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The interactome of Knr4/Smi1, a protein involved in coordinating cell wall assembly with cellular growth in yeast

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Part 1 : Isolation of Knr4/Smi1 and its phenotypic characteristics

Part 2 : Knr4 is involved in Mpk1/Slt2-cell wall integrity pathway

Part 3 : Interactomic map of Knr4

KNR4/SMI1 was isolated by three different genetic screens

- → Genetic screen for proteins that bind MAR (matrix-associated regions) on DNA. Use a *lacZ* construct and check for blue colonies
(Fishel et al., PNAS, 90, 5623-5627, 1993)
- → Genetic screen for mutants that acquire resistance to the Killer toxin K9.
(Hong et al., MCB, 14, 1017-1025, 1994)
- → Genetic screen for suppressors of several Calcofluor white hypersensitive mutant (*cwh*).
(Martin et al. Microbiology, 145, 249-258, 1999)

❖ At the gene level

- ✓ single gene, not essential
- ✓ specific to fungal kingdom

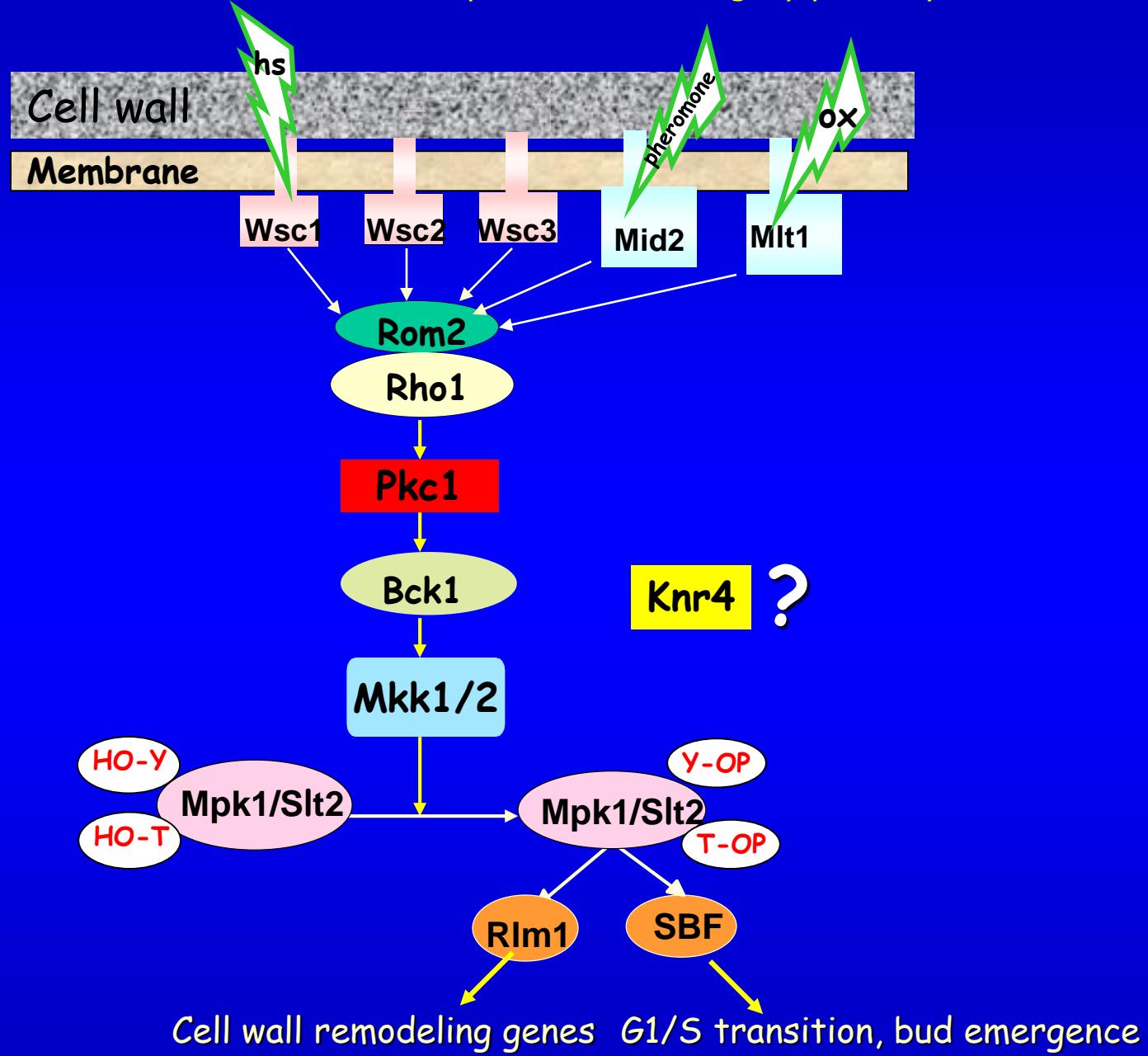
■ main phenotypes of *knr4Δ* mutant

- Hypersensitive to cell wall drugs
- Hypersensitive to caspofungin and cercosporamide
- Stop growth > 38°C as with small buds, osmotically remediable
- 50% reduction of cell wall β -1,3 glucans in vegetative cells
- Synthetic lethal with *pkc1Δ*, *slt2Δ*, *rlm1Δ*.....

■ main phenotypes of *KNR4* overexpression

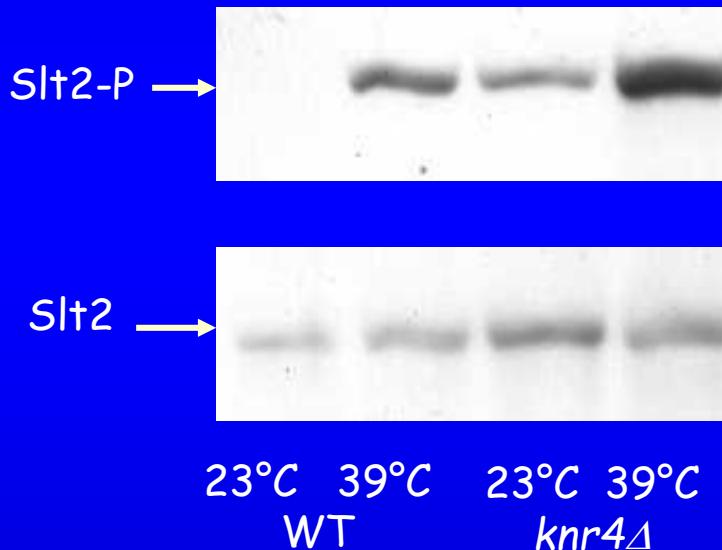
- suppress several *cwh* mutants
- repression *CHS1*, *CHS2*, *CHS3*

Part 2: Knr4 is involved in Mpk1-cell wall integrity pathway

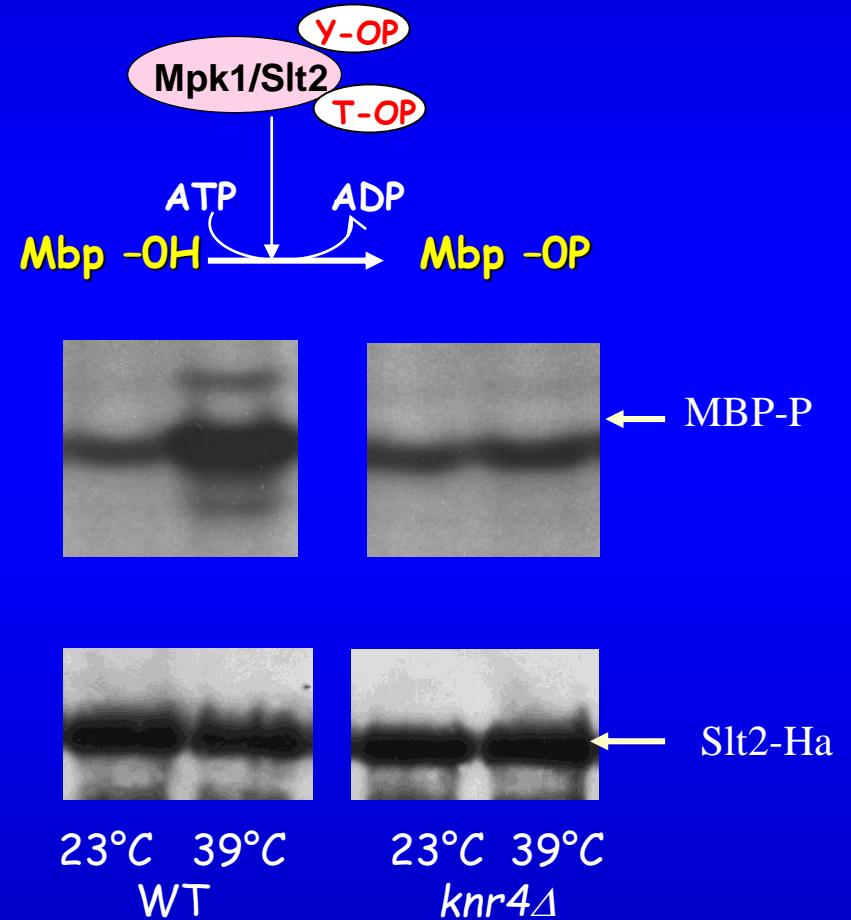


1: KNR4 is required for the heat shock activation Mpk1/Slt2

a) *knr4Δ* does not prevent signaling to Slt2 activation



b) Phosphorylated Slt2 is inactive in a *knr4Δ*



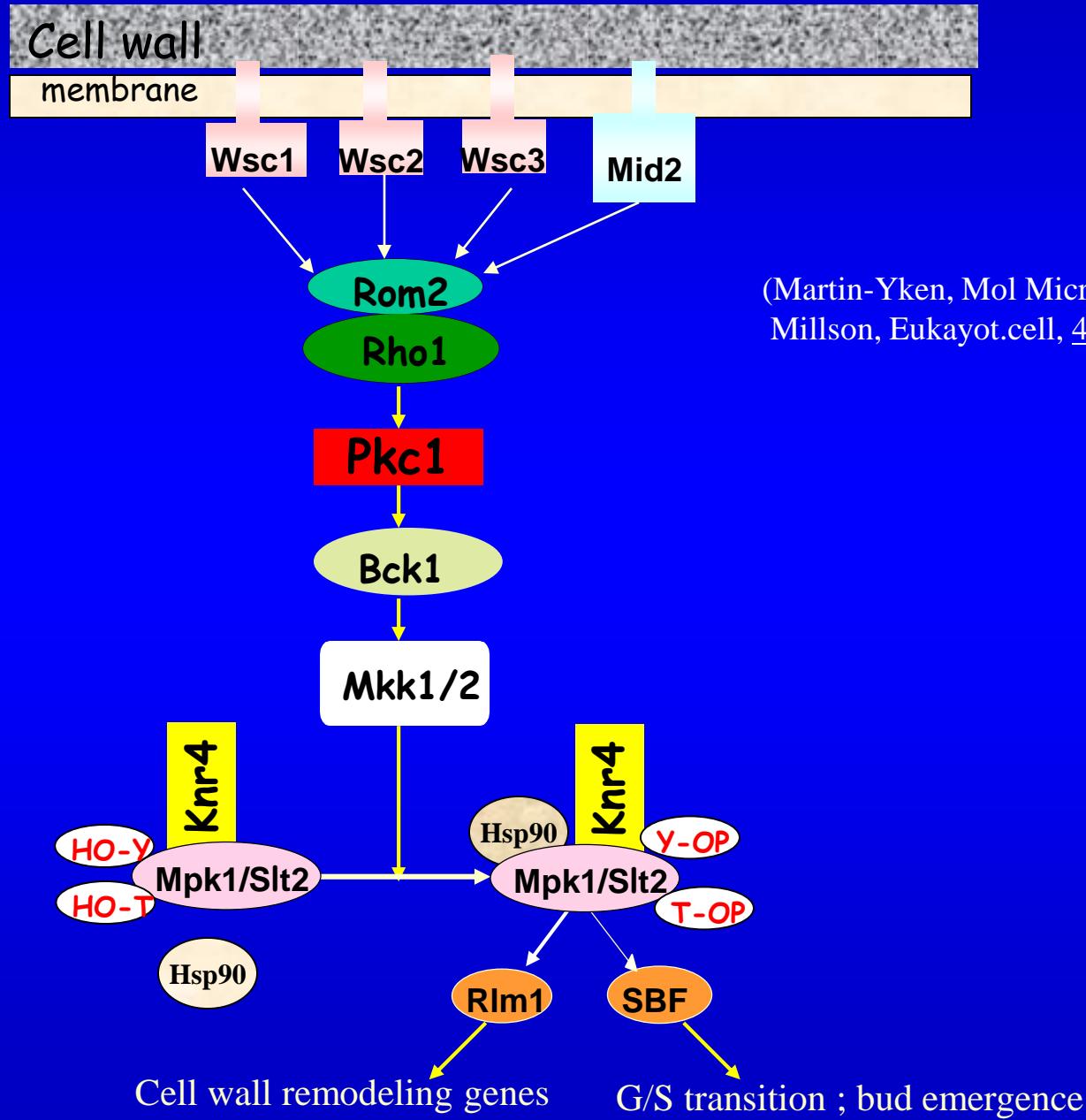
3: Transcriptional activity of Rlm1 is strongly reduced in a *knr4Δ*

Strain	Relevant genotype	β-galactosidase
W303-1A	<i>KNR4 SLT2</i>	448
HM13	<i>knr4Δ SLT2</i>	44
JF1366	<i>rlm1Δ SLT2</i>	434
W303Δ <i>slt2</i>	<i>slt2Δ</i>	< 1

Assay of Rlm1 activity :

Use of a reporter plasmid containing a *lexA-RLM1ΔN* fusion and a *lacZ* reporter gene with *lexA* binding sites in the promoter.

Part 2: Knr4 is involved in Slt2-cell wall integrity pathway



(Martin-Yken, Mol Microbiol, 49, 23, 2003;
Millson, Eukaryot.cell, 4, 849, 2005)

Three strategies

Synthetic Lethal

- genetic
- functional
- not temporal
- not spatial
- not [prot]
- no false +/-

Two Hybrid

- physical
- not temporal
- spatial
- not [prot]
- some false +/-

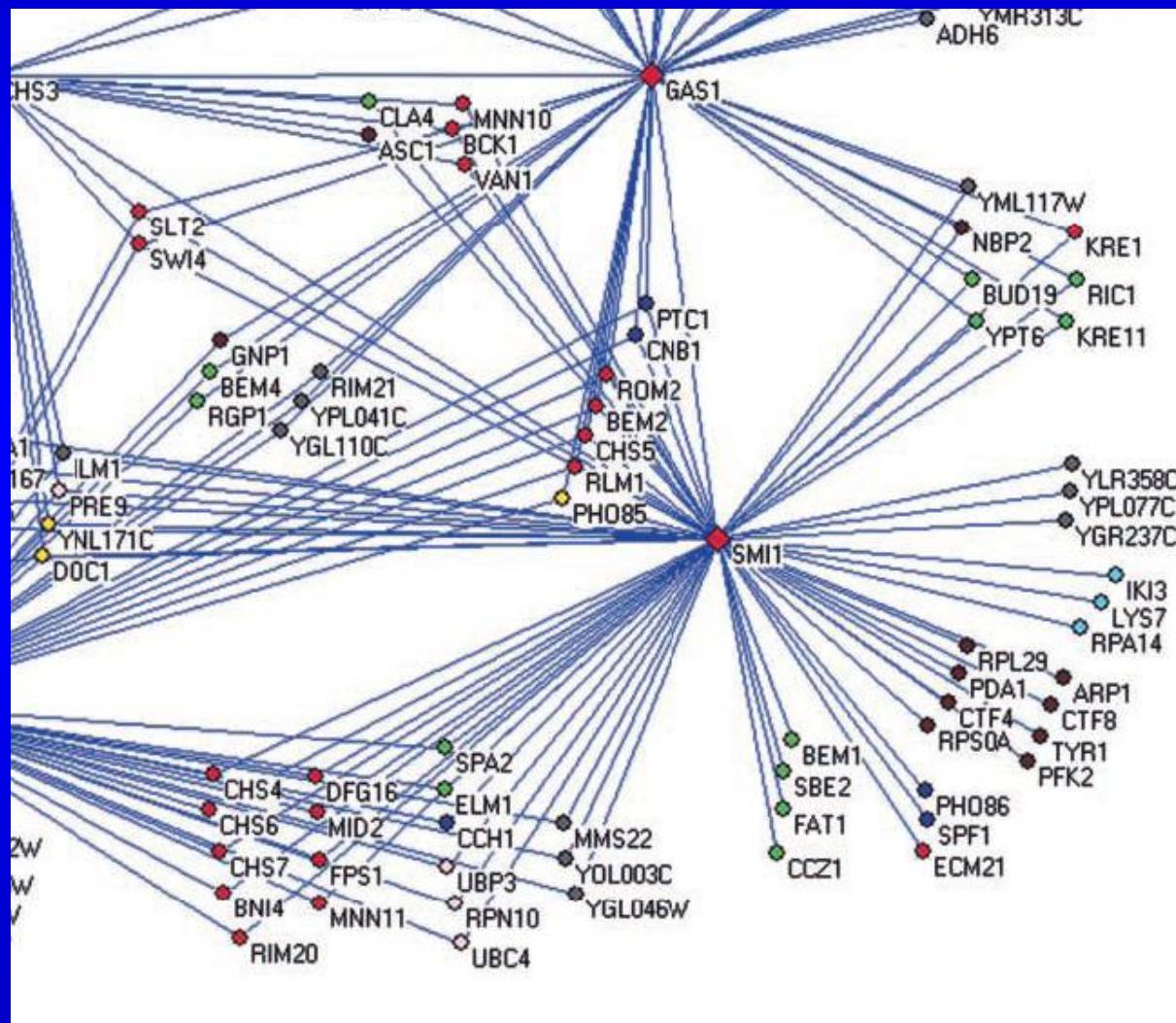
TAP-Tag

- physical
- functional
- temporal
- spatial
- [protein]
- lot false +

The 'most probable interaction maps'

Part 3: Interatomic map of Knr4

► Synthetic interactions with *knr4Δ*: 82



Functional categories

Cell wall 33%

Polarisome 22%

Cell cycle 5 %

Transcription 7 %

Others (metab.) 22 %

Unknown 11 %

(from Lesage et al., *Genetics*, 167, 35-49, 2004)

Part 3: Interactomic map of Knr4

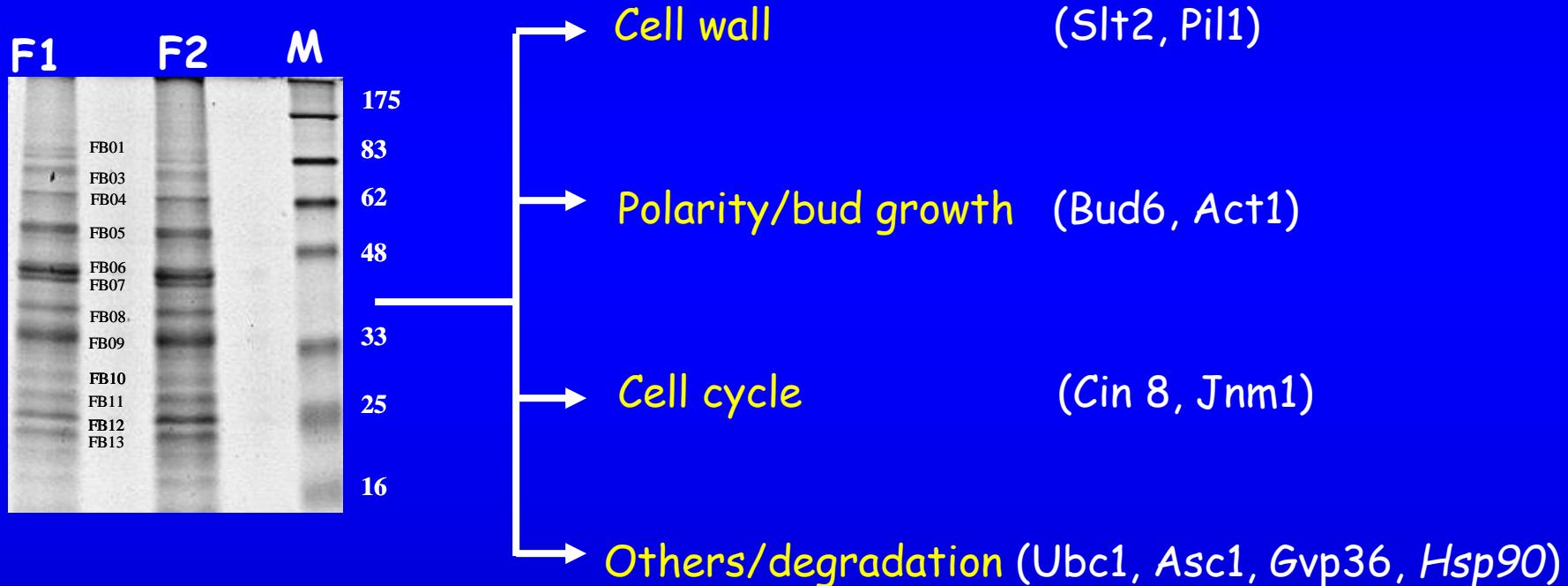
➤ 10 two-hybrid interactions :



(From Uetz et al., Nature, 403, 2000; Ito et al., PNAS, 98, 2001; Dagkessamanskaia et al. FEMS Lett, 2001; Martin-Yken et al., Mol. Microbiol., 2003; Basmaji et al., 2006)

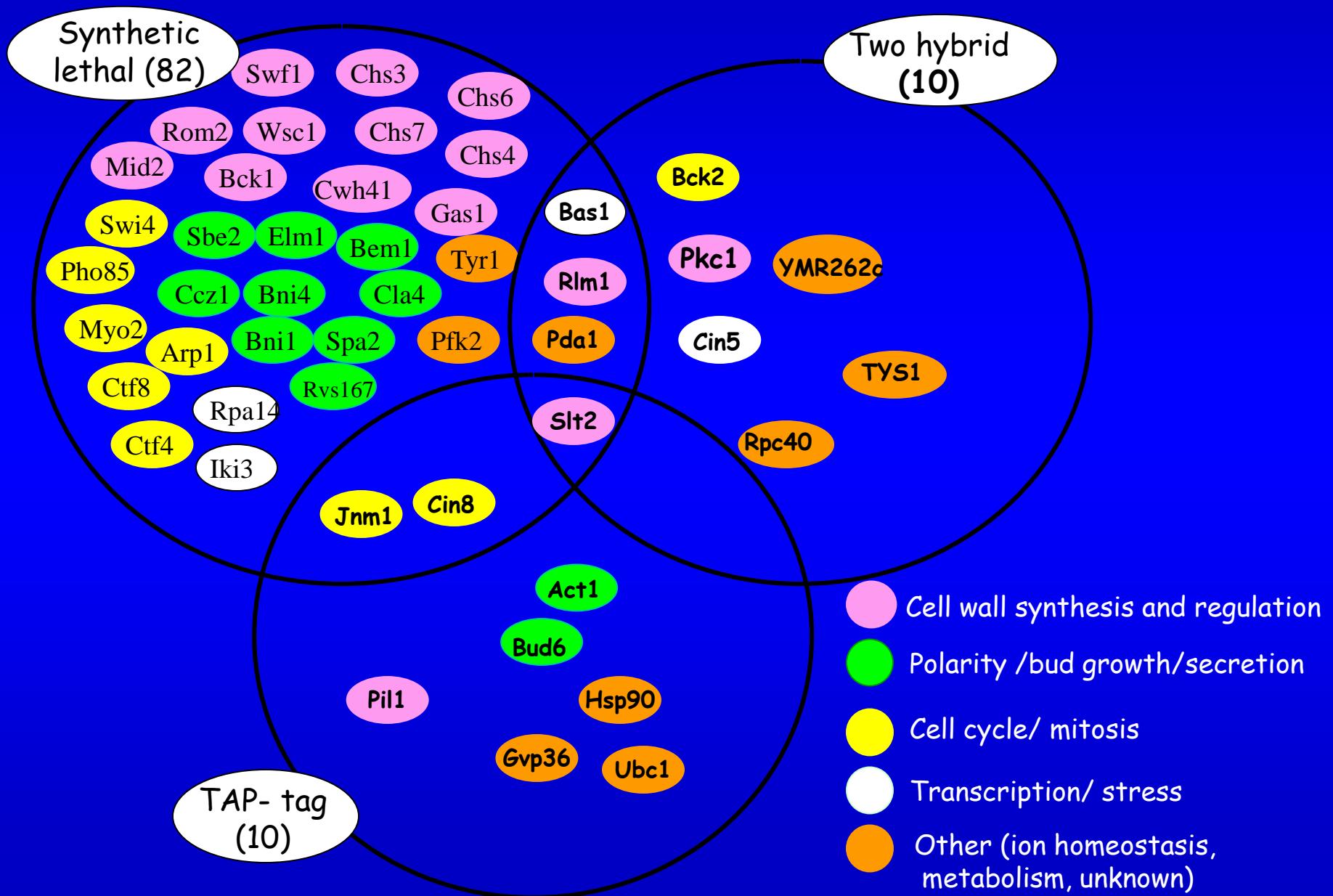
TAP-TAG Results

- 3 criteria for filtering data
- high score & > 3 matches/protein
 - abundance similar to Knr4
 - localization

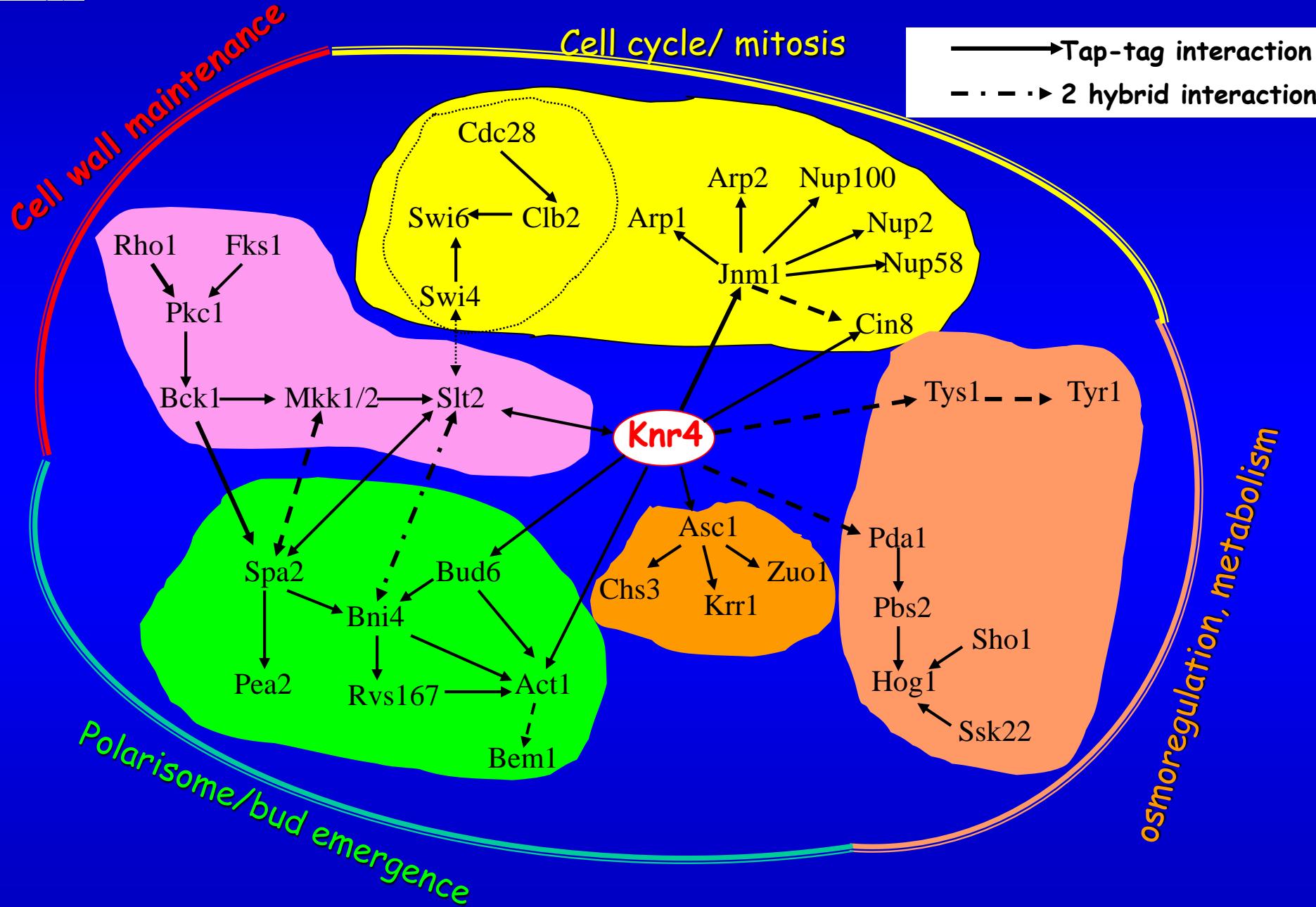


(Note: data gathered from 3 indep.expt)

Part 3: Interactomic map of Knr4



Part 3: Interactomic map of Knr4



How Knr4 can have so many partners ? Is there any physical explanation ?

Hypothesis:

Unfolded /unstructured proteins have lots of partners, are often implicated in regulatory aspect and get their folding upon binding to their partners or substrates

Knr4 may be such a protein, since :

Criteria:

- Prediction of disordered protein using various algorithms
- non-globular protein; no secondary structure
- high net charge (E, K, R, G, Q, S; I, L, V, W, F, Y)
- relatively unstable protein /high turnover

Thanks to

M. Molina & H. Martin (Madrid)
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P. Briza (Univ Salzburg)

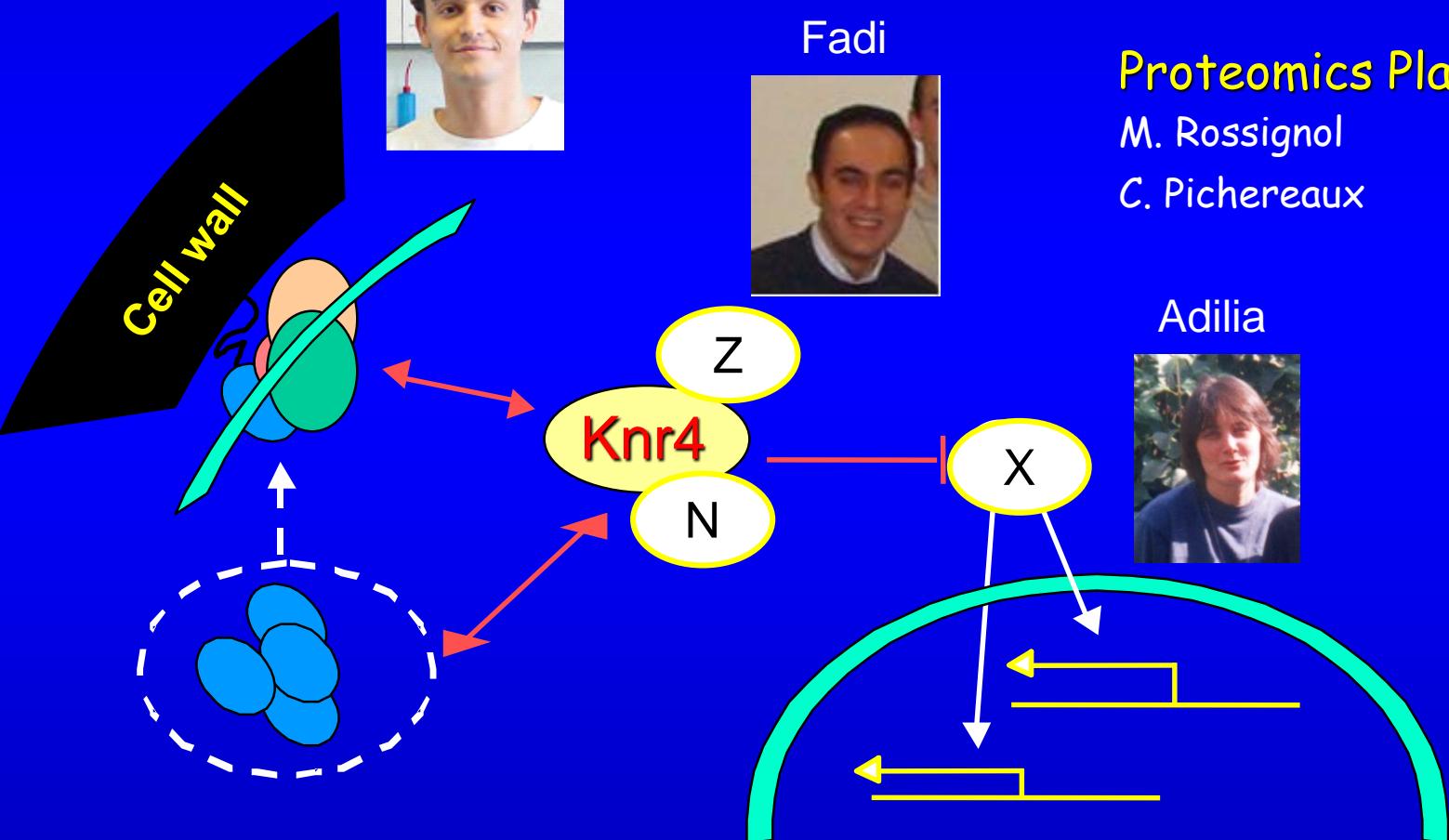
Proteomics Platform

M. Rossignol
C. Pichereaux

Adilia



Hélène



Fabien



Fadi



