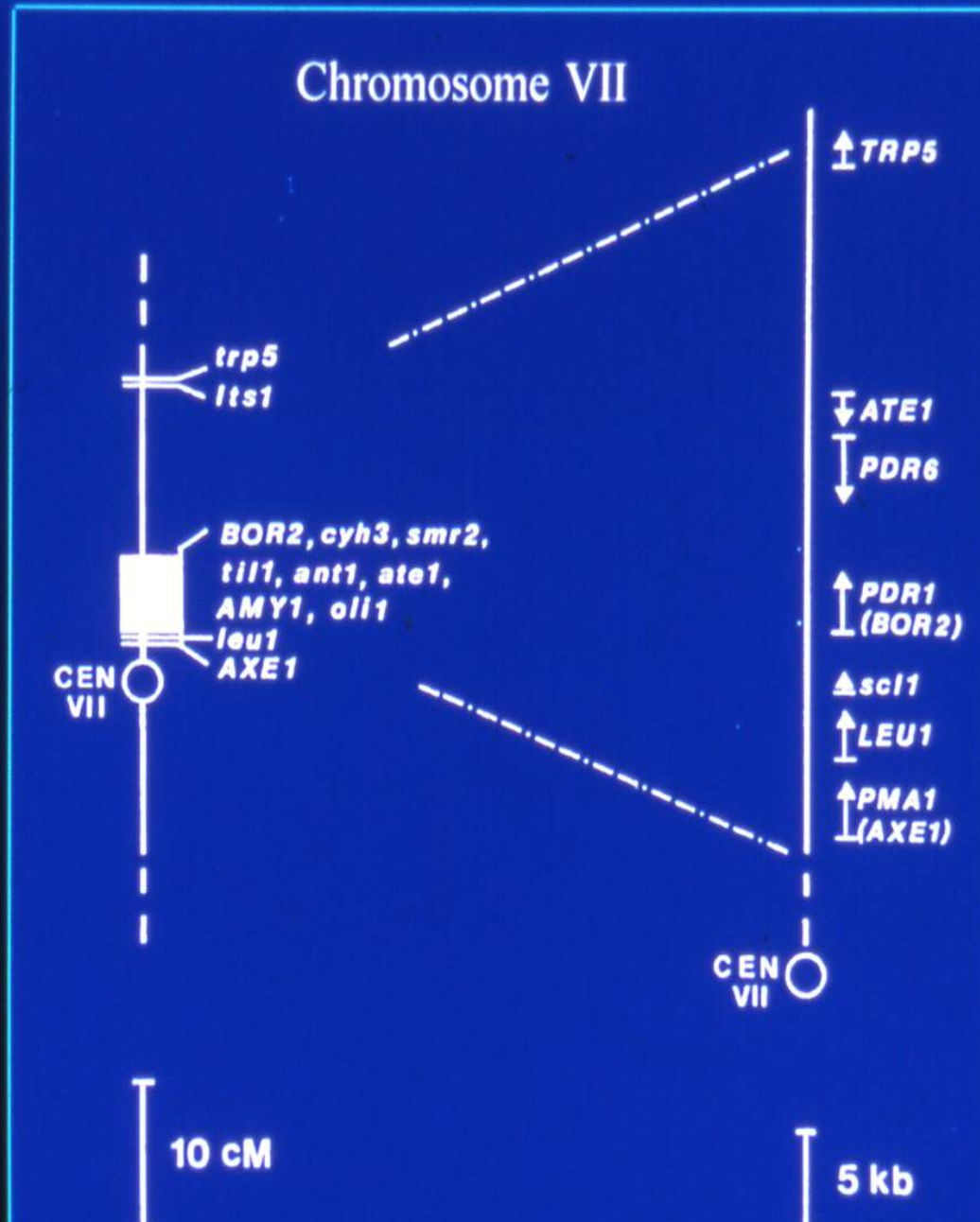


**PLEIOTROPIC  
DRUG TRANSPORTERS  
in  
Fungi**

**André Goffeau, Bruxelles, 2012**

# The first multidrug resistance locus in yeast : *PDR1*



*PDR*

RANK & BECH-HANSEN

MGG 1973

*PDR1*

BALZI, CHEN

ULASZEWSKI, BOUTRY,

DI PIETRO

JBC 1987

# of the Zn<sub>2</sub> Cys<sub>6</sub> family

**PDR1**

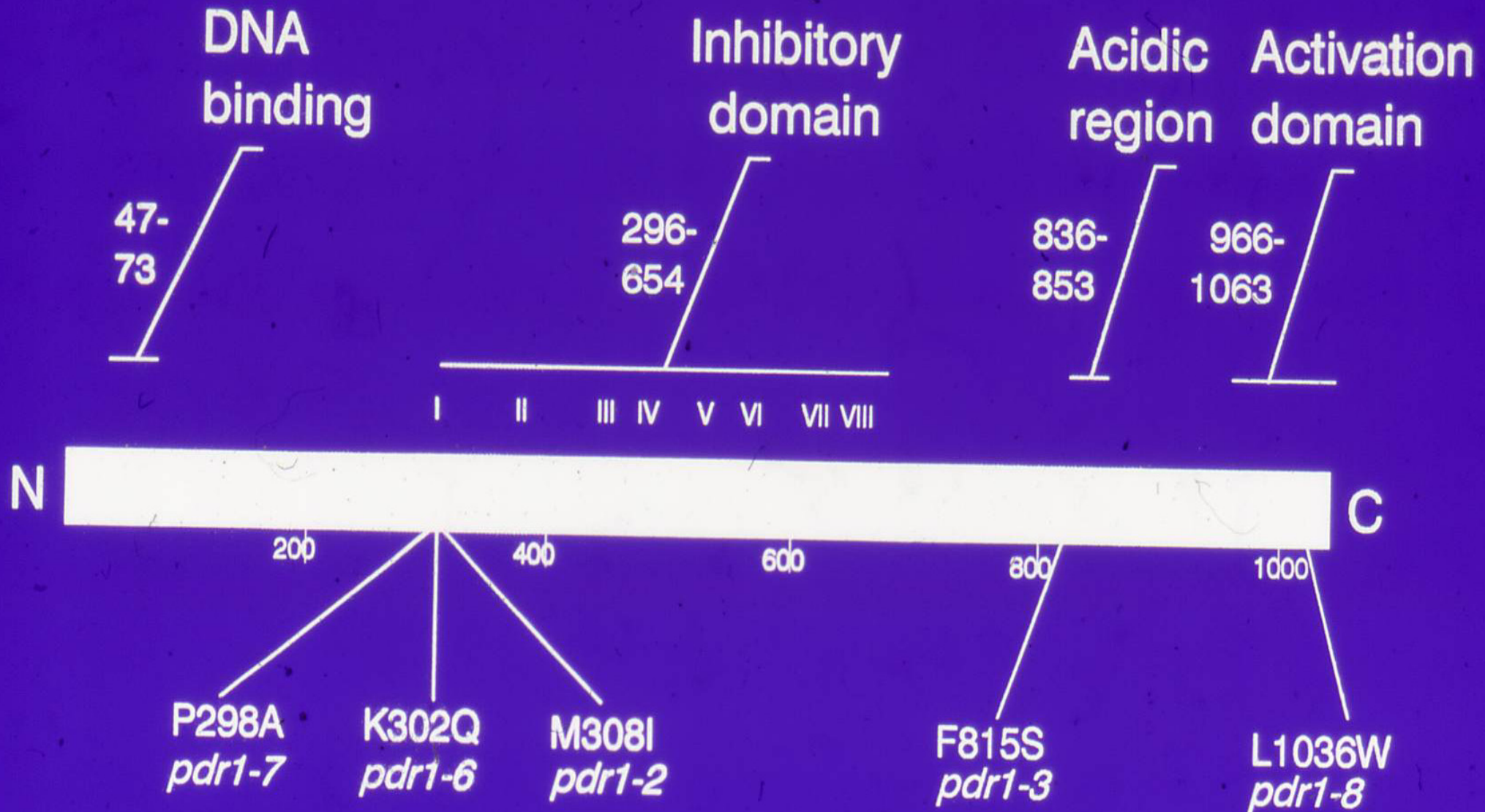
A**C**DN**C**RKRRIK**C**NGK - FP - **C**AS**C**E I YS **C**EC

**GAL4**

A**C**DI**C**RLLK**C**KLK**C** - SKEKPK**C**AK**C**LKNN**C**W**C**E**C**



# Point mutations in PDR1



CYH

SMU

Point mutations

PDR 1 (chr VII)

R

R

Multicopy plasmid

PDR 5 (chr XV)

R

R

*PDR5*

GOLIN 1992

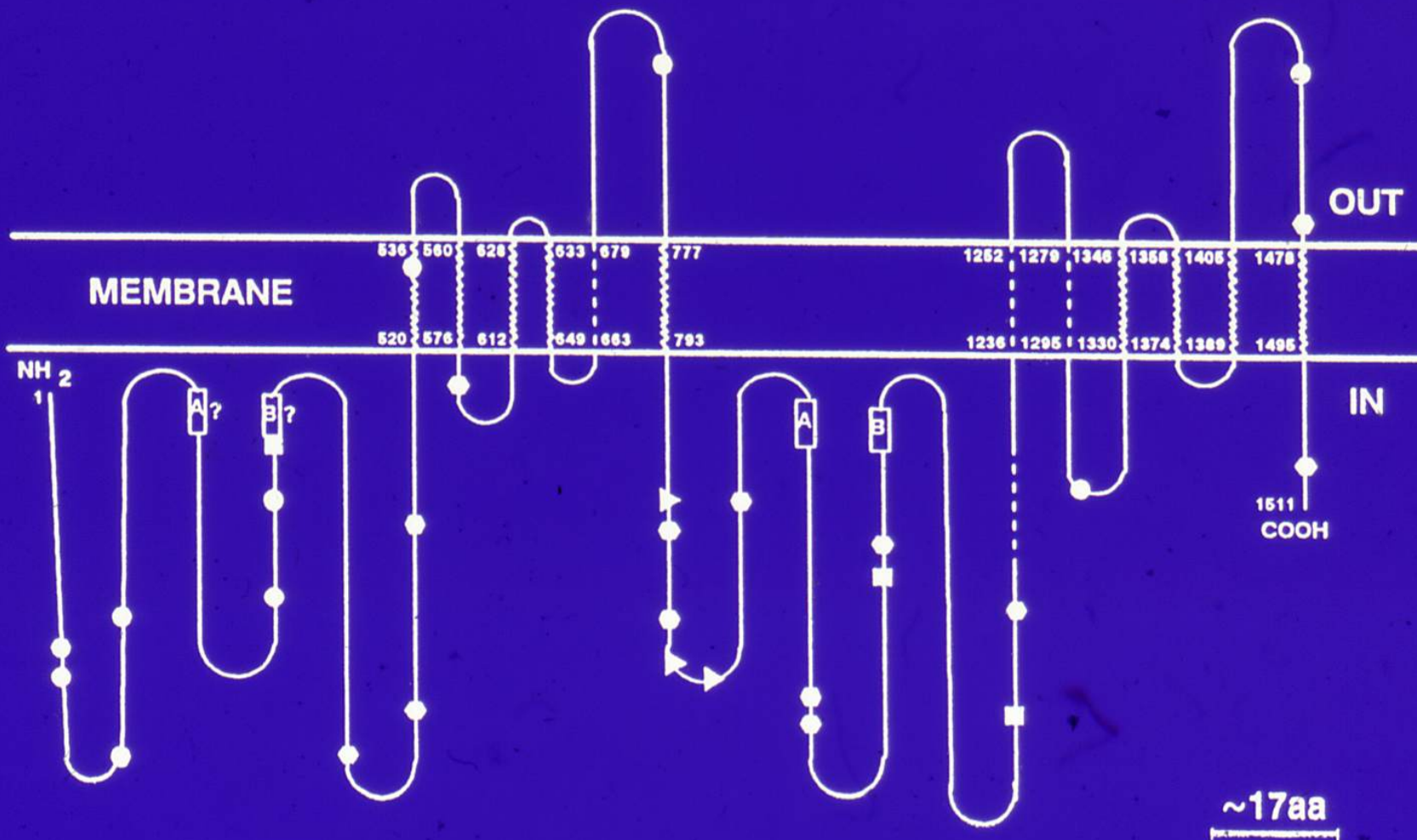


*PDR5*

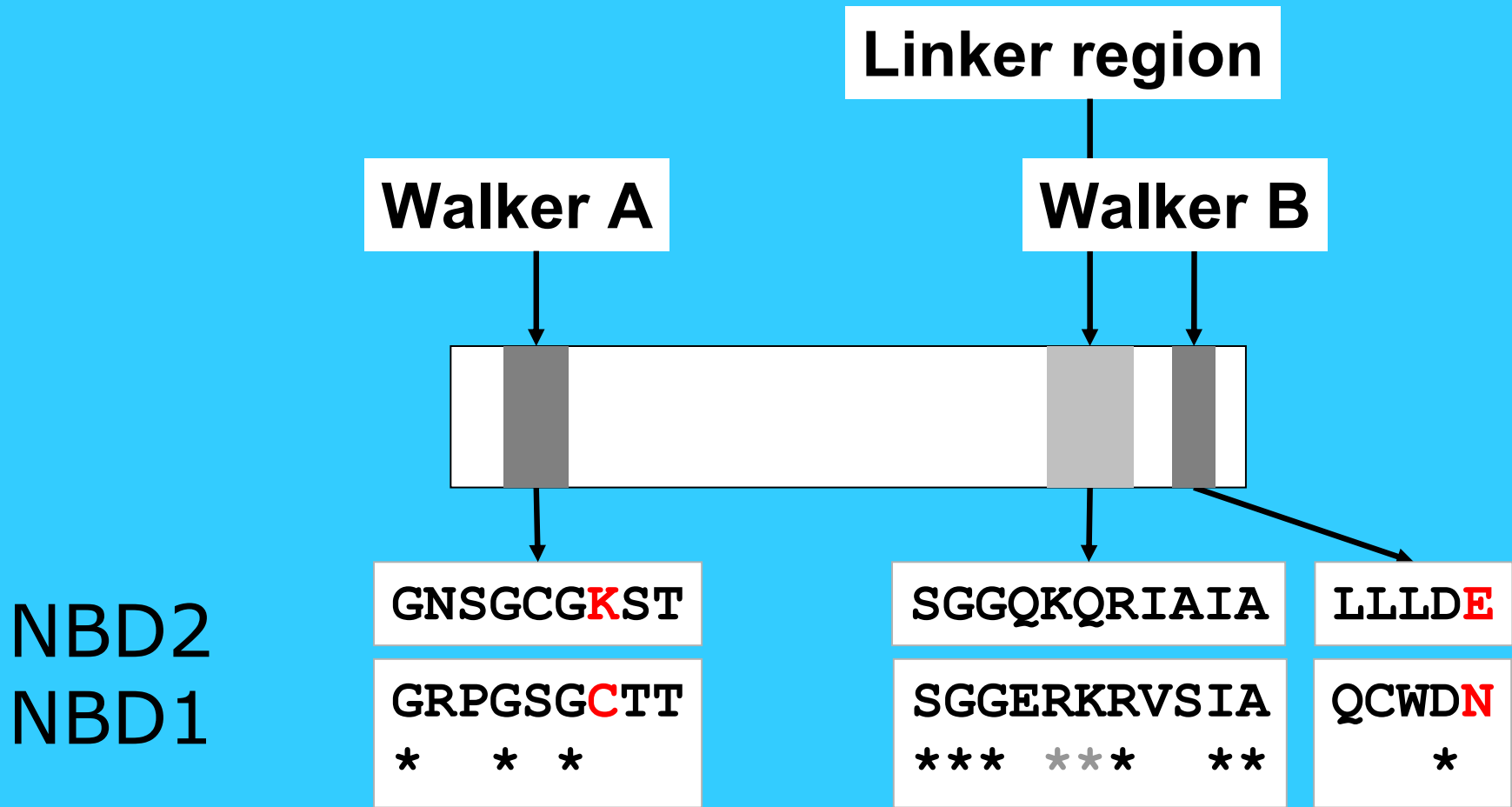
*BALZI et al,*

JBC 1994

# PDR5 IS A NEW ABC TRANSPORTER



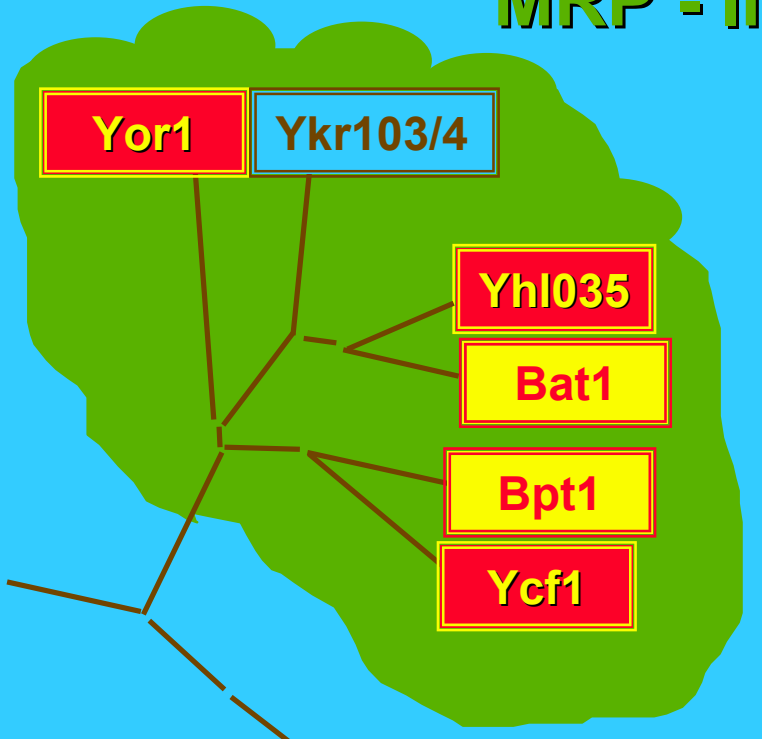
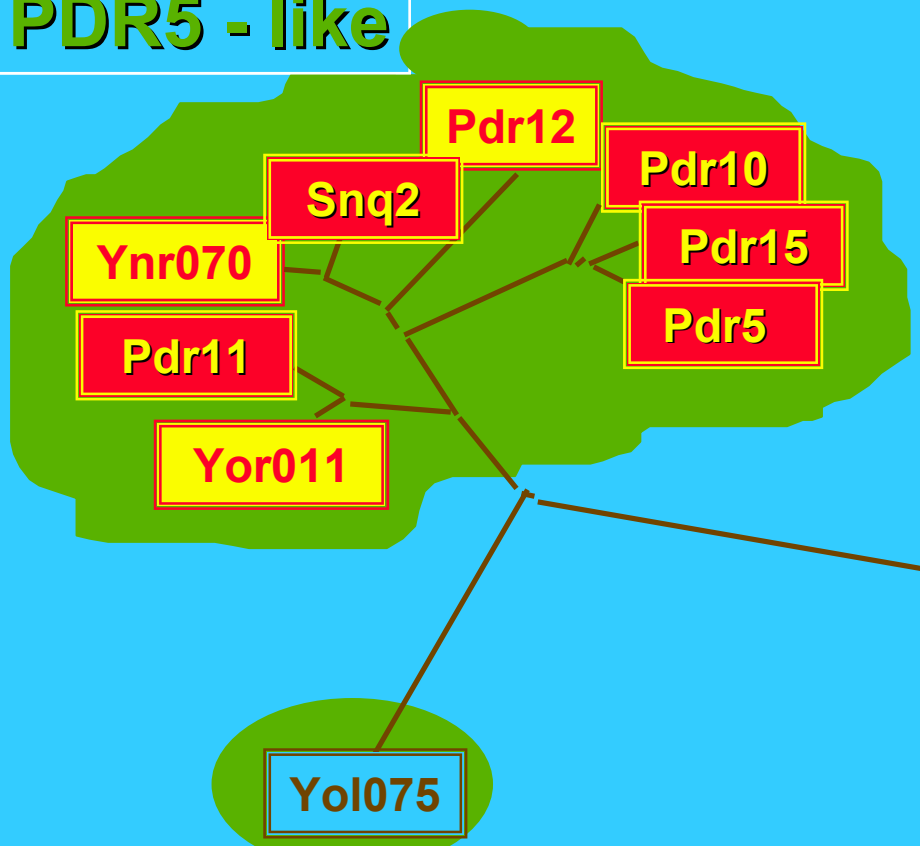
# ASYMMETRIC NBD's in Pdr5p



# The 16 "full - size" ABC transporters

**PDR5 - like**

**MRP - like**



**NEW**

**(NBF-TM)<sub>2</sub>**

**(TM-NBF)<sub>2</sub>**

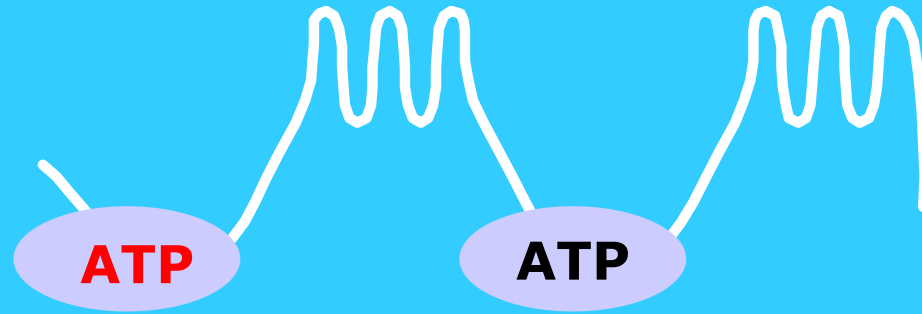
**MDR - like**



**MULTIPLE disruptions**

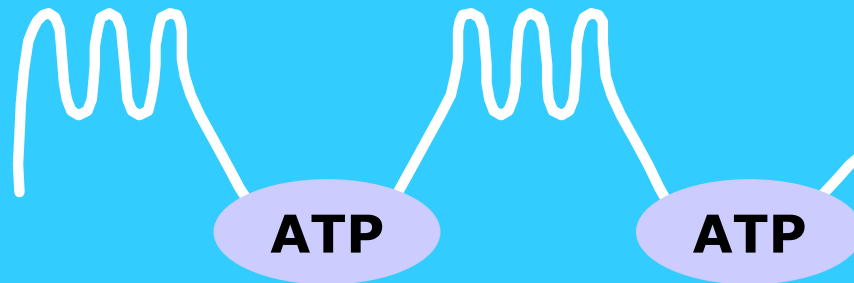
**SINGLE disruptions**

**NO disruptions yet**



**PDR-like:**

**Pdr5p**



**MRP/MDR-like:**

**Yor1p**

*pdr1-3*

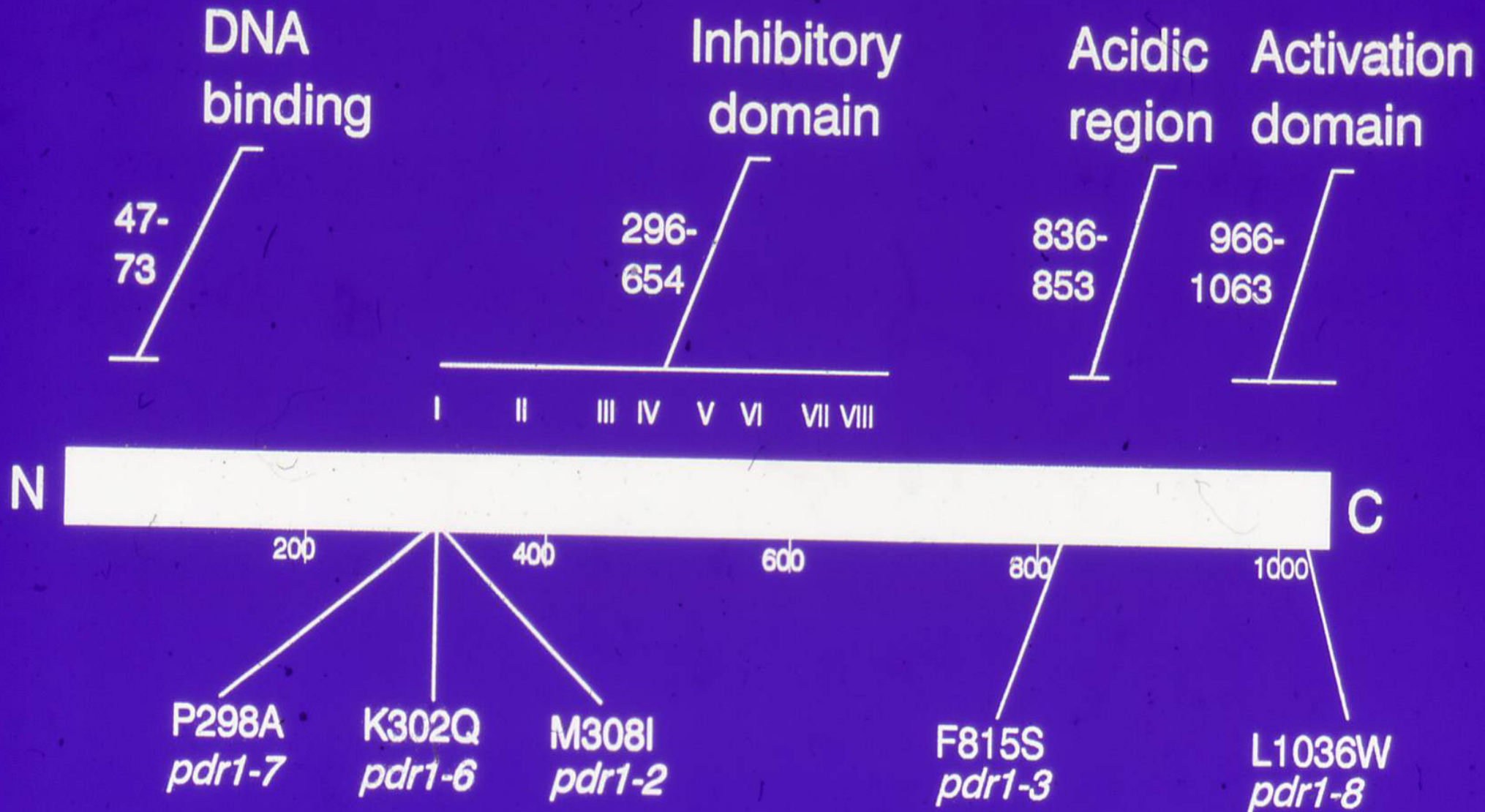
CARVAJAL *et al*

MGG 1997

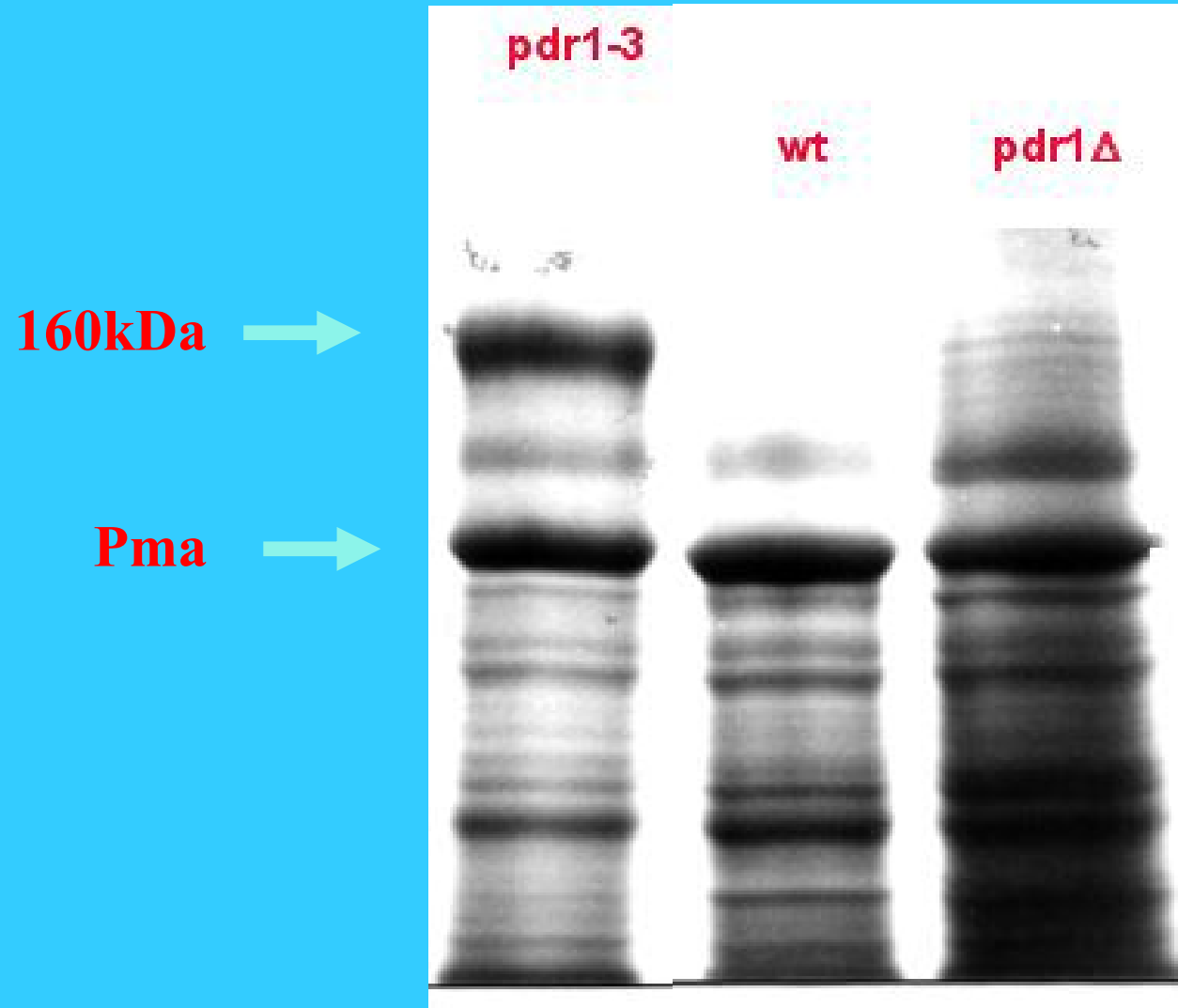




# Point mutations in PDR1



# *The pdr1-3 mutant overexpresses a new protein band in the plasma membrane*

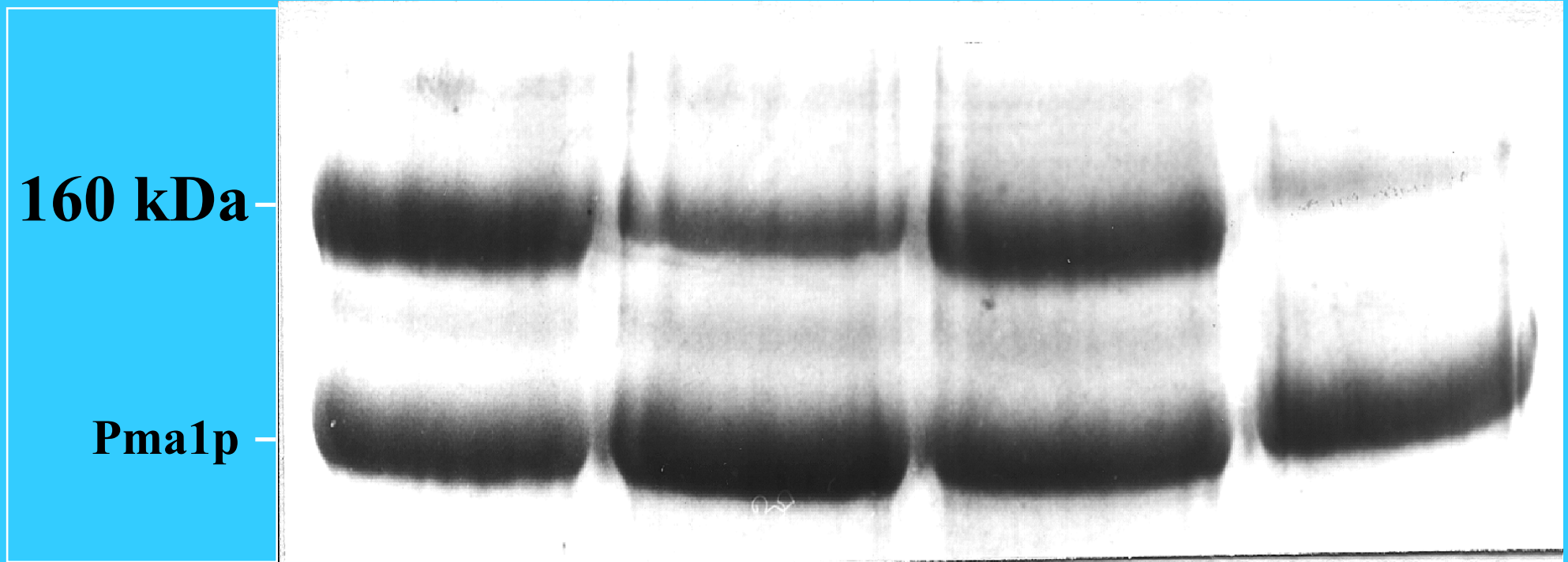


# GENETIC PURIFICATION

**Snq2p**

**Pdr5p**

**Yor1p**

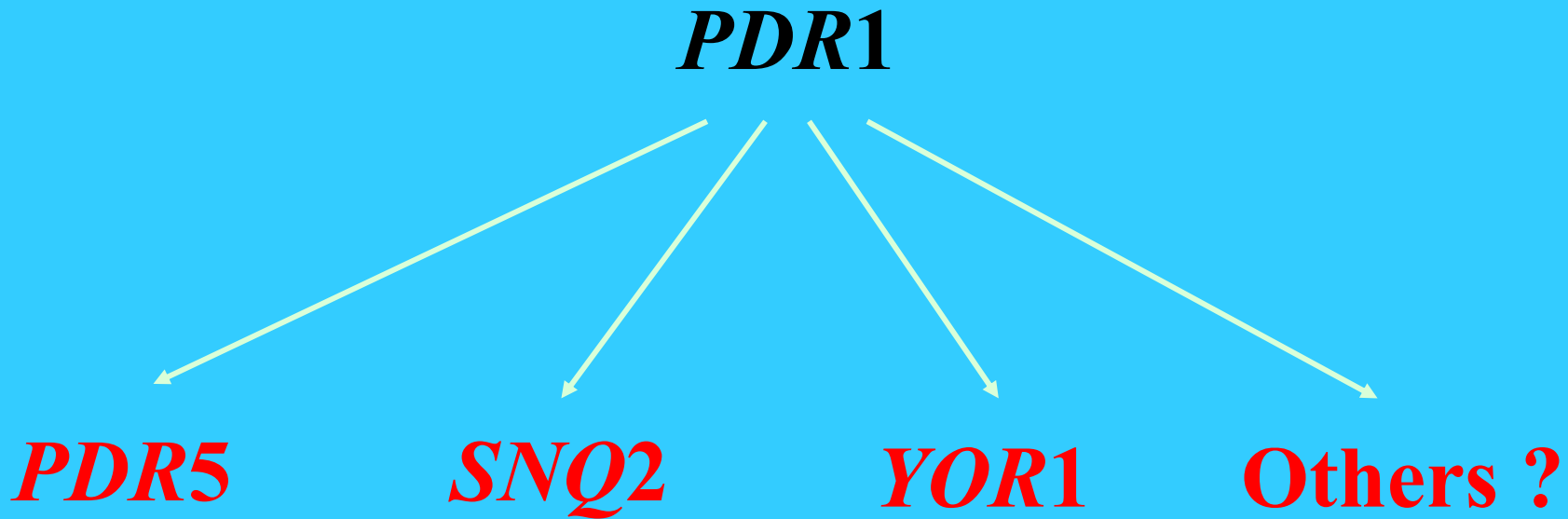


*$\Delta pdr5$*

*$\Delta snq2$*

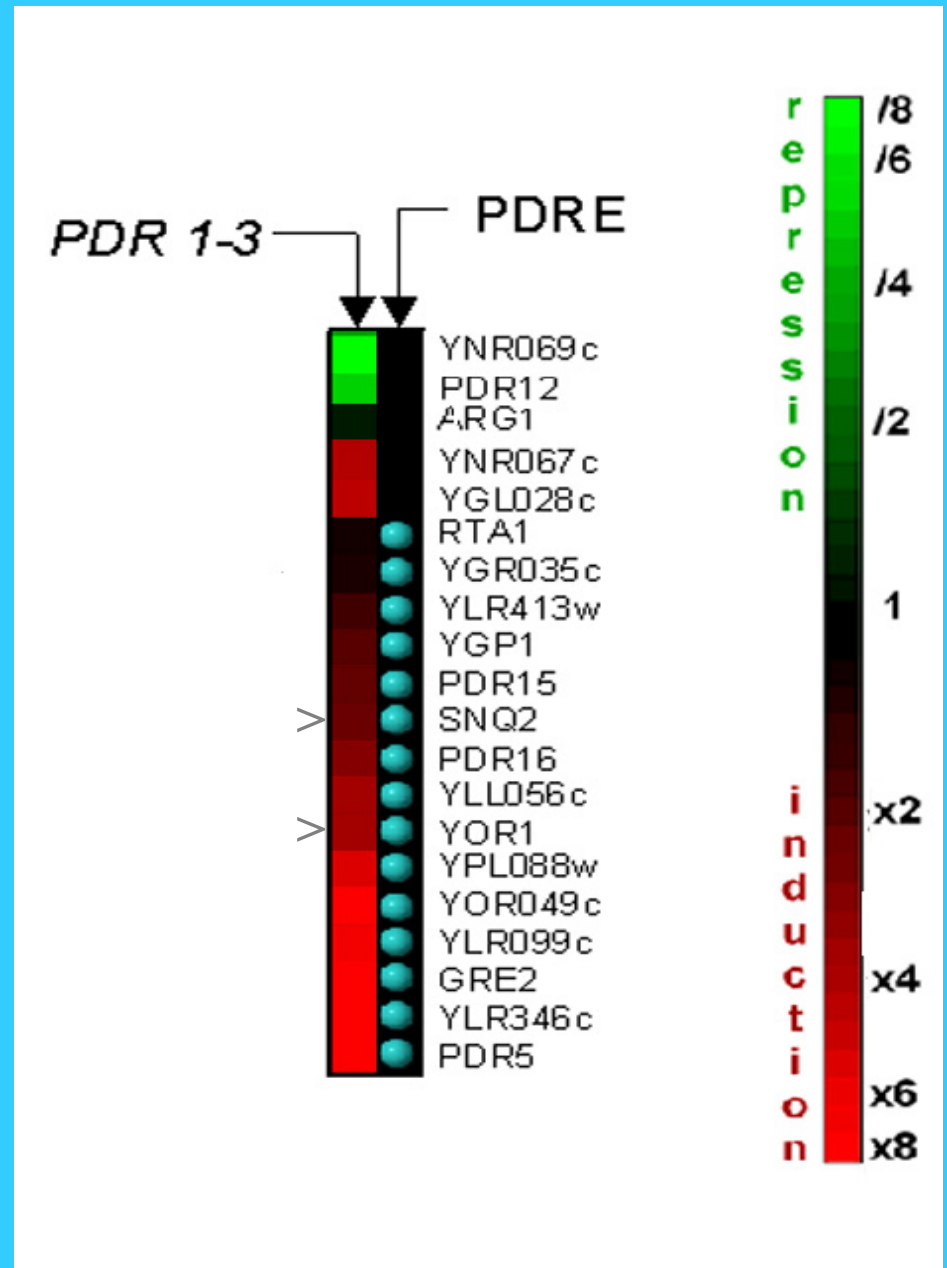
*$\Delta pdr5, \Delta snq2$*

The mutant *pdr1-3* in the regulatory gene  
*PDR1* activates expression of  
several ABC transporters



# Elements of the PDR Network responsive to the *pdr1-3* Transcription Regulator

*De RISI et al, 2002*





# GENE TARGETS OVEREXPRESSED BY THE *pdr1-3* DRUG-RESISTANCE REGULATORY MUTATION

## 1. ABC

*PDR5 / SNQ2 / YOR1 / PDR10 / PDR15*

## 2. MFS

*TPO1 / RTA1 / YOR049c*

## 3. LIPIDS

*PDR16 / IPT1 / YLR099c*

## 4. WALLS

*YPL088w / YGL028c / YLR413w / YNR067c*

## 5. STRESS

*GRE2 / MET17 / FRE4 / REV1 / YGR243w*

## 6. UNKNOWN

*YLR346c / YGR035c / COS1*

*PDR3/YAP1/YRR1*

- JULIUS SUBIC
- CLAUDE JACQ
- BERNARD TURCOTTE



# TRANSPORT SPECIFICITY

of Pdr5p

Kolaszkowski *et al*

JBC 1996

# THE YEAST ABC TRANSPORTER PDR5 MEDIATES RESISTANCE TO MANY DIFFERENT COMPOUNDS

## Anticancer drugs chemosensitizers

tamoxifen  
daunorubicin  
doxorubicin  
trifluoperazine

## Antibiotics

chloramphenicol  
tetracycline  
anisomycin  
antimycin A1  
cycloheximide  
lincomycin  
oligomycin  
tunicamycin  
thiolutin

## Fluorescent dyes

rhodamine 6G  
rhodamine 123

## Detergents

tetradecyltrimethyl-  
-ammonium bromide  
hexadecyltrimethyl-  
-ammoniumbromide  
zwittergent 3-10  
zwittergent 3-12  
zwittergent 3-14  
brij 58  
brij 35  
n octylglucoside  
n dodecylglucoside  
n dodecylmaltoside

## Herbicides

sulfomethuron methyl  
fluometuron  
chloroxuron  
difenoxuron  
chlorbromuron  
diuron

## Ionophores

valinomycin  
nigericin  
monensin  
A23187

## Others

2,3,5 triphenyltetrazoliumchloride  
4 nitroquinoline n oxide  
8 hydroxyquinoline  
diphenylamine  
p aminodiphenylamine  
n decylamine  
4 nitrophenol  
aniline  
anthron  
tetranitrotetrazoliumchloride blue  
resazurine  
sporidesmin  
staurosporine  
fluphenazine  
cerulenin  
compactin

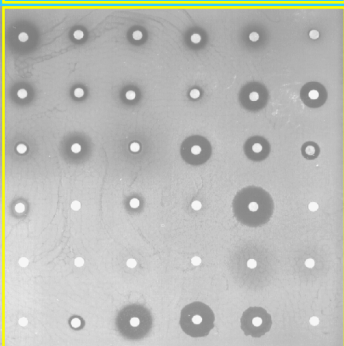
## Steroids and their structural analogues

5  $\beta$  androstan 3  $\alpha$  ol 17 one  
5  $\beta$  androstan 3  $\beta$  ol 17 one  
5  $\beta$  androstan 17 $\beta$  ol 3 one  
5  $\alpha$  androstane 3,17 dione  
5  $\beta$  androstane 3,17 dione  
1,4 androstadiene 3,17 dione  
5  $\beta$  pregnan 3  $\beta$  ol 20 one  
4 pregnen 20  $\alpha$  ol 3 one  
trans dehydroandrosterone  
4 androstene 3,17 dione  
epiandrosterone acetate  
epiandrosterone  
estriol 16, 17 diacetate  
17  $\alpha$  ethynyl estradiol  
deoxycorticosterone  
pregnenolone  
progesterone  
testosterone  
diethylstilbestrol  
hexestrol  
4 hydroxytamoxifen

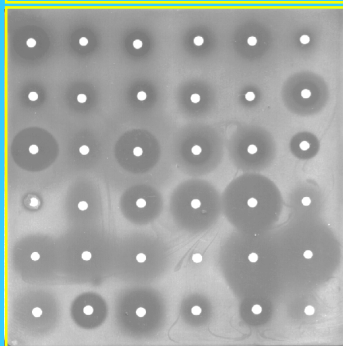
## Fungicides

itraconazole  
ketoconazole  
econazole  
miconazole  
triadimenol  
bitertanol  
nuarimol  
fenarimol  
carbendazim  
diclobutrazol  
phenapronil  
chlorothalonil  
cyprodinil  
dodine  
imazalil  
prochloraz  
azoxystrobin  
flusilazol  
tebuconazole  
CGA64251  
2 mercapto-  
-benzothiazole  
krexoxim methyl  
nystatin  
soraphen A  
epoxiconazole

**PDR5**

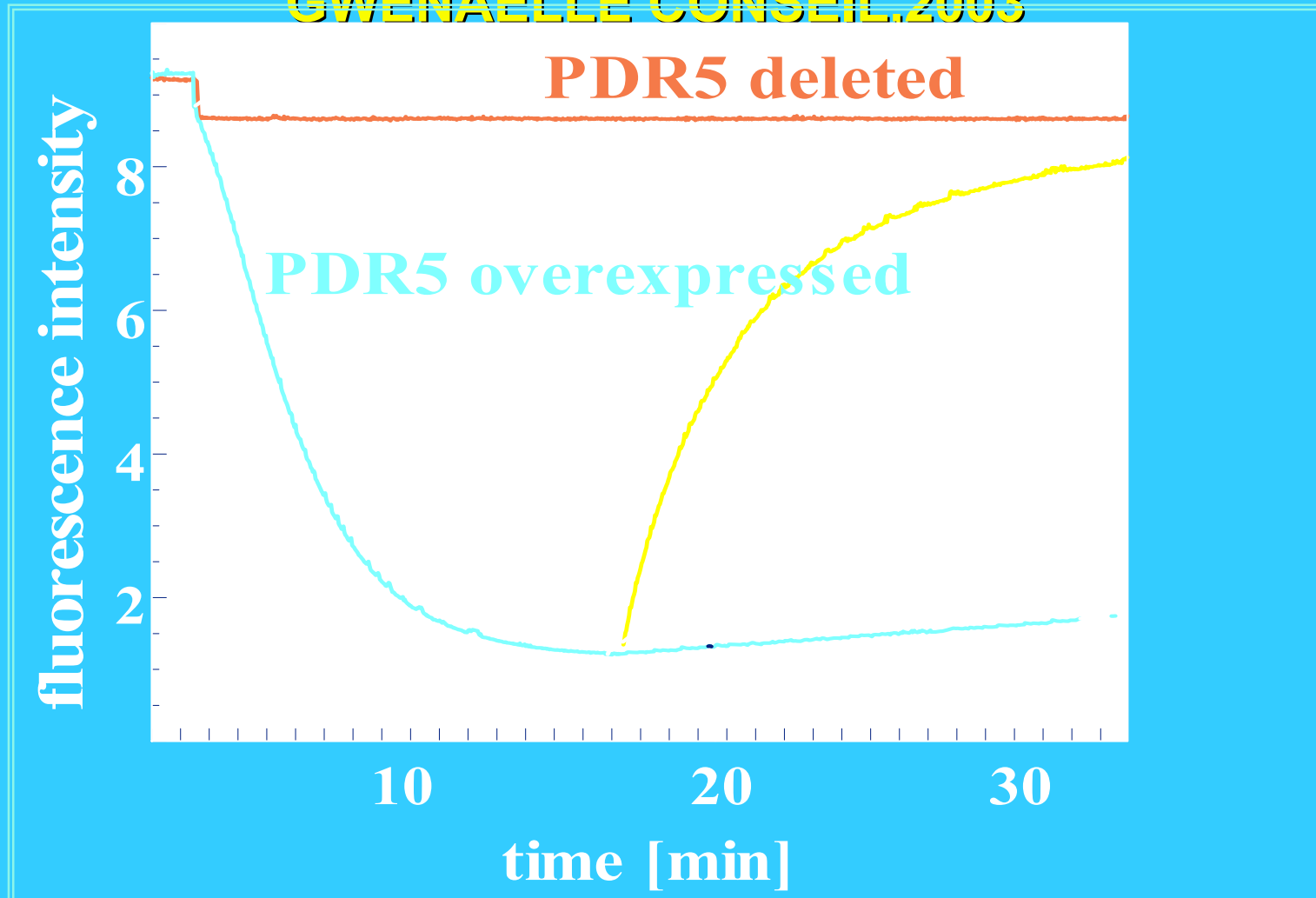


**$\Delta$  PDR5**



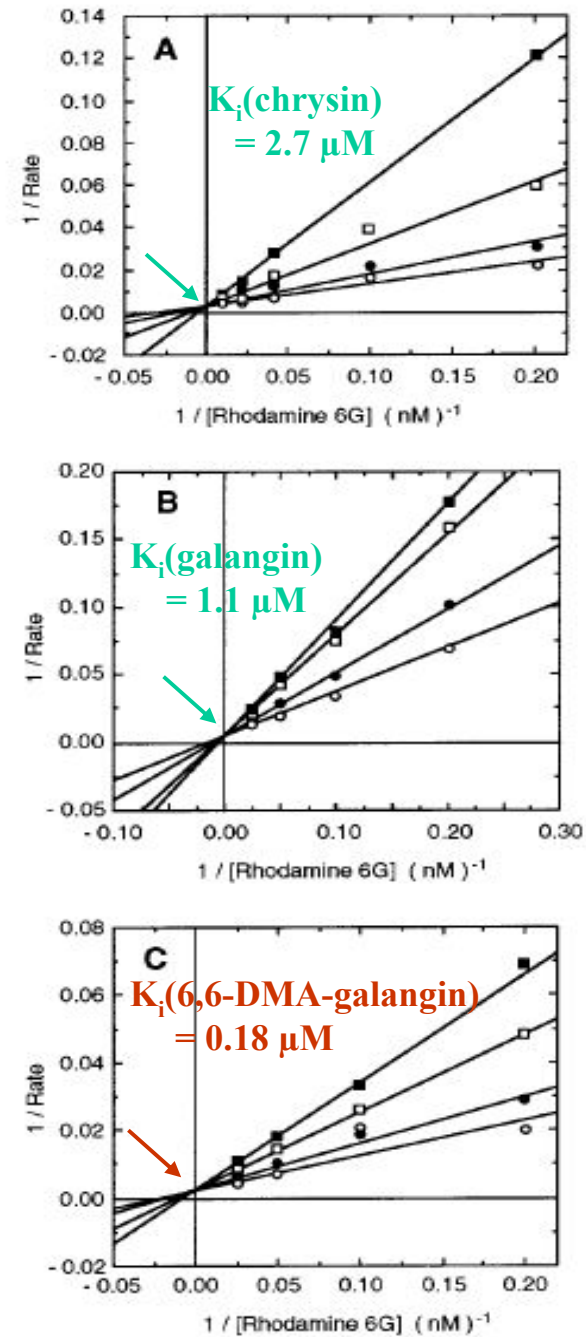
# Membranes with overexpressed Pdr5p show ATP-dependent fluorescence quenching of Rhodamine 6G

GWENAELE CONSEIL, 2003

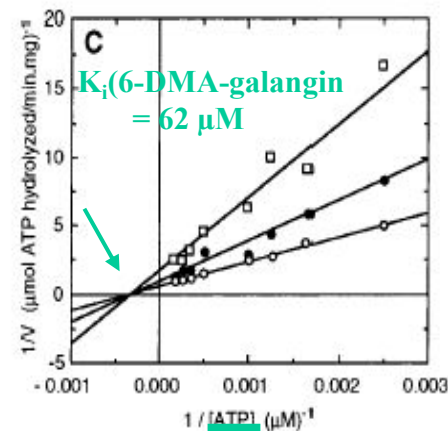
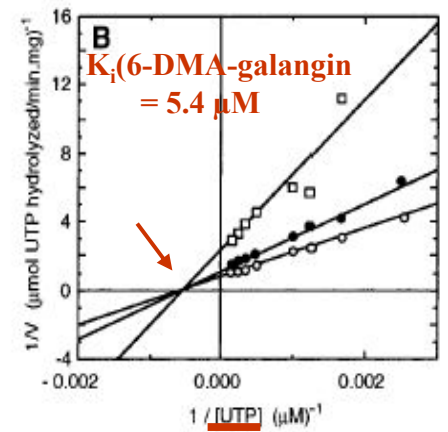
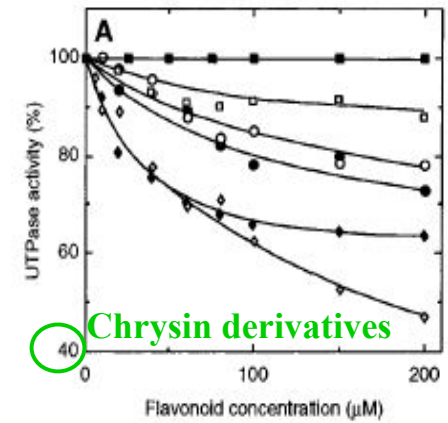


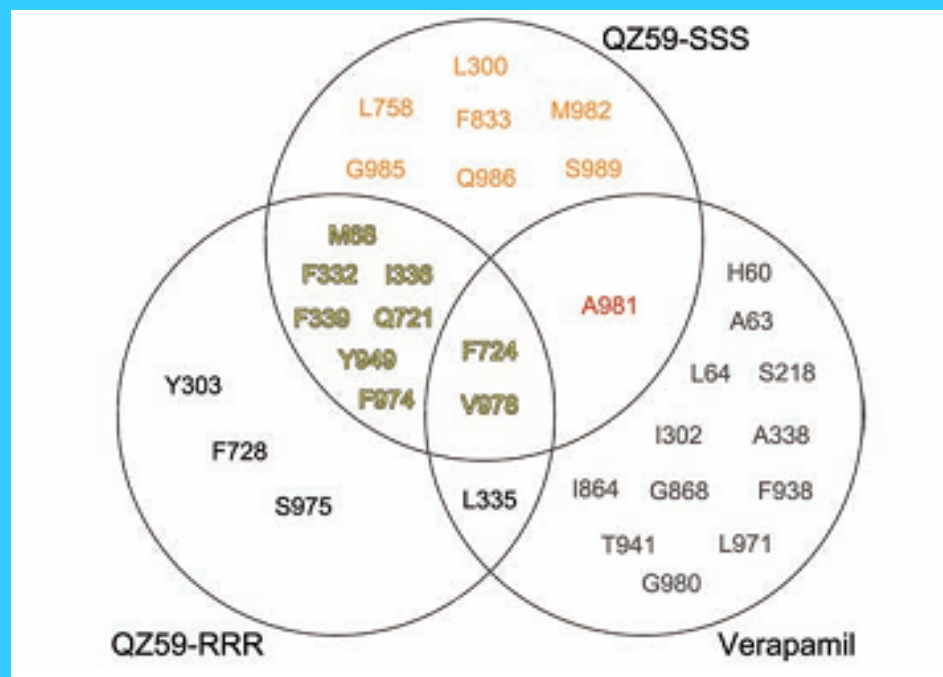


# Competitive inhibition of prenyl-flavonoids on rhodamine 6G interaction



Partial and non competitive inhibition of the nucleotide hydrolytic activity of Pdr5p-enriched yeast plasma membranes





# HETEROLOGOUS EXPRESSION OF MEMBRANE PROTEINS

A.DECOTTIGNIES/B.MONK

# MULTIPLE DELETIONS OF YEAST ABC TRANSPORTERS AND REGULATORS

*pdr1-3*



# HYPERSENSITIVITY TO DRUGS OF THE YEAST STRAIN AD1-9, DELETED OF MULTIPLE ABC TRANSPORTERS

<b>INHIBITOR</b>	<b>WILD-TYPE MIC (µg/ml)</b>	<b>AD1-9 MIC (µg/ml)</b>	<b>Fold- Difference</b>
<i>Brefeldin A</i>	100	6.25	16
Clotrimazole	1.56	0.0012	128
Crystal Violet	0.073	0.049	1.5
Cycloheximide	0.049	0.012	4.1
Fluconazole	12.5	0.391	32
Fluphenazine	12.5	6.25	2
8-Hydroxyquinoline	25	6.25	4
Imazalil	0.78	0.025	31
Ketokonazole	0.781	0.006	130
Miconazole	0.391	0.0031	126
Nocodazole	6.25	0.781	8
Propiconazole	1.56	0.003	5.2
Rhodamine 6G	25	0.098	255
Sanguinarine	3.13	0.781	4
Staurosporine	0.781	0.024	33
Sulfometuron methyl	6.25	1.56	4
Thiabendazole	>100	14	7.2

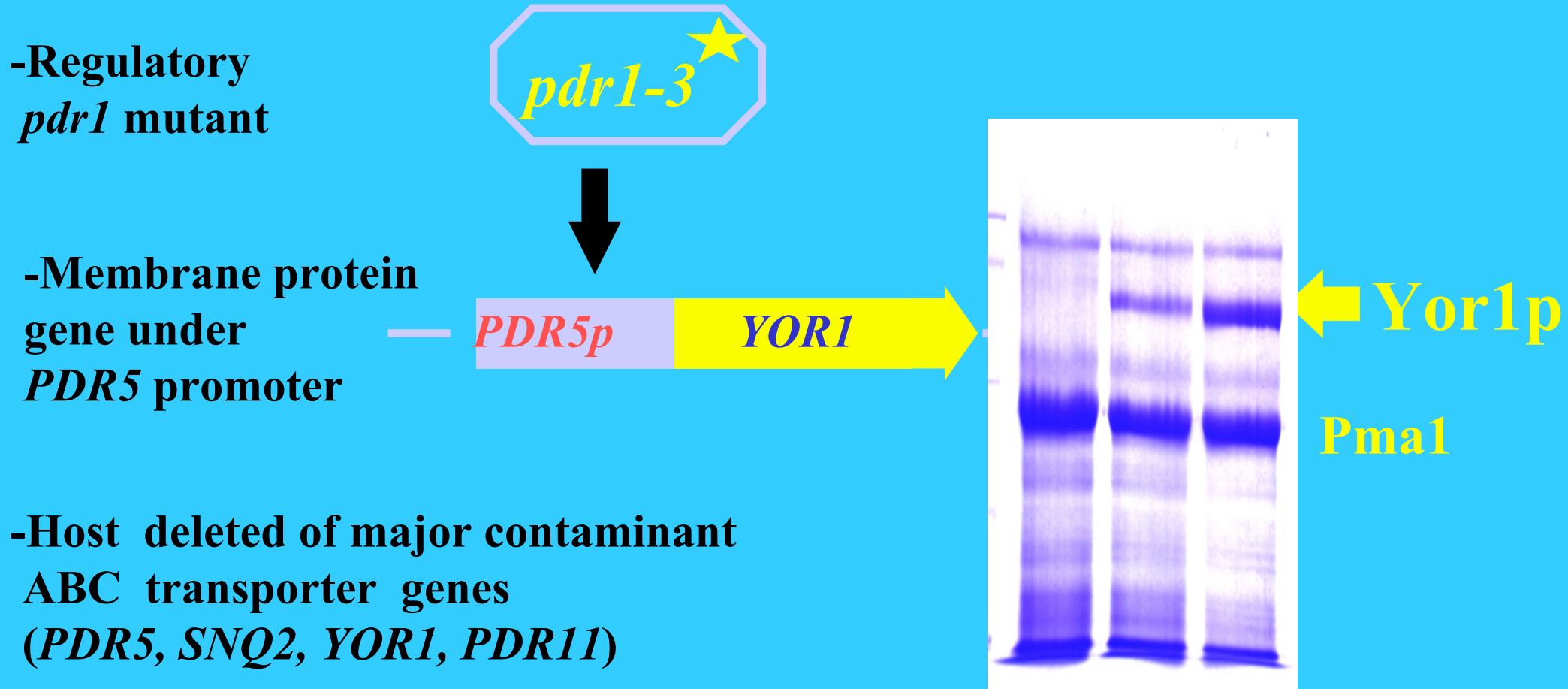


BOR2-XI	<i>MATa, pdr1-6, ade1, his1</i>
US54-17B	<i>MAT<math>\alpha</math>, pdr1-2, ura3, his4</i>
D1-2	<i>MAT<math>\alpha</math>, pdr1-<math>\Delta</math>1::URA3, his4</i>
JG200	<i>MATa, PDR1, leu2, his4, can1-100</i>
JG204	<i>MATa, pdr1-7, leu2, his4, can1-100</i>
US50-D5 <sup>a</sup>	<i>pdr5-<math>\Delta</math>1::URA3</i>
US50-D2 <sup>a</sup>	<i>snq2::Tn10-LUK</i>
US50-D25 <sup>a</sup>	<i>pdr5-<math>\Delta</math>1::URA3, snq2::Tn10-LUK</i>
AD1 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG</i>
AD2 <sup>a</sup>	<i><math>\Delta</math>snq2::hisG</i>
AD3 <sup>a</sup>	<i>pdr5-<math>\Delta</math>2::hisG</i>
AD4 <sup>a</sup>	<i><math>\Delta</math>pdr10::hisG-URA3-hisG</i>
AD5 <sup>a</sup>	<i><math>\Delta</math>pdr11::hisG-URA3-hisG</i>
AD6 <sup>a</sup>	<i><math>\Delta</math>ycf1::hisG-URA3-hisG</i>
AD10 <sup>a</sup>	<i><math>\Delta</math>yap1::hisG-URA3-hisG</i>
AD12 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG</i>
AD23 <sup>a</sup>	<i><math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG</i>
AD13 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, pdr5-<math>\Delta</math>2::hisG</i>
AD26 <sup>a</sup>	<i><math>\Delta</math>snq2::hisG, <math>\Delta</math>ycf1::hisG</i>
AD34 <sup>a</sup>	<i>pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>pdr10::hisG-URA3-hisG</i>
AD36 <sup>a</sup>	<i>pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>ycf1::hisG-URA3-hisG</i>
AD123 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG</i>
AD124 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, <math>\Delta</math>pdr10::hisG</i>
AD126 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, <math>\Delta</math>ycf1::hisG-URA3-hisG</i>
AD136 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>ycf1::hisG-URA3-hisG</i>
AD1234 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>pdr10::hisG</i>
AD1236 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>ycf1::hisG-URA3-hisG</i>
AD1237 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, pdr3-<math>\Delta</math>2::hisG</i>
AD1246 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, <math>\Delta</math>pdr10::hisG, <math>\Delta</math>ycf1::hisG-URA3-hisG</i>
AD12346 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>pdr10::hisG, <math>\Delta</math>ycf1::hisG-URA3-hisG</i>
AD12367 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>ycf1::hisG, pdr3-<math>\Delta</math>2::hisG</i>
AD12467 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, <math>\Delta</math>pdr10::hisG, <math>\Delta</math>ycf1::hisG, pdr3-<math>\Delta</math>2::hisG</i>
AD23456 <sup>a</sup>	<i><math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>pdr10::hisG, <math>\Delta</math>pdr11::hisG, <math>\Delta</math>ycf1::hisG</i>
AD123467 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>pdr10::hisG, <math>\Delta</math>ycf1::hisG, pdr3-<math>\Delta</math>2::hisG</i>
AD123456 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>pdr10::hisG, <math>\Delta</math>pdr11::hisG, <math>\Delta</math>ycf1::hisG</i>
AD124567 <sup>a</sup>	<i><math>\Delta</math>yor1::hisG, <math>\Delta</math>snq2::hisG, <math>\Delta</math>pdr10::hisG, <math>\Delta</math>pdr11::hisG, <math>\Delta</math>ycf1::hisG, pdr3-<math>\Delta</math>2::hisG</i>
AD234567 <sup>a</sup>	<i><math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>pdr10::hisG, <math>\Delta</math>pdr11::hisG, <math>\Delta</math>ycf1::hisG, pdr3-<math>\Delta</math>2::hisG</i>
AD1234567 <sup>a</sup>	<i><math>\Delta</math>yap1::hisG, <math>\Delta</math>snq2::hisG, pdr5-<math>\Delta</math>2::hisG, <math>\Delta</math>pdr10::hisG, <math>\Delta</math>pdr11::hisG, <math>\Delta</math>ycf1::hisG, pdr3-<math>\Delta</math>2::hisG</i>

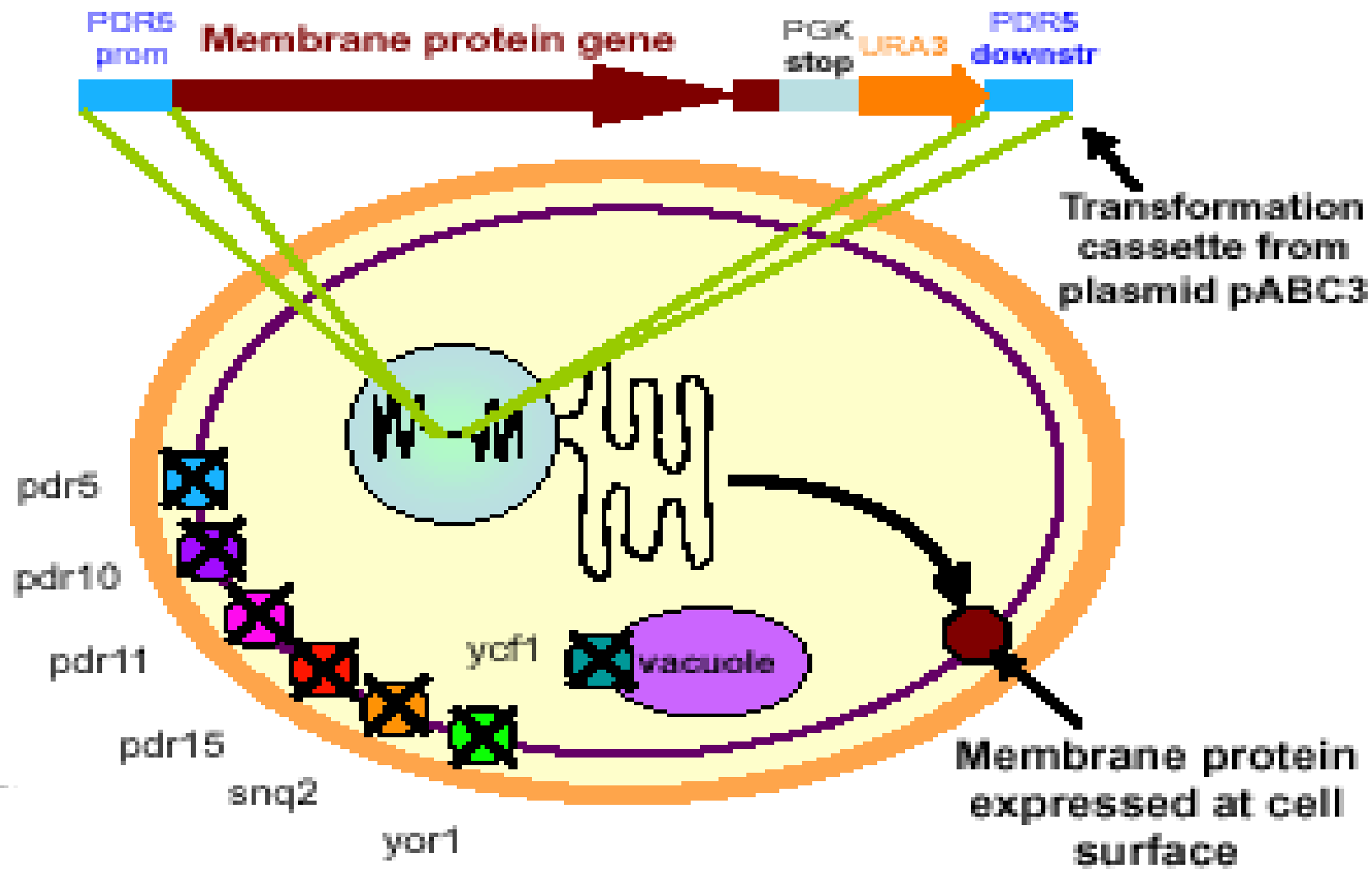
STRAINS made by Anabelle Decottignies  
and Elvira Carvajal

STRAINS curated by Stan Ulaszewski  
and Michel Ghislain  
[michel.ghislain@uclouvain.be](mailto:michel.ghislain@uclouvain.be)

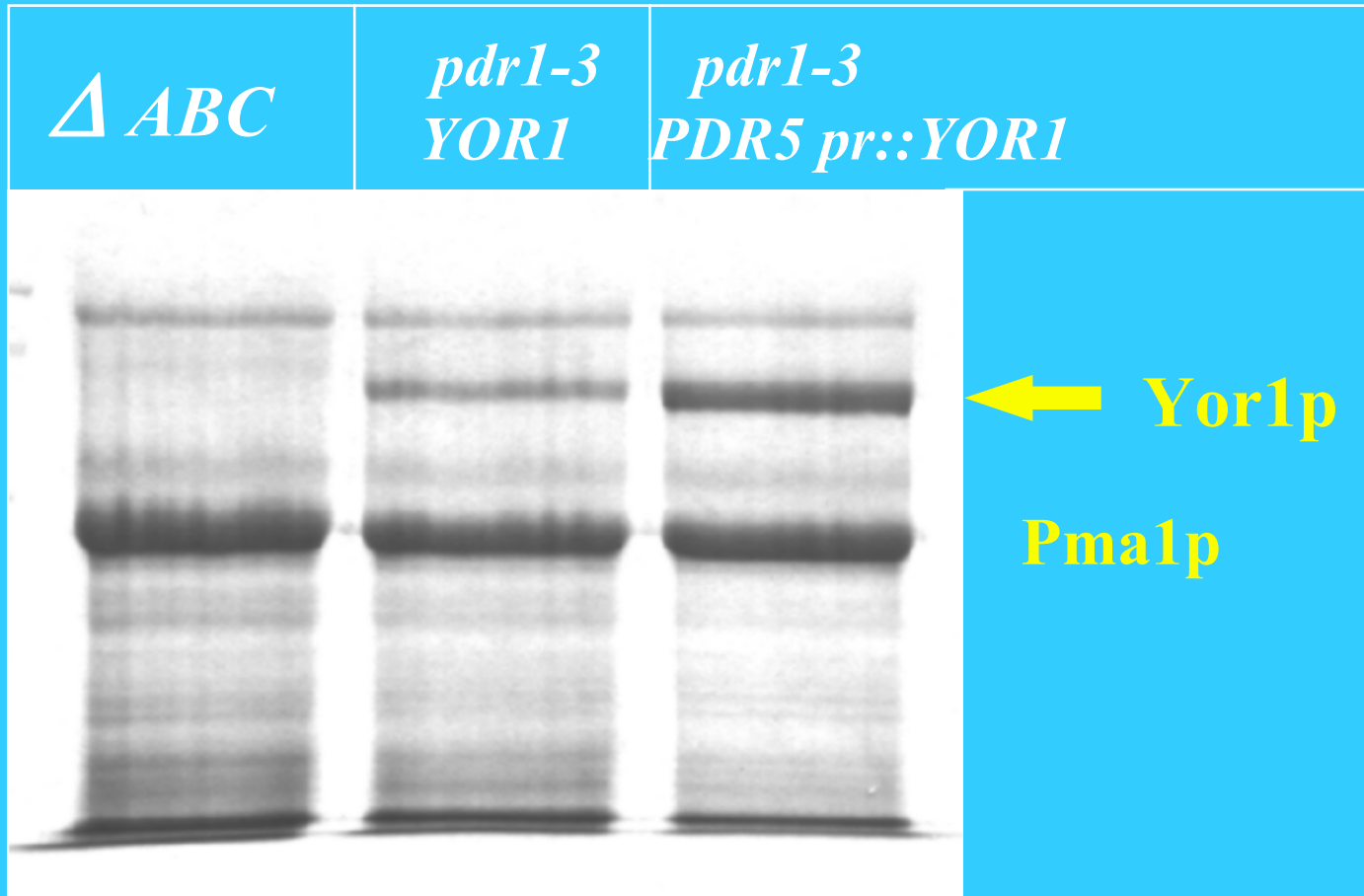
# YEAST PDR AS A SYSTEM FOR OVEREXPRESSION OF MEMBRANE PROTEINS



# The pdr1-3 Regulated PDR5 Hyper-expression System



# OVERPRODUCTION OF YOR1p



OLIGOMYCIN  
RESISTANCE

0.1

4

30  $\mu\text{g/ml}$

*Decottignies et al., 1997*

# MEMBRANE TRANSPORTERS OVEREXPRESSED IN S.CEREVISIAE

## ABC

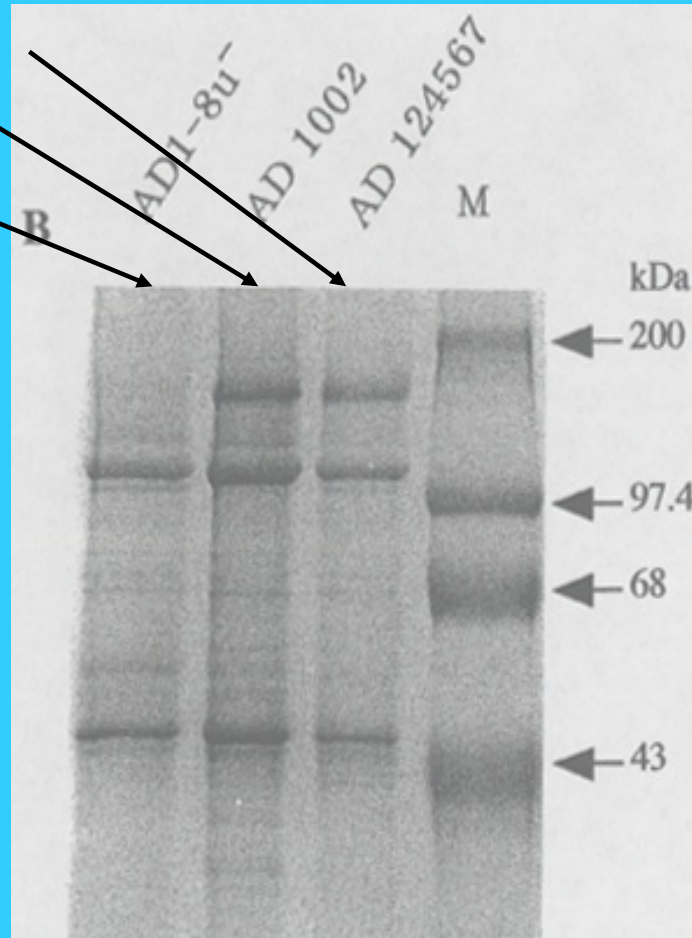
- CaCDR1p
- CaCDR2p
- CgCDR1p
- CgCDR2p
- ABCA1

## MFS

- Ben<sup>R</sup>p

# SUPER CDR1 and Super PDR5 in *S.cerevisiae* plasma membranes

**SUPER PDR5**  
**SUPER CDR1**  
**MULTIDELETIONS**



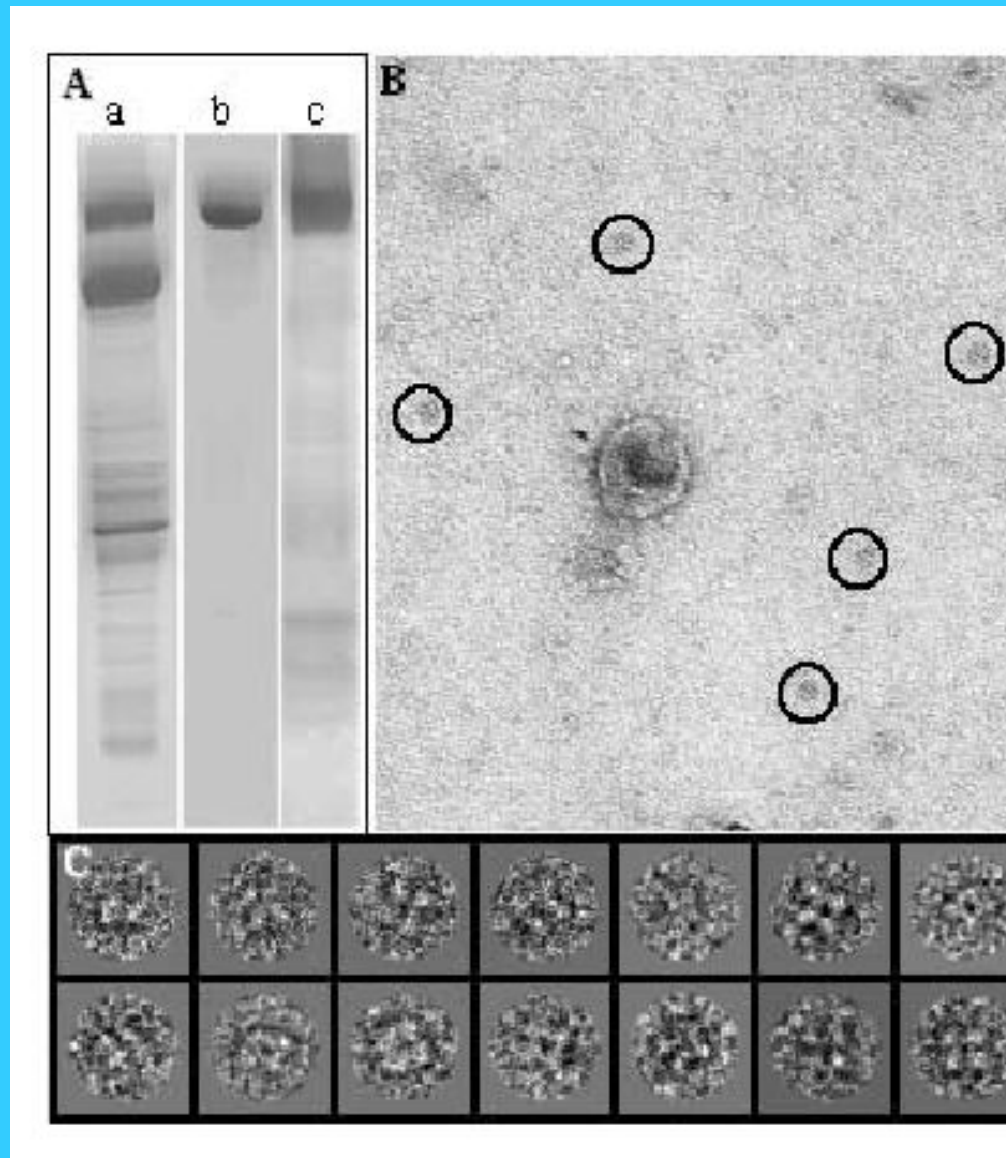
**B.MONK 2001**

EM structural analysis  
of the *S.cerevisiae*  
Pdr5p

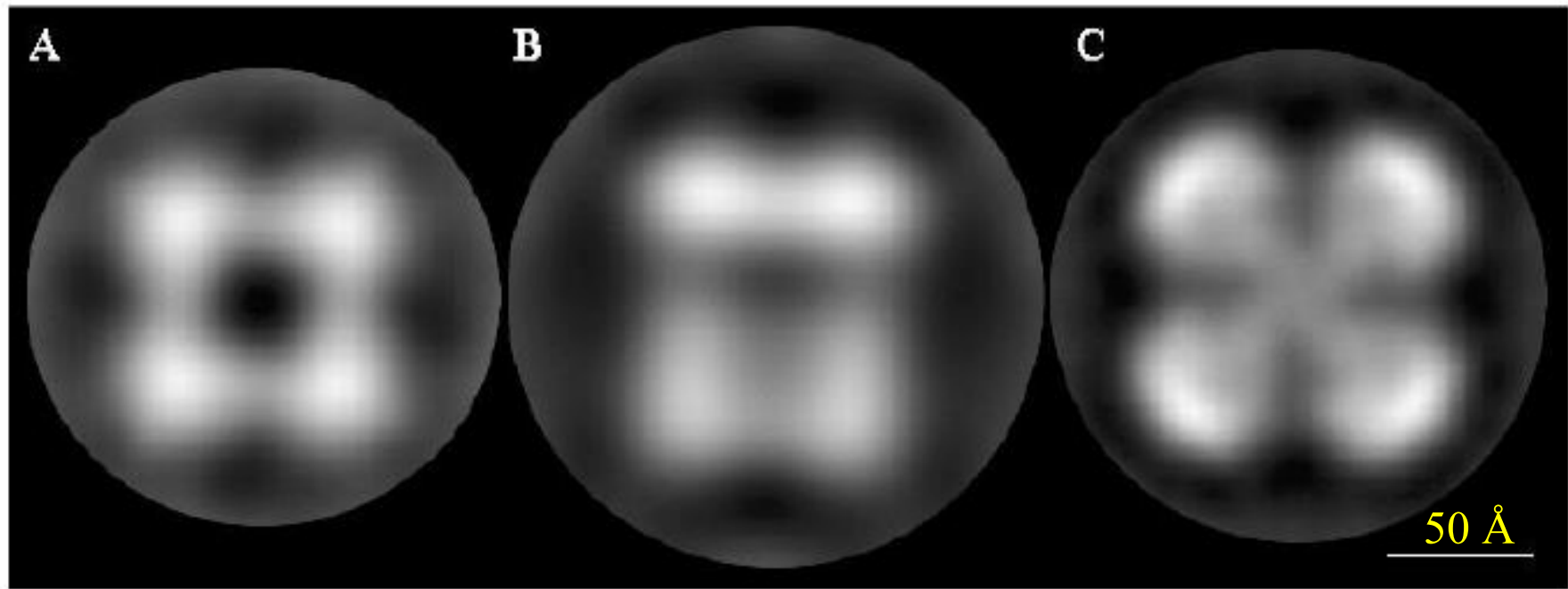
MARCO/RIGAUD



# PURIFIED Pdr5p PARTICLES

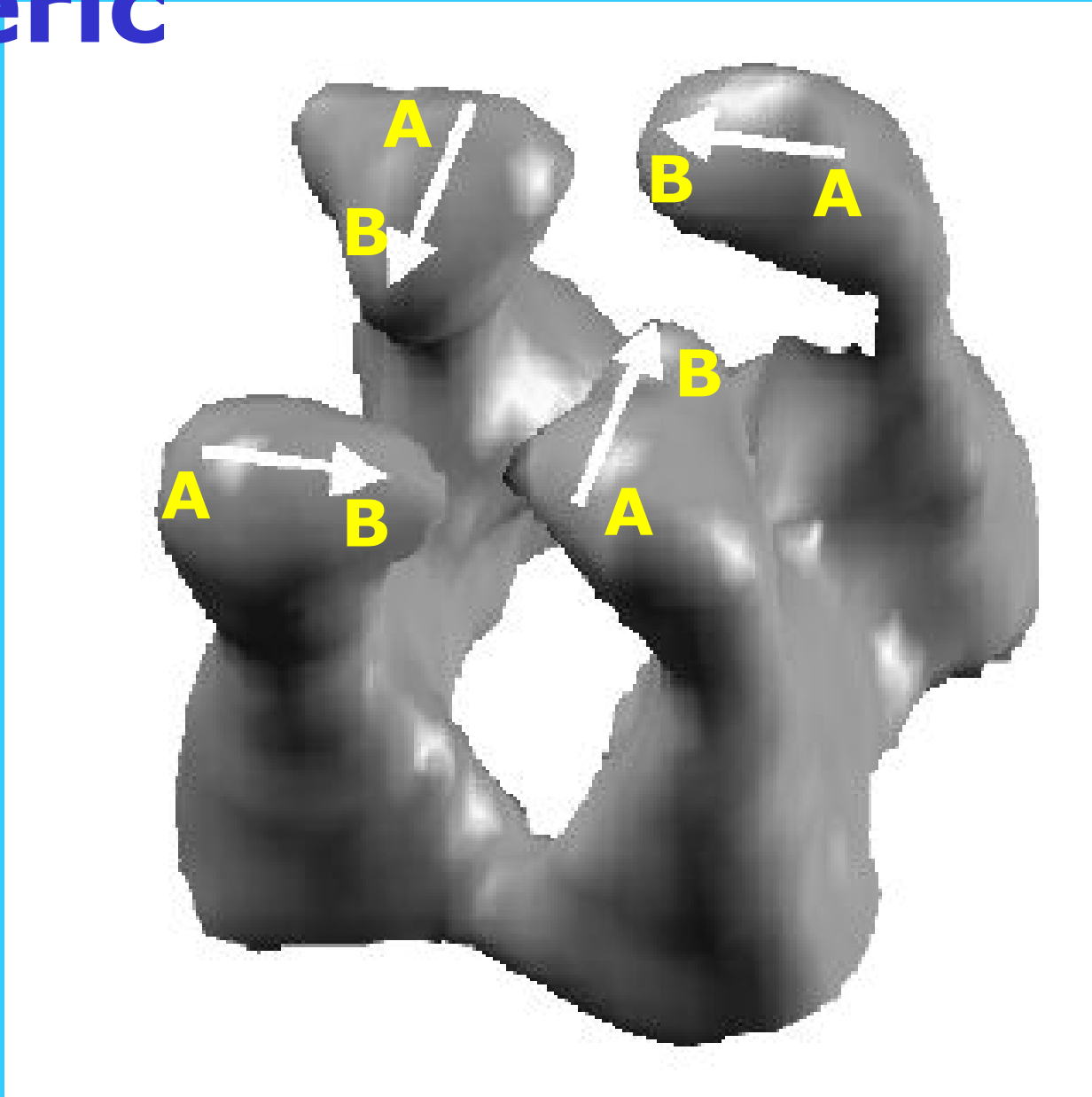


# Pdr5p Particles Projection

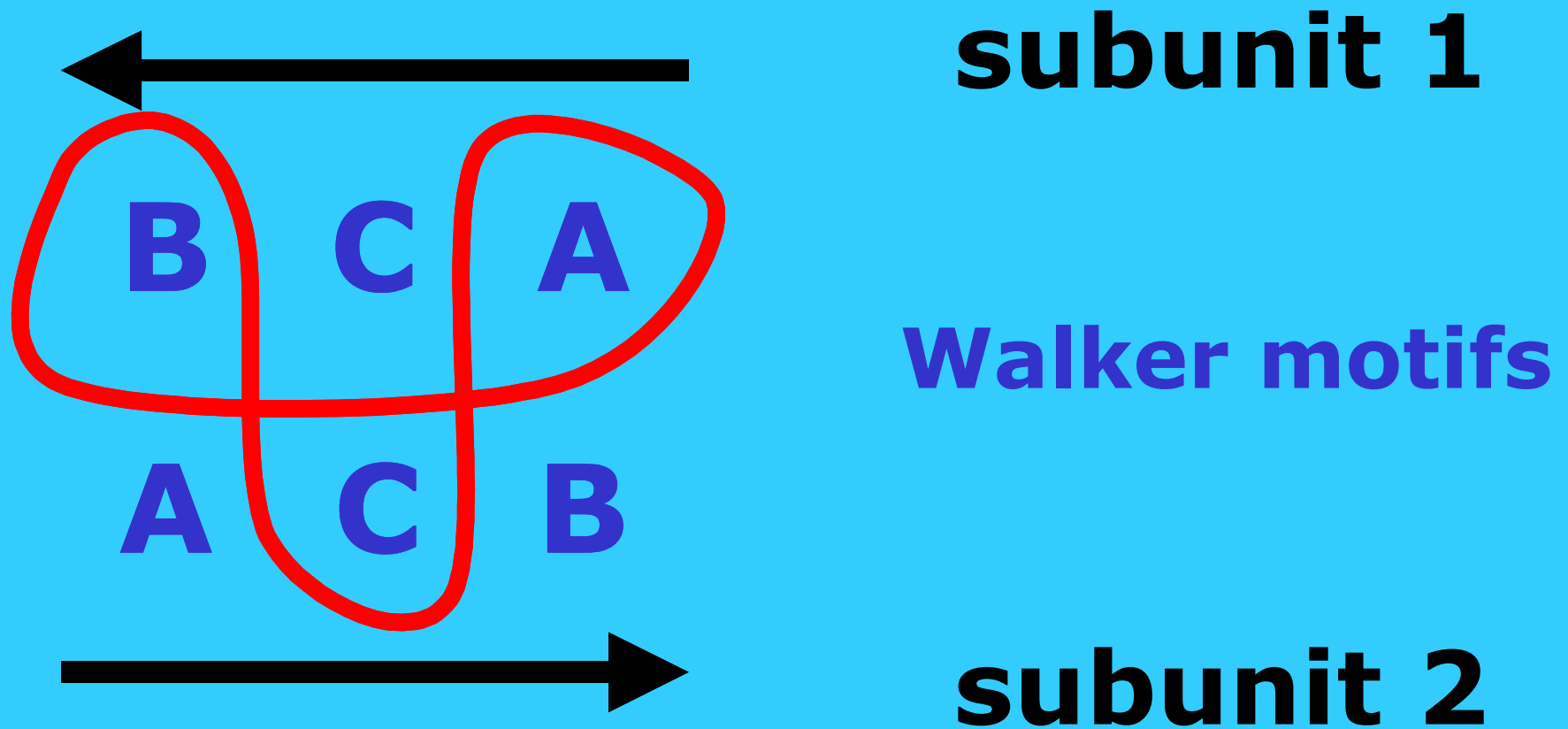


**Dimeric**

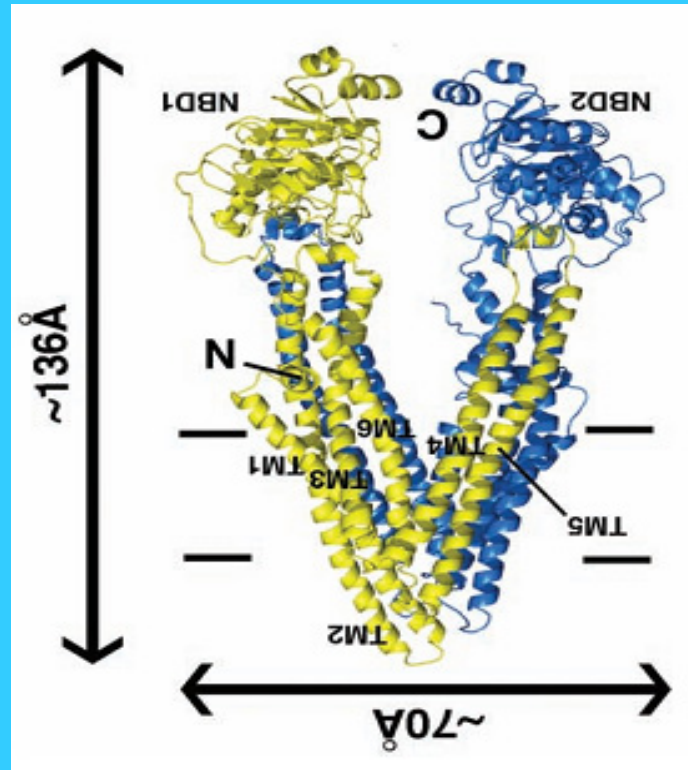
**Pdr5p**



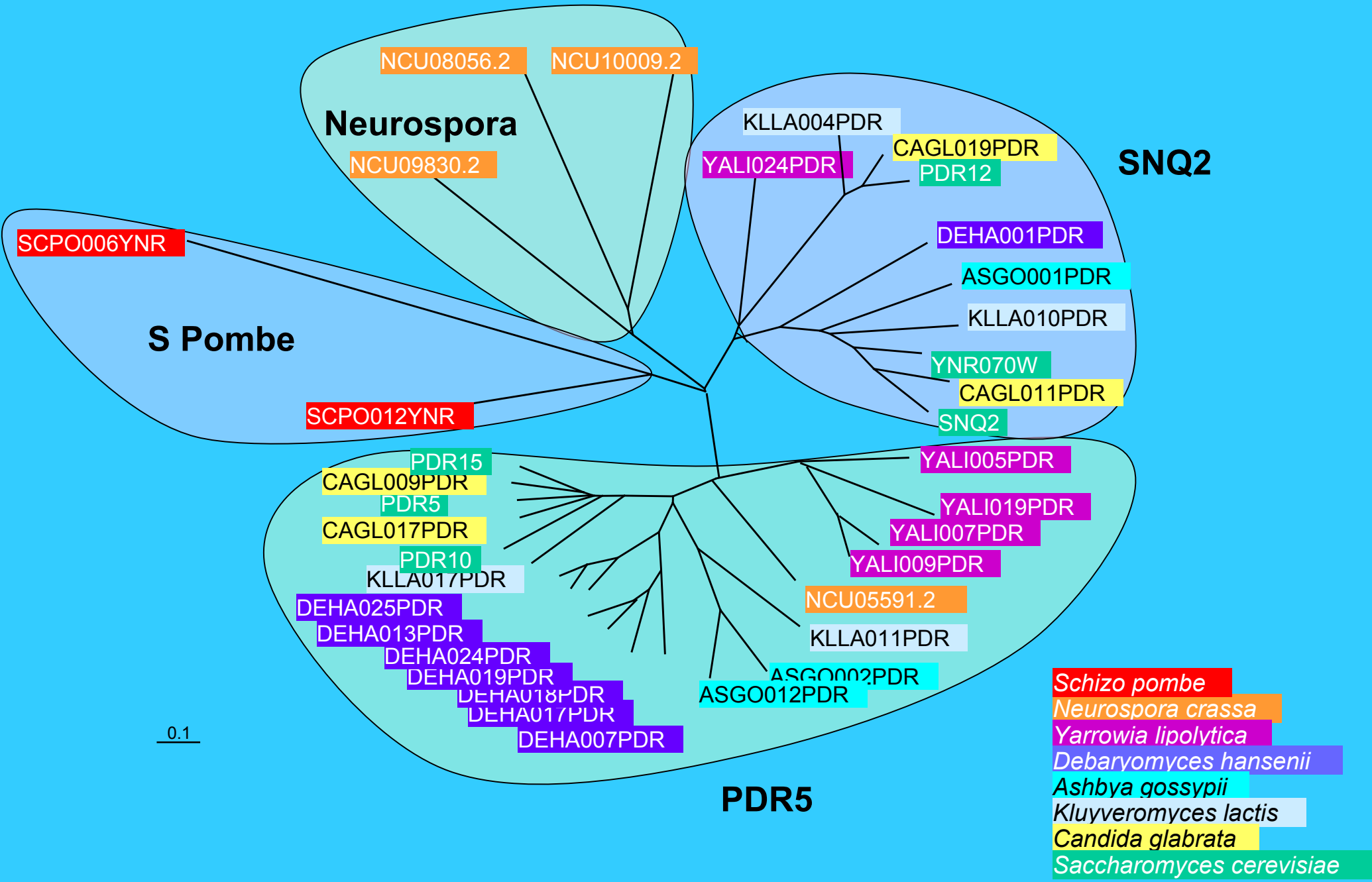
# ATP binding requires two « head to tail » subunits



# Pgp structure, Chang et al ,2009



# PHYLOGENY



# Conclusion

We can now envisage

- crystallization of each catalytic step of Pdr5p
- overexpression, purification, crystallization and phenotypic characterization of each subfamily of fungal and plant's Pdr



FYSA / PDR MEMBERS (1980-1996)

Elisabetta BALZI

Elvira CARVAJAL

Maryse CHARBONNIER

Sister SUSAN CRONIN

Anna CYBULARZ

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Jean-Luc JONNIAUX

Zhao JUN

Marcin KOLACZKOWSKI

Laurence LAMBERT

Yannick MAHE

Rajendra PRASAD

Wren SCHAUWER

Julius SUBIK

Stanislaw ULASZEWSKI

Bart VAN DEN HAZEL

Min WANG

ANDRE GOFFEAU

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- NIMMI kyoko

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- ULASZEWSKI stan

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- DE HERTOG benoit
- DIFFELS julie
- HANCY frederic

## PARIS ENS

- JACQ claude
- MARC philippe
- DEVAUX Frédéric

## IOWA

- MOYE-ROWLEY scott

## ODENSE

- FEY stephen
- MOSE LARSEN peter
- NAWROCKI arkadiusz

## PARIS Curie

- RIGAUD jean-louis
- MARCO sergio

# STILL GOING ON

Degradation of Pdr5p by GHISLAIN michel

Control by kinases and phosphatase by MONTERO monica

Crystallisation of Pdr5p by XIA di

Plant Inhibitors of Pdr5p by PEIRERA antonio

Crystallisation of Yor1p by Falson pierre

Vmr1p a novel vacuolar yeast MRP1 by WAWRZYCKA donata

Plant PDRs by BOUTRY marc *et al*