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# Knr4/Smi1 Family: Conserved Fungal Chaperones of Puzzling Origin

***Hélène MARTIN-YKEN***

***Fungal Cell Wall Biogenesis Meeting,  
Primösten, June 6-9th, 2012.***

**Physiologie et Génomique Fonctionnelle des Eucaryotes  
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Email: [helene.martin@insa-toulouse.fr](mailto: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***

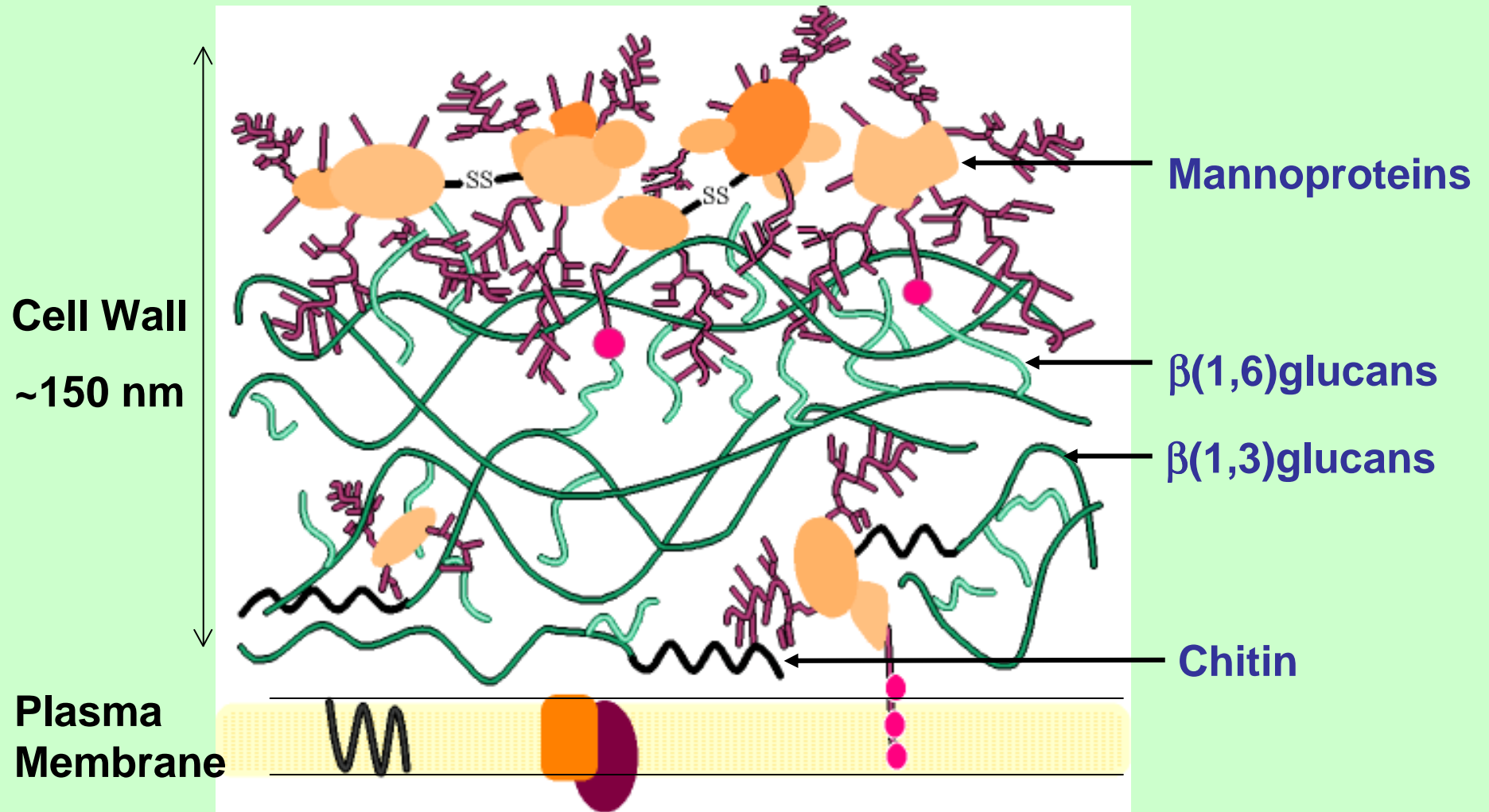
**The role of ScKnr4/Smi1 in cell signaling  
So many partners !  
A very peculiar structure...**

## ***III Gene and function conserved among fungi***

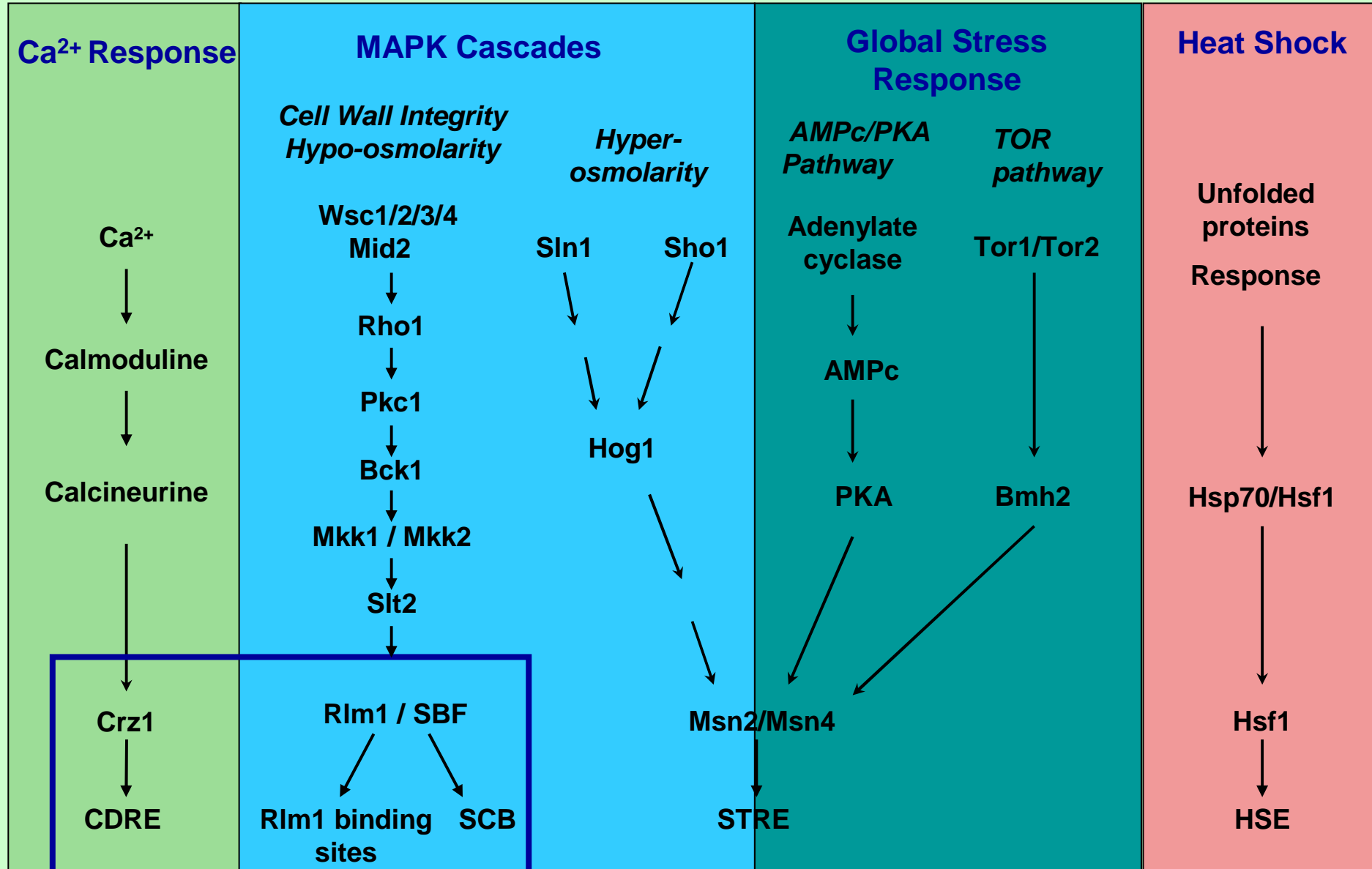
**Complementation of *S. cerevisiae* mutant  
*Candida albicans* Smi1 and Smi1B  
*Neurospora crassa* GS-1.**

## ***IV Integrative Overview and Perspectives***

# Cell Wall Architecture in *S. cerevisiae* :



# Signaling pathways involved in coping with Cell Wall Stress :



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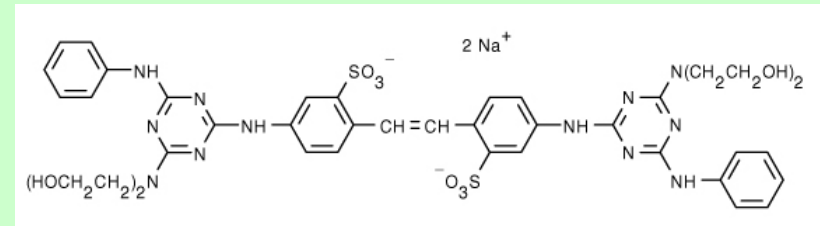
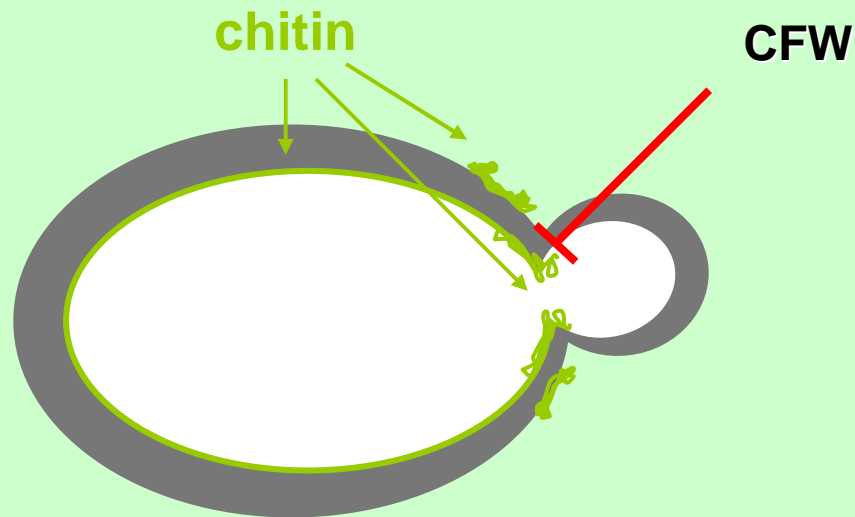
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# Screen for Calcofluor White hypersensitive mutants



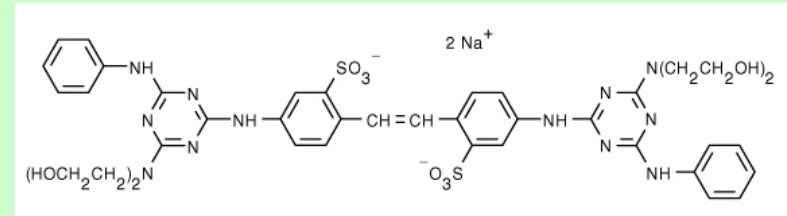
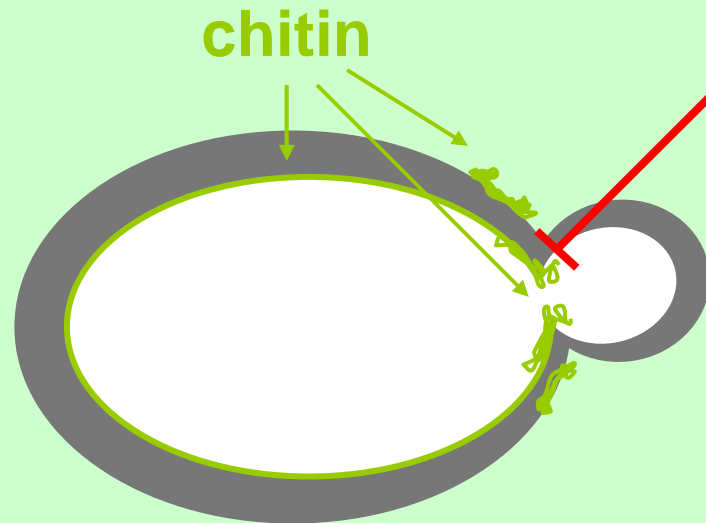
*Calcofluor White*

- **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



CFW

→ **Suppressor genes :**  
***RHO1, BCK2,***  
***and KNR4***

*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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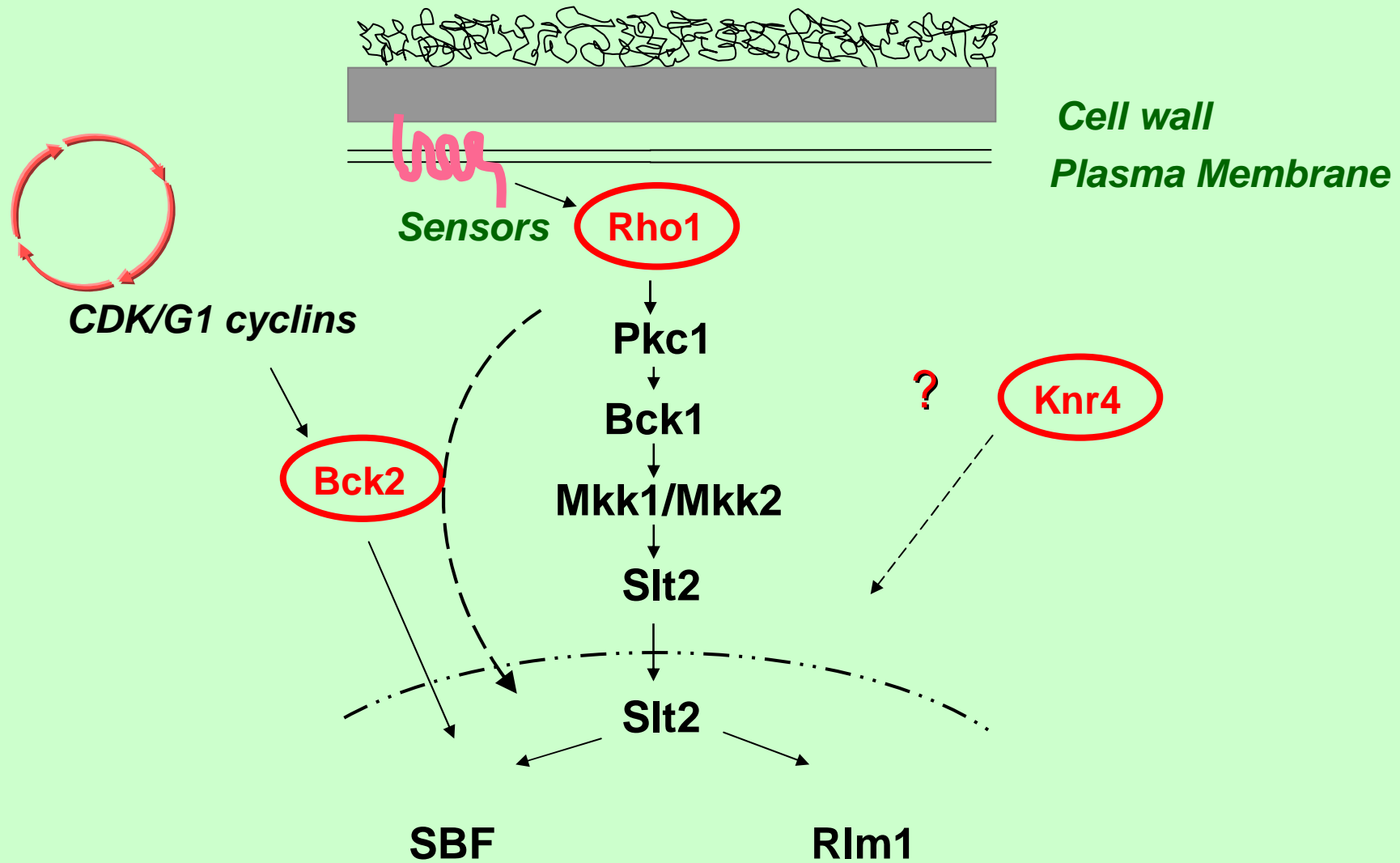
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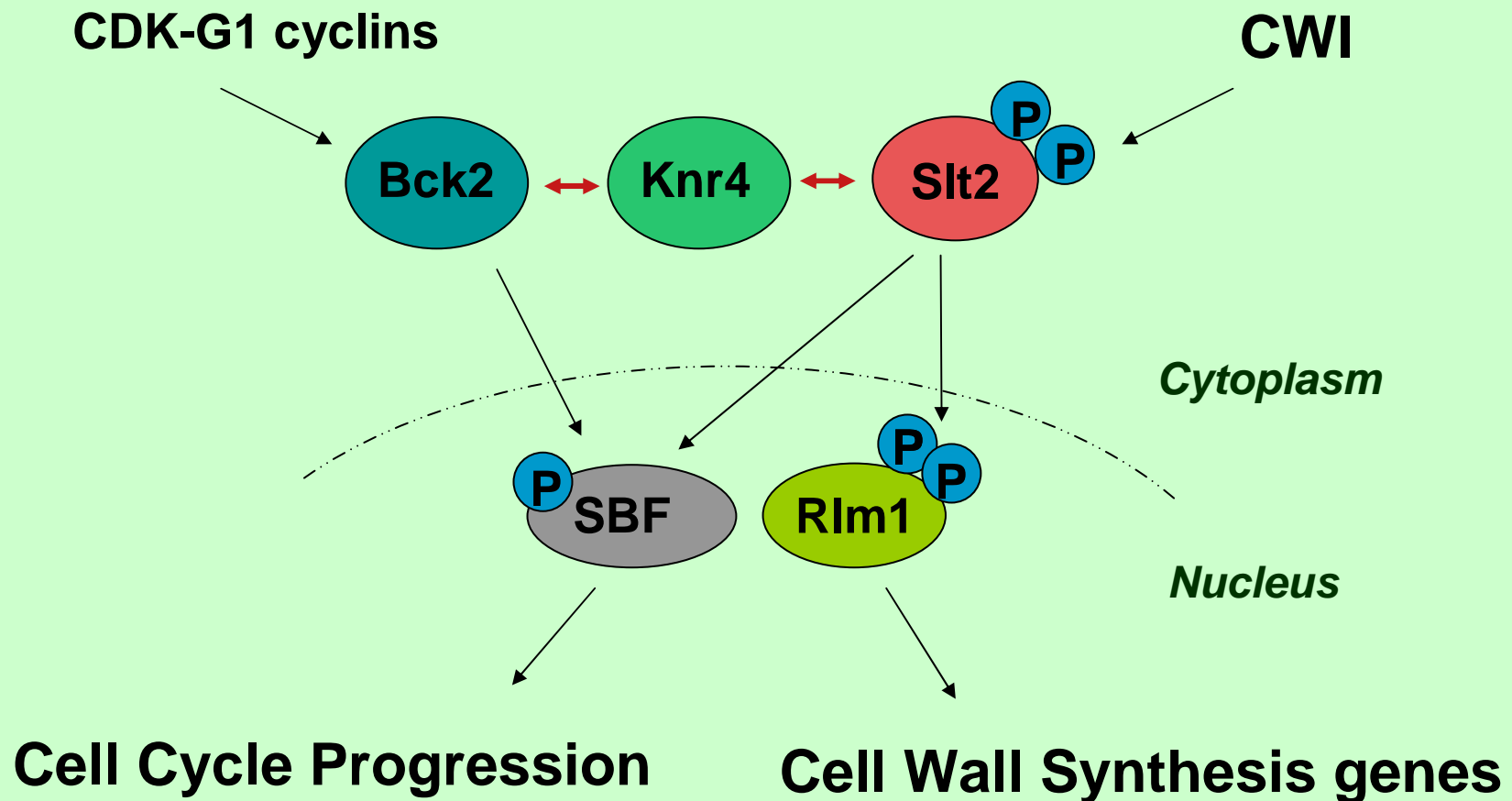
## **IV *Integrative Overview and Perspectives***

# Position of the suppressor genes isolated in cell signaling pathways :



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

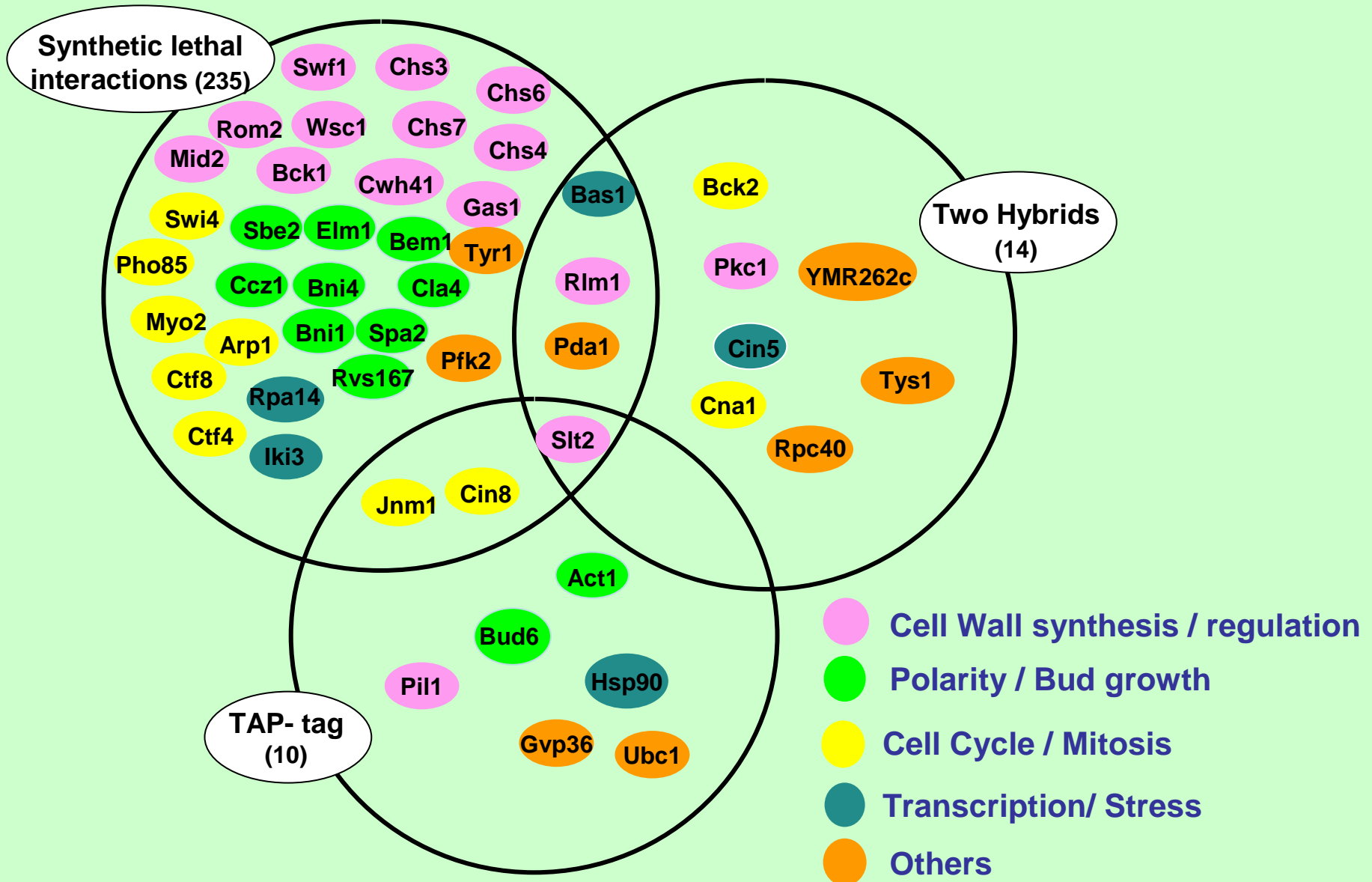
# Knr4 affects transcriptional Control of Cell Cycle and Cell Wall Synthesis genes



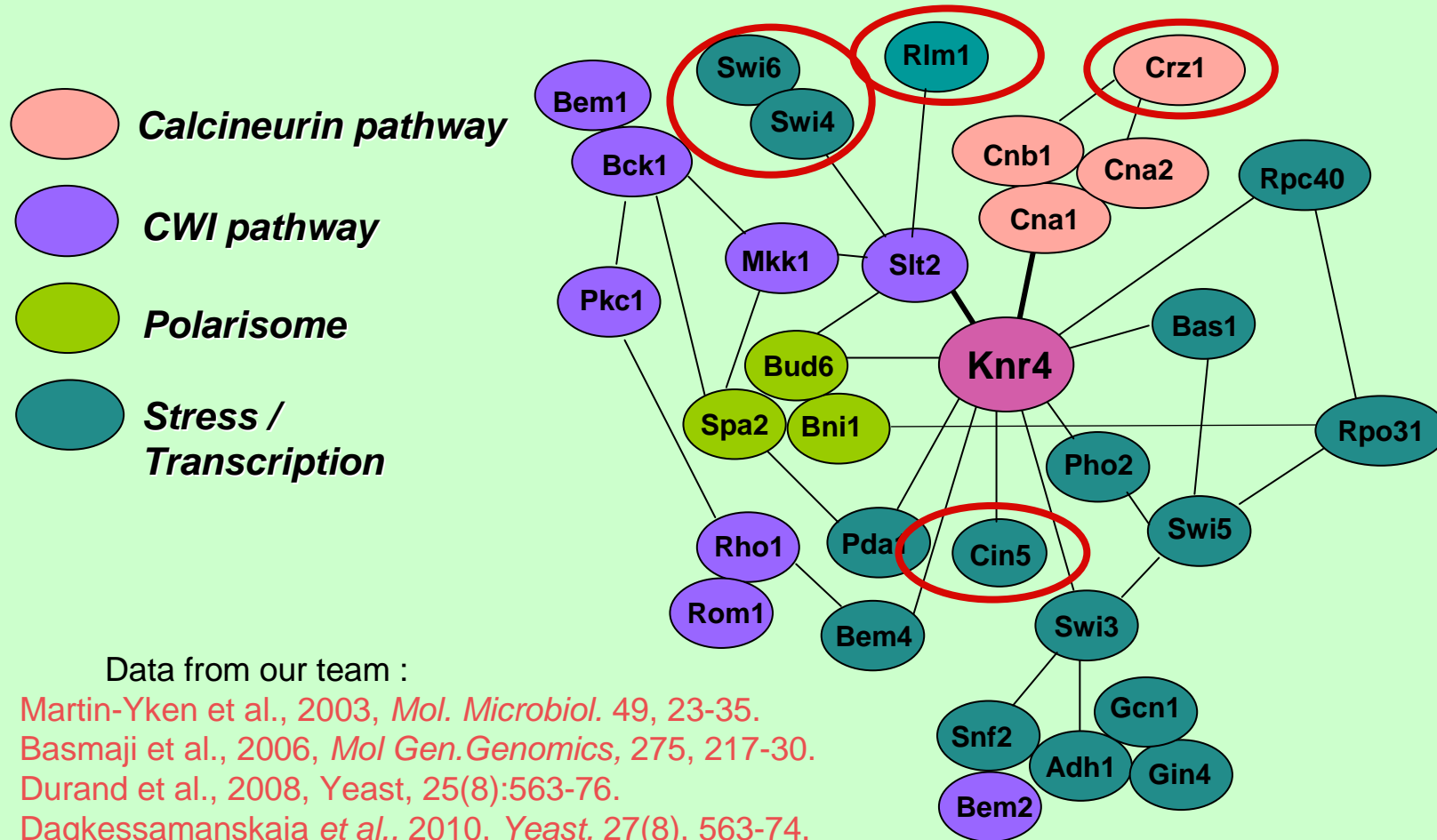
Martin-Yken H., et al., 2002, *Current Genetics*, 41: 323-332.

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 :

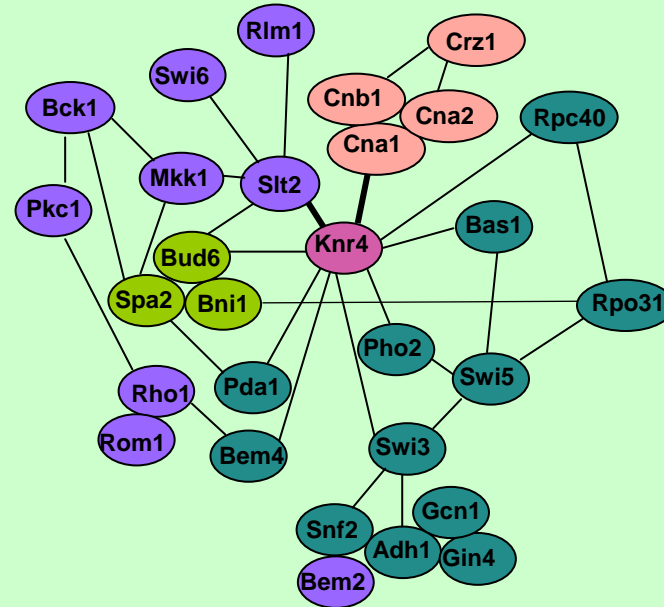
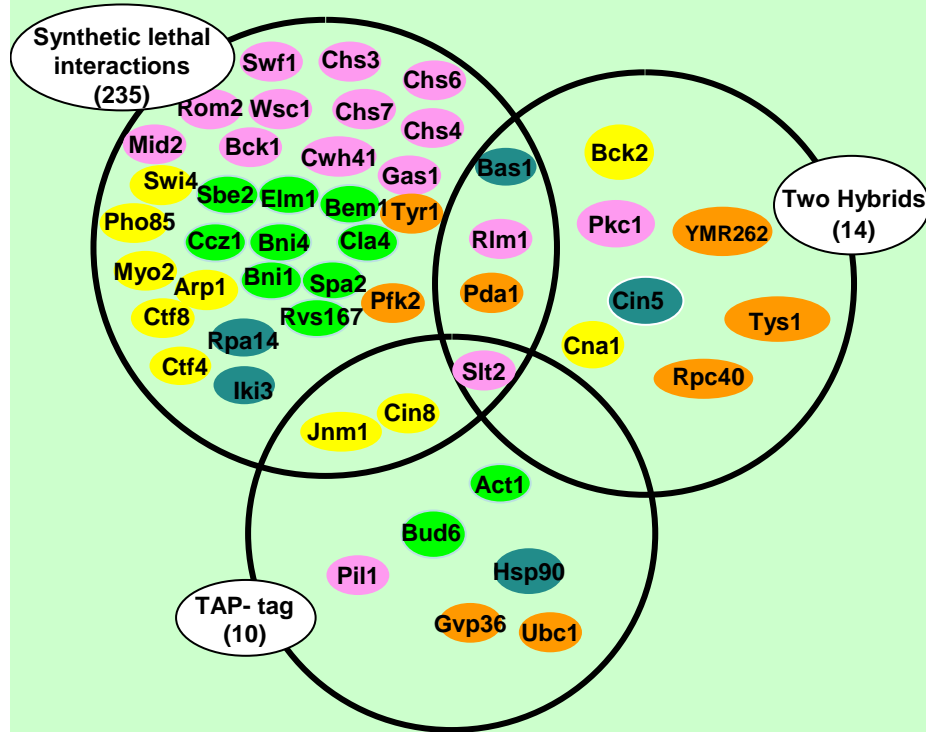
Ito T. et al., 2001 *PNAS U S A.*; 98, 4277-8.

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 :

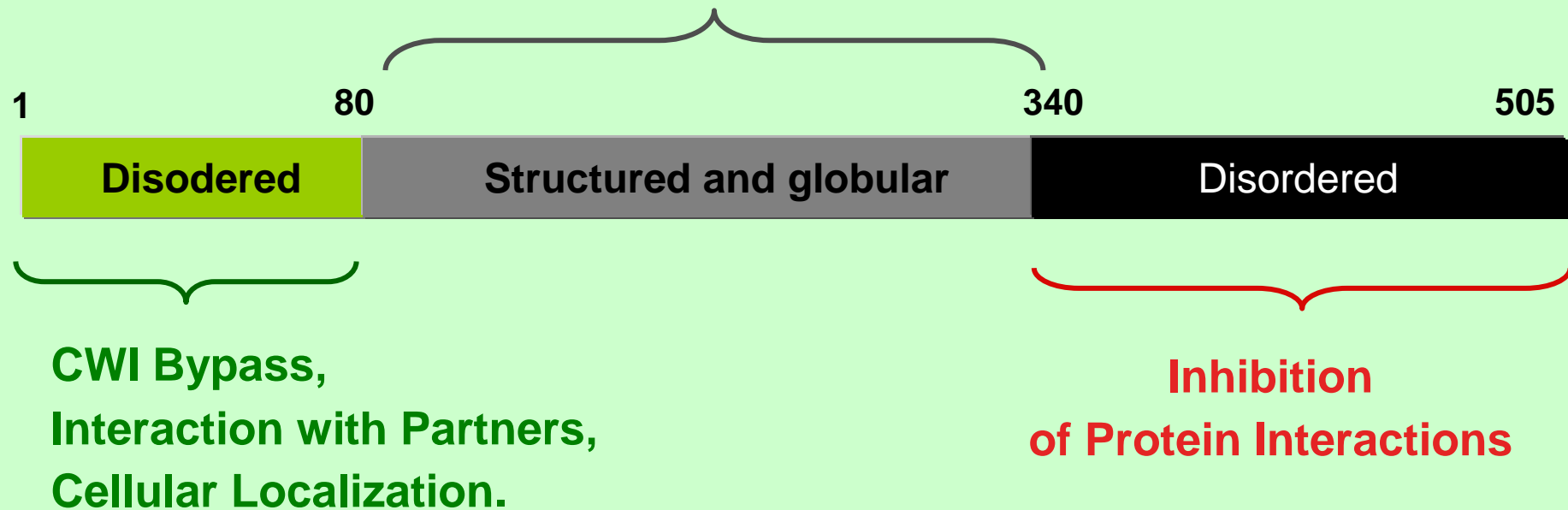


→ **Genetic : why so many Synthetic Lethals ?**

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

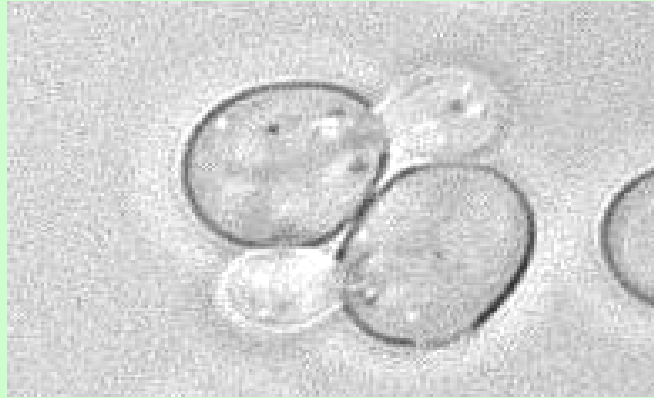
## Different functions of the distinct Knr4 domains :

Complements the *knr4* $\Delta$  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



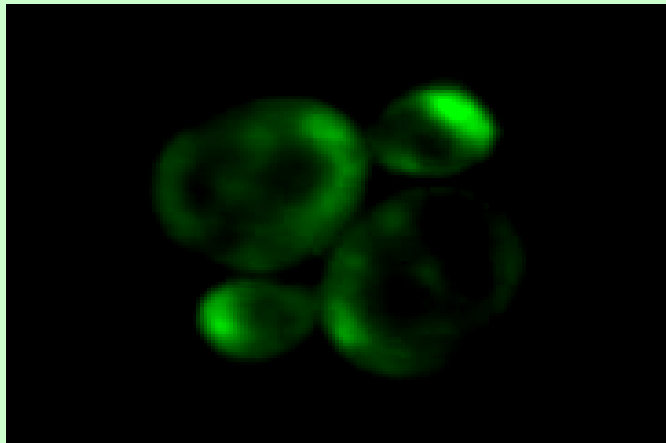
**Axial budding pattern**

a or  $\alpha$  Haploid cells



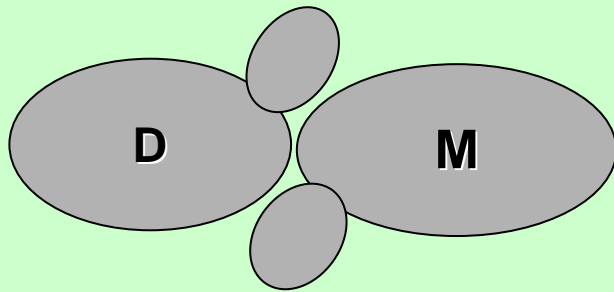
**Bipolar budding pattern**

a/ $\alpha$  Diploid cells



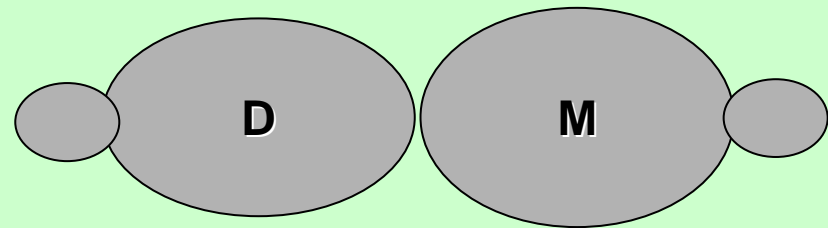


***S.cerevisiae* haploids and diploids cells  
budding patterns :**



**Axial budding pattern**

**a or  $\alpha$  Haploid cells**



**Bipolar budding pattern**

**a/ $\alpha$  Diploid cells**

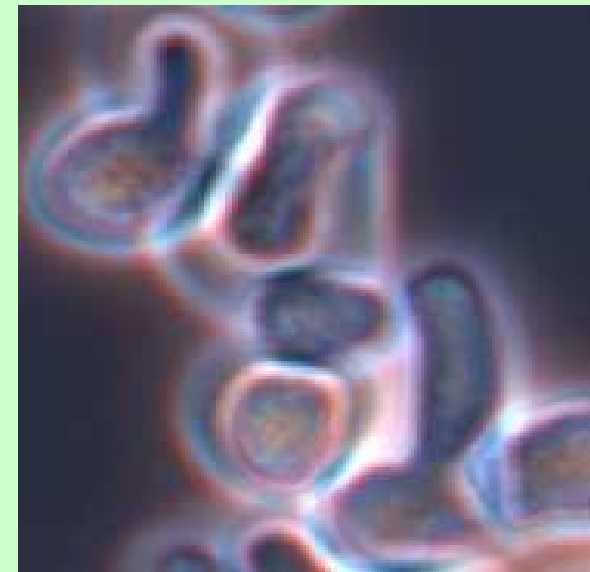
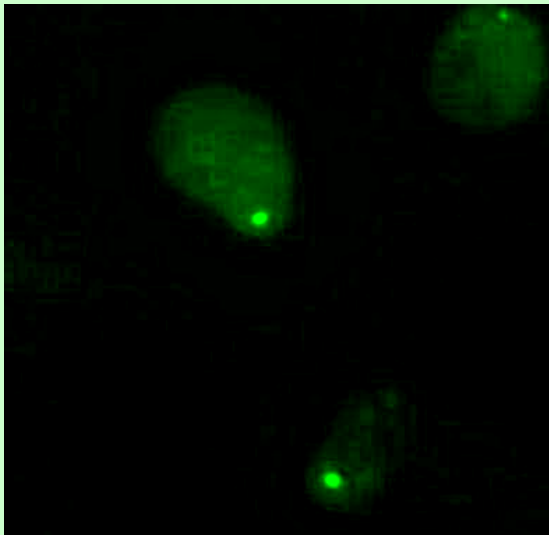
# Cellular Localization of Knr4 in Mating Projections

Haploid cells of a mating type, exposed to  $\alpha$  pheromone for:

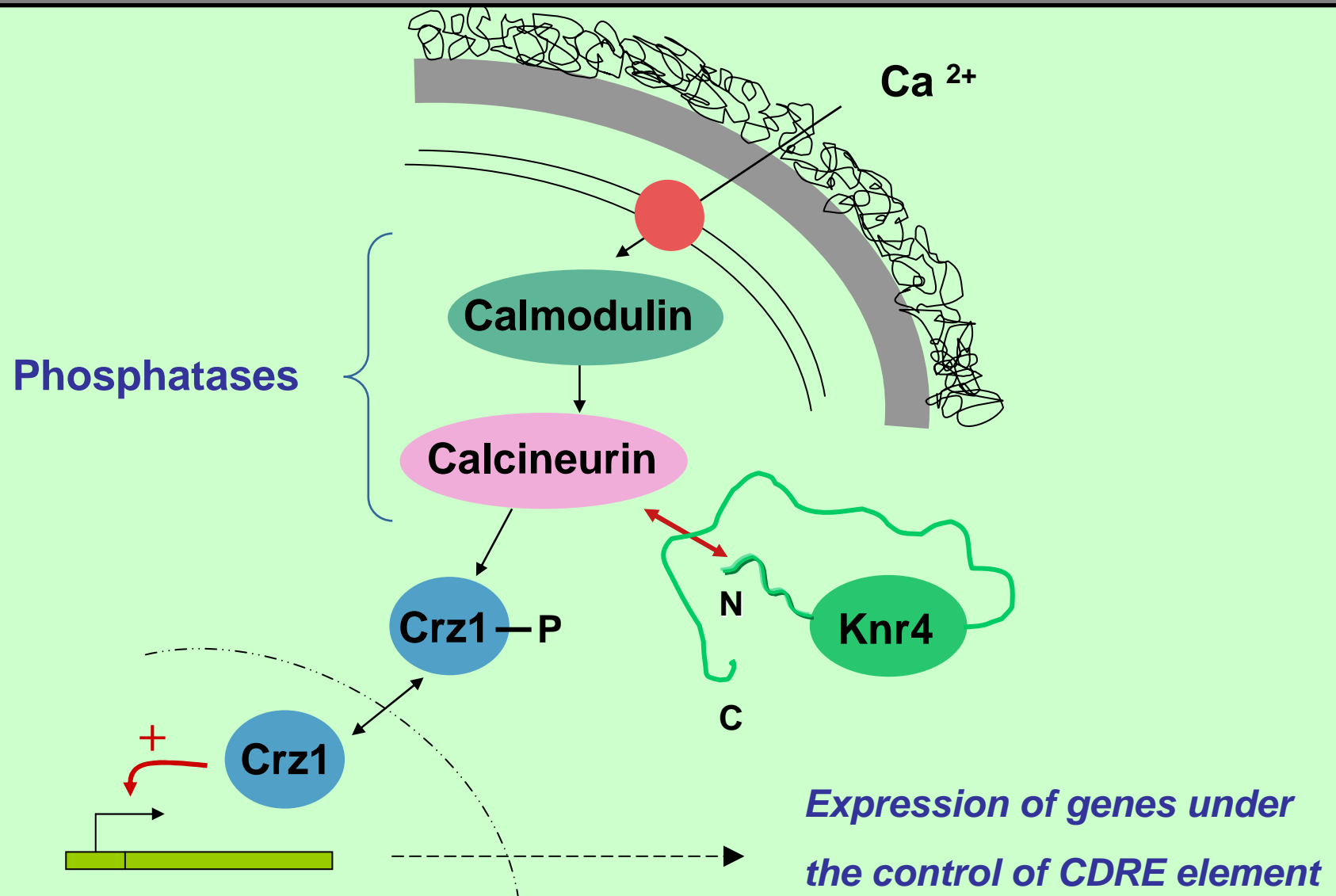
1 hour

2 hours

3 hours

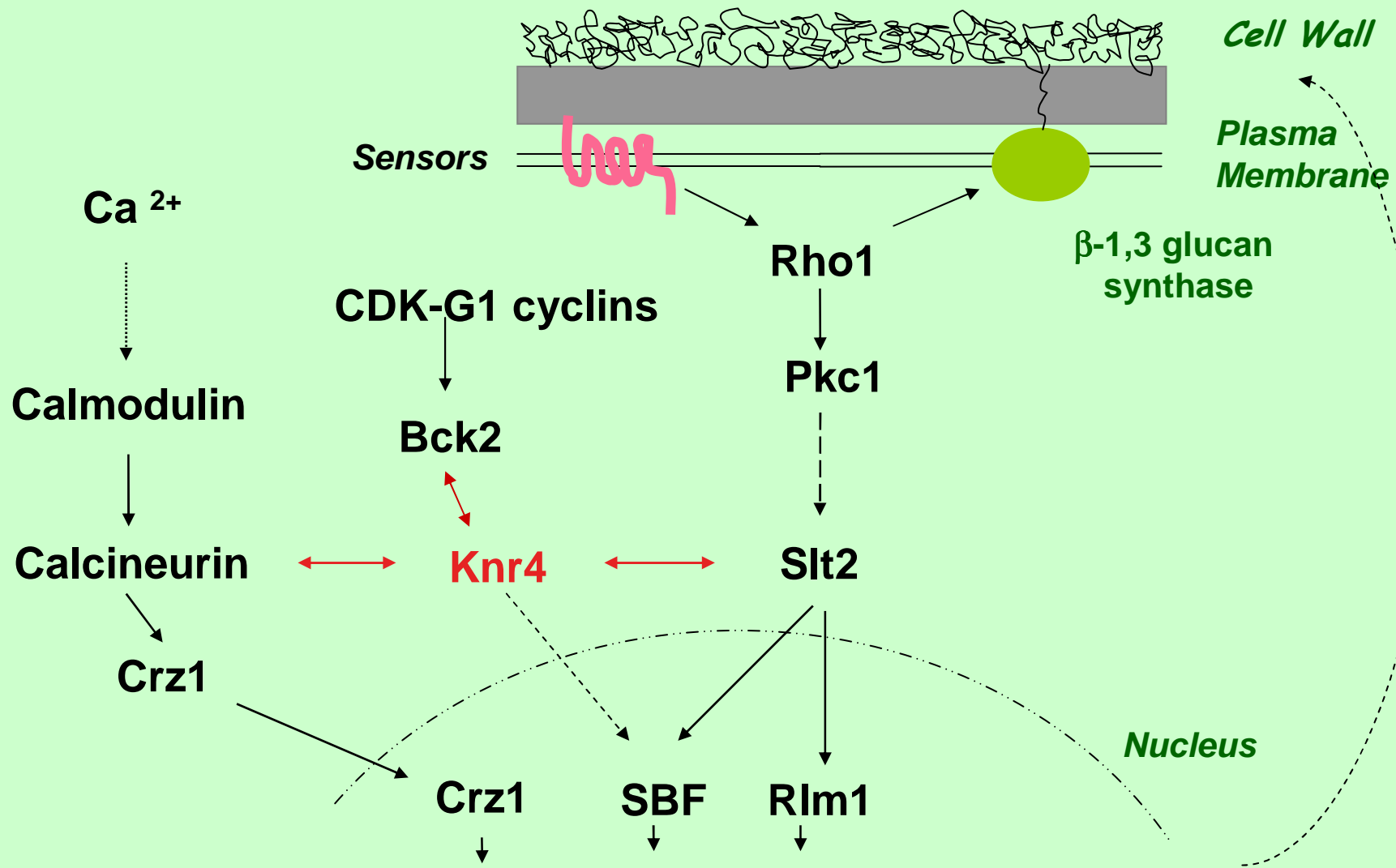


# Knr4 interacts with the Calcineurin signaling pathway :



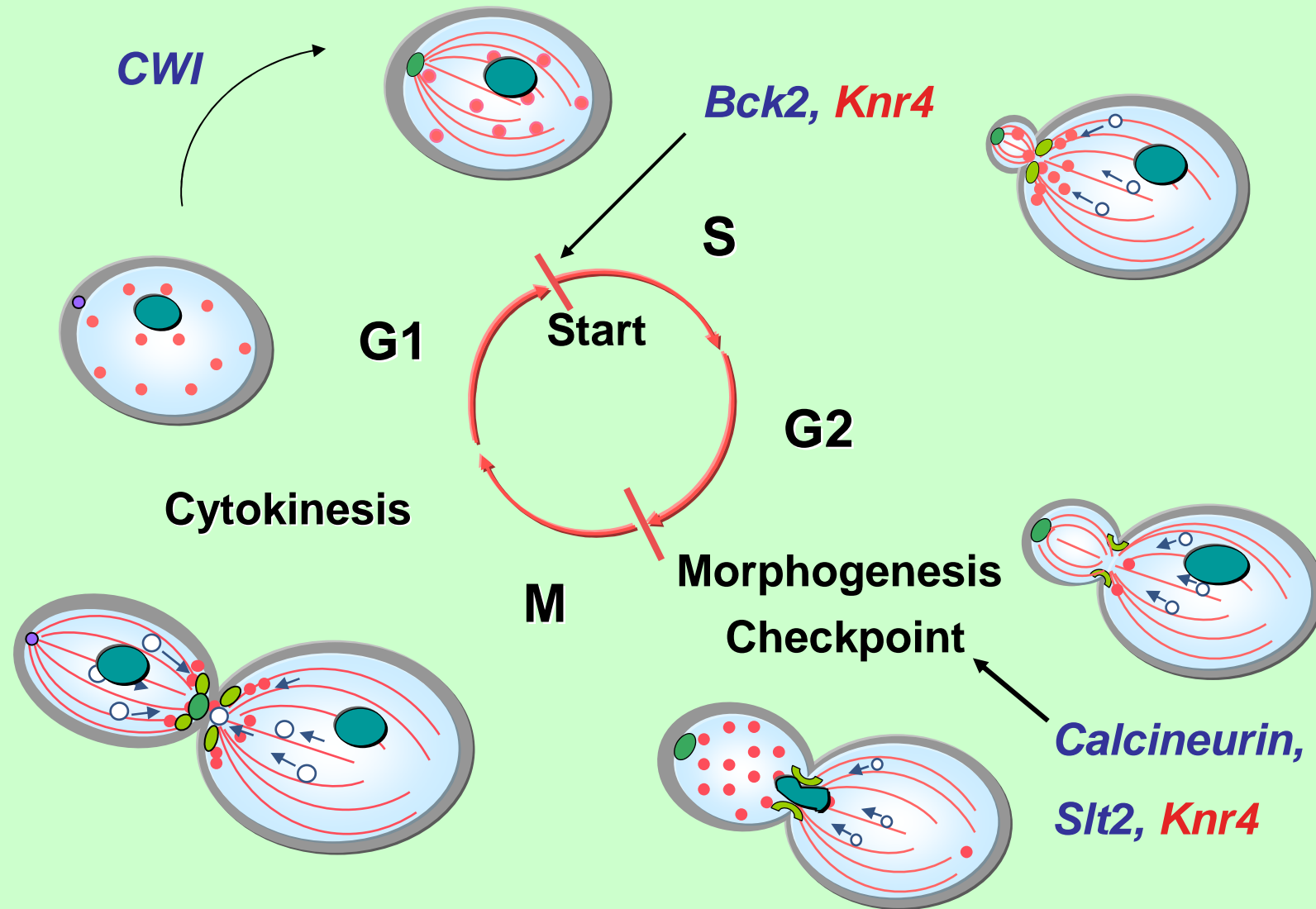
Dagkessamanskaia A., 2010. *Yeast*, 27(8), 563-74.

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



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

# Involvement of *Knr4* and partners in Cell Cycle progression :



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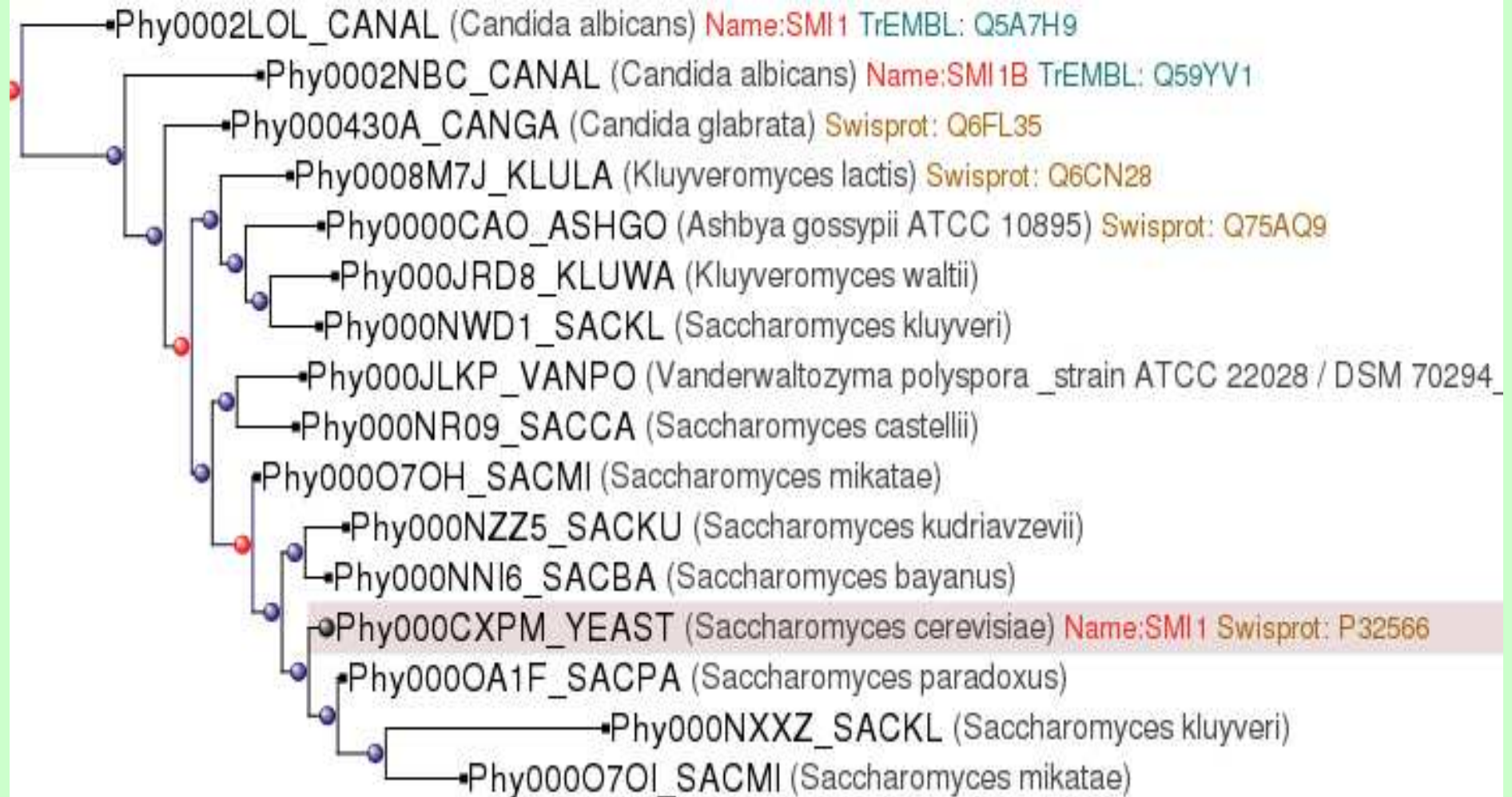
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# Fungal homologs of *KNR4/SMI1*



0.59

# Fungal homologs of *KNR4/SMI1*

|                                       | Amino acids      |                | Suppression of<br><i>S. cerevisiae knr4Δ</i><br>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>Kluveromyces 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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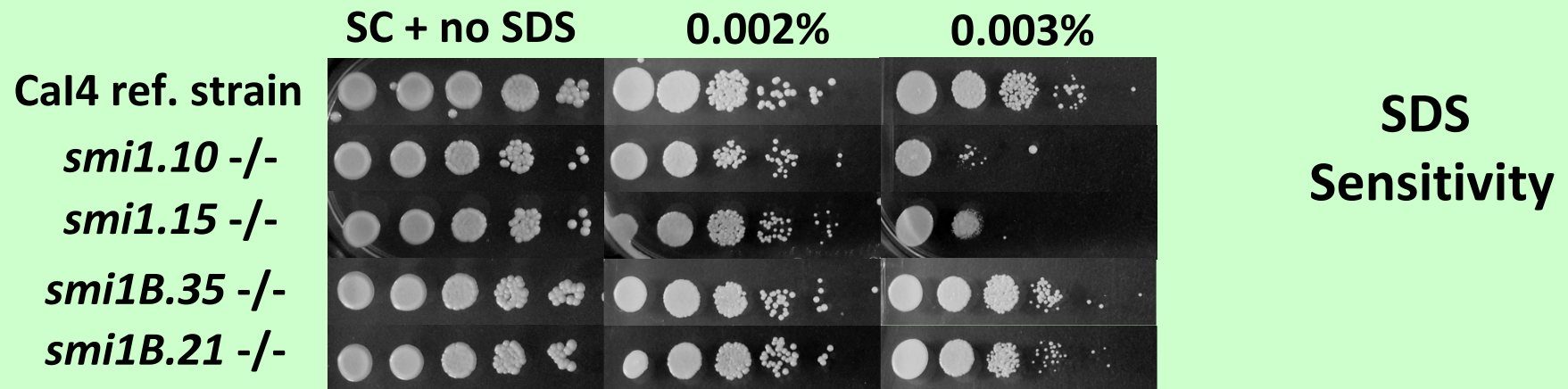
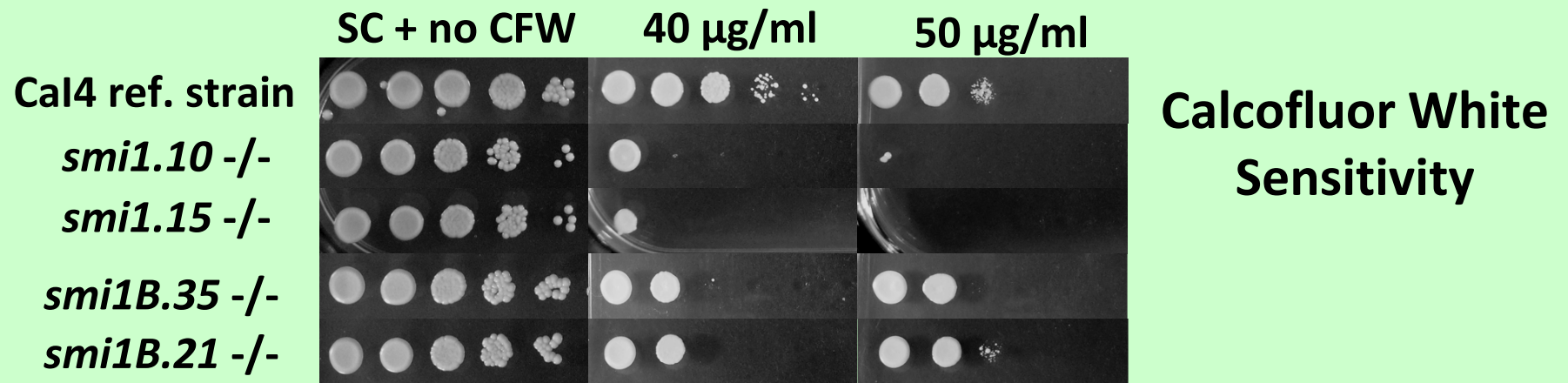
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# *C. albicans* KNR4/SMI1 homologs



## *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  $\beta$ -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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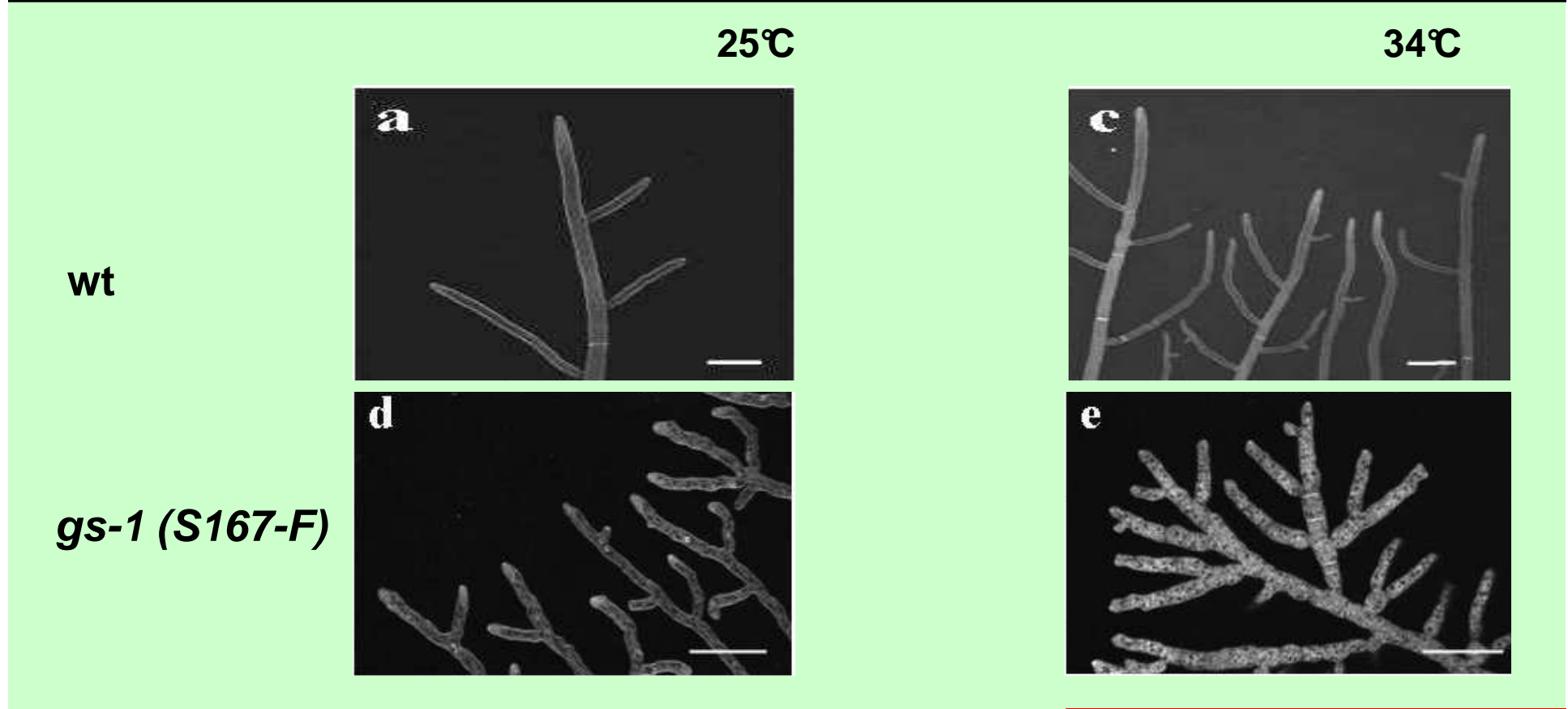
# Neurospora crassa KNR4/SMI1 homolog : GS-1

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

|             |  |
|-------------|--|
| GS-1p       | MAGLFDKIWHAMTSYDRHAGIDS PYRTGRHVPLNRNSGLAGVTTASDSRADINSPYLQGDGRGSTMSFDI  |
| Smilp/Ktr4p | M-DLFDK-----RKVKEWVYSLSTDDHYAEYNPDETFT                                   |
| GS-1p       | AYGGRAISPMPSPANGGPFYSPGLVSRQCSVHQDAFDVHSPTGETPHONFQNGGPPPPVAVSSWEKIDRWA  |
| Smilp/Ktr4p | FNMGKRLNSNNGQVNPSSQMHLNSVDEEMSMGFQNGVPSNEDINIDEFTSTESNDGVSETLLAWRHIDFMT  |
| GS-1p       | EENYPELFDQLGEGCTVNDLNELEYQLDCTLPODLRISLQIHDGQERGGGLPTGIIFFSSMLLDCEEMVQEW |
| Smilp/Ktr4p | SEHNPELNAITLSDPCTVNDITHAEELLEVSFPNPVKASPKIHDGQEDLESMTGTSGLFYGFQQLMTLDQVV |
| GS-1p       | ENNKTVNQEFMLDPVLVKRQSQAFAAQASSSKDAPNRNQNWRQELLNKQDSUPPAATOKAYAHPAWIPLV   |
| Smilp/Ktr4p | AMTQAWRNVAKNLNKRSQQGLSHVTSTGSSSSMERLNGNKFKLPNIPDQKSIIPNAVQPVYAHPAWIPLI   |
| GS-1p       | RDWGGNLAVDLAPGPGHGWGQIILFGRDYDTKVVARSNAHFLAMVAEDLSSGMEVDEDIN-----        |
| Smilp/Ktr4p | TDNAGNHIGVDLAPGPGKYAQIILFGRDFDTKEVIAENNGEFLLSFANDLEAGNMLVDDNDYFSGDG      |
| GS-1p       | ELKLRERKATRVESYFEDLRWRMDQKYGRTANKRKSMAPSMASASGMRSPPTPGSPYQSPTEHNEPRGR    |
| Smilp/Ktr4p | ELVFRDKKENGPIQDYFEWLKRRWIKYQENLRSQQQKSQPDTSLOEQKYVPASQKKVAABEQPSTLNAES   |
| GS-1p       | SLHRLTGTSPMSSPIRPGYCKPSPPLARVAEEAPPTTSLTASNALEKKAADNLMELNTPRPTSGEHSKEDI  |
| Smilp/Ktr4p | IKGEDSGSADVQSVQDHESVKIKVKTEPSEAEITTVNTESLGOASHEKKAONVDIKQESERKEDEKQPKVE  |
| GS-1p       | KVNEDSPAKERTSEDKKPKPETEANGKATESKGGKQTTVEDAEDMKDIEI                       |
| Smilp/Ktr4p | EKEHVENEHVTESAKDDOVNKQTEEMNKKEENEIRSDDAKVEEAREEFENLAL                    |

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

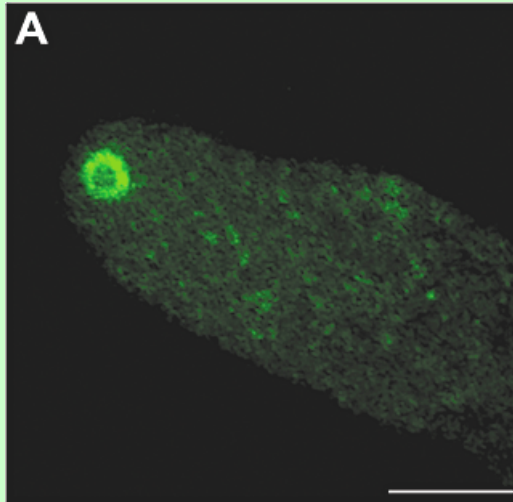
# Morphogenetic defects of a point mutant in GS-1



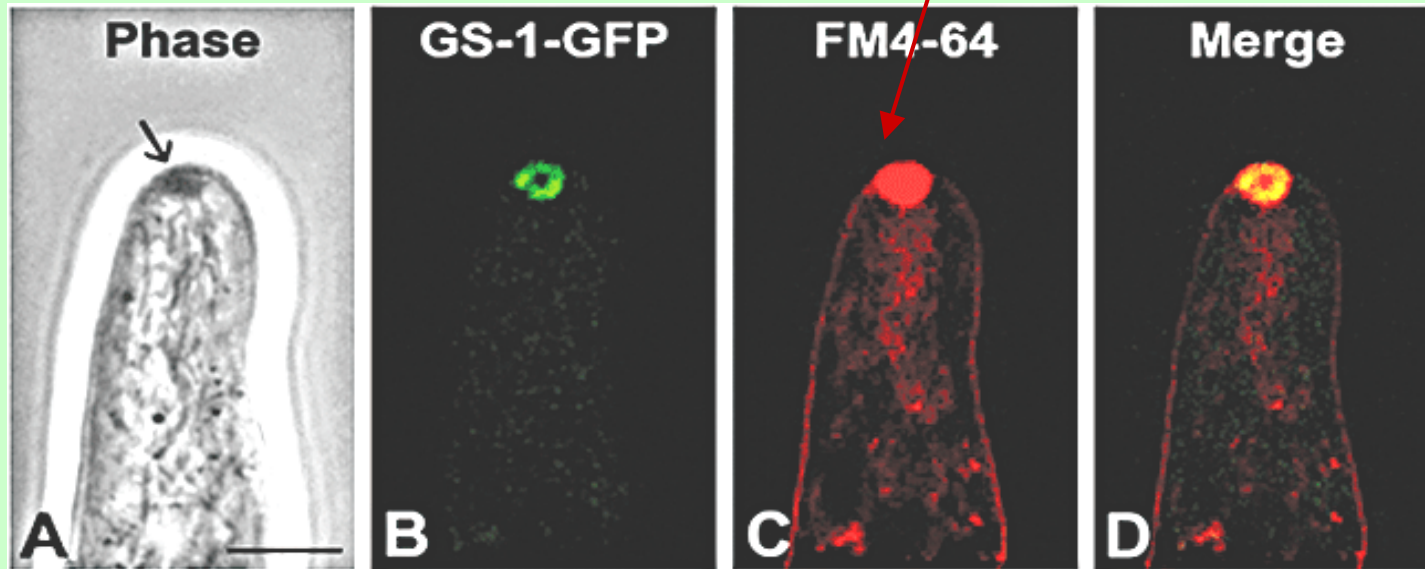
| Strain       | Average distance (um) between branches at 25° C | Average distance (um) between branches at 34° C | Increase in branching frequency (%) at 34° C |
|--------------|---|---|--|
| <i>wt</i>    | 180±4.0   | 176±14.1  | ≈0   |
| <i>cot-2</i> | 110±7.0   | 30±1.8  | 360%   |

(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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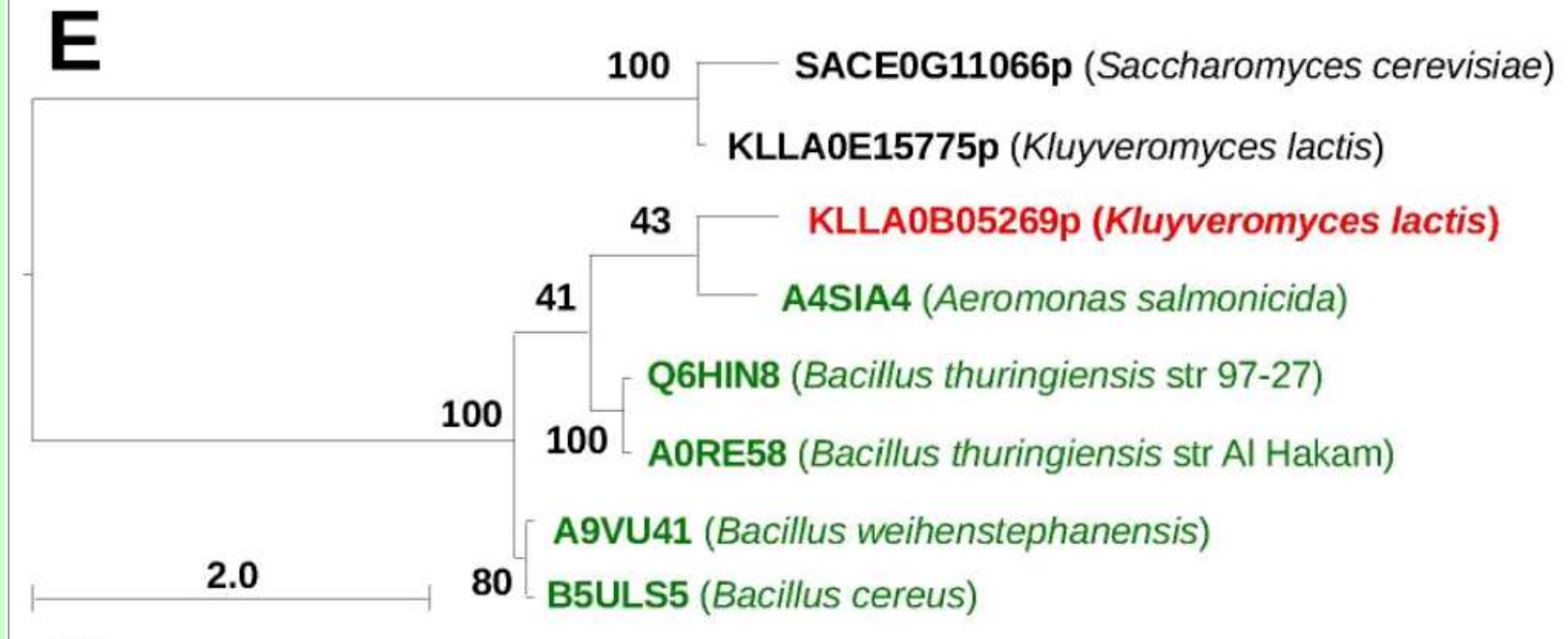
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## ***Conserved features of the Knr4/Smi1 homologs***

- ***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 :***

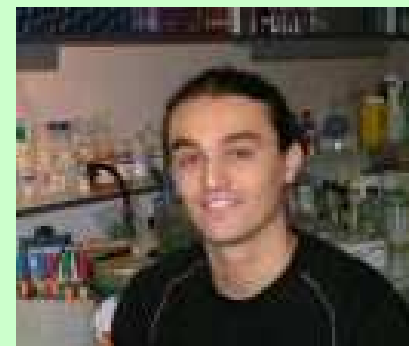
A possible role in Virulence ?

*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)

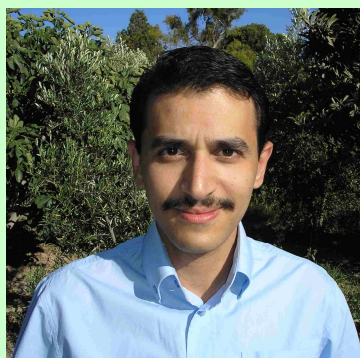


Fadi Basmaji  
(Master I, II, PhD)



A. Dagkessamanskaia  
(*Post Doc*)

**Karim El Azzouzi**  
**(Master II)**



Pr. Jean-Marie François.

***And thank you for your attention !***

***Questions ?***