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François

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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

Positioning 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

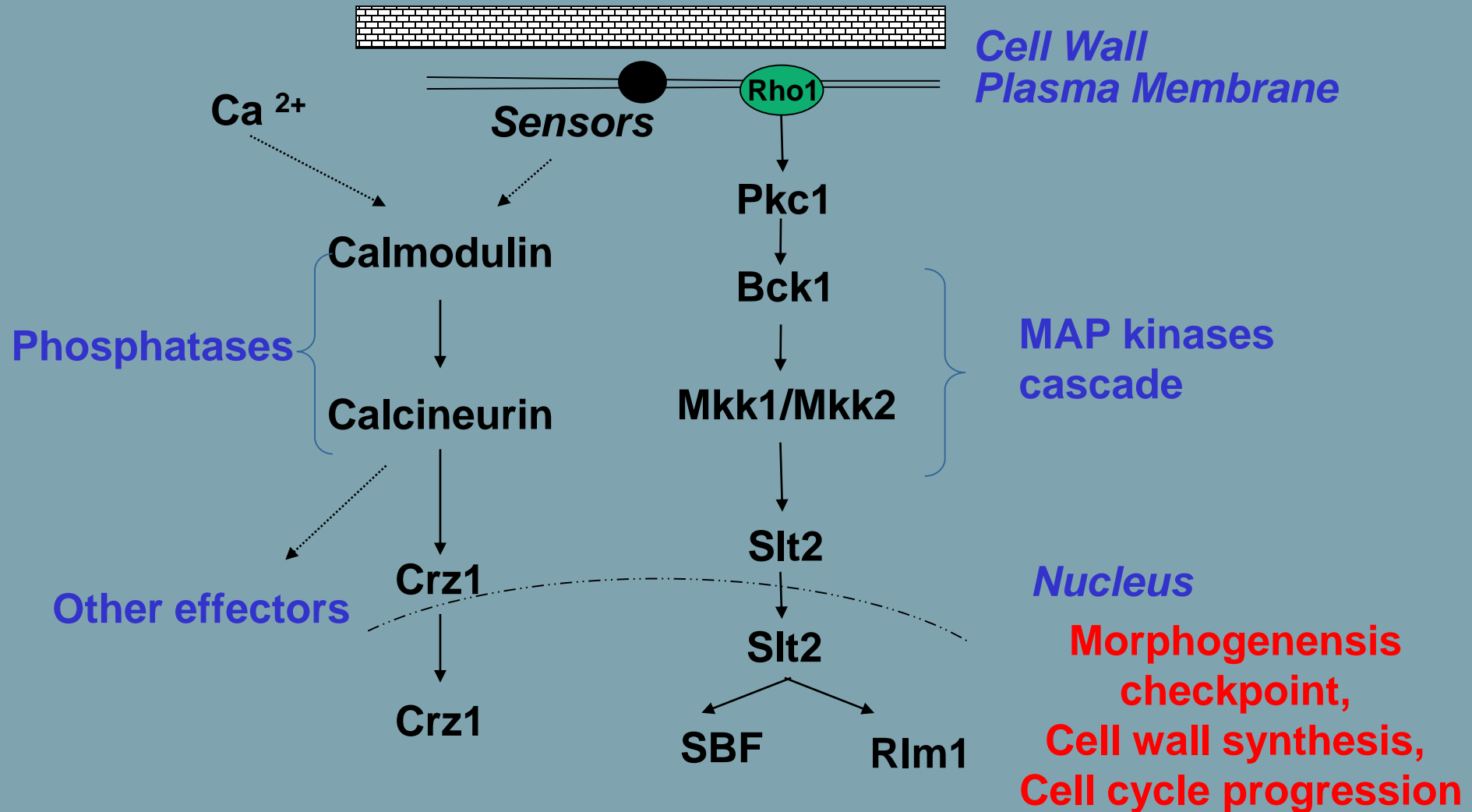
Connections with the Calcineurin Pathway

Effect on the Morphogenesis Checkpoint

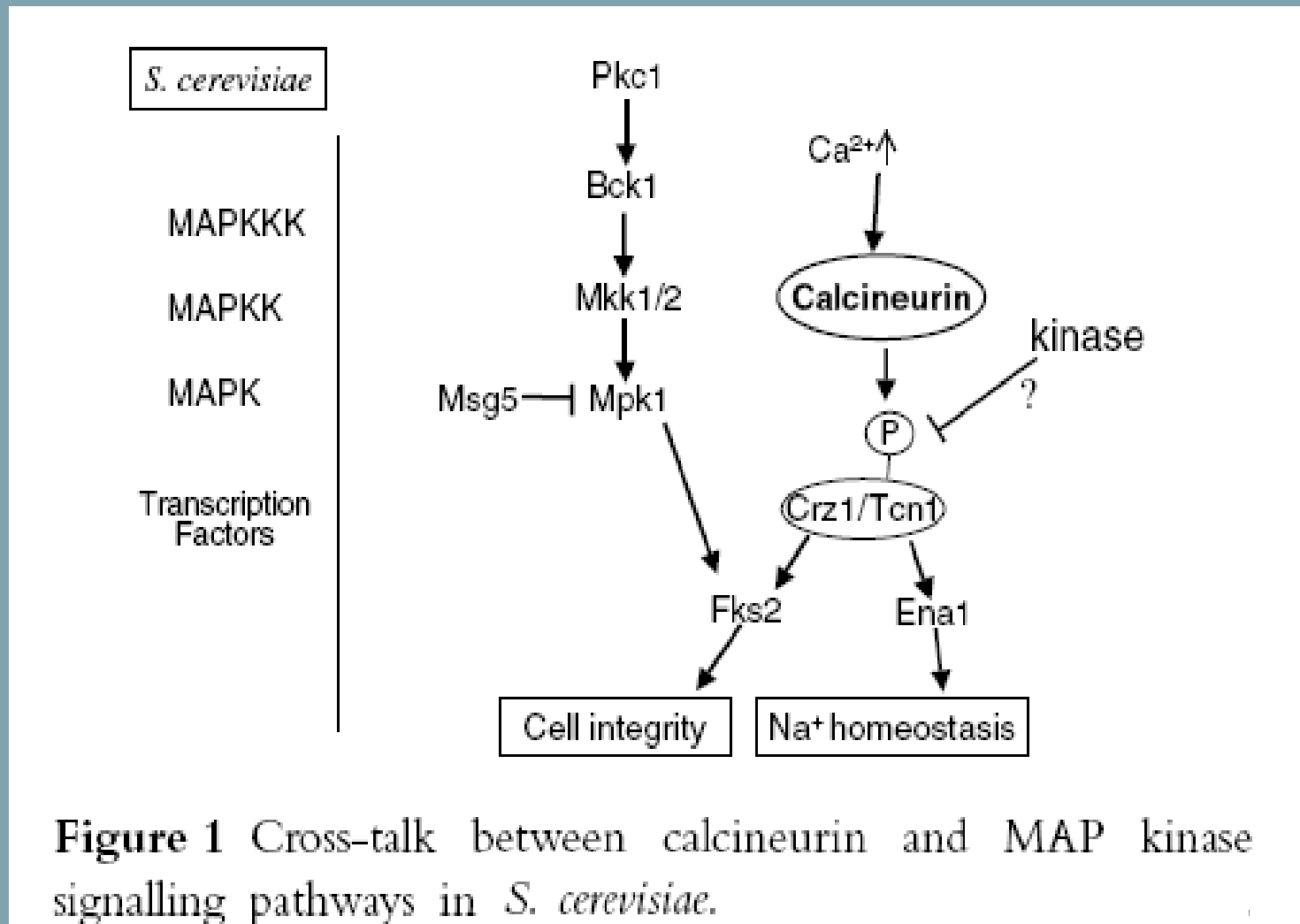
Conclusions

Introduction :

Cell integrity and morphogenesis mechanisms under stress conditions

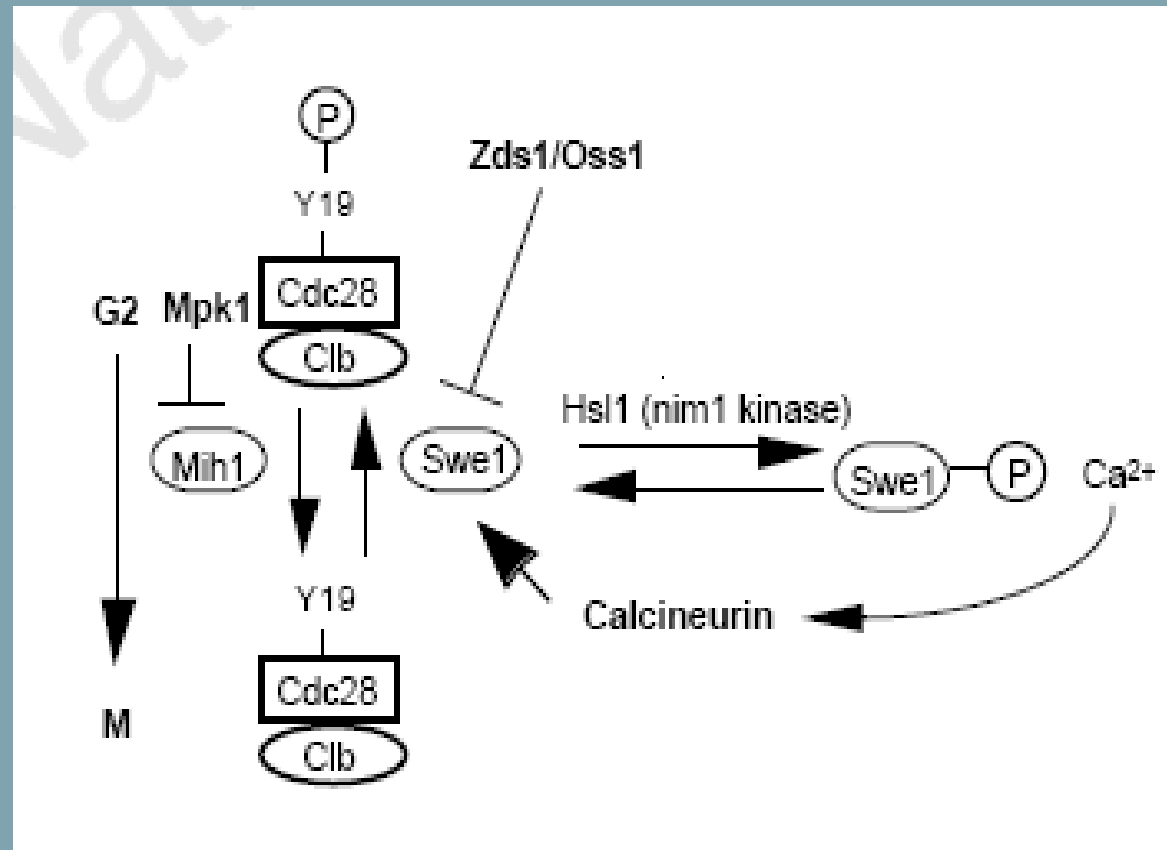


Slt2/Mpk1 and calcineurin dependant pathways share at least one essential function ... But which one ???

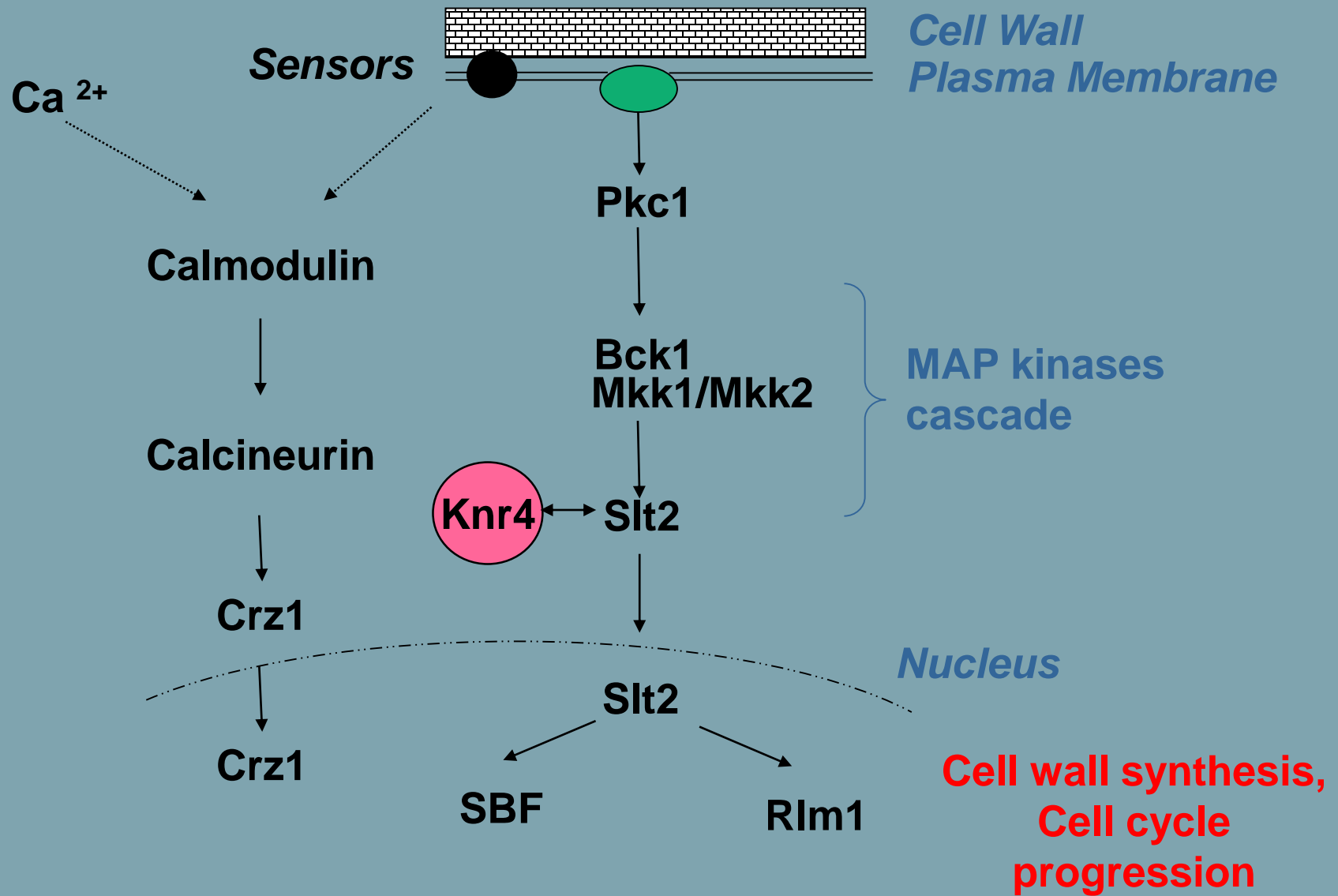


A common function of Slt2/Mpk1 and calcineurin, essential in case of cell wall stress, the « Morphogenesis checkpoint »

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

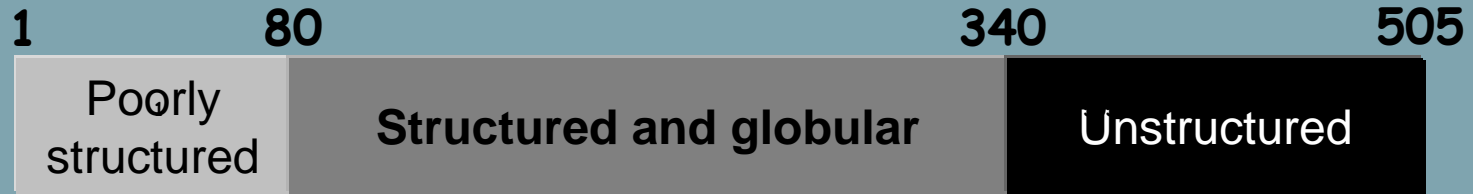


Minuzuma et al., 1998, Nature 392
Corrected by Harrison *et al*, 2001,
Nature Cell Biol, 3, 417-420.

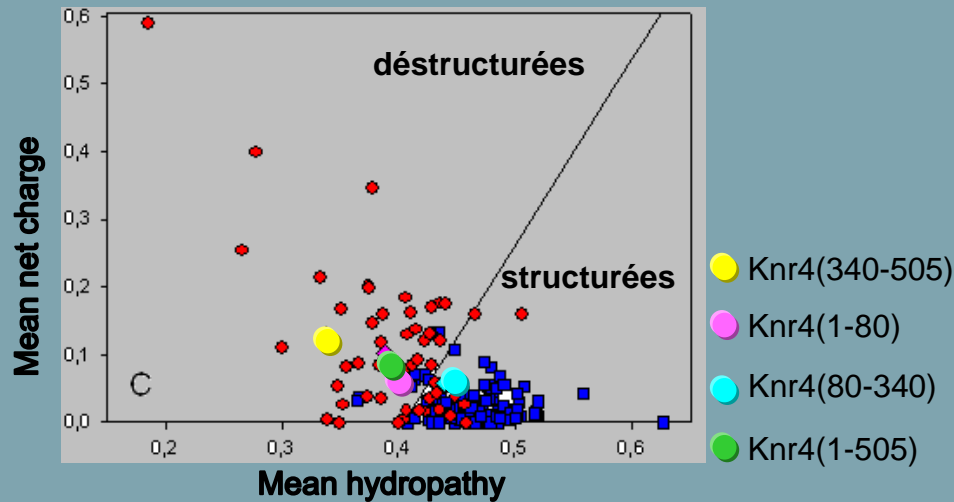


In silico Structure Predictions

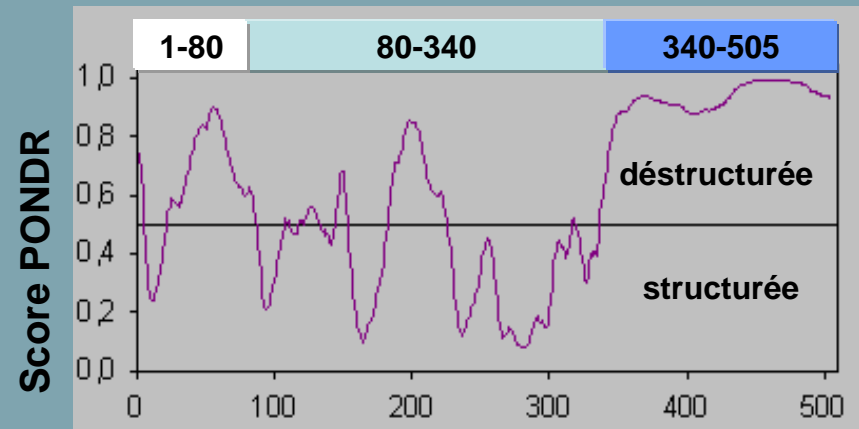
3 Identified Domains in Knr4p :



C-H Plot



PONDR Analysis



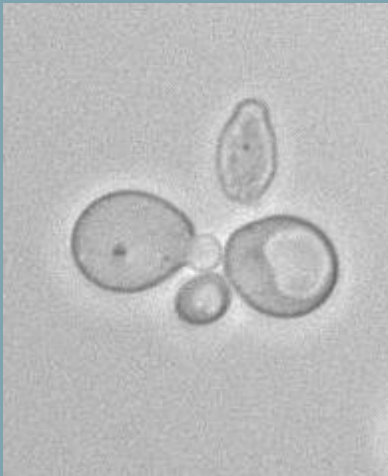
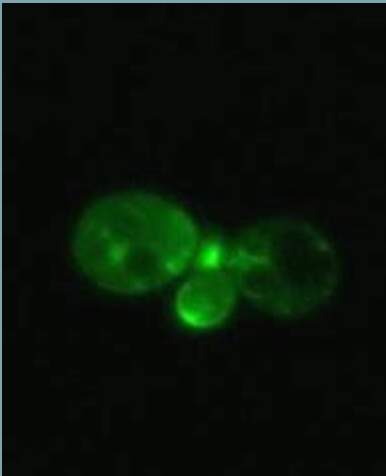
These *in silico* predictions have been confirmed by *in vitro* experiments.

Collaboration with Pr. Vladimir_Uversky

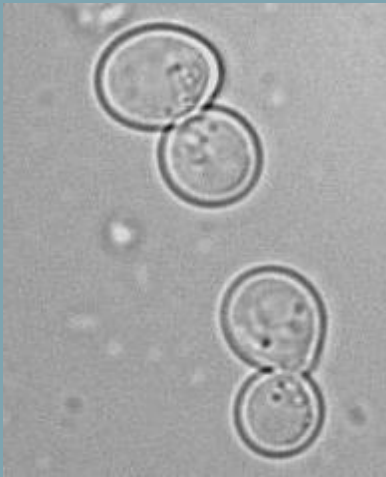
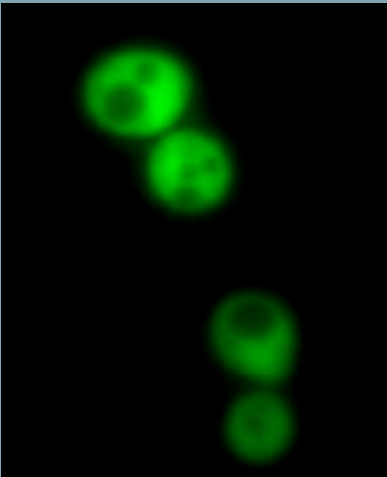
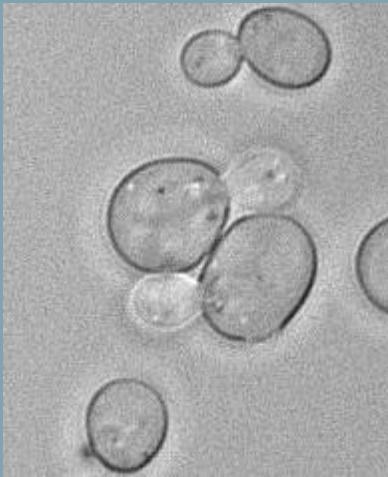
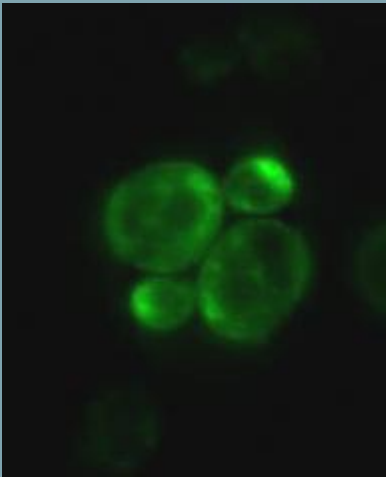
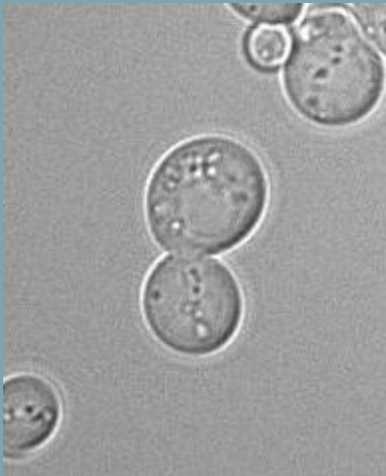
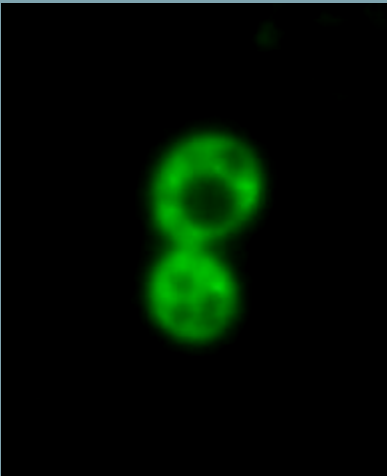
(*F. Durand et al. Yeast, 2008*)

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

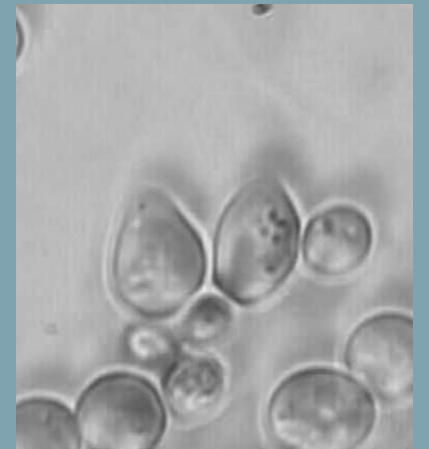
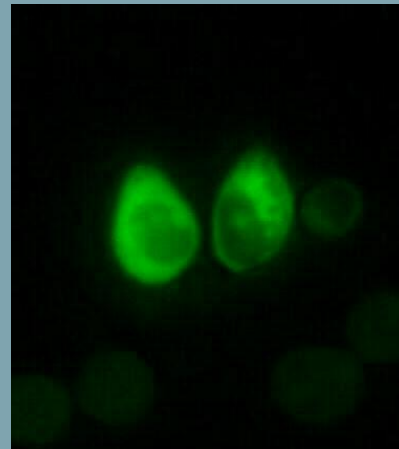
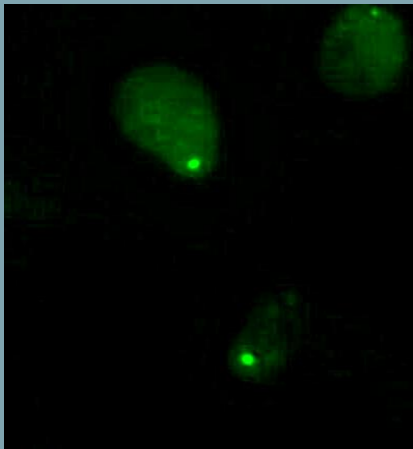
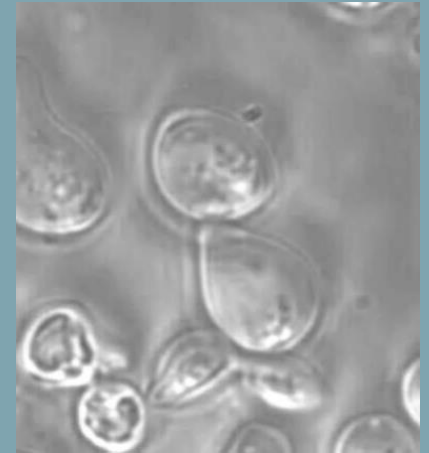
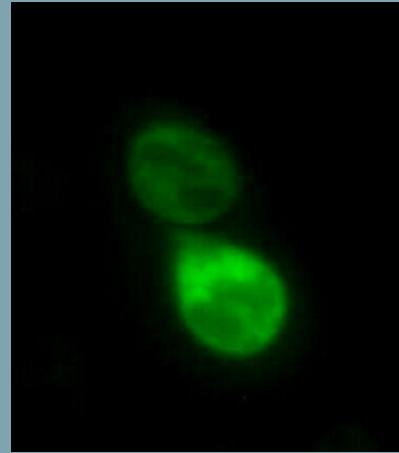
GFP alone

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

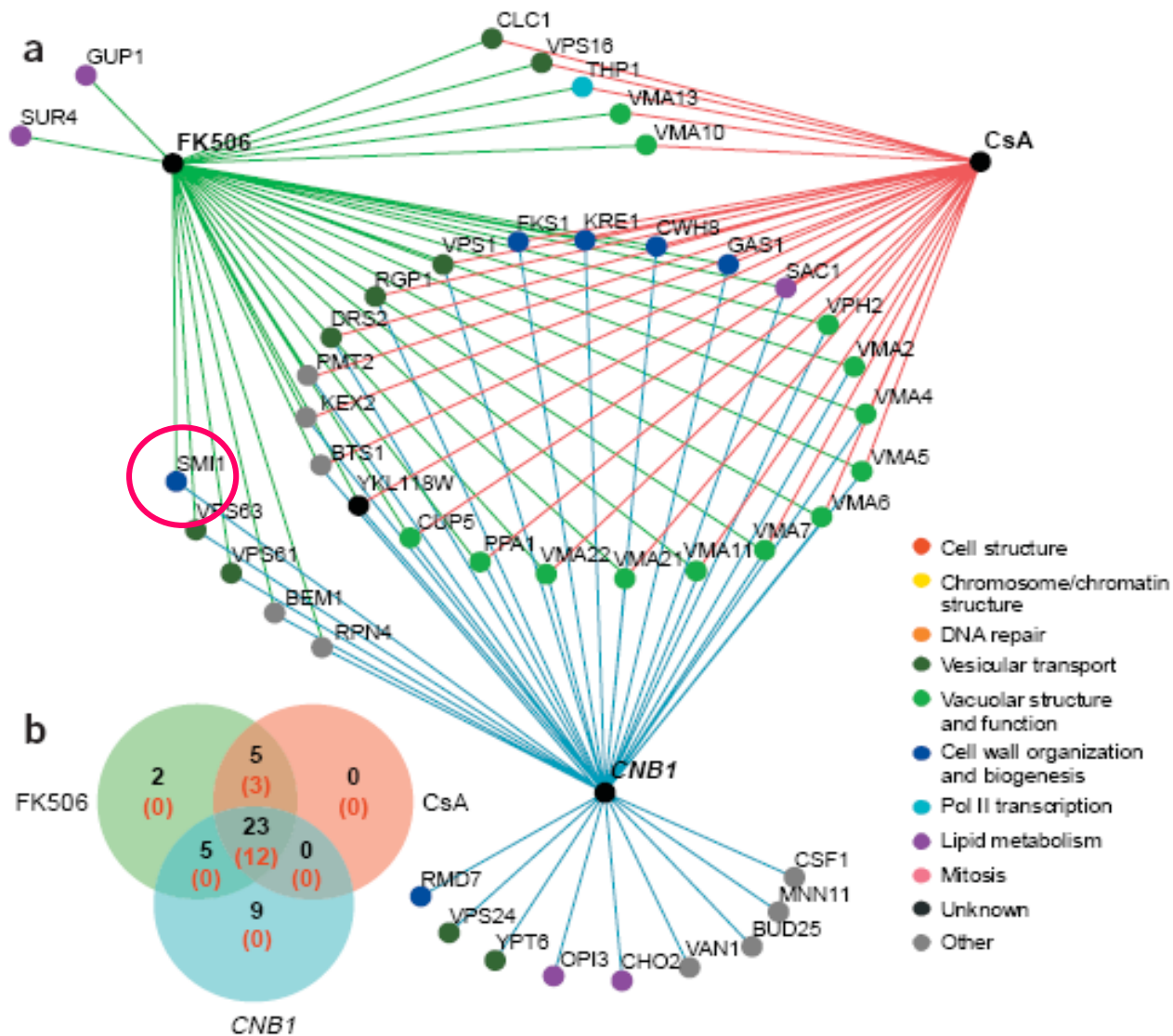
GFP alone

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

	% Unbudded cells Shmoos	% Budding cells	%
<i>Knr4</i> Δ-pRS315	5	77	18
<i>Knr4</i> Δ-pRS315- <i>KNR4</i> (1-505)	7	17	76
<i>Knr4</i> Δ-pRS315- <i>KNR4</i> (1-340)	9	16	75
<i>Knr4</i> Δ-pRS315- <i>KNR4</i> (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*, *Kluyveromyces 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.

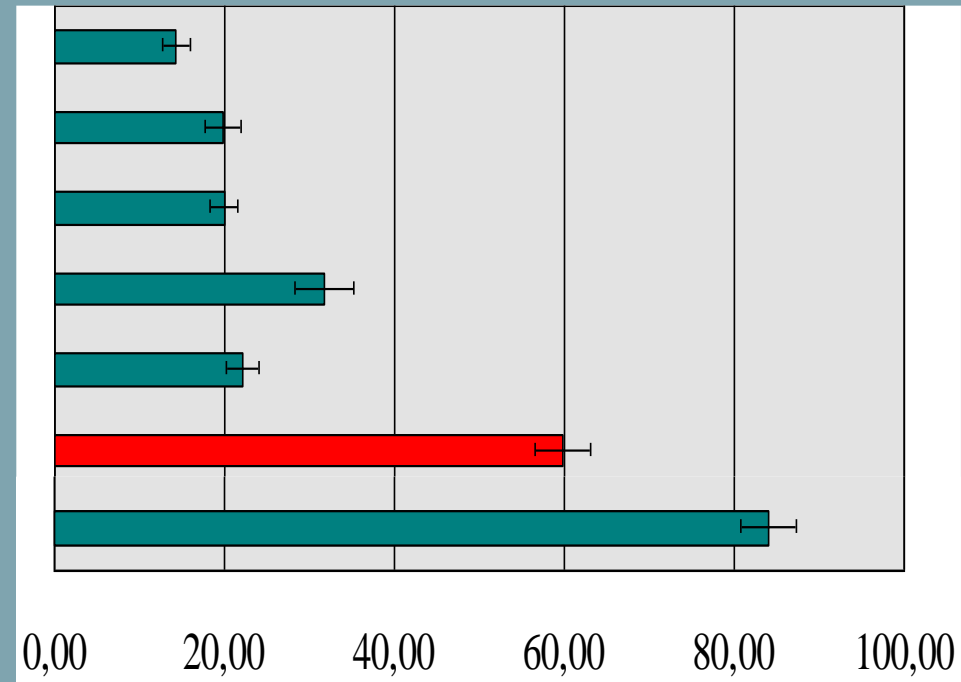


A « Chemical-genetic » screen closely links *KNR4* and Calcineurin

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.

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)

Knr4 physically interacts with calcineurin

pGAD2f / pOBD2 (negative control)

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

pGAD2f-*CNB1* / pPOBD2

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

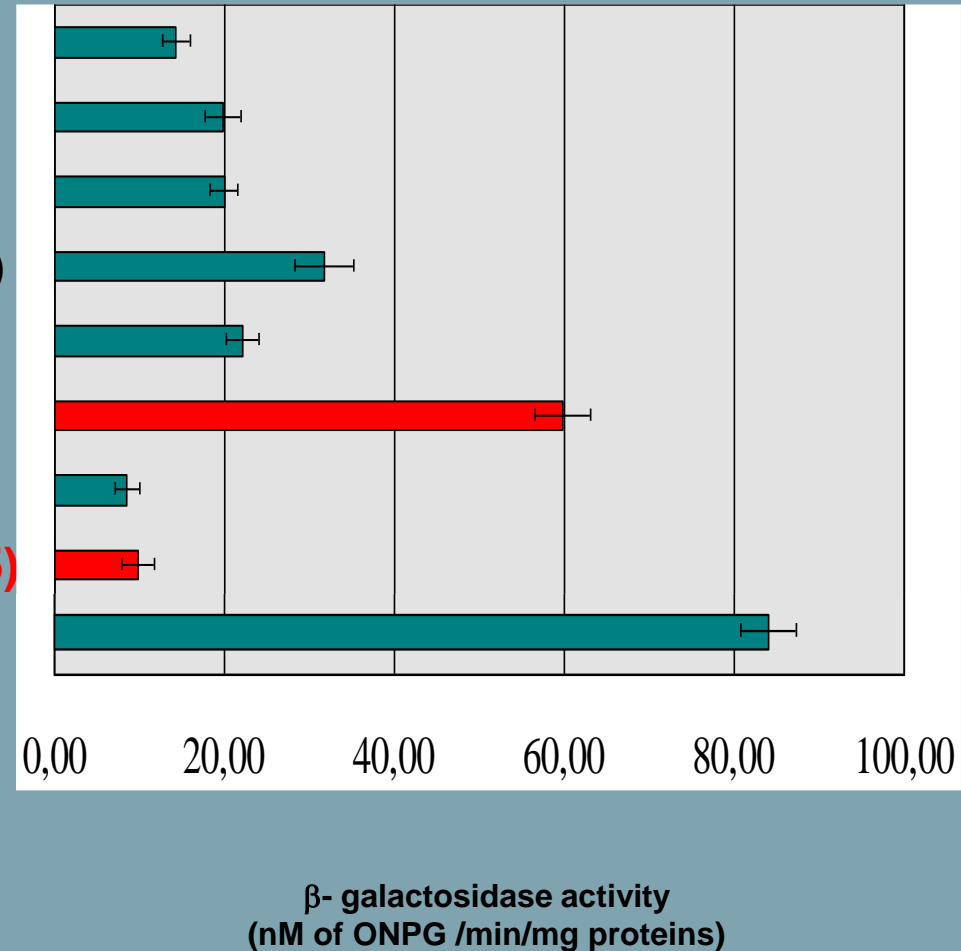
pGAD2f-*CNA1* / pOBD2

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

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

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

Positive control



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)

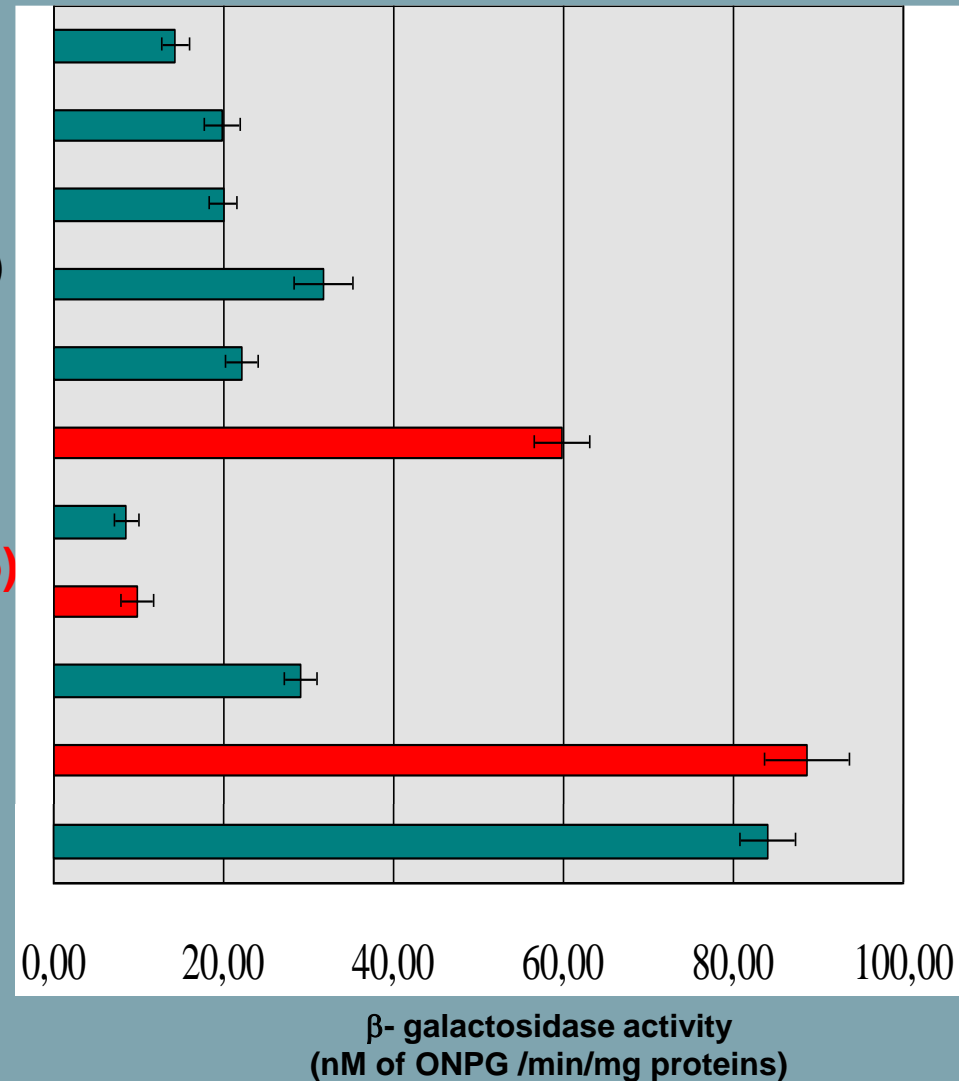
pGAD2f / pOBD2-KNR4(80-505)

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

pGAD2f / pOBD2-KNR4(1-340)

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

Positive control



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)

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

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

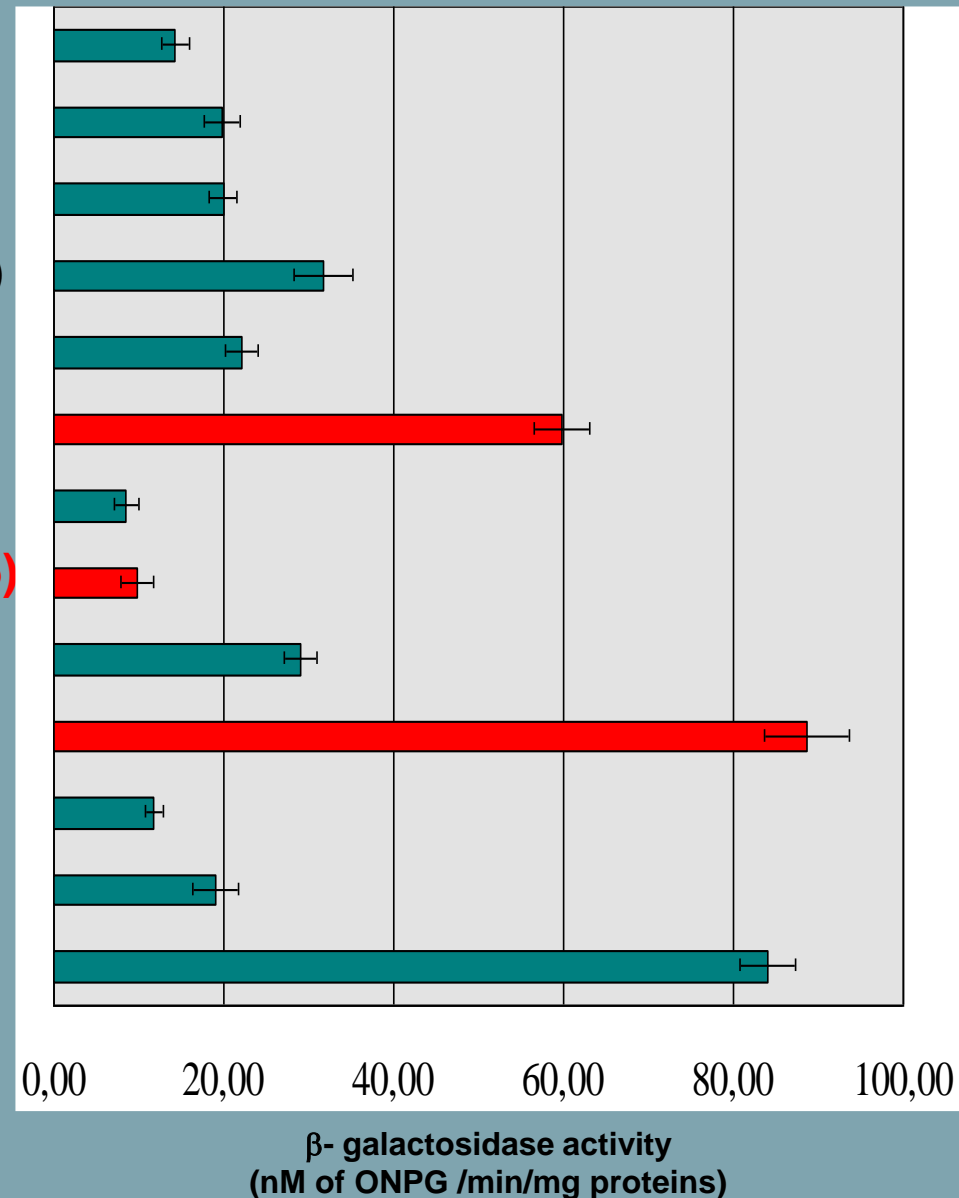
pGAD2f / pOBD2-*KNR4*(1-340)

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

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

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

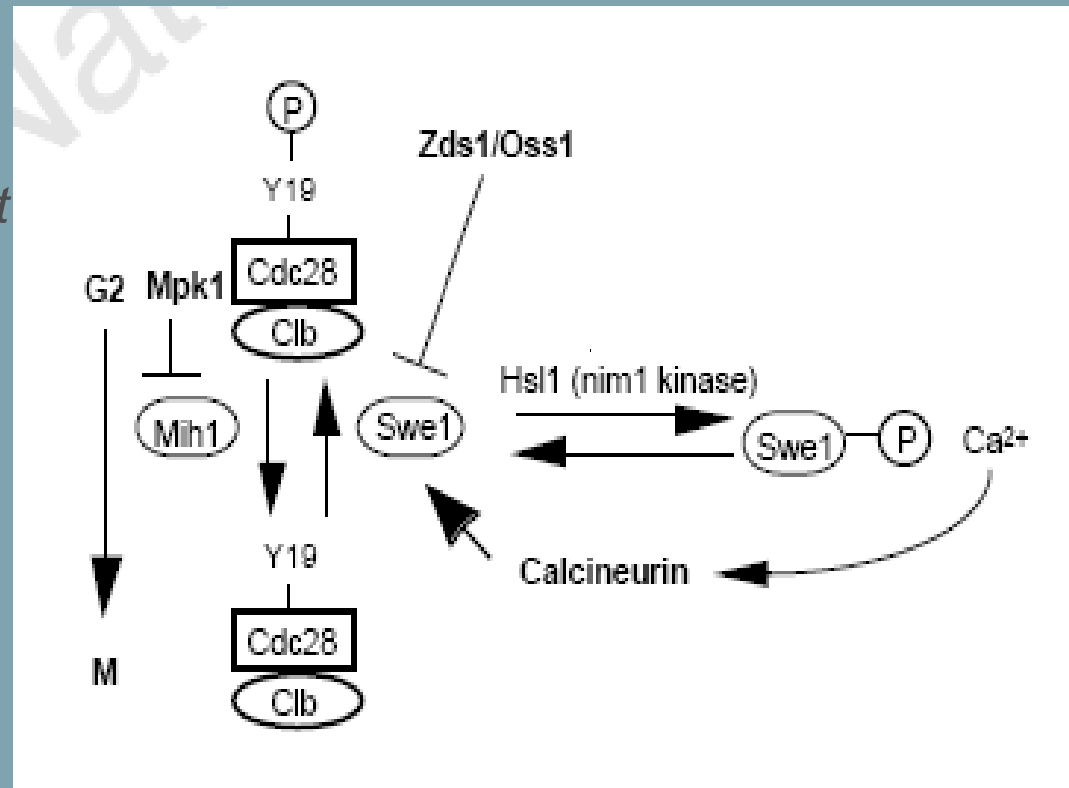
Positive control



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.

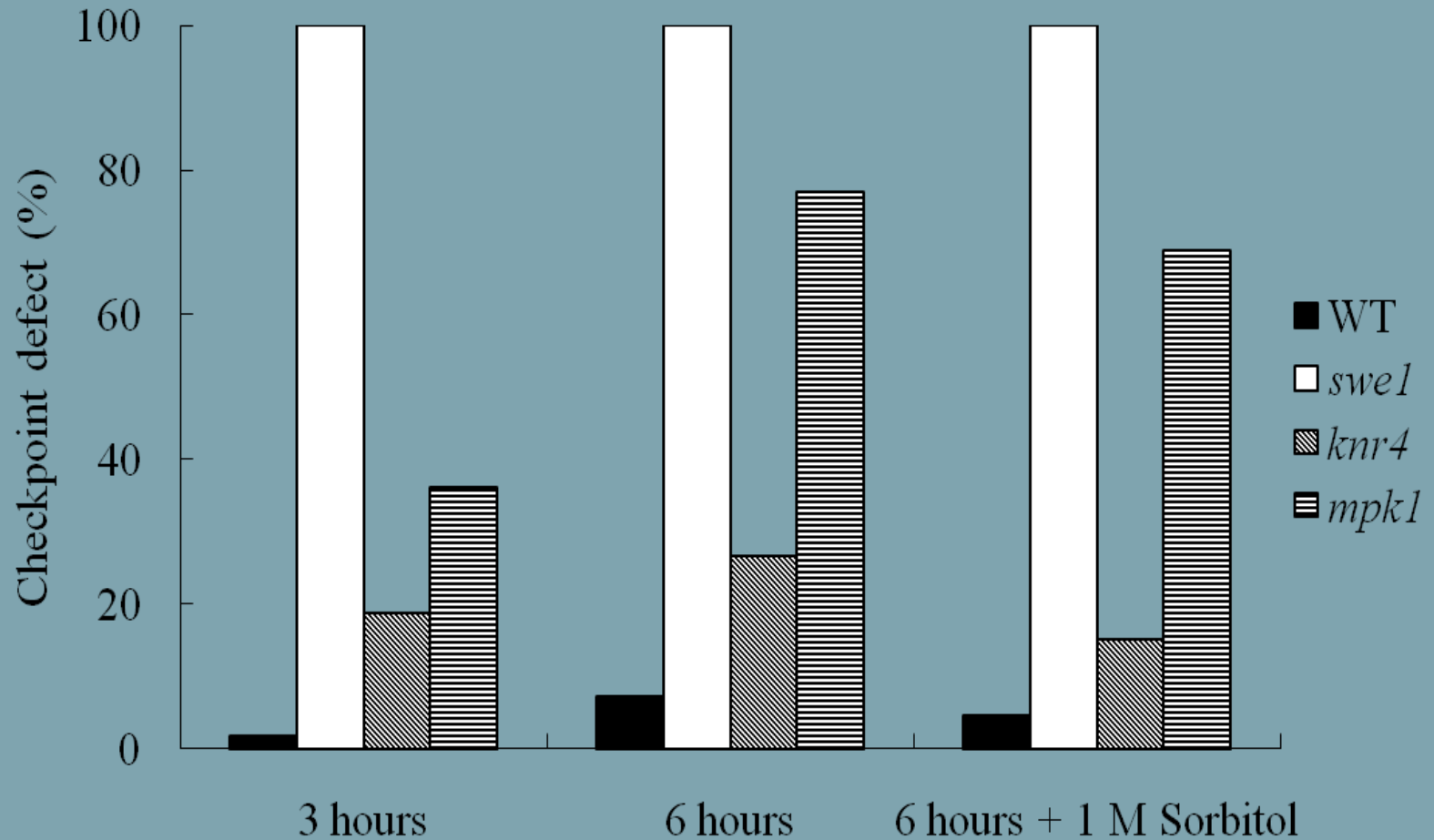
Is this checkpoint functioning in the *knr4Δ* mutant?
(cf : several cell cycle defect phenotypes in this mutant...)



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

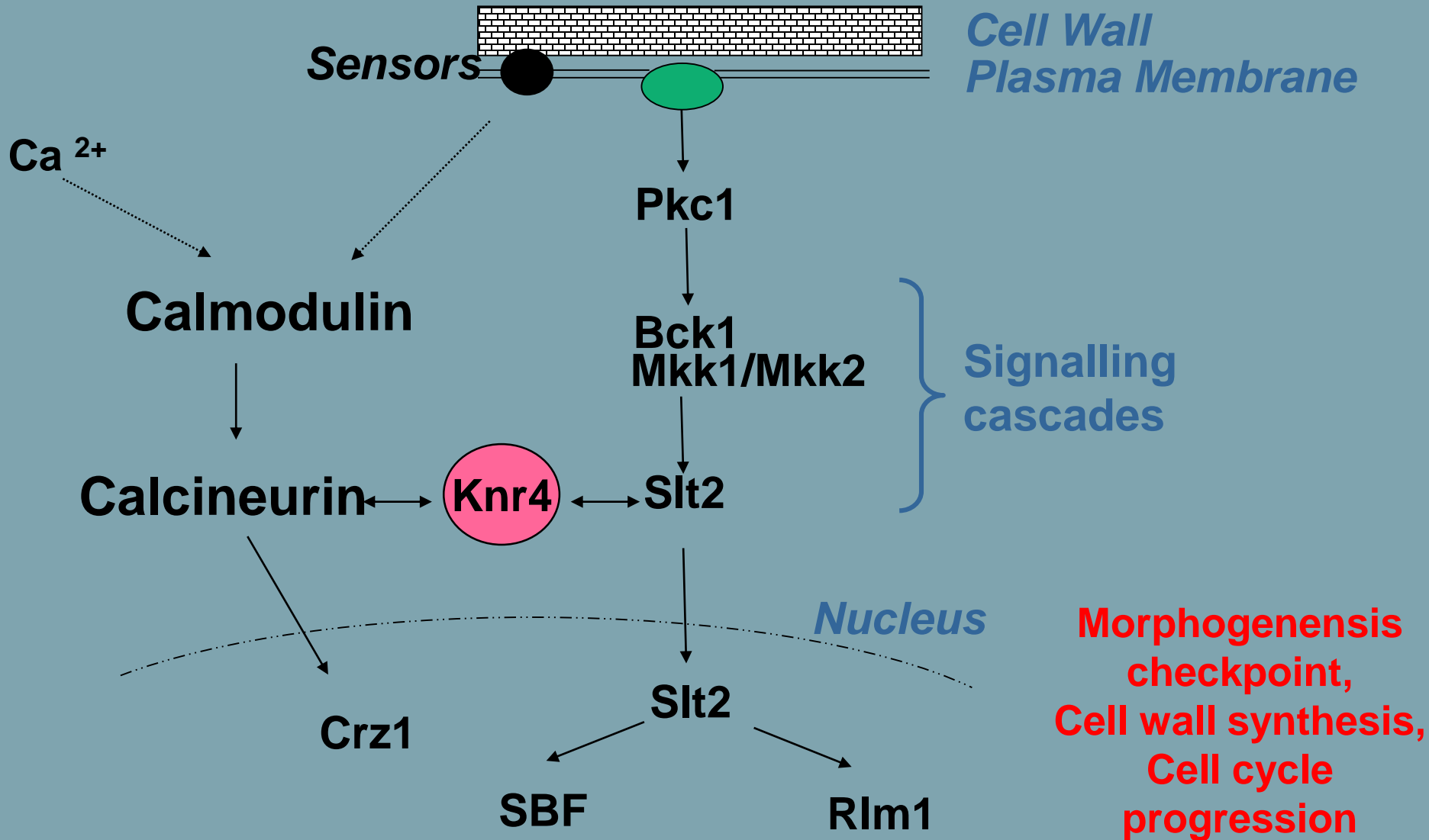
Minuzuma et al., 1998, Nature 392
Corrected by Harrison et al, 2001,
Nature Cell Biol, 3, 417-420.

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)

1

80

340

505

N-term:
unstructured

Structured and globular

C-term: unstructured

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 + pK_{NR4}(1-505) + pSG2

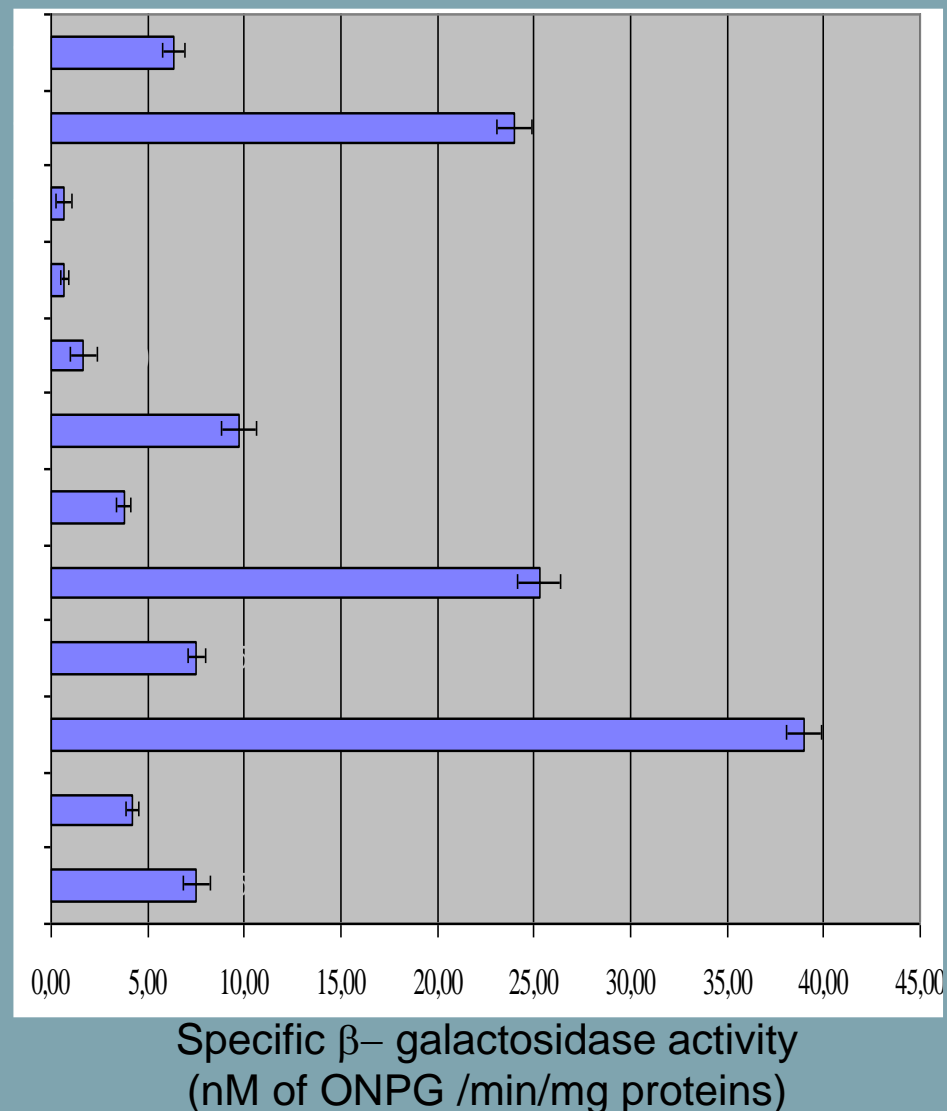
*knr4*Δ + pK_{NR4}(1-505) + pSG2 / CFW

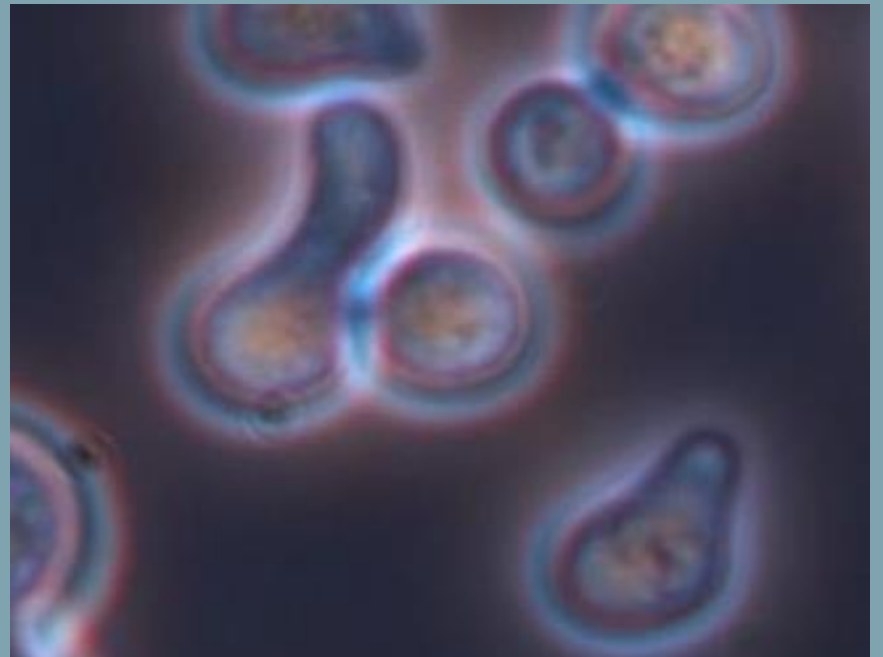
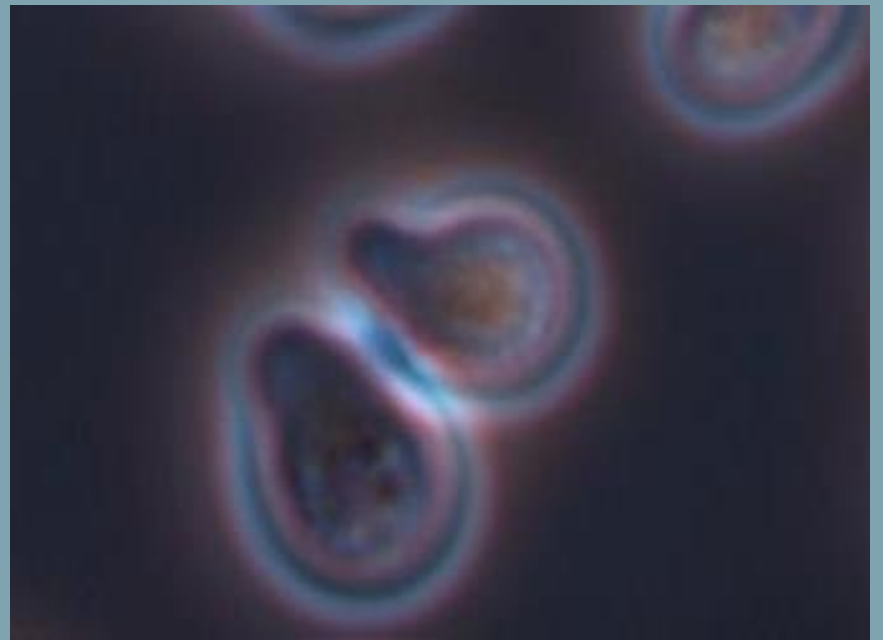
*knr4*Δ + pK_{NR4}(1-340) + pSG2

*knr4*Δ + pK_{NR4}(1-340) + pSG2 / CFW

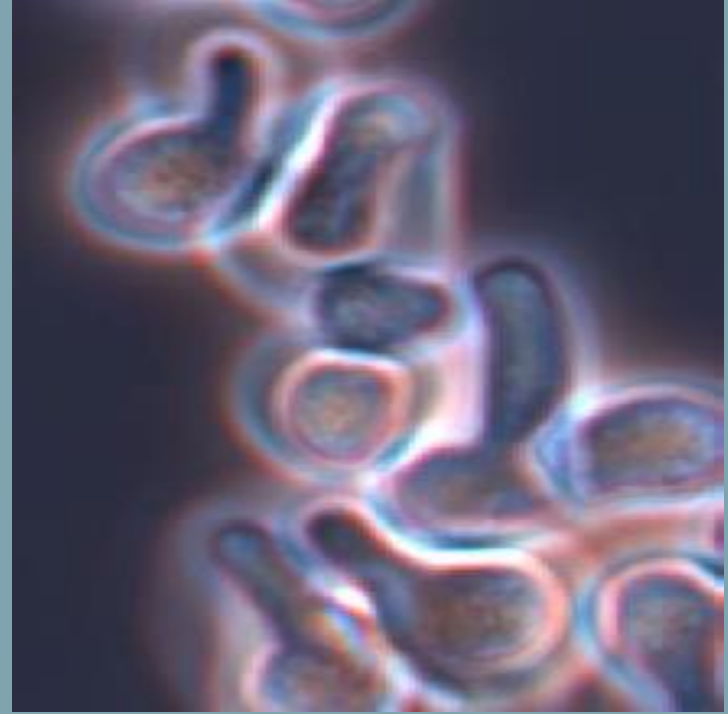
*knr4*Δ + pK_{NR4}(80-505) + pSG2

*knr4*Δ + pK_{NR4}(80-505) + pSG2 / CFW

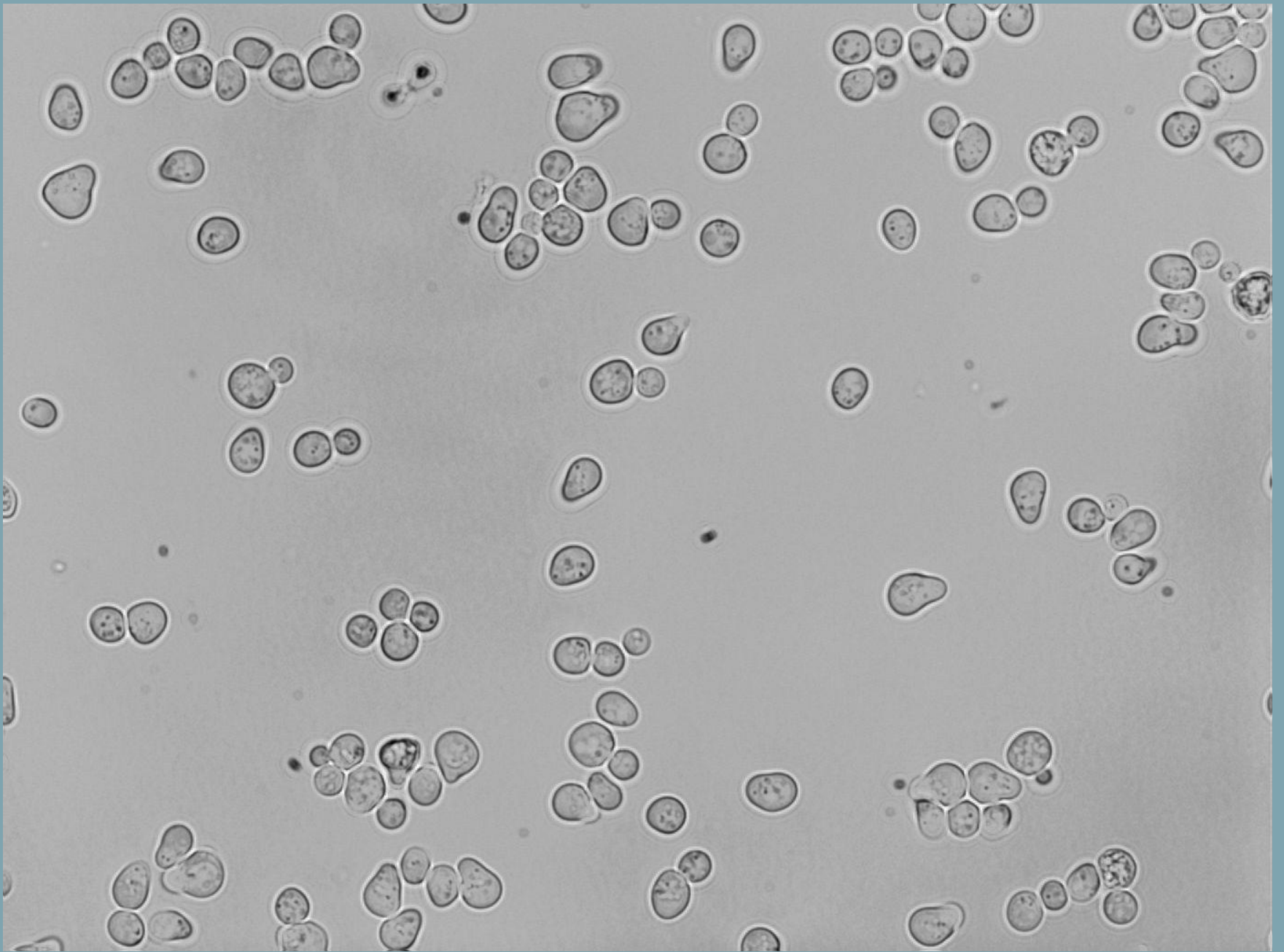




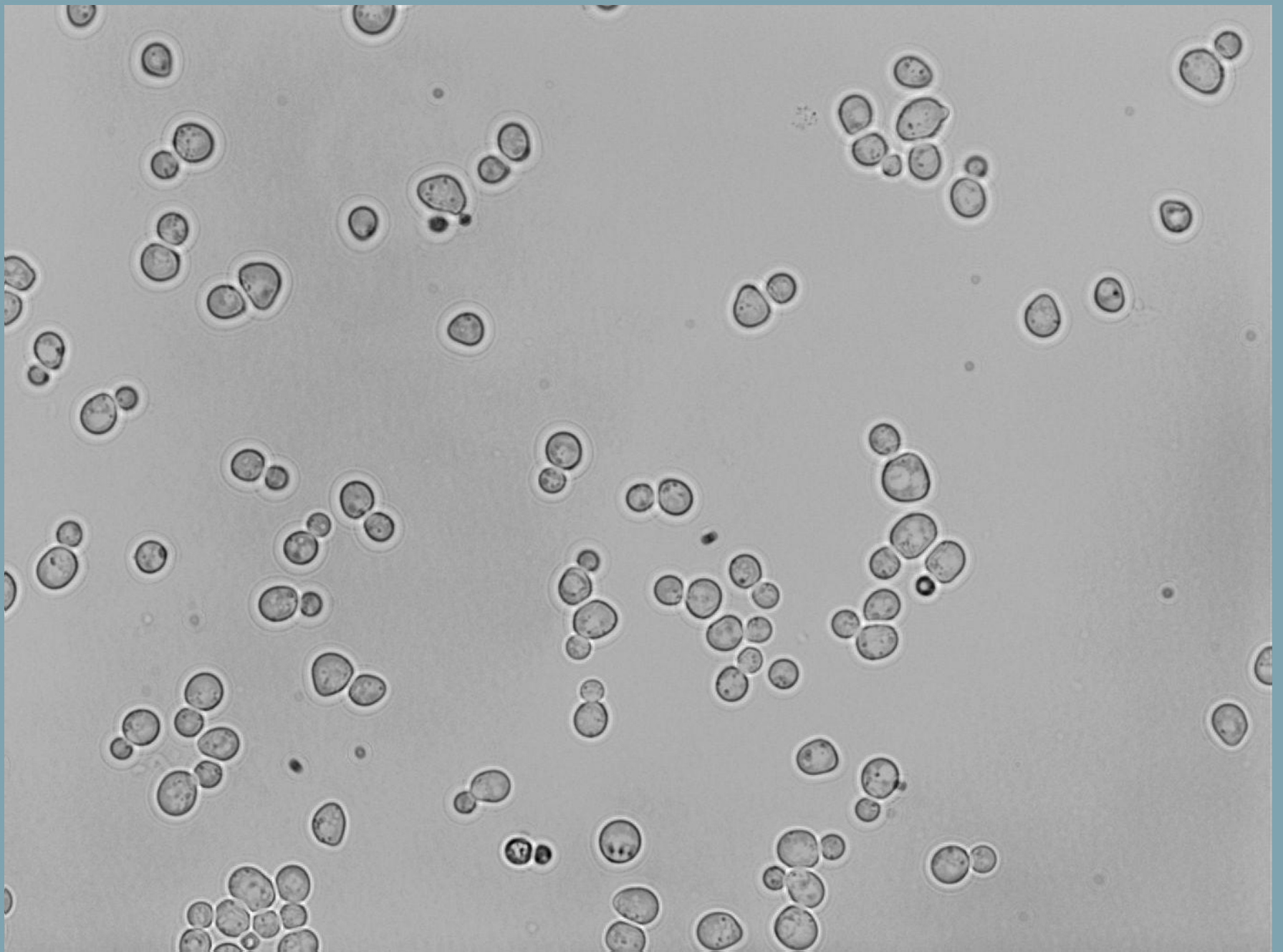
Knr4 (1-505)-GFP, α factor 2 hours



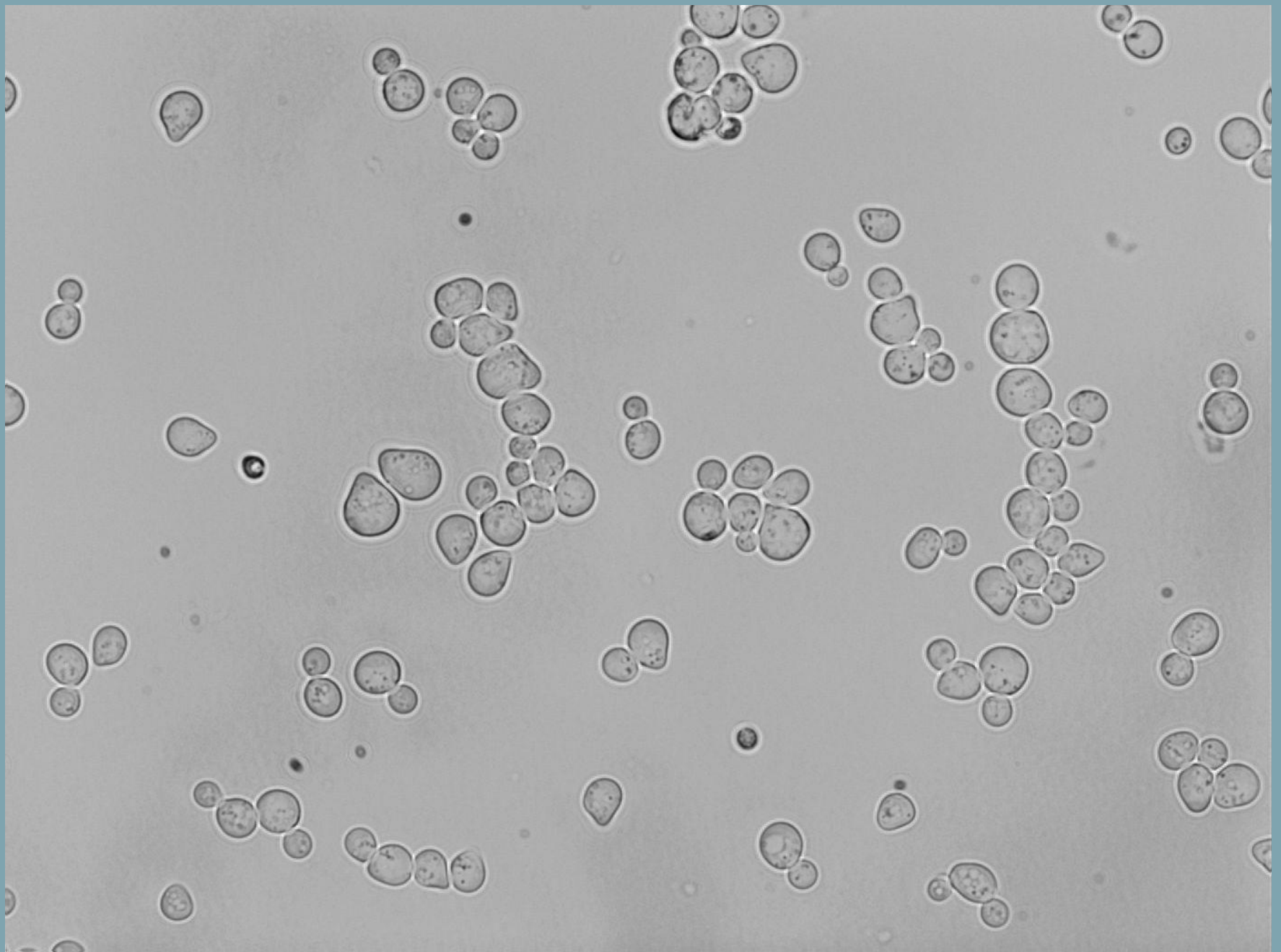
Knr4 (1-505)-GFP, α factor 3 hours



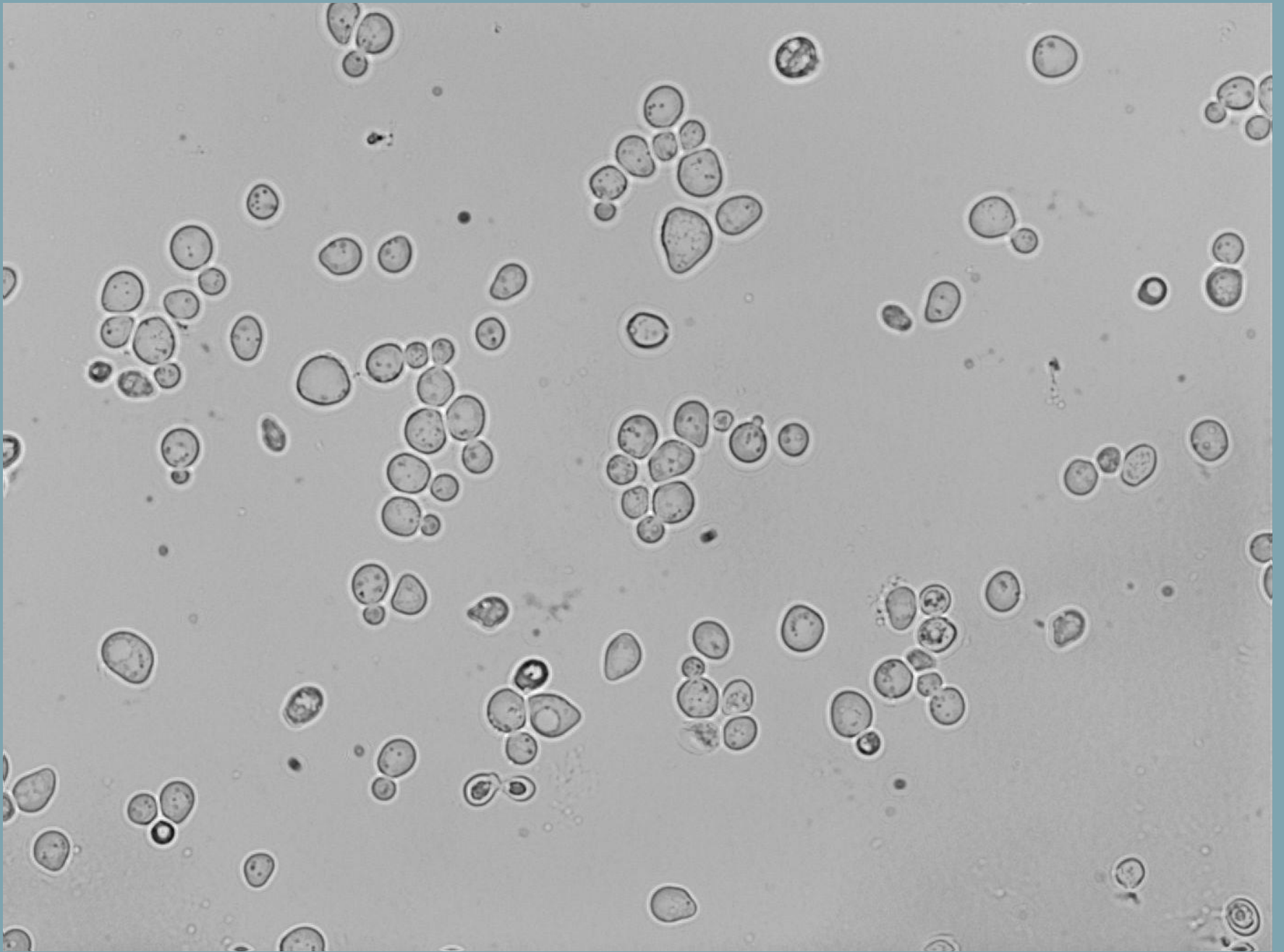
***knr4*Δ mutant + pRS315 Knr4 1-505, α-factor 1h30**



***knr4*Δ mutant + pRS315 1h30**



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



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