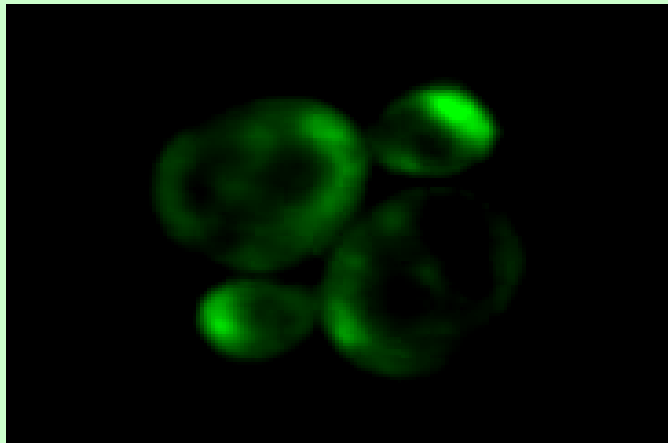
Axial budding pattern

a or α Haploid cells

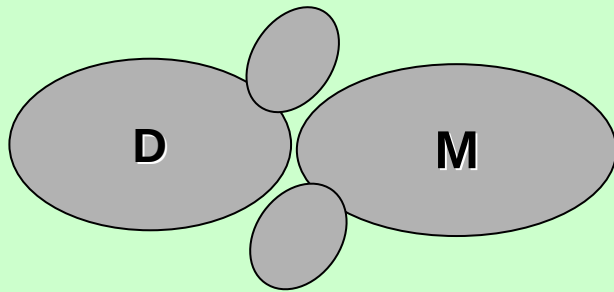


Bipolar budding pattern

a/ α Diploid cells

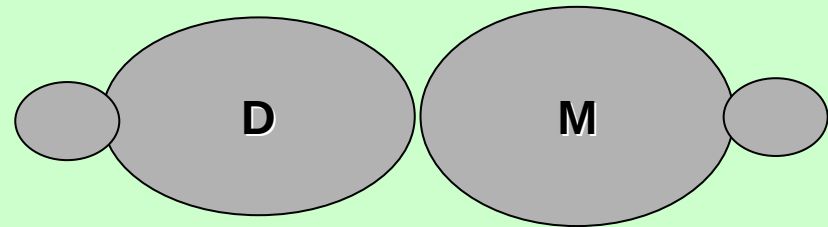


***S.cerevisiae* haploids and diploids cells
budding patterns :**



Axial budding pattern

a or α Haploid cells



Bipolar budding pattern

a/ α Diploid cells

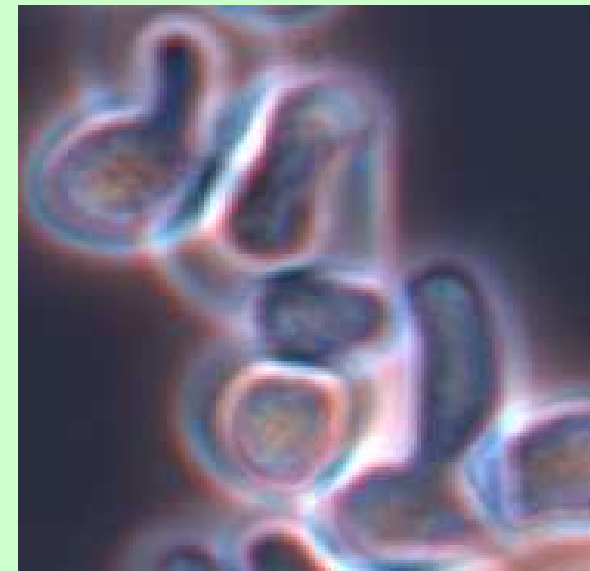
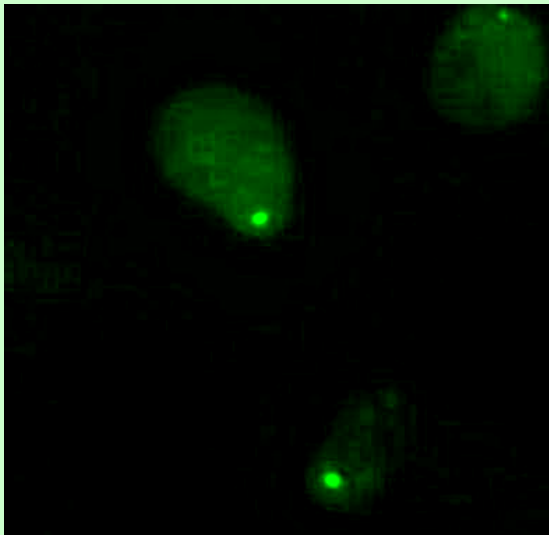
Cellular Localization of Knr4 in Mating Projections

Haploid cells of a mating type, exposed to α pheromone for:

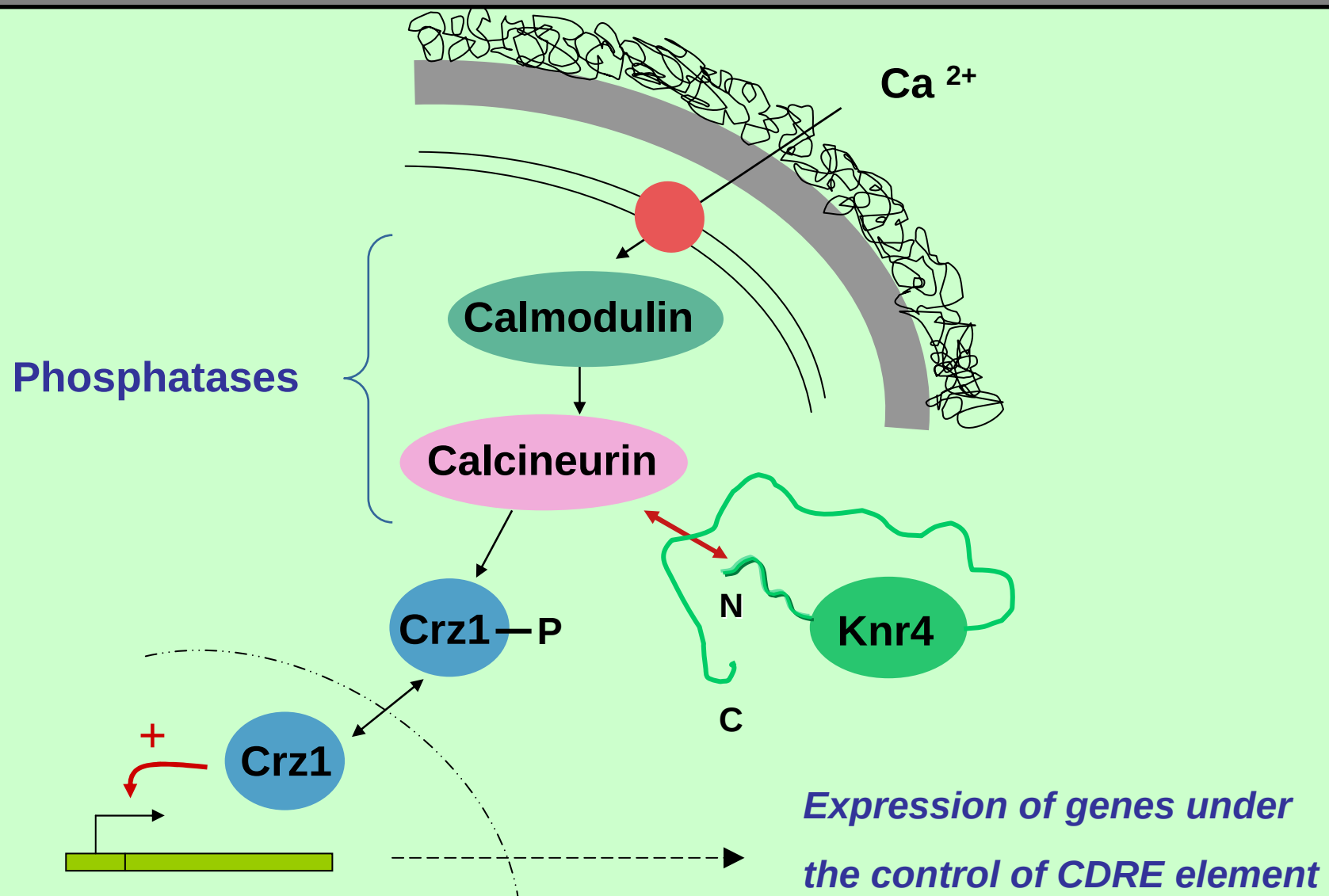
1 hour

2 hours

3 hours

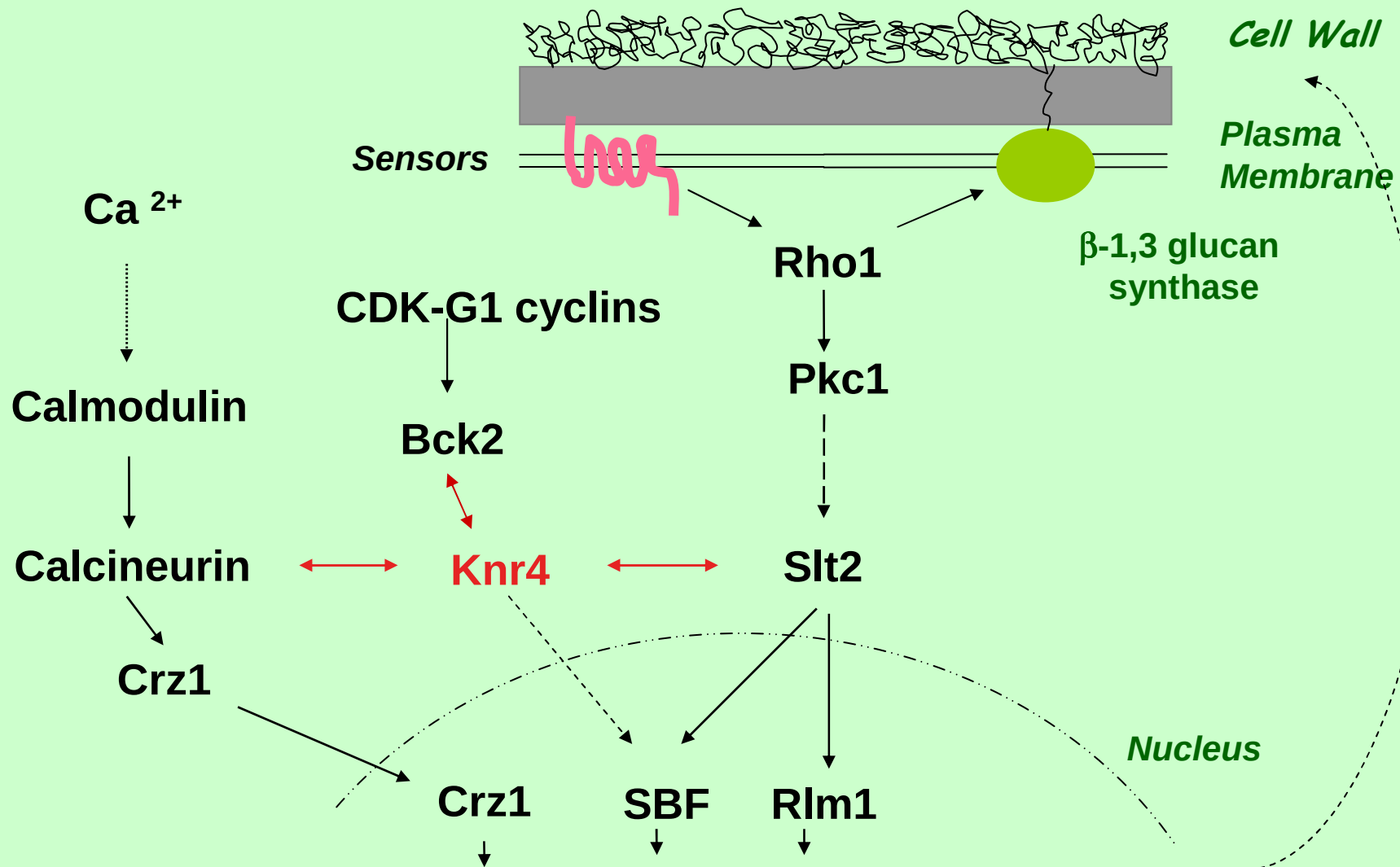


Knrr4 interacts with the Calcineurin signaling pathway :



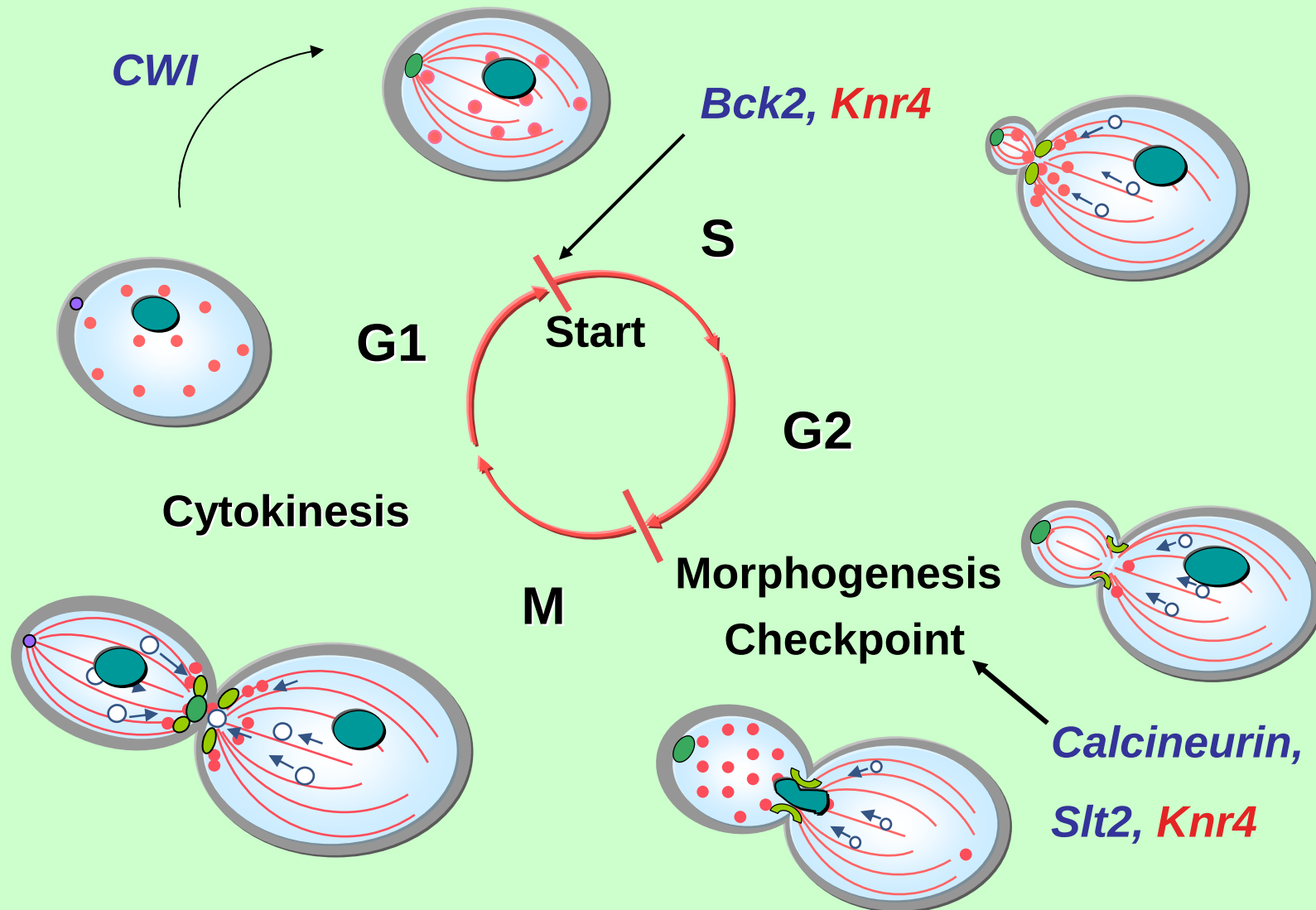
Dagkessamanskaia A., 2010. *Yeast*, 27(8), 563-74.

Knr4: coordinator of two major Cell Wall Synthesis Control Pathways.



Cell wall synthesis, Bud emergence, and G1/S transition genes.

Involvement of *Knrr4* and partners in Cell Cycle progression :



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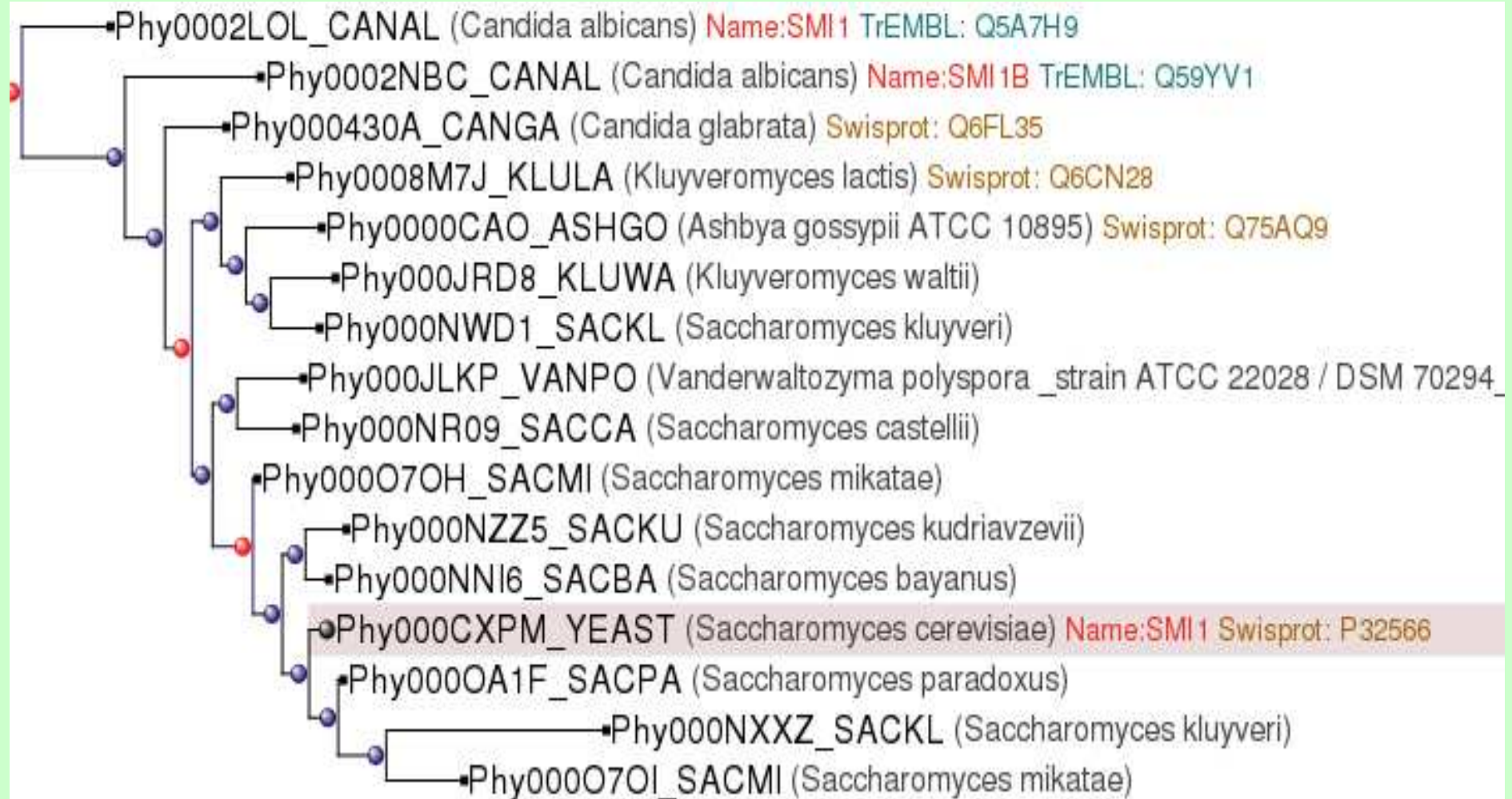
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Fungal homologs of *KNR4/SMI1*



0.59

Fungal homologs of *KNR4/SMI1*

	Amino acids		Suppression of <i>S. cerevisiae knr4Δ</i> mutant phenotype	
	<i>identical</i>	<i>similar</i>	/to CFW	/to HS
<i>Candida albicans</i> CaSMI1	29	38	+++	+++
<i>Candida albicans</i> CaSMI1B	23	30	+++	+++
<i>Ashbya (Eremothecium) gossypii</i>	33	40	++	+
<i>Neurospora crassa</i> (GS1)	23	34	-	-
<i>Aspergillus fumigatus</i>	25	38	-	-
<i>Kluyveromyces lactis</i>	39	49	<i>Not Determined</i>	
<i>Yarrowia Lipolytica</i>	20	30		ND
<i>Magnaporthe grisea</i>	23	35		ND
<i>Schizosaccharomyces pombe</i>	23	34		ND

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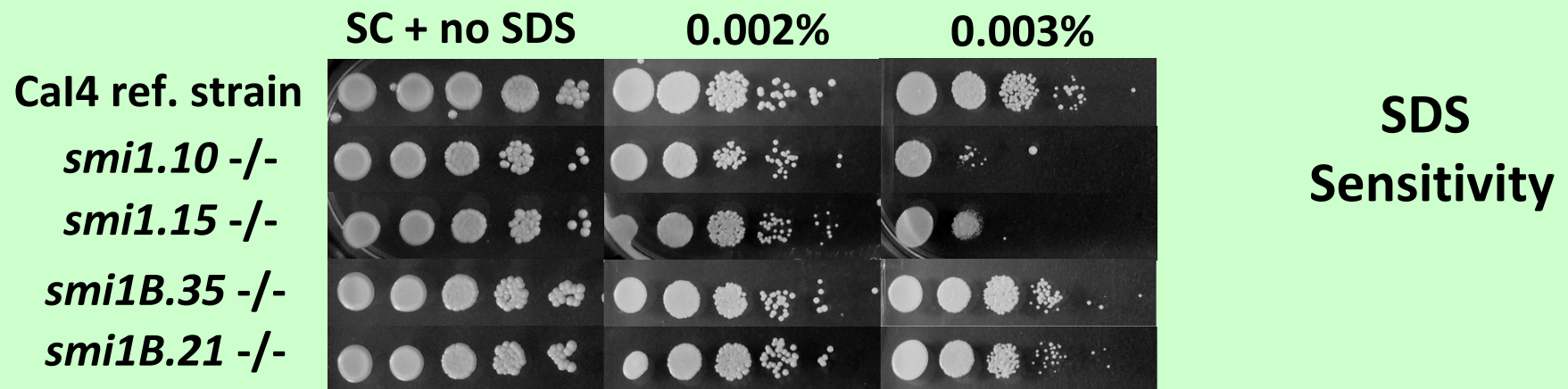
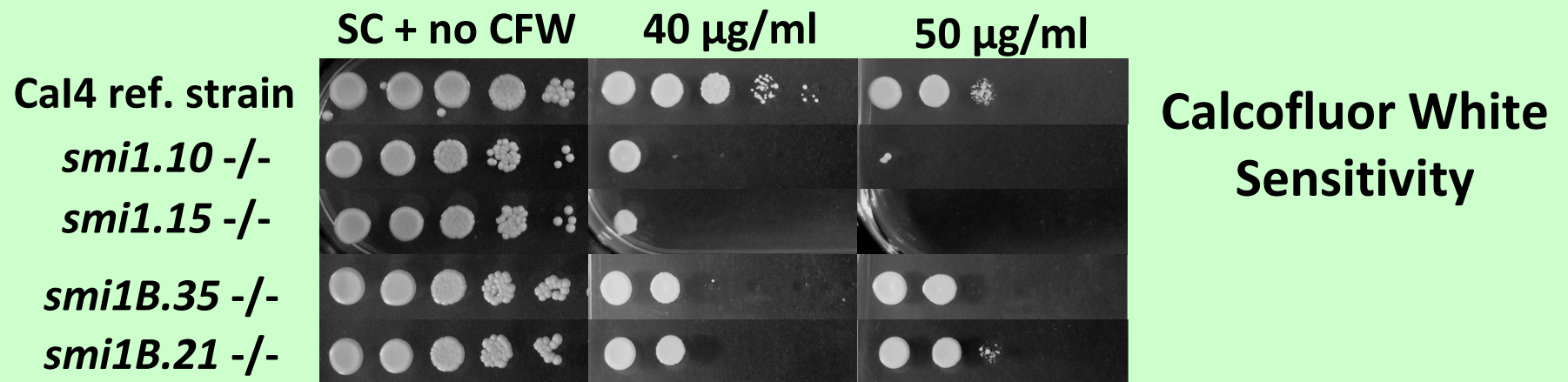
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C. albicans *KNR4*/*SMI1* homologs



C. albicans KNR4/SMI1 homologs

SMI1

Cell wall biosynthesis protein; Hap43p-, caspofungin-repressed; Cyr1p-induced in hyphal cells; planktonic and biofilm-induced; **null mutant shows hypersensitivity to CFW, SDS, reduced biofilm formation, cell wall β -glucan production, and drug (flukonazole) resistance.**

SMI1B

Putative cell wall assembly regulatory protein; transcription is negatively regulated by Rim101p;

<http://www.candidagenome.org/>, our results,

and Nett et al., *Eukaryotic Cell*, 10.1128/EC.05126-11

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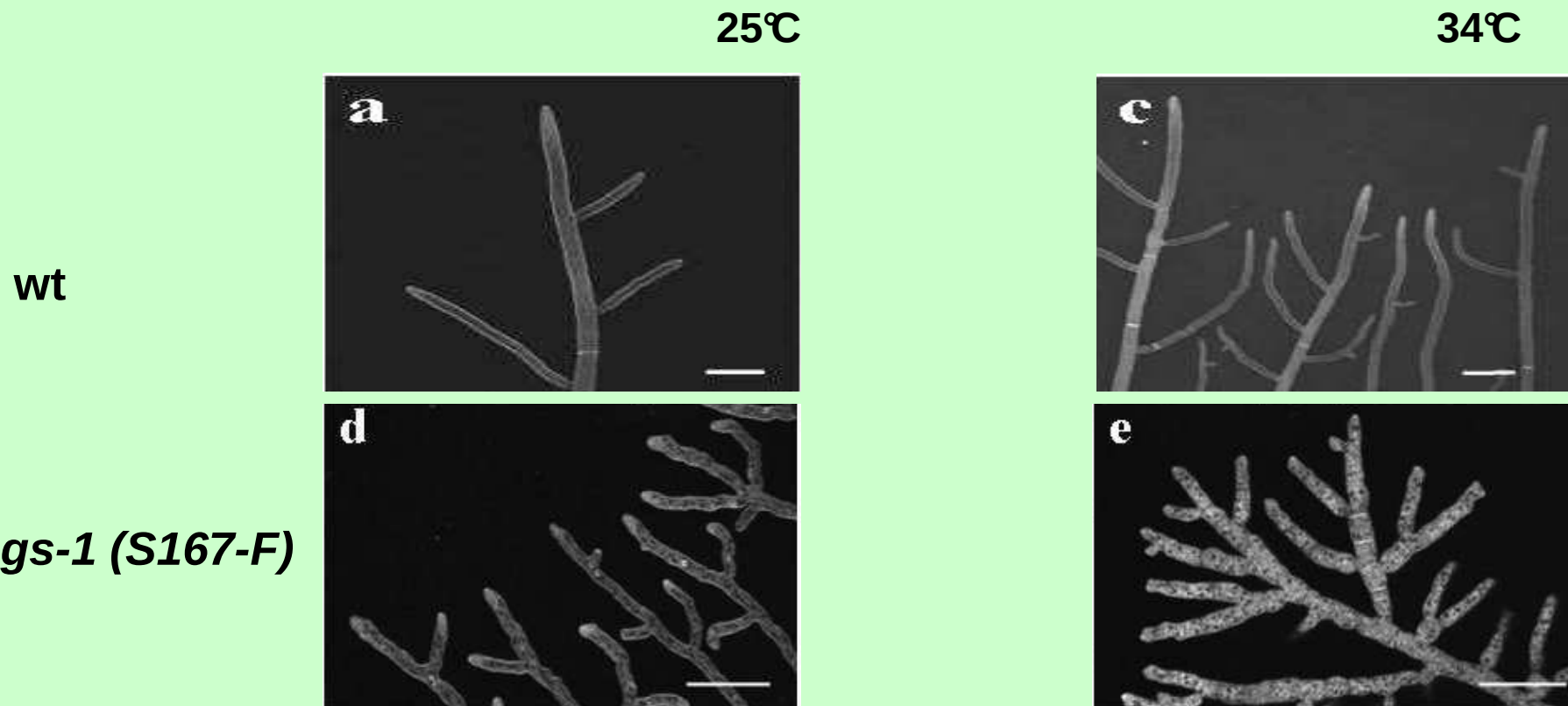
Neurospora crassa KNR4/Smi1 homolog : GS-1

- Involved in β -(1,3) glucan synthesis (GS for Glucan Synthase)
- Sequence comparison of GS-1 and ScKnr4/Smi1 proteins :

GS-1p	MAGLFKDIWHAMTSYDRHAGIDSPYRTGRHVPLNRNSGLAGVTTASDSRADINSPYLQGDGRGSTMSFDIT
Smi1p/Ktr4p	M-DLFK-----RKVKEWVYSLSTDDHYAEYNPDETFT
GS-1p	AYGGRRAISPMFSPANGGPYSPGLVSRQSVHQDAFDVHSPTGEIPMONFQNGGPPPPPVASSWEKIDRWA
Smi1p/Ktr4p	FNMGKRLNSNNGQVNPSSQMHLNSVDEEMSGFQNGVPSNEDINIDEFTSTESNDGVSETLLARHIDFWT
GS-1p	EENYPELFDQLGEGCTVNDLNELEYQLDCTLPODLRISLQIHDGQERGGGLPTGIIFFSSMLLDCEEMVQEW
Smi1p/Ktr4p	SEHNPDLNATLSDPCTQNDITHAEELLEVSPNPVKASEKIHDGQEDLESMTGTSGLFYGFQQLMTLDQVV
GS-1p	ENNKTVNQEFMLDPVLVKRQSQAFAAQASSSKDAPNRNQNWQELLNKQDSUPPAATOKAYAHPAWIPLV
Smi1p/Ktr4p	AMTQAWRNVAKNLNKRSQQGLSHVTSTGSSSSMERLNGNKFCLPNIPOKSIIPPAVQPVYAHPAWIPLI
GS-1p	RDWGGNNLAVDLAPGPNKGHWGQIILFGRDYDTKIVVARSNAHFLAMVAEDLSSGMEVDEDIN-----
Smi1p/Ktr4p	TDNAGNHIGVDLAPGPNKYAQIILFGRDFTKIVDAENGGEFLSPANDLEAGNMLVLDNDQDYFSGDG
GS-1p	ELKLREFKATRVESYFEILRWKMDQKYGRTANKRKSMAPSMAASAGMRSPTPTGSPYQSPTEHNEPRGR
Smi1p/Ktr4p	ELVFRDKKENGPIQDYFEWLKRRWIKYQENLRSQQQKSQPDTSLOEQKYVPASQKKVAABEQPSTLNAES
GS-1p	SLHRLTGTSPMSSPIRPGYCKPSPLARVAEEAPPTTSLTASNALEKKAADNLMELNTPRTSGEHSKEDI
Smi1p/Ktr4p	IKGEDSGEADVQSVQDHESVKIVKTEPSEAEITTVNTESLGOASHEKKAONVDIKQESERKEDEKQPKVE
GS-1p	KVNEDSPAKERTSEDKKKPETEANGKATESKKGKQTTVEDAEDNKDIEI
Smi1p/Ktr4p	EKEHVENEHVTESAKKDDOVNKQTEEMNKKEENEIRSDDAKVEEARREEFENIAL

(Enderlin and Selitrennikoff, *PNAS USA* 91, 9500-4, 1994).

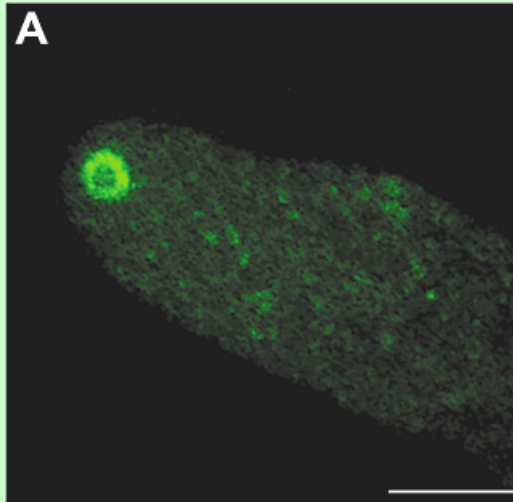
Morphogenetic defects of a point mutant in GS-1



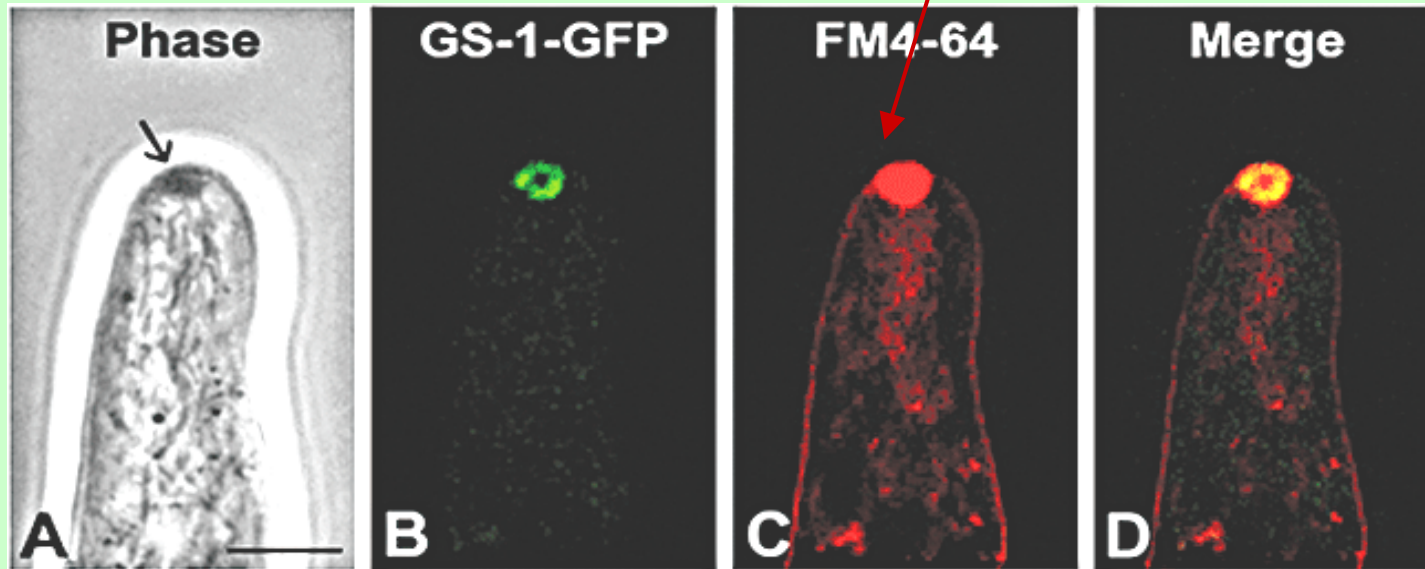
Strain	Average distance (um) between branches at 25° C	Average distance (um) between branches at 34° C	Increase in branching frequency (%) at 34° C
<i>wt</i>	180±4.0	176±14.1	≈0
<i>cot-2</i>	110±7.0	30±1.8	360%

(Fungal Genetics Report 55:32-36, Resheat-Eini et al., 2008)

KNR4/SMI1 homolog in *Neurospora crassa* : GS-1



*Localized at the hyphae tip,
around the Spitzenkörper.*



(Verdin et al., 2009. Mol Microbiol 74, 1044-53).

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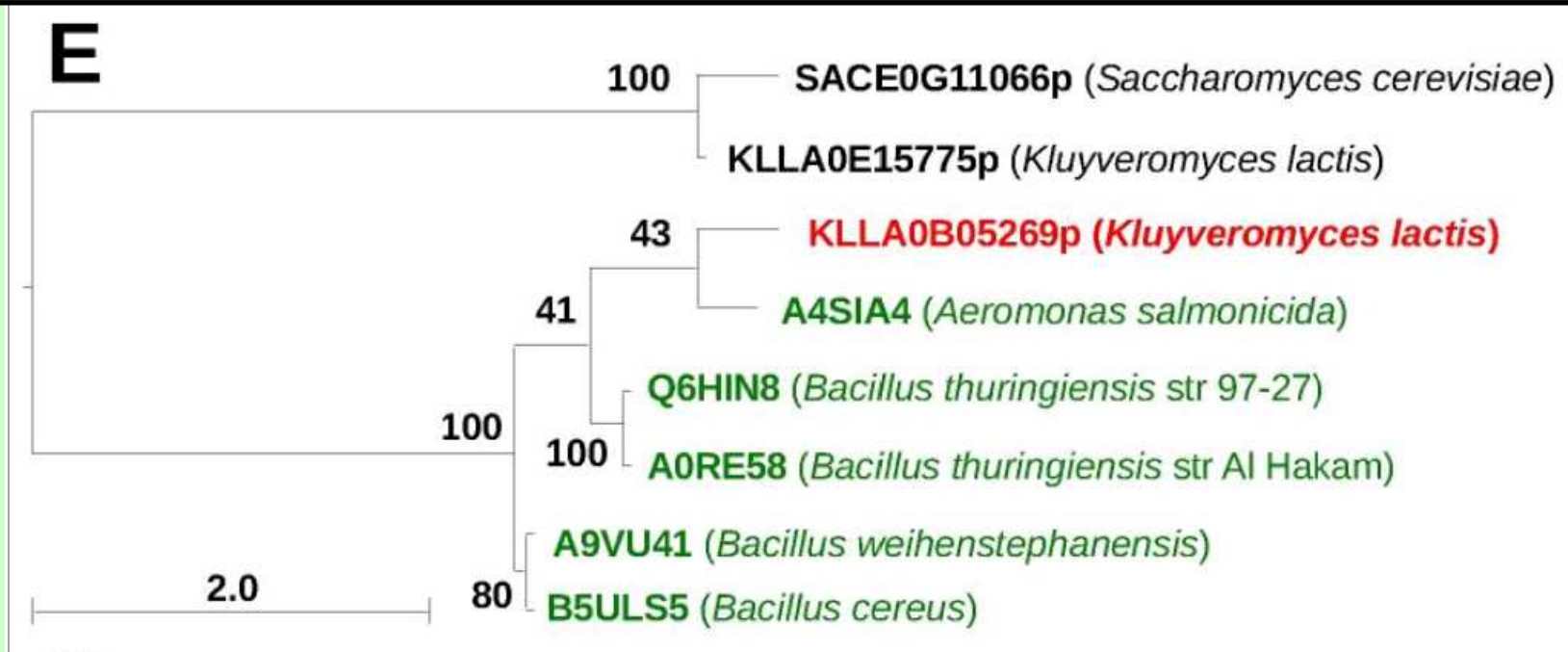
Conserved features of the Knr4/Smi1 homologs

- ***Transcriptional control of cell wall synthesis genes*** notably chitin and $\beta(1,3)$ glucan synthases

- ***Localization : at sites of active polarized growth*** dependant on cellular landmarks

- ***Disordered domains*** conferring ability to interact with multiple partners.

The origin of the *KNR4/SMI1* gene family : bacterial ?



(Rolland et al., 2009, PLoS One, 2009 Aug 5;4(8):e6515)

- Smi1/Knr4 homologs could derive from bacteria
- Conserved domain in the Smi1/Knr4 superfamily **able to bind structurally diverse protein partners** re-used by eukaryotes to recruit targets to peptide-modifying systems (ubiquitin, polyglutamylase systems)

(Zhang et al., 2011, *N.A.R.*, 39(11): 4532-4552)

Perspectives

Tridimensional structure of Knr4 :

Currently under investigation, for N-term and central domain. (aa 1-340, *with L. Mourey, IPBS, Toulouse*).

Knr4 connections with Morphogenesis :

Knr4 localization dependence on cell polarity landmarks,
Confirm and map Knr4 - Act1 physical interaction
(*PhD Ran Liu, INSA-China Scientific Council*).

Investigate the C. albicans homologues of Knr4 :

A possible role in Virulence ?

with M. Lavie-Richard (AgroParisTech, Grignon).

New information on the specific function of this fungal protein family (ex : in hyphae, Polarisome or Spitzenkörper ?)



Arnaud Lagorce
(PhD)



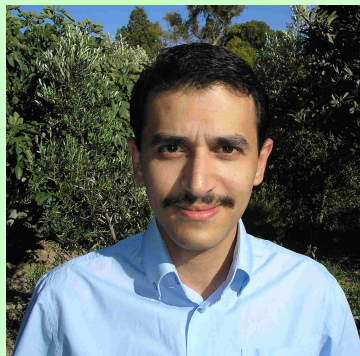
Fabien Durand
(PhD)



Fadi Basmaji
(Master I, II, PhD)



A. Dagkessamanskaia
(*Post Doc*)



Karim El Azzouzi
(Master II)



Pr. Jean-Marie François.

And thank you for your attention !

Questions ?