A disordered protein domain involved in morphogenesis and cell cycle progression.

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Levures Modèles et Outils IX, Strasbourg 2010.

Introduction

Cell integrity and morphogenesis mechanisms under stress conditions

Positionning of Knr4 protein in these mechanisms

Knr4 protein structural information

New Results

Role of N-term in
-protein localization during mitosis and shmoo formation
-Morphogenesis / cell cycle control

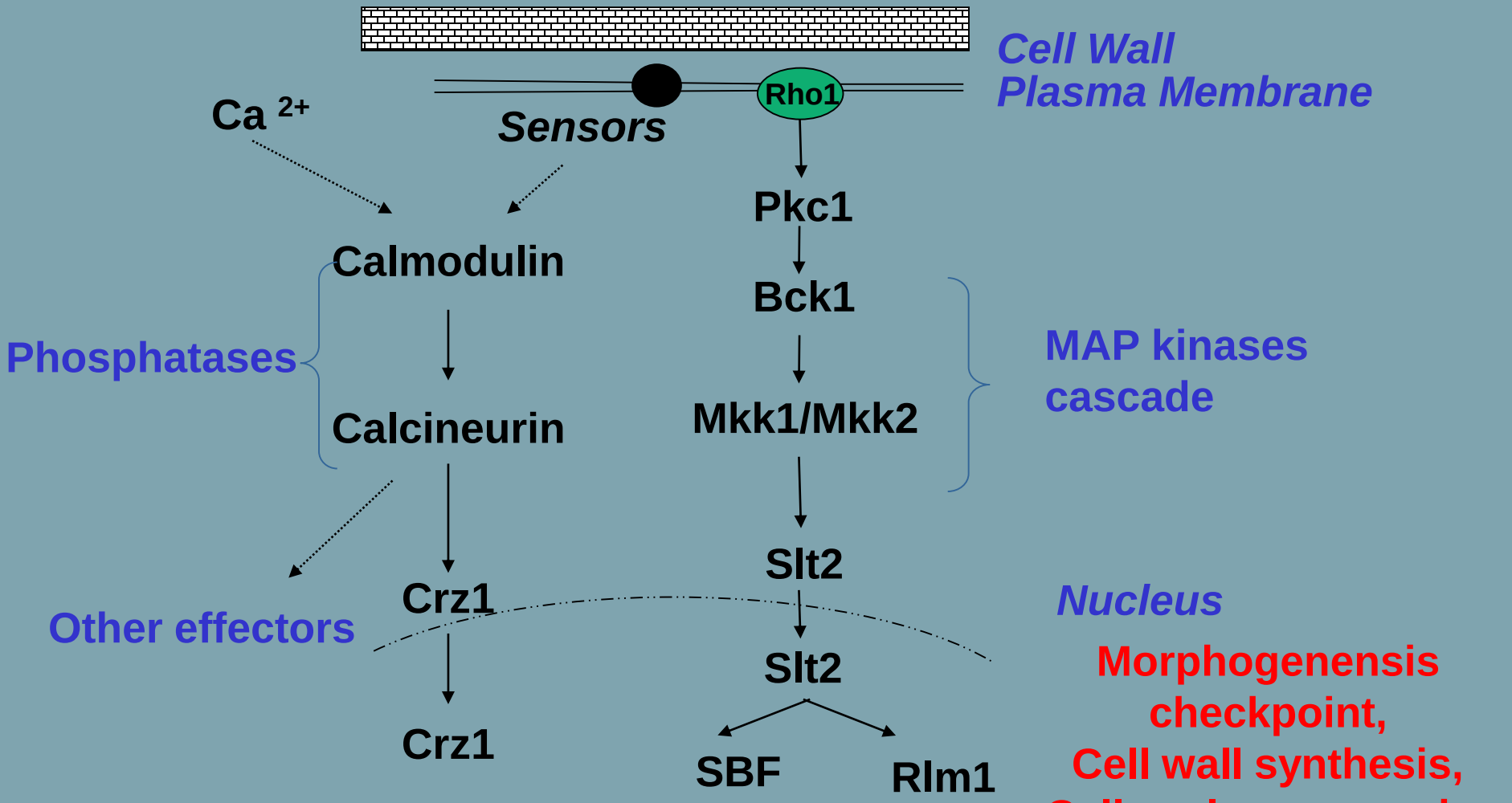
Connections with the Calcineurin Pathway

Effect on the Morphogenesis Checkpoint

Conclusions

Introduction :

Cell integrity and morphogenesis mechanisms under stress conditions



Slt2/Mpk1 and calcineurin dependant pathways sha at least one essential function … But wich one ???

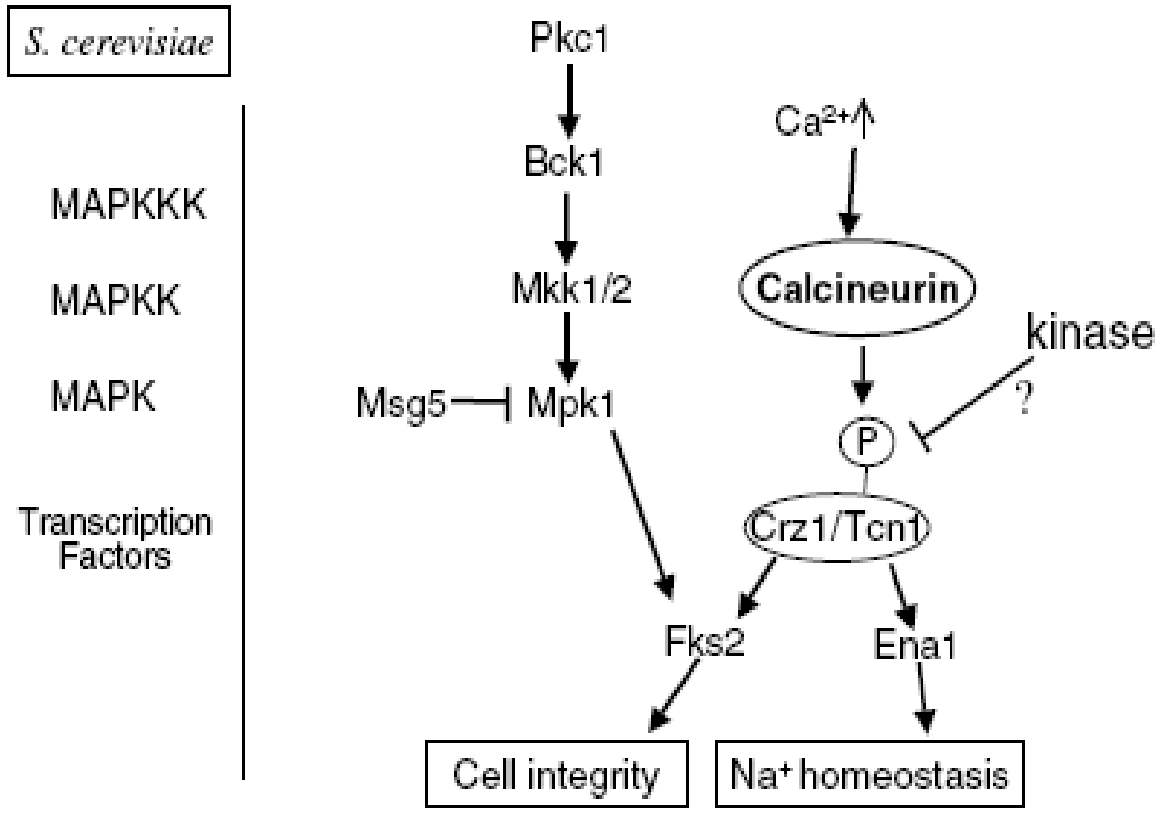
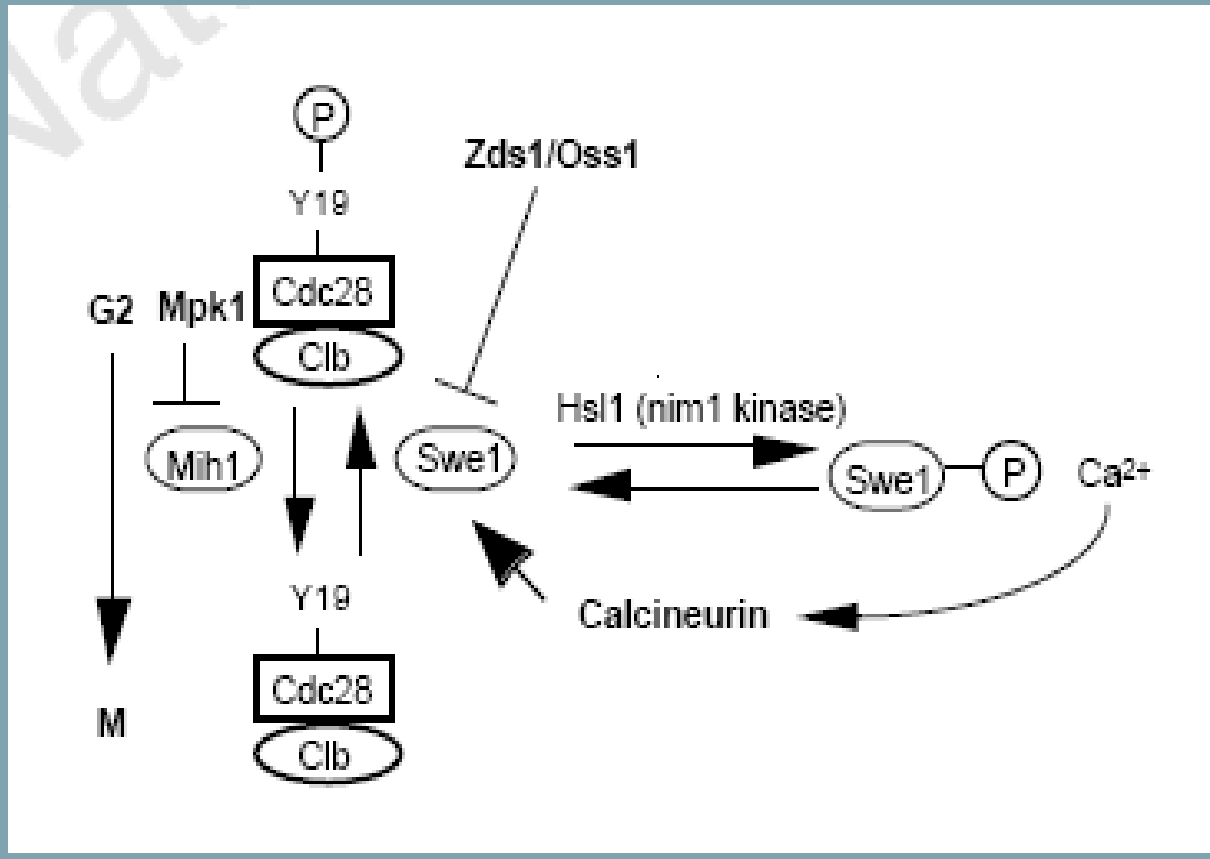


Figure 1 Cross-talk between calcineurin and MAP kinase signalling pathways in *S. cerevisiae*.

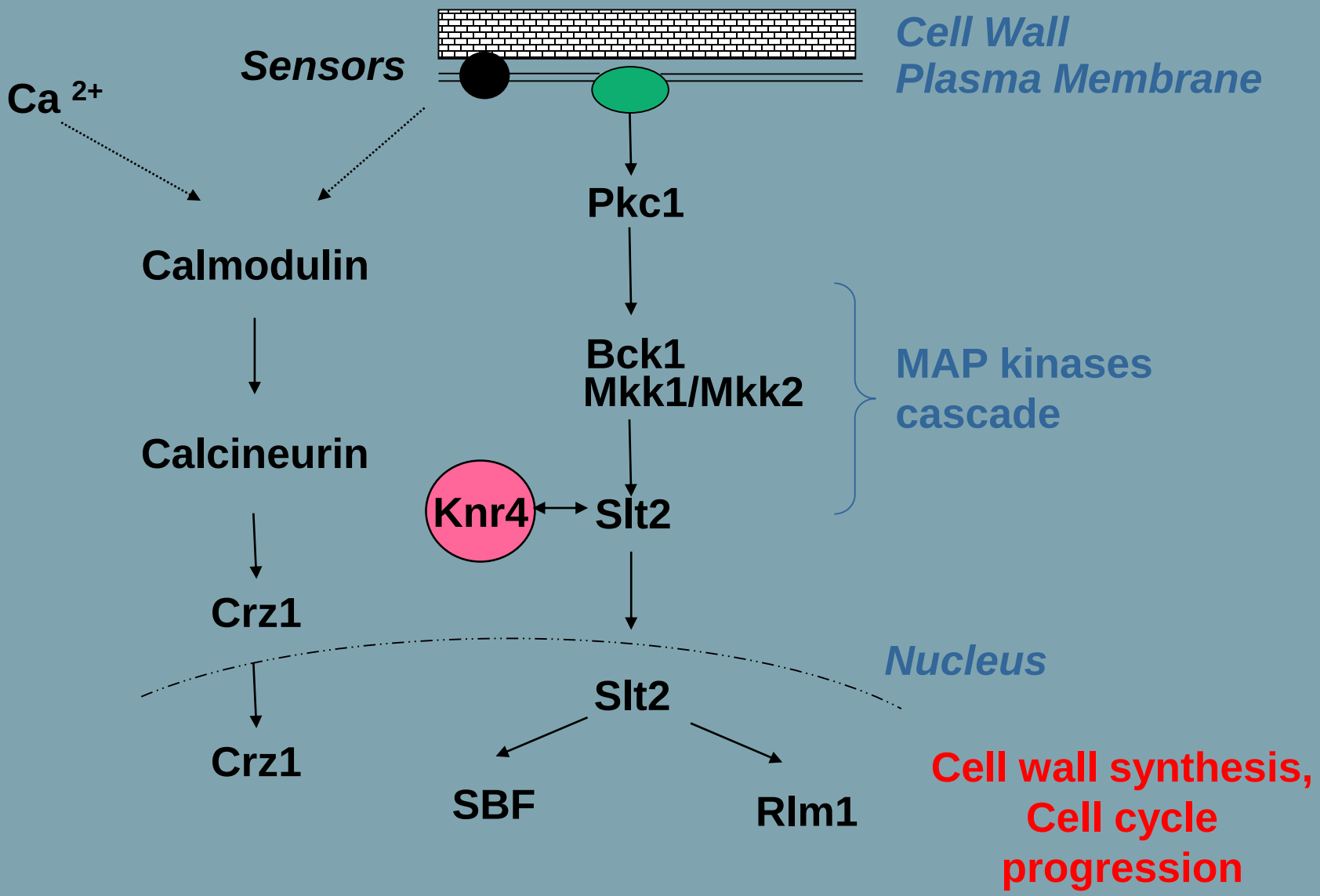
(from Sugiura et al., Genes to Cells,7, 619-627, 2007)

A common function of Slt2/Mpk1 and calcineurin, essential in case of cell wall stress, the « Morphogenesis checkpoint »

Upon cell wall synthesis or actin polarization defect, this checkpoint maintains cells in G2 until they adapt and become able to bud correctly.

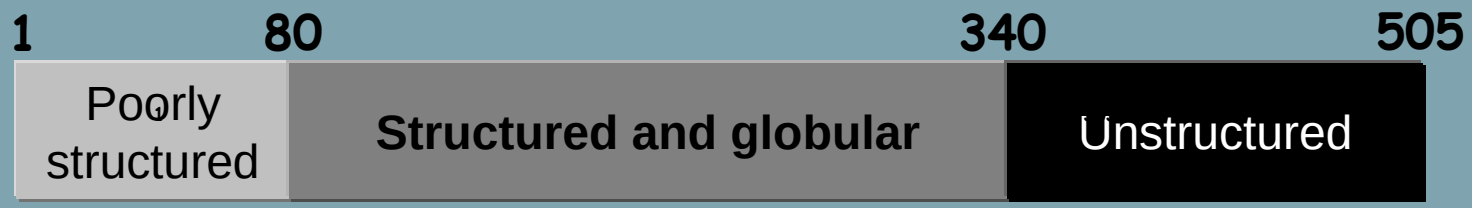


Minuzuma et al., 1998, Nature 392
Corrected by Harrison *et al,* 2001, Nature Cell Biol, 3, 417-420.

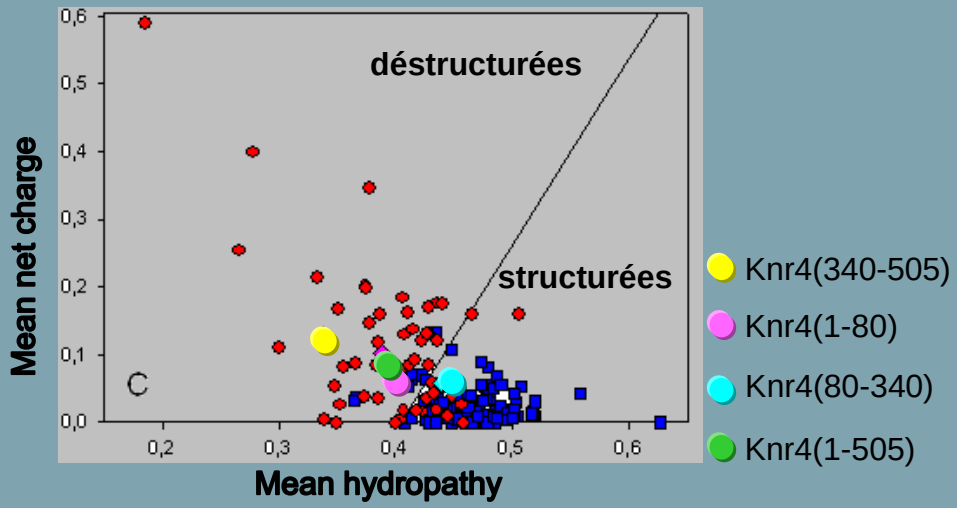

Ca 2+
Sensors
Cell Wall
Plasma Membrane
Pkc1
Calmodulin
Bck1
Mkk1/Mkk2
MAP kinases
cascade
Calcineurin
Knr4
Slt2
Crz1
Nucleus
Slt2
Crz1
SBF
Rlm1
Cell wall synthesis,
Cell cycle
progression

In silico Structure Predictions

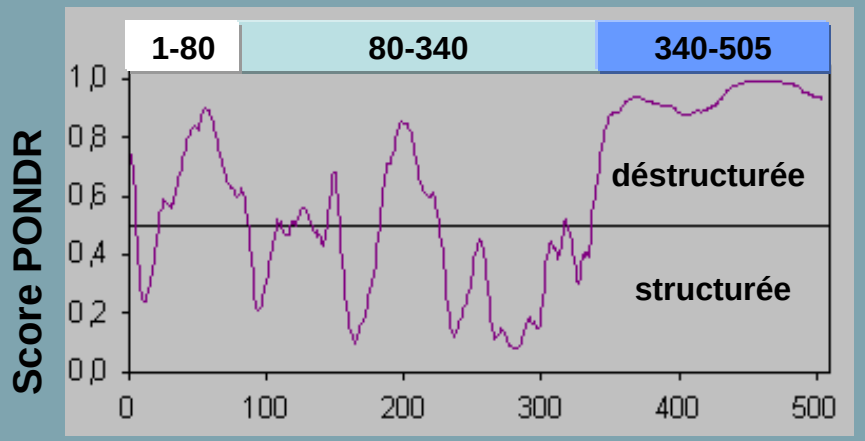
3 Identified Domains in Knr4p :



C-H Plot



PONDR Analysis



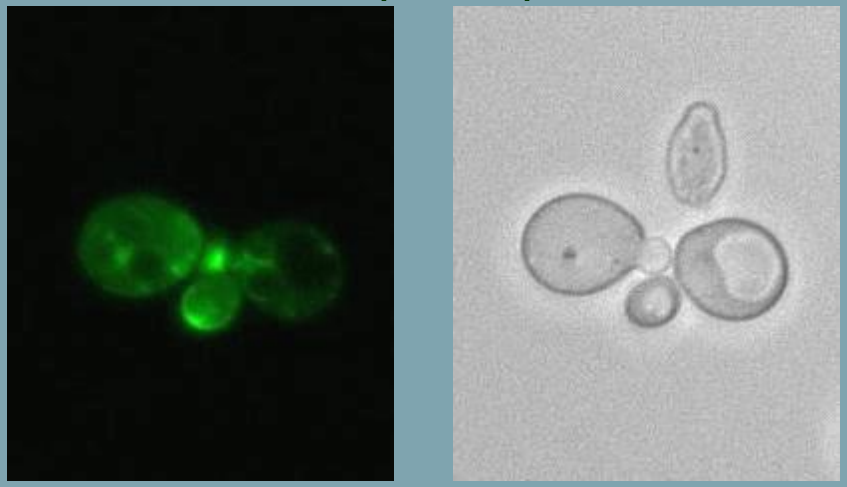
These *in silico* predictions have been confirmed by *in vitro* experiments.

Collaboration with Pr. Vladimir Uversky

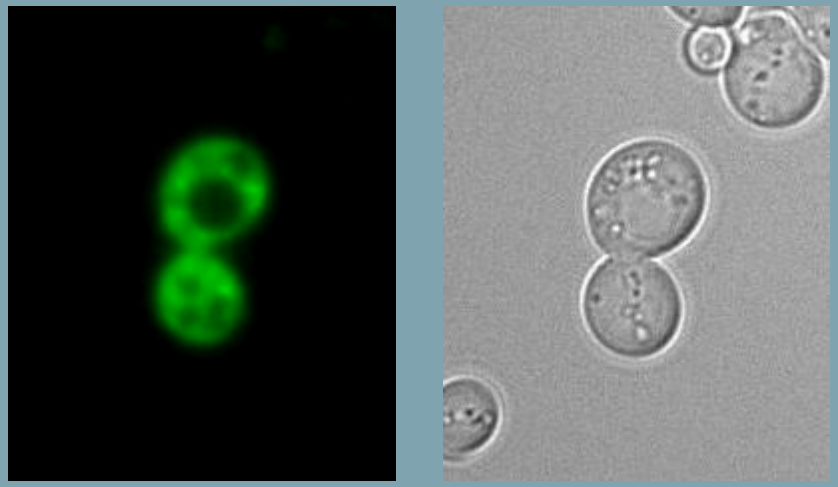
(F. Durand et al. Yeast, 2008)

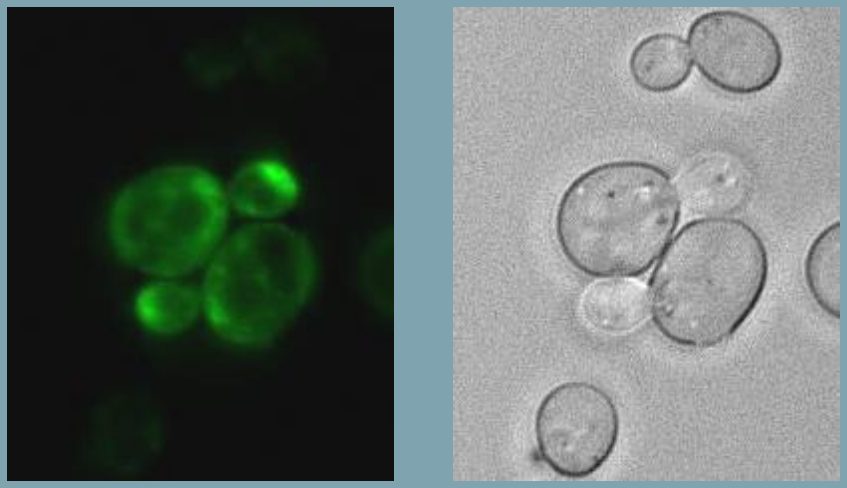
Knr4 N-term is required for its localization during vegetative growth

Knr4 full (1-505)-GFP

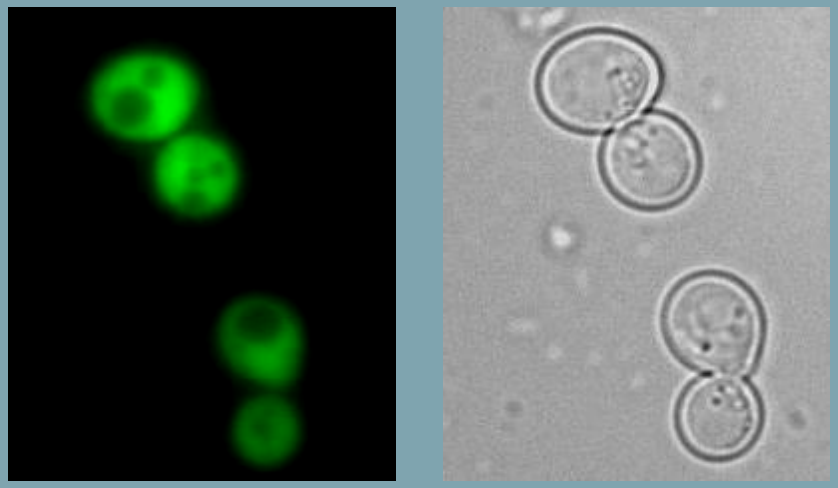


Knr4 without N-term (80-505)





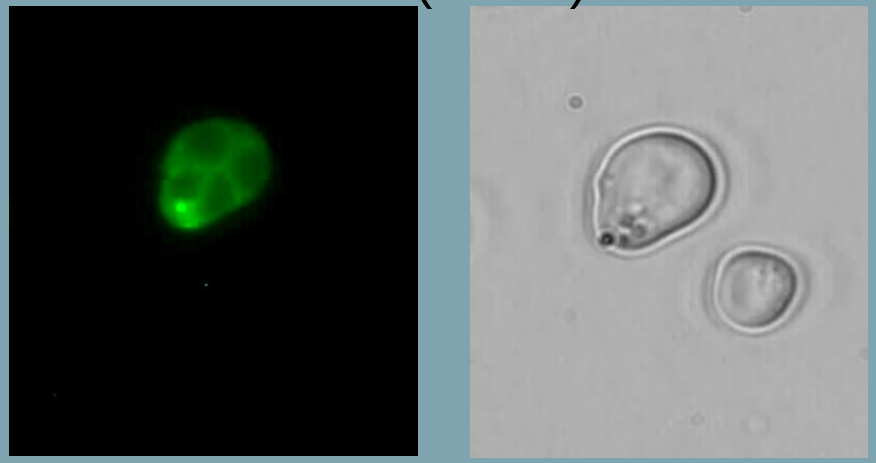
Knr4 without C-term (1-340)-GFP



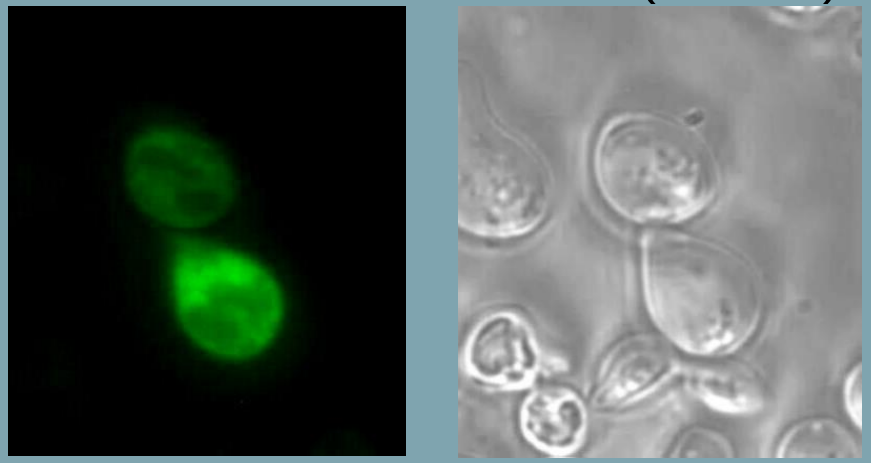
GFP alone

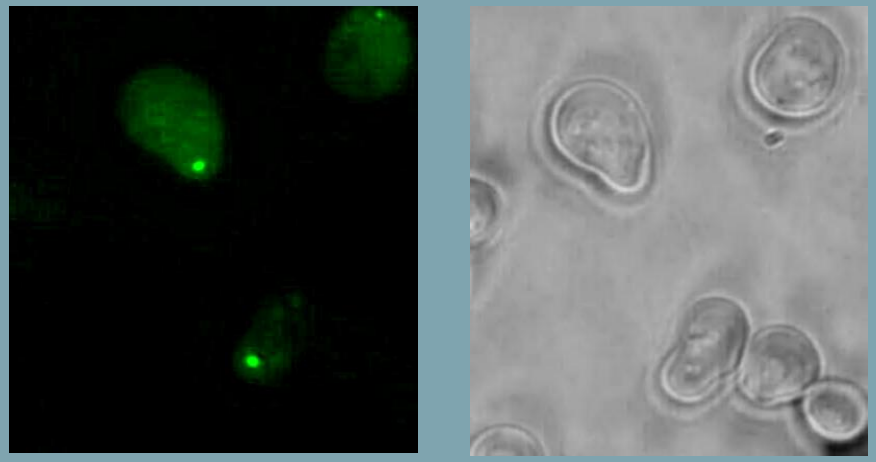
Knr4 N-term is required for its localization at the shmoo tip

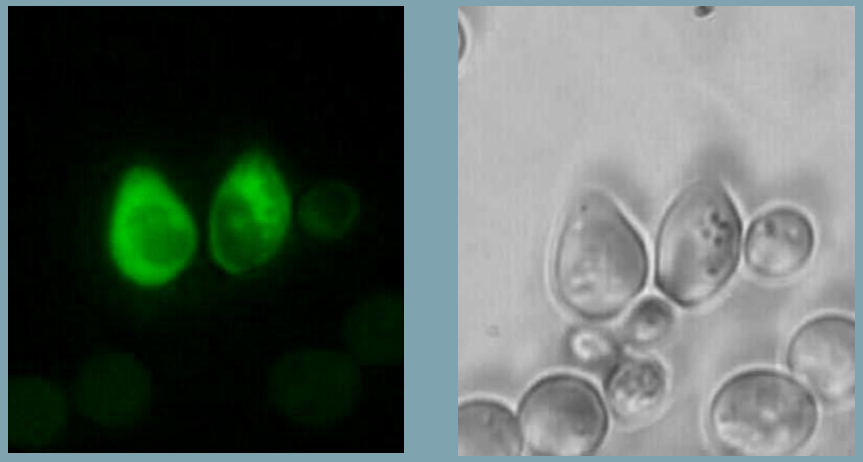
Knr4 full (1-505)-GFP



Knr4 without N-term (80-505)







Knr4 without C-term (1-340)-GFP

GFP alone

Knr4 N-term is required for cell cycle arrest and shmoo formation kinetics

	% Unbudded cells	% Budding cells	% Shmoos
*Knr4*Δ-pRS315	5	77	18
*Knr4*Δ-pRS315-*KNR4* (1-505)	7	17	76
*Knr4*Δ-pRS315-*KNR4* (1-340)	9	16	75
*Knr4*Δ-pRS315-*KNR4* (80-505)	4	75	21

KNR4 and the Calcineurin pathway

- *KNR4* and calmodulin were first isolated together by Fishel *et al.,* 1993, and they show similar cellular localization.

- Knr4 and several of its homologs from other species (*Aspergillus fumigatus, Kluveromyces lactis, Cryptococcus neoformans,…*) share a « Calcineurin like phosphoesterase » domain.

- *Knr4Δ* is SL with *PKC1/SLT2* pathway members and with those of the calcineurin signalling pathway, as well as with drugs that inhibit calcineurin.

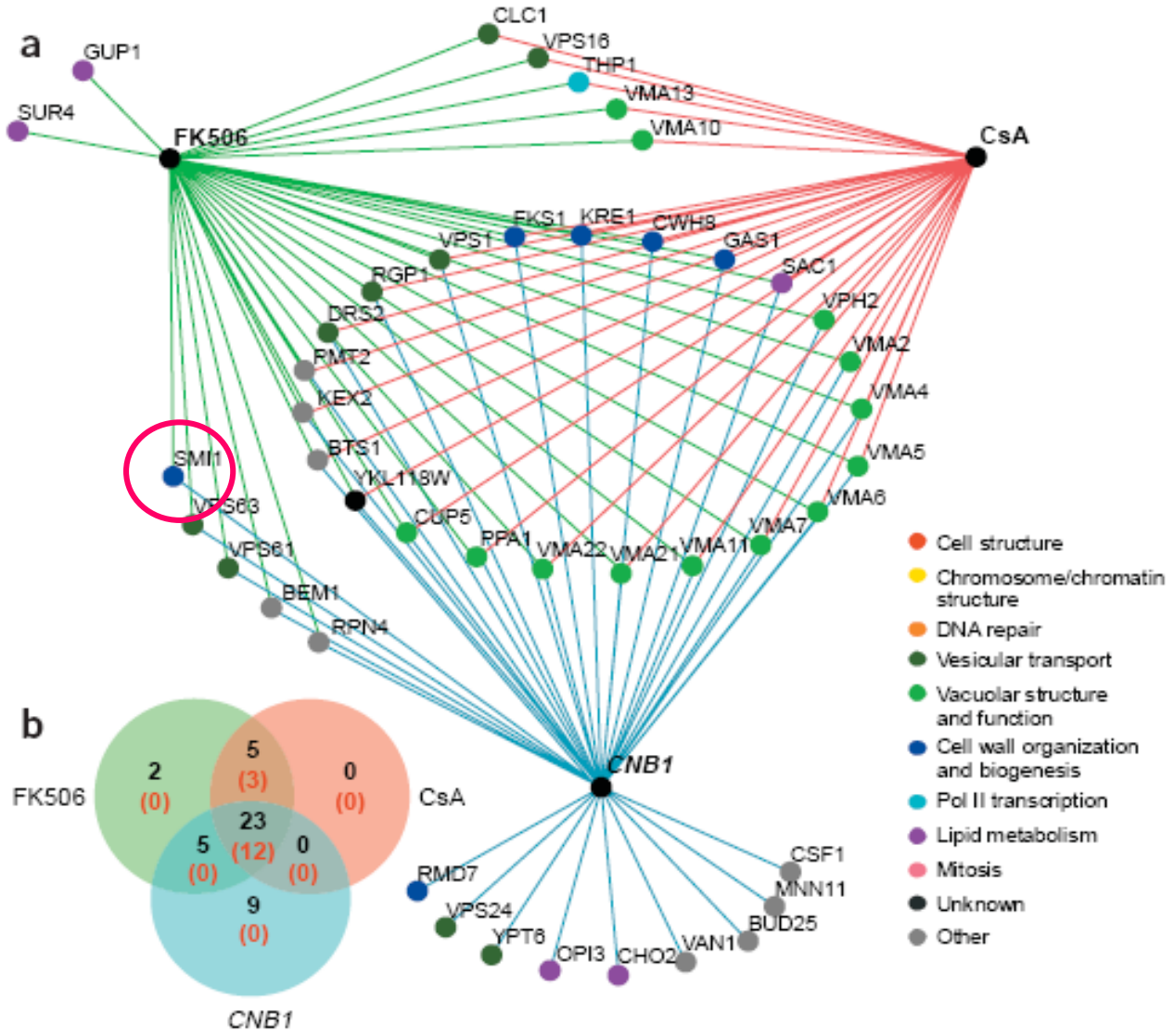


Figure 5 Overlap between the chemical-genetic profiles of FK506 and CsA and the genetic interaction profile of *CNB1*. Edges indicate either a chemical-genetic or a genetic interaction and nodes represent either compounds or genes, with the gene nodes color-coded from a defined subset of GO functional attributes. (**a**) Network of the chemical-genetic interactions of FK506 and CsA and the genetic interactions with *CNB1*. (**b**) Venn diagram summarizing that 35 genes showed a chemical-genetic interaction with FK506, 28 genes showed a chemical genetic interaction with CsA and 38 genes showed a genetic interaction with *CNB1*, with 24 interactions common to all three screens. Red bracketed numbers indicate the number of genes, in each group, classified as multidrug resistant.

A « Chemical-genetic » screen closely links *KNR4* and Calcineurin

Parsons *et al.*, 2004, Nature Biotech, 22(1) 37-8

Knr4 physically interacts with calcineurin

pGAD2f / pOBD2 (negative control)

pGAD2f / pOBD2-*KNR4*(1-505)

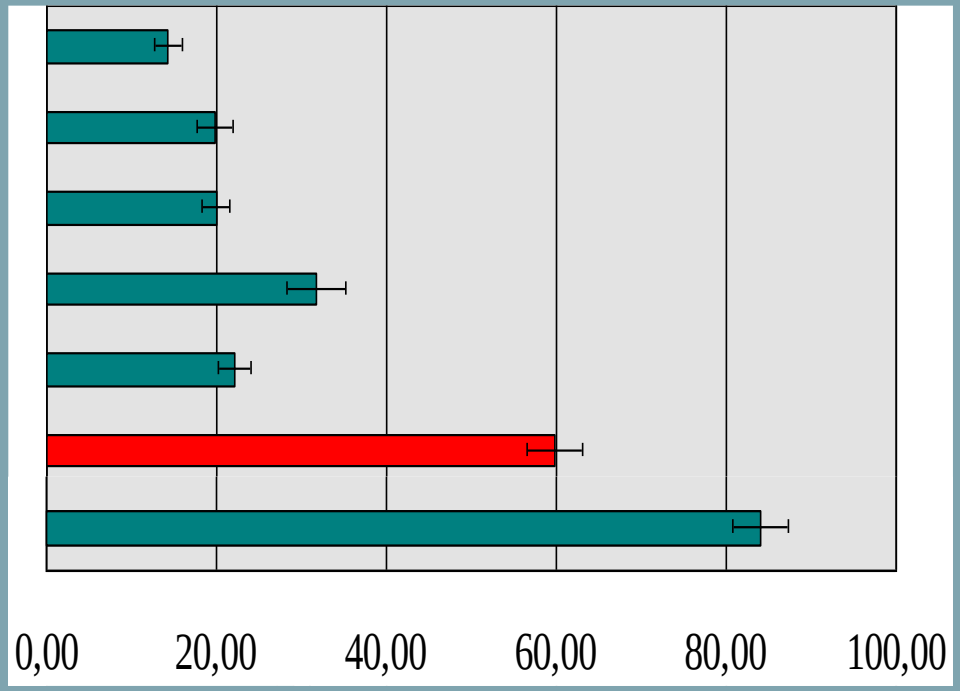
pGAD2f-*CNB1* / pPOBD2

pGAD2f-*CNB1* / pPOBD2-*KNR4* (1-505)

pGAD2f-*CNA1* / pOBD2

pGAD2f-*CNA1* / pOBD2-*KNR4* (1-505)

Positive control



β- galactosidase activity
(nM of ONPG /min/mg proteins)

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pGAD2f / pOBD2-*KNR4*(1-505)

pGAD2f-*CNB1* / pPOBD2

pGAD2f-*CNB1* / pPOBD2-*KNR4* (1-505)

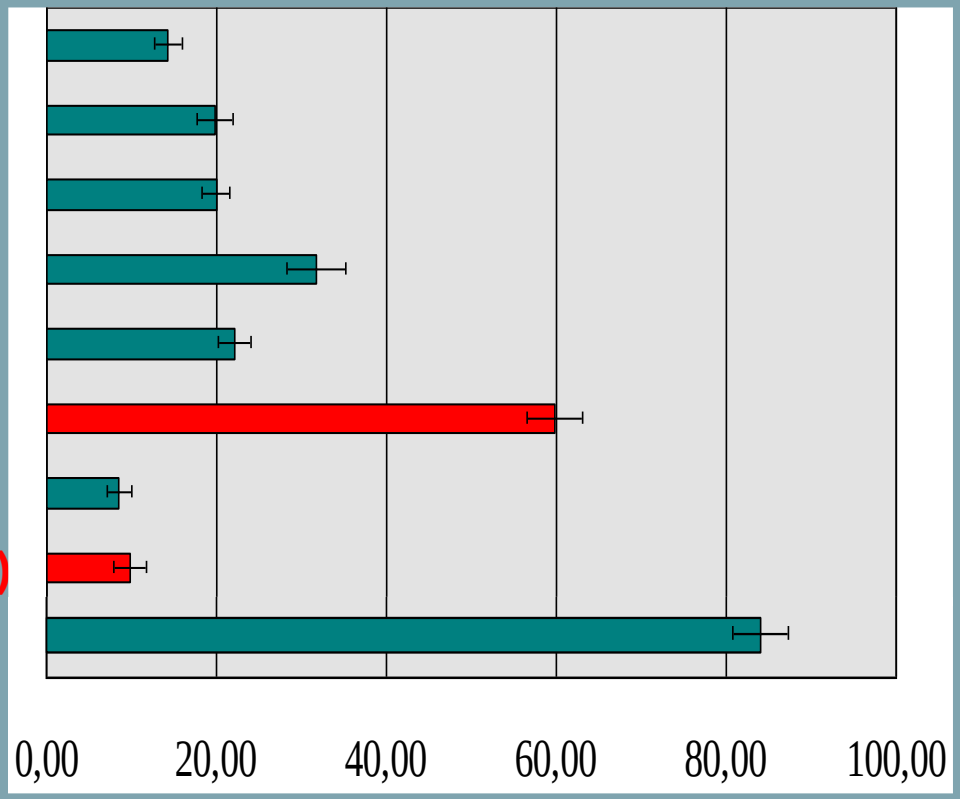
pGAD2f-*CNA1* / pOBD2

pGAD2f-*CNA1* / pOBD2-*KNR4* (1-505)

pGAD2f / pOBD2-*KNR4*(80-505)

pGAD2f-*CNA1* / pOBD2-*KNR4* (80-505)

Positive control



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pGAD2f-*CNA1* / pOBD2

pGAD2f-*CNA1* / pOBD2-*KNR4* (1-505)

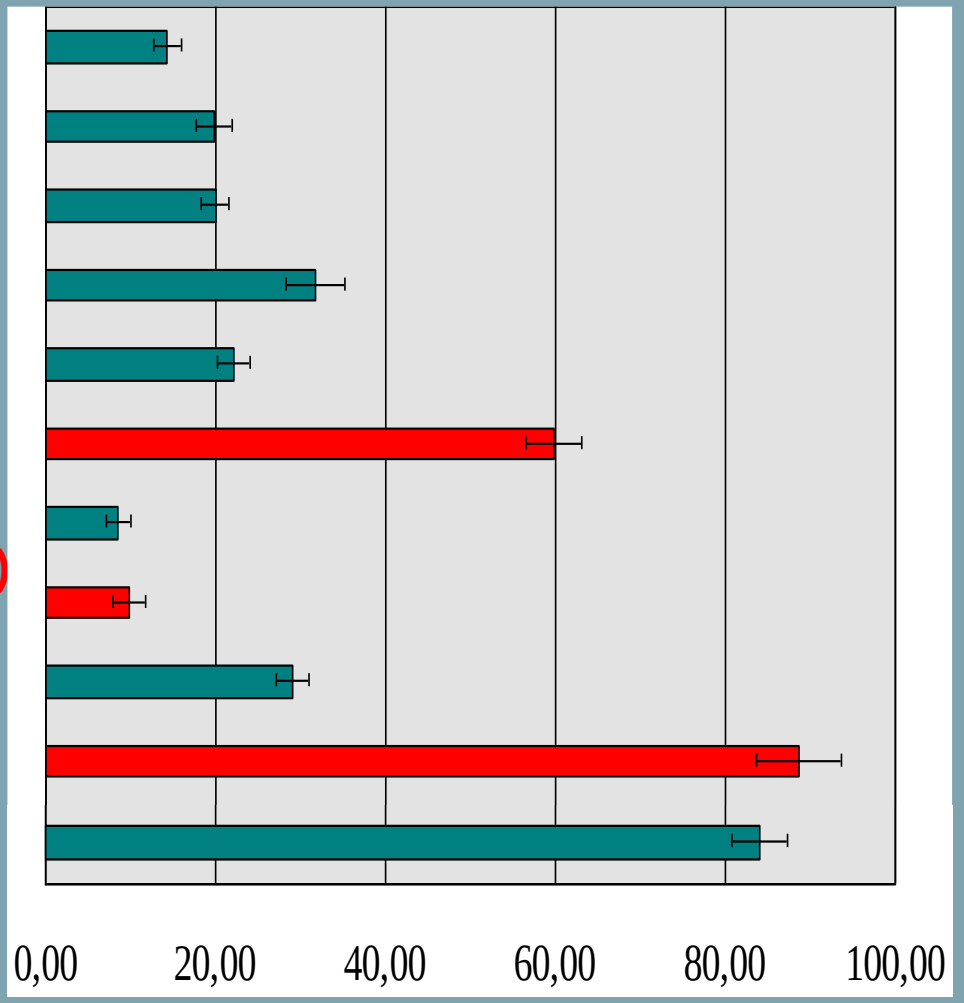
pGAD2f / pOBD2-*KNR4*(80-505)

pGAD2f-*CNA1* / pOBD2-*KNR4* (80-505)

pGAD2f / pOBD2-*KNR4*(1-340)

pGAD2f-*CNA1* / pOBD2-*KNR4* (1-340)

Positive control



β- galactosidase activity
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Knr4 physically interacts with calcineurin

pGAD2f / pOBD2 (negative control)

pGAD2f / pOBD2-*KNR4*(1-505)

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pGAD2f-*CNB1* / pPOBD2-*KNR4* (1-505)

pGAD2f-*CNA1* / pOBD2

pGAD2f-*CNA1* / pOBD2-*KNR4* (1-505)

pGAD2f / pOBD2-*KNR4*(80-505)

pGAD2f-*CNA1* / pOBD2-*KNR4* (80-505)

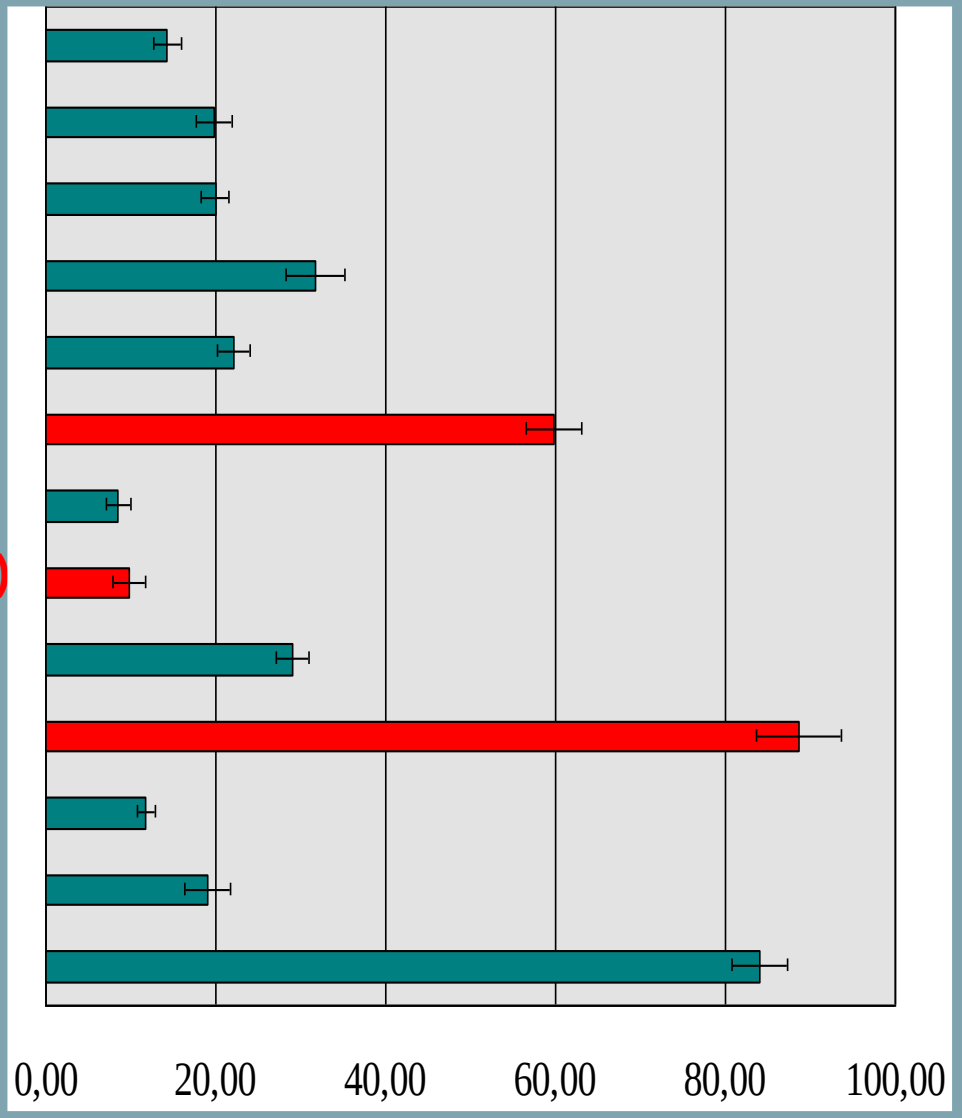
pGAD2f / pOBD2-*KNR4*(1-340)

pGAD2f-*CNA1* / pOBD2-*KNR4* (1-340)

pGAD2f / pOBD2-*KNR4*(1-80)

pGAD2f-*CNA1* / pOBD2-*KNR4* (1-80)

Positive control

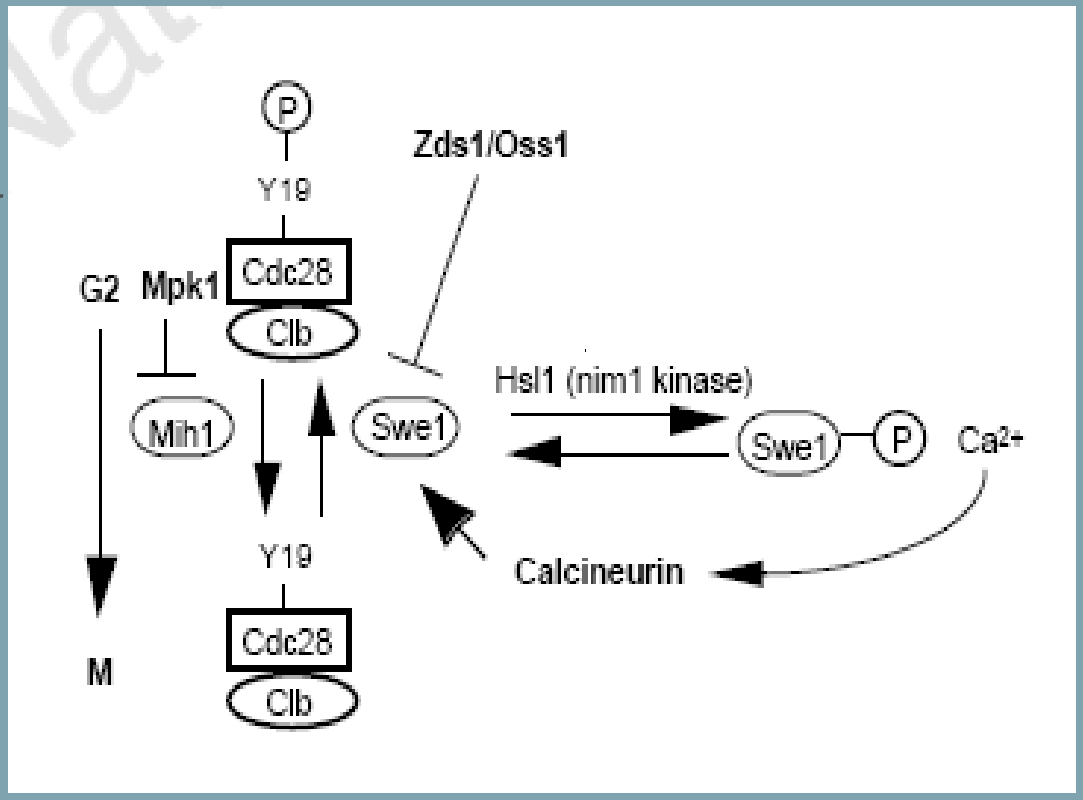


β- galactosidase activity (nM of ONPG /min/mg proteins)

A common function of Slt2/Mpk1 and calcineurin, essential in case of cell wall stress, the « Morphogenesis checkpoint »

Upon cell wall synthesis or actin polarization defect, this checkpoint maintains cells in G2 until they adapt and become able to bud correctly.

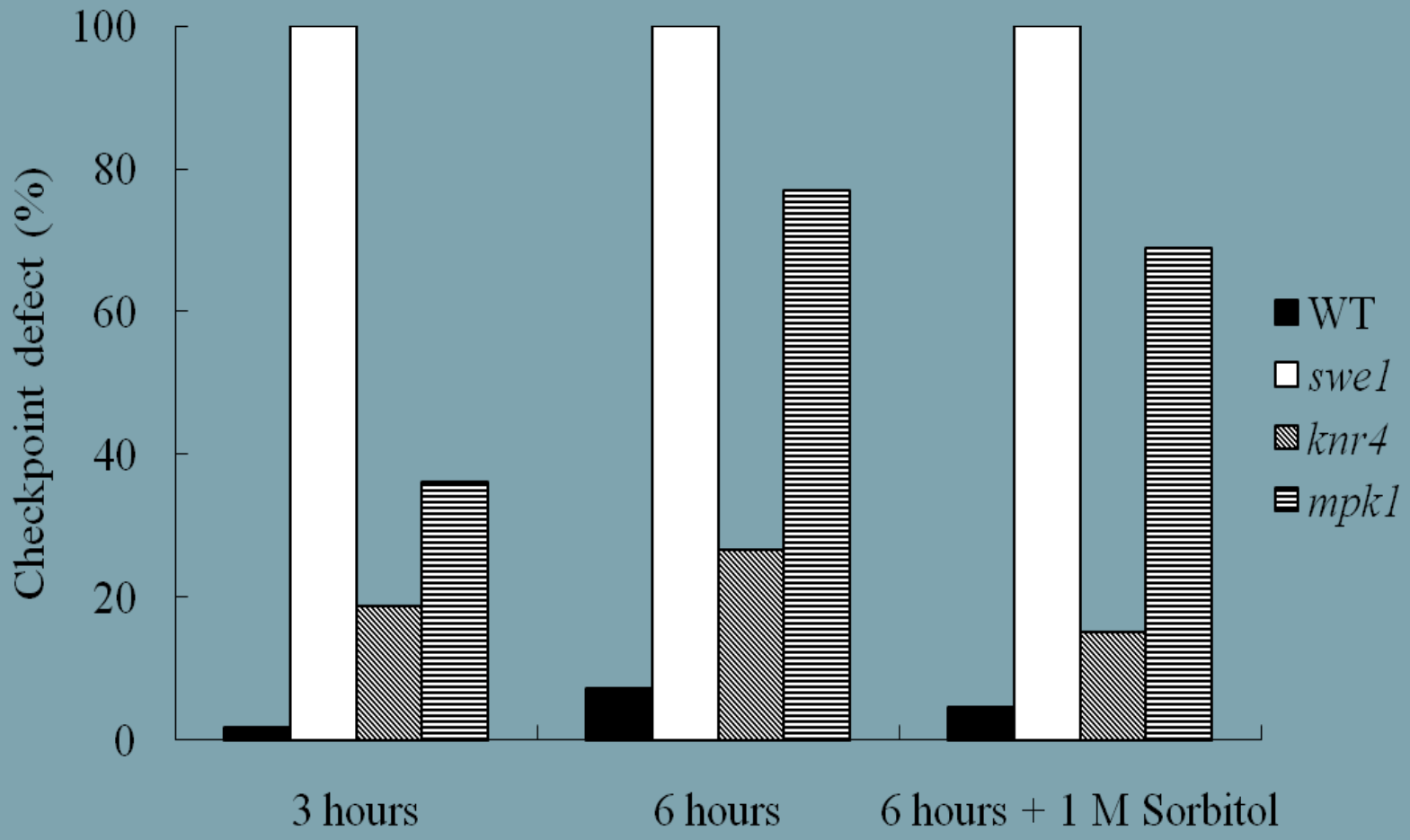
Is this checkpoint functioning in the *knr4Δ* mutant?
(cf : several cell cycle defect phenotypes in this mutant…)



→ *Tested in Tokyo, in the lab of Pr Yoshikazu Ohya.*

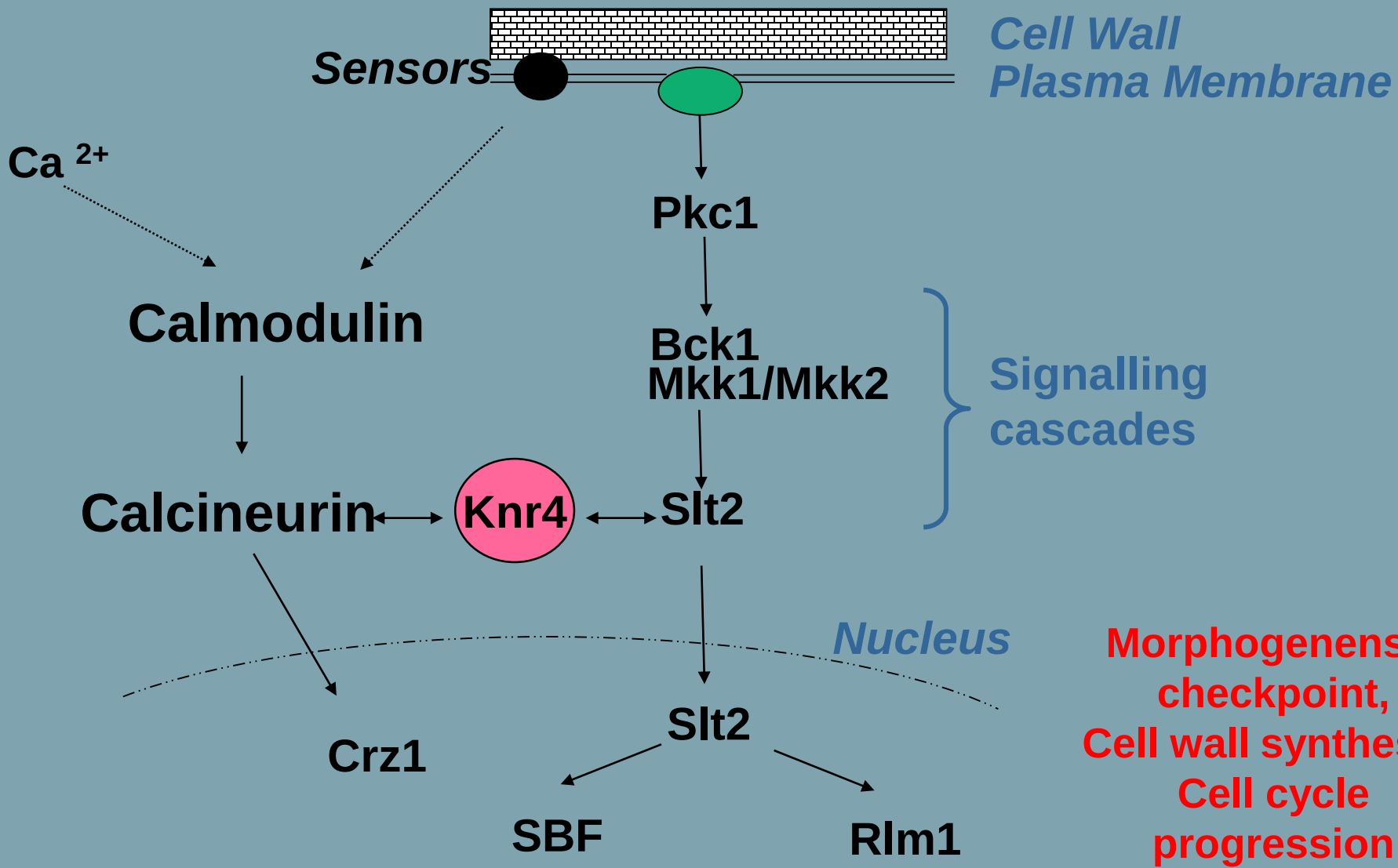
Minuzuma et al., 1998, Nature 392
Corrected by Harrison *et al,* 2001, Nature Cell Biol, 3, 417-420.

Knr4 participates in the morphogenesis checkpoint



Conclusions :

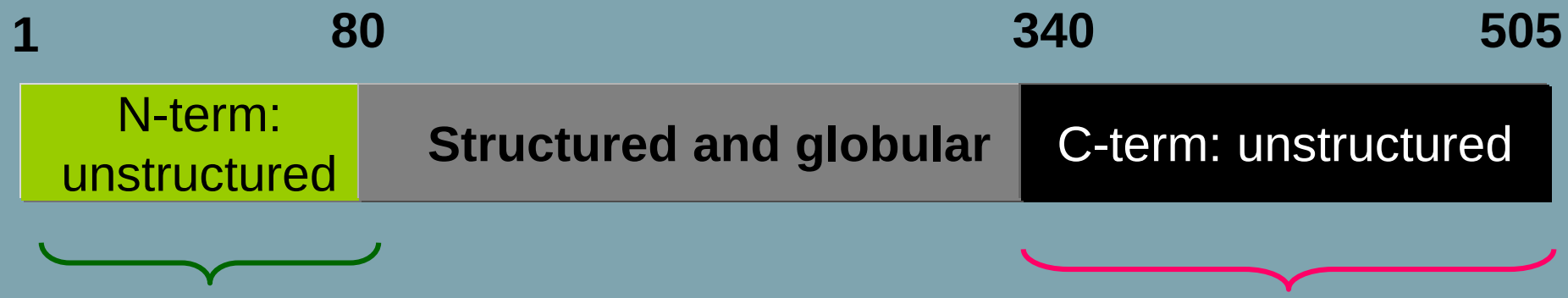
Knr4 links two parallel signalling pathways



Conclusions :

Two disordered domains with very different properties

(most of the protein biological function)



Essential :
- for protein localization
- when PKC1 pathway is disrupted,
- for interaction with calcineurin,
- *and calcineurin activity***.**

Interactions inhibition, PEST sequences.

Thank you for your attention !

For more data :

- *Poster session.*

- *Dagkessamanskaia et al., 2010. Yeast, July 2.*
  Knr4 N-terminal domain controls its localization and function during sexual differentiation and vegetative growth,
- *Dagkessamanskaia et al., 2010. Protein Science, 19(7):1376-85.*
  Functional dissection of an intrinsically disordered protein: understanding the roles of different domains of Knr4 protein in protein-protein interactions.

Crz1 transcriptional activity upon CFW induction is reduced in the absence of Knr4 protein or its N-terminal domain.

BY4741+ pSG2

BY4741 + pSG2 / CFW

*crz1*Δ + pSG2

*crz1*Δ + pSG2 / CFW

*knr4*Δ + pSG2

*knr4*Δ + pSG2 / CFW

*knr4*Δ + p*KNR4*(1-505) + pSG2

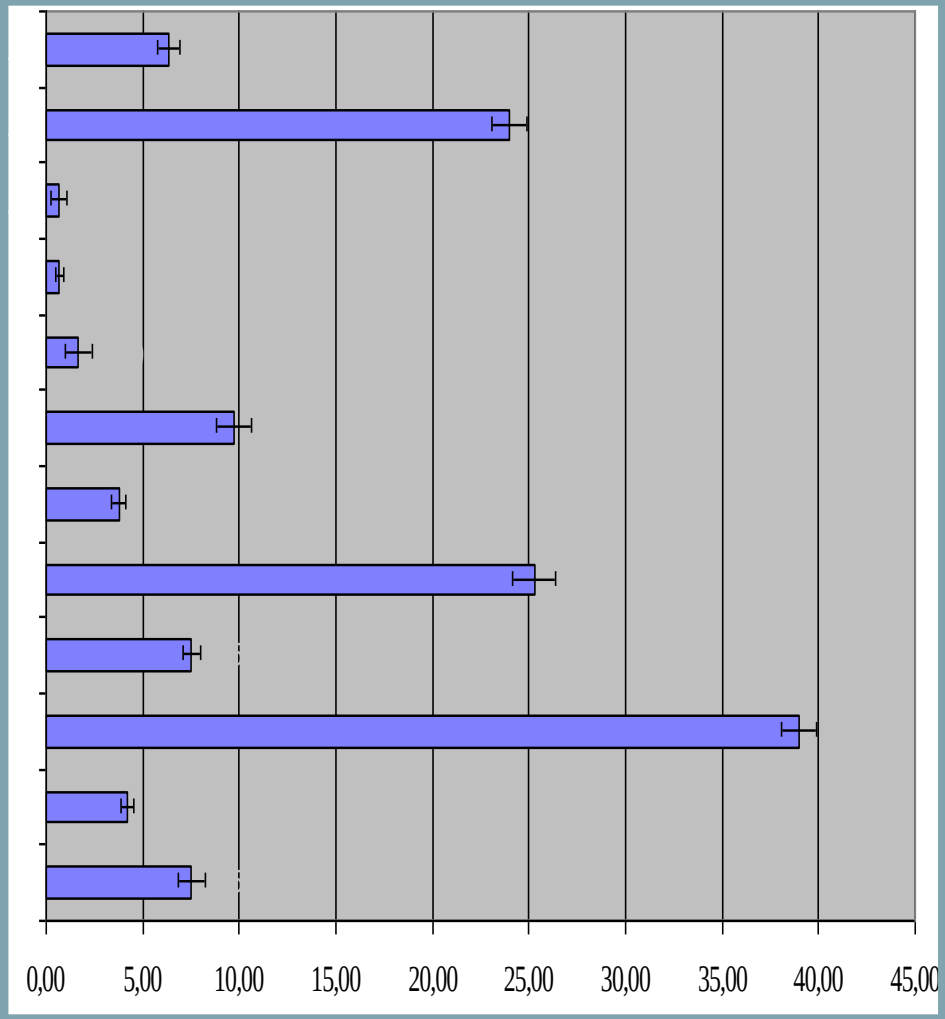
*knr4*Δ + p*KNR4*(1-505) + pSG2 / CFW

*knr4*Δ + p*KNR4*(1-340) + pSG2

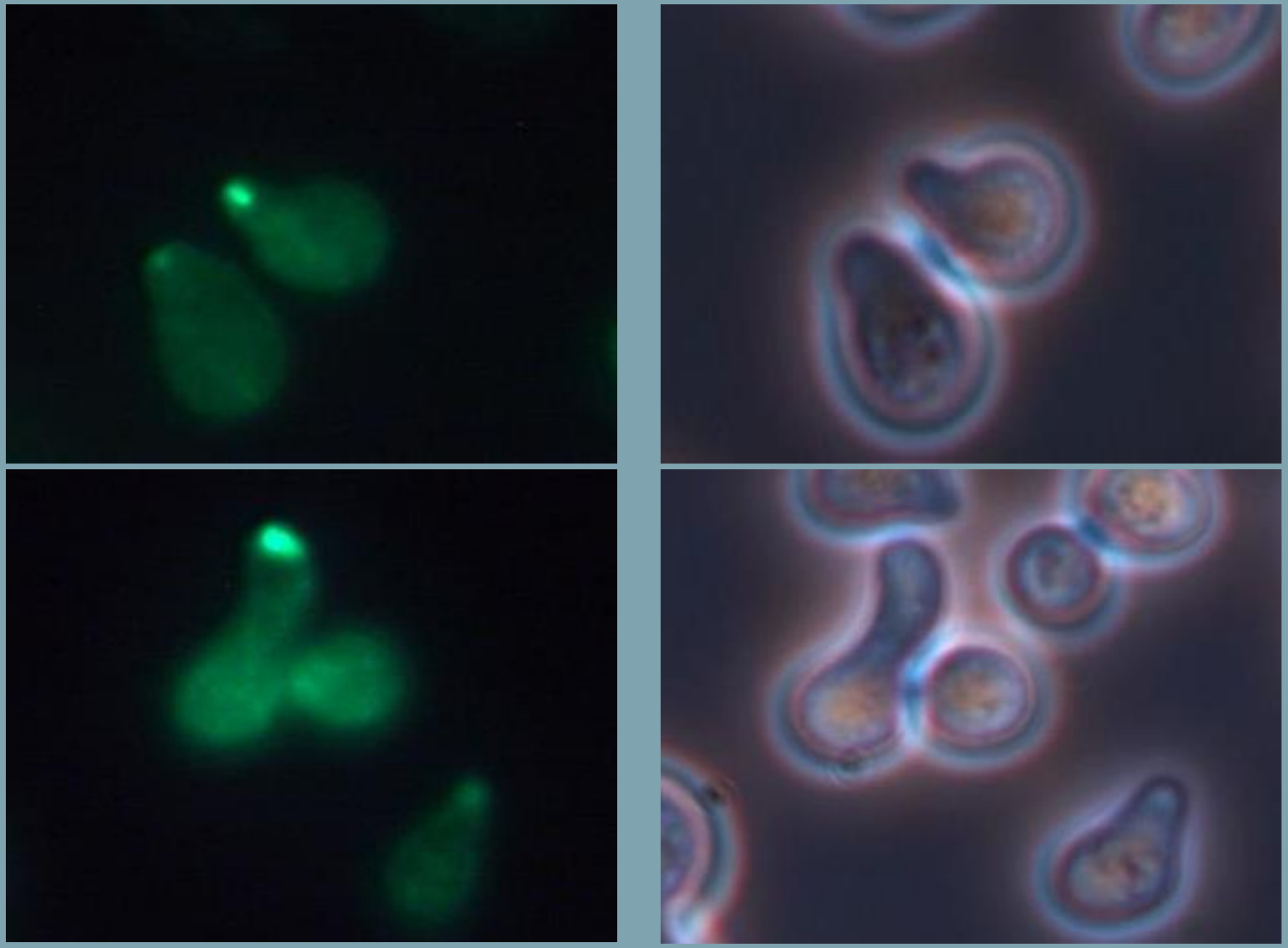
*knr4*Δ + p*KNR4*(1-340) + pSG2 / CFW

*knr4*Δ + p*KNR4*(80-505) + pSG2

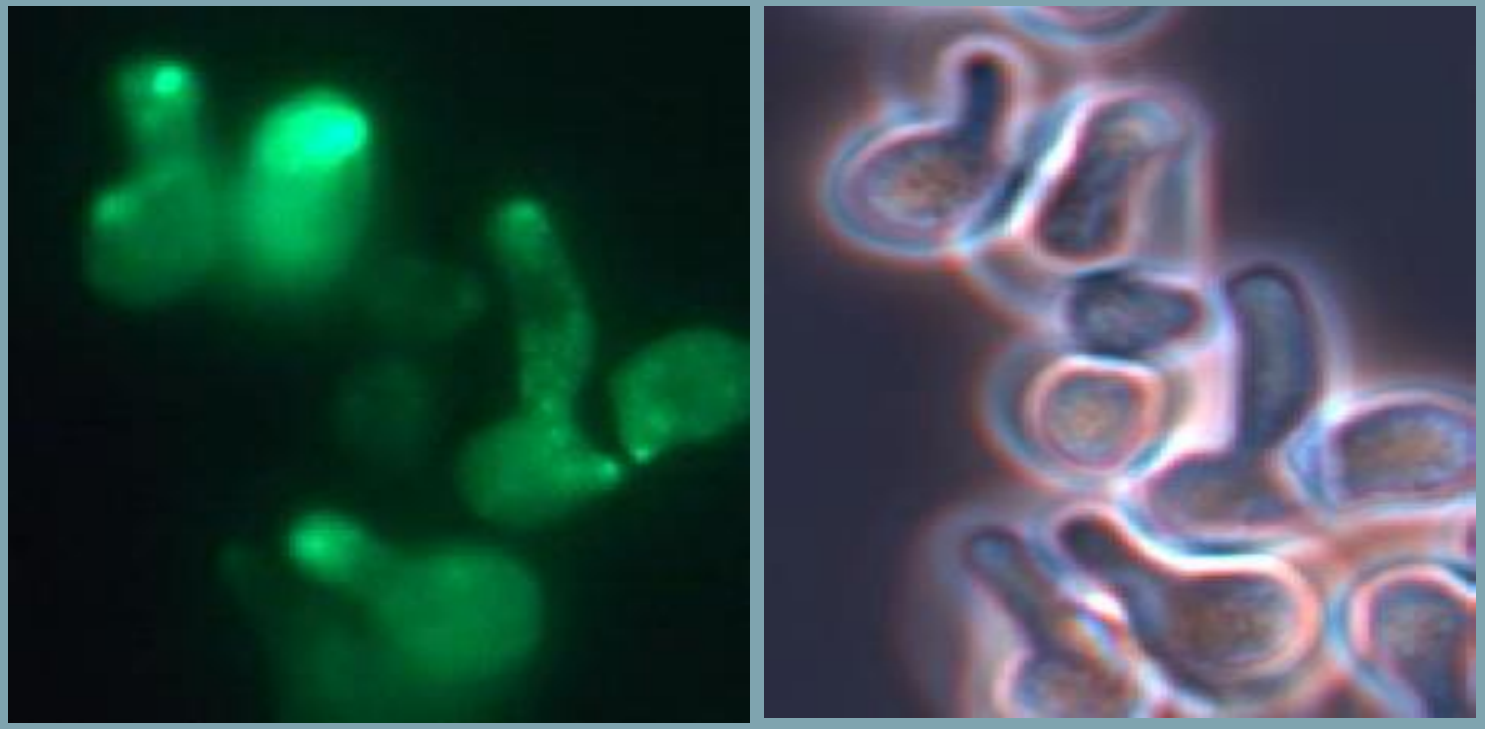
*knr4*Δ + p*KNR4*(80-505) + pSG2 / CFW



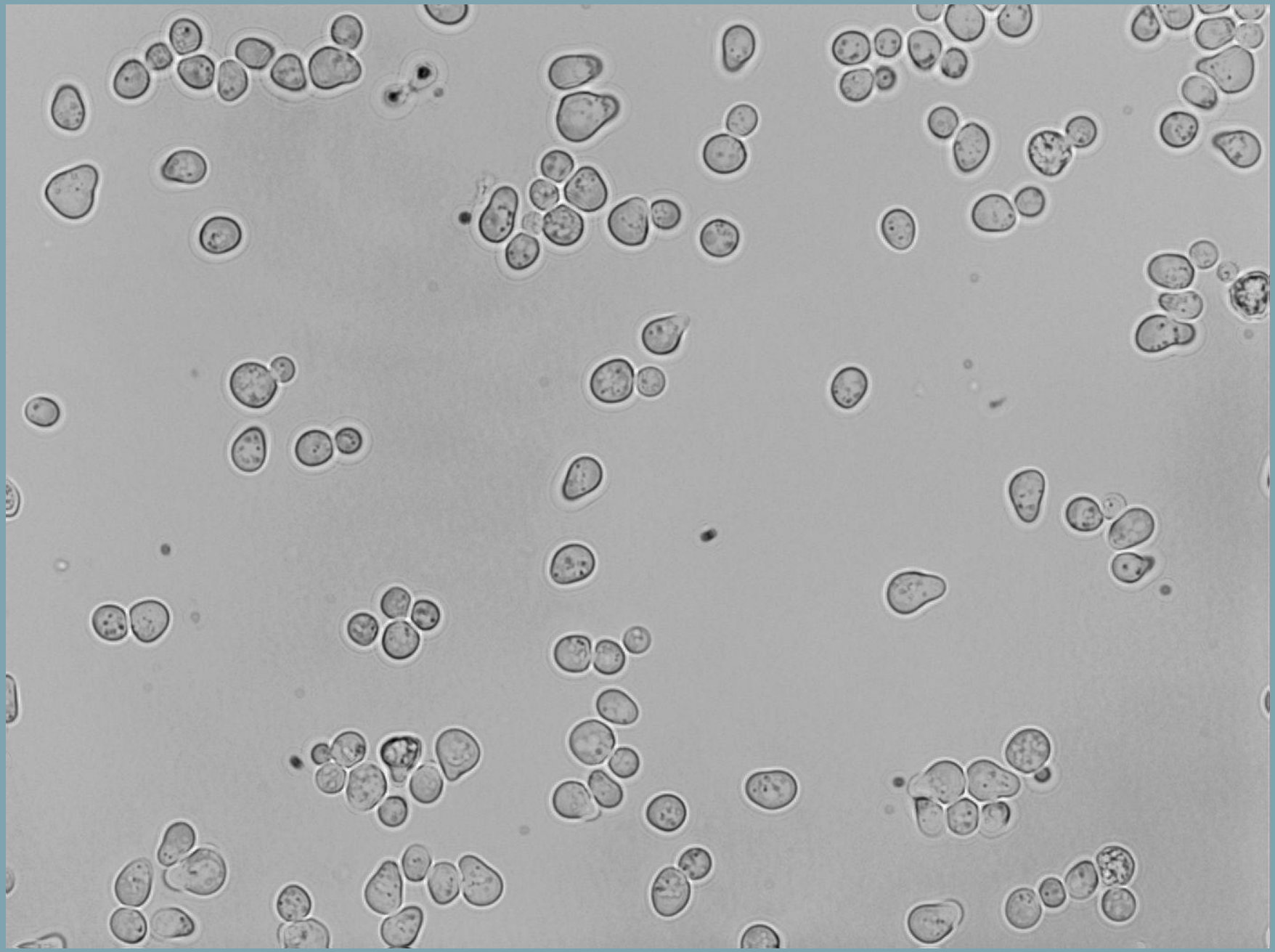
Specific β– galactosidase activity
(nM of ONPG /min/mg proteins)



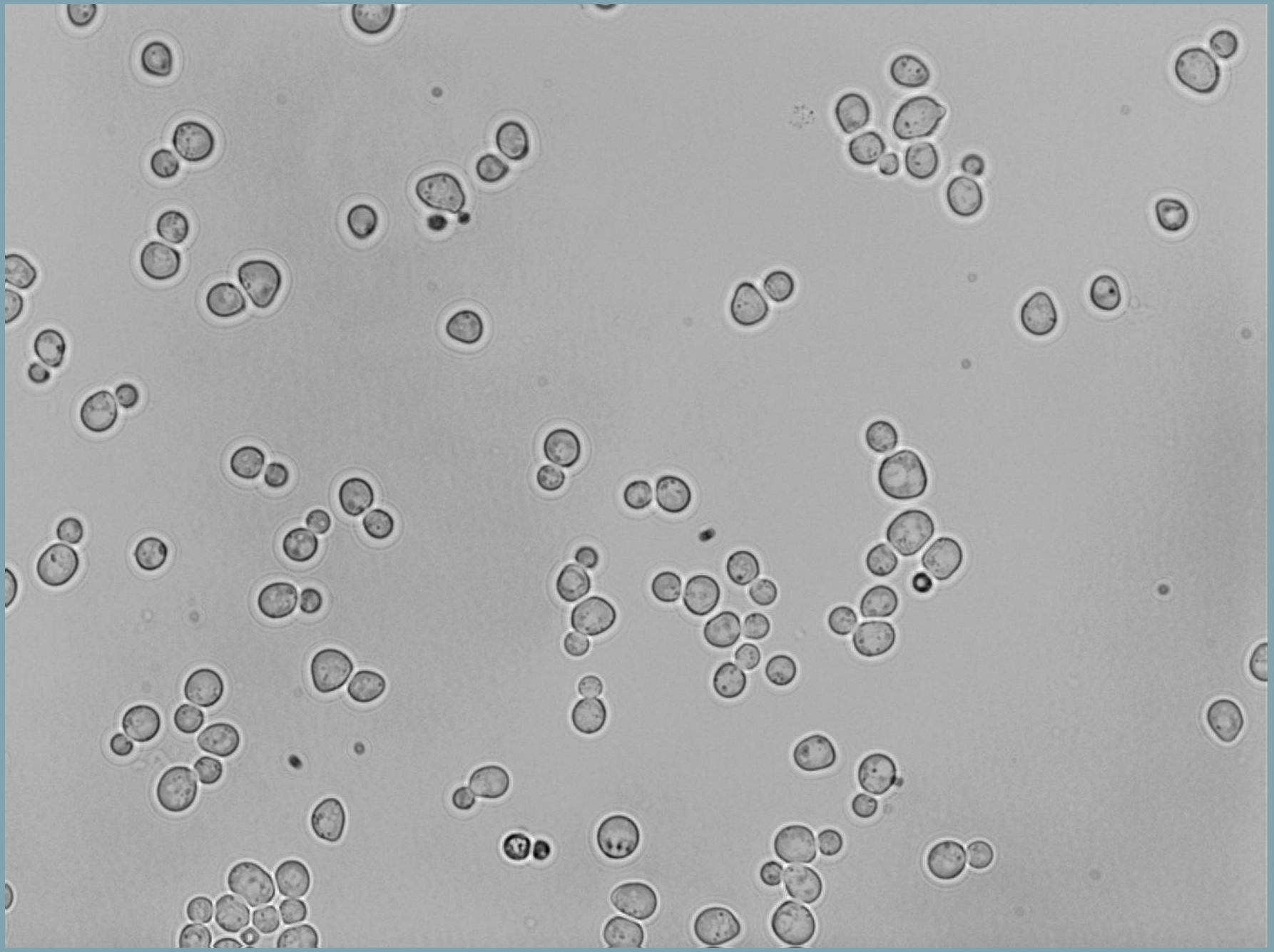
Knr4 (1-505)-GFP, α factor 2 hours



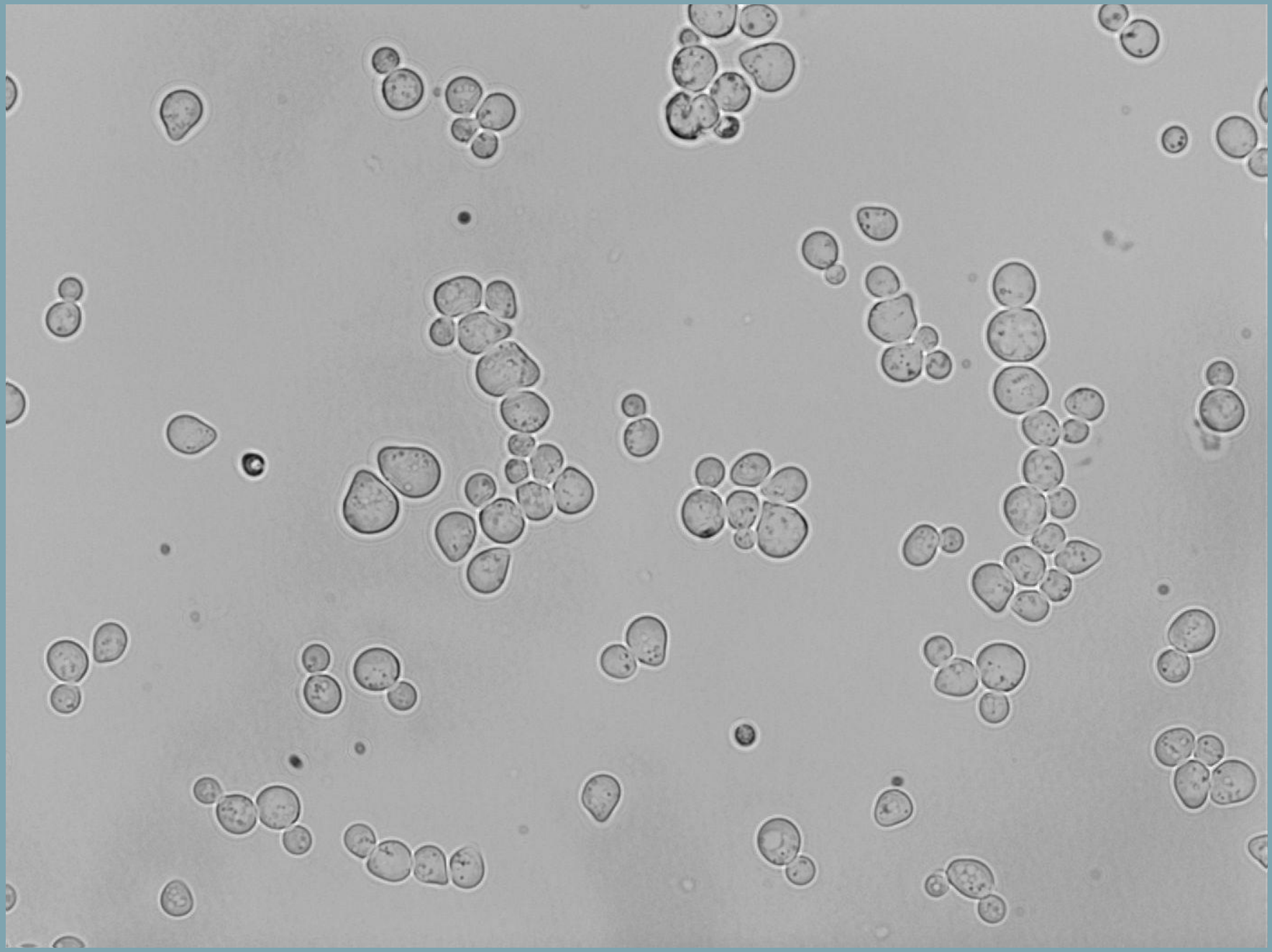
Knr4 (1-505)-GFP, α factor 3 hours



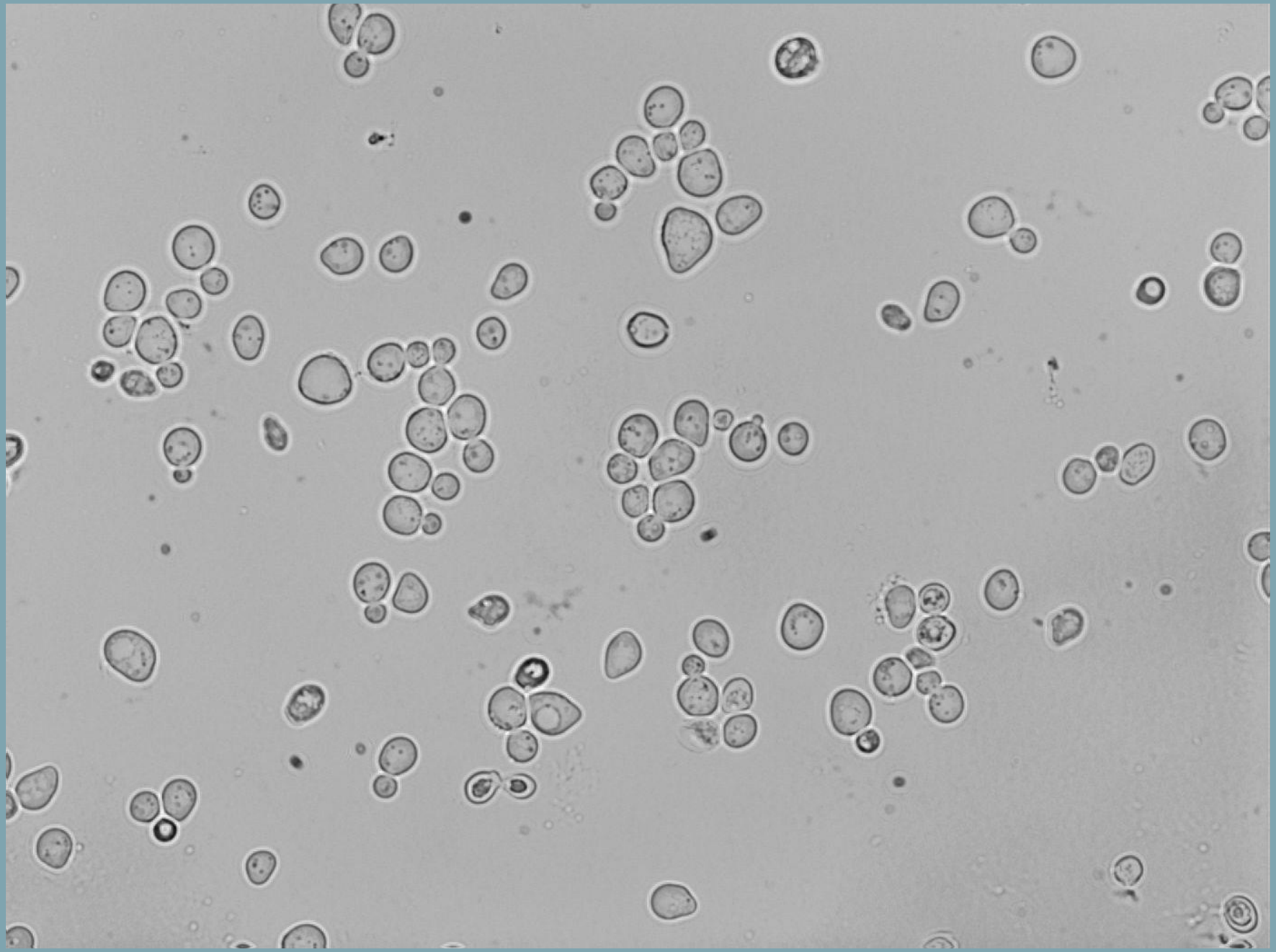
***knr4*Δ mutant + pRS315 Knr4 1-505, α-factor 1h30**



***knr4*Δ mutant + pRS315 1h30**



***knr4*Δ mutant + pRS315 Knr4 1-340, 1h30**



***knr4*Δ mutant + pRS315 Knr4 80-505 1h30**