Phenotypic analysis and transcriptome profiling of Saccharomyces response to medium chain fatty acids

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Phenotypic ANALYSIS and transcriptome PROFILING of Saccharomyces RESPONSE to medium chain fatty acidS

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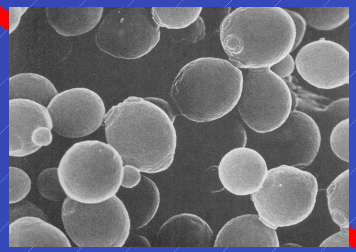
INRA UMR SVQV, Colmar,
INRA UMR SPO Montpellier,
UHA LVBE, Colmar



Fermentation

nn metabolites (esters …)

toxic compounds :

Medium chain fatty acids …

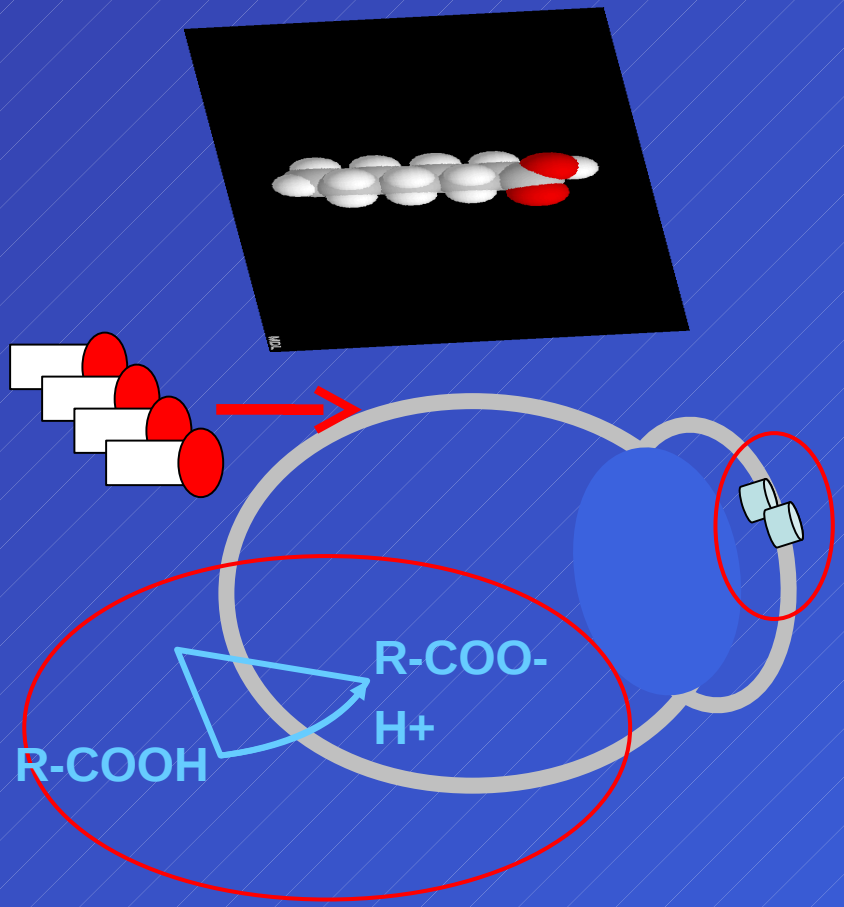
MCFA (medium chain fatty acids)

C8 : octanoïc acid

CH**3**–(CH**2**)**6**-COOH

C10 : decanoïc acid

CH**3**–(CH**2**)**8**-COOH



- Toxics for *S. cerevisiae:*
fermentation inhibitors

- *S. cerevisiae* can adapt to MCFA

- PDR12 induced by C8 (Hatzixanthis et al., Yeast 2003) but not involved in resistance (Holyoak et al., j Bact. 1999)

=> no clear mechanism of cell resistance

Wine yeast Resistance polymorphism to MCFA

C10 Resistance

80 wine strains tested
on YPD
containing AGCM

C8 Resistance

=> 2 partially overlapping mechanisms

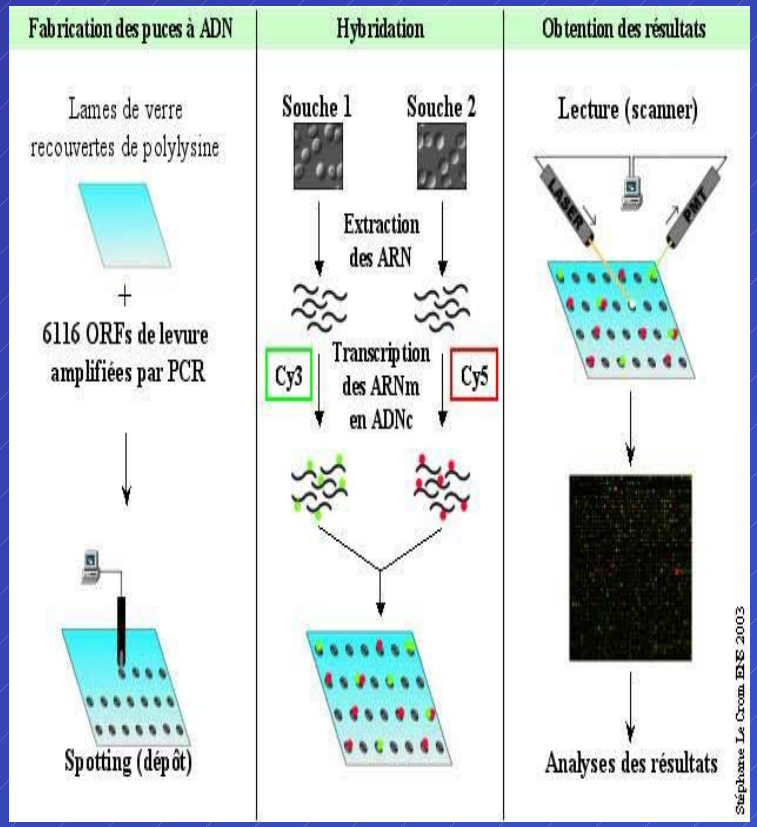
Identification of *S. cerevisiae* genes involved in MCFA resistance

(Legras et al 2010 AEM)

Search of genes involved in resistance

Transcriptome

Analysis of all mRNA in cell at a specific time in a specific condition.

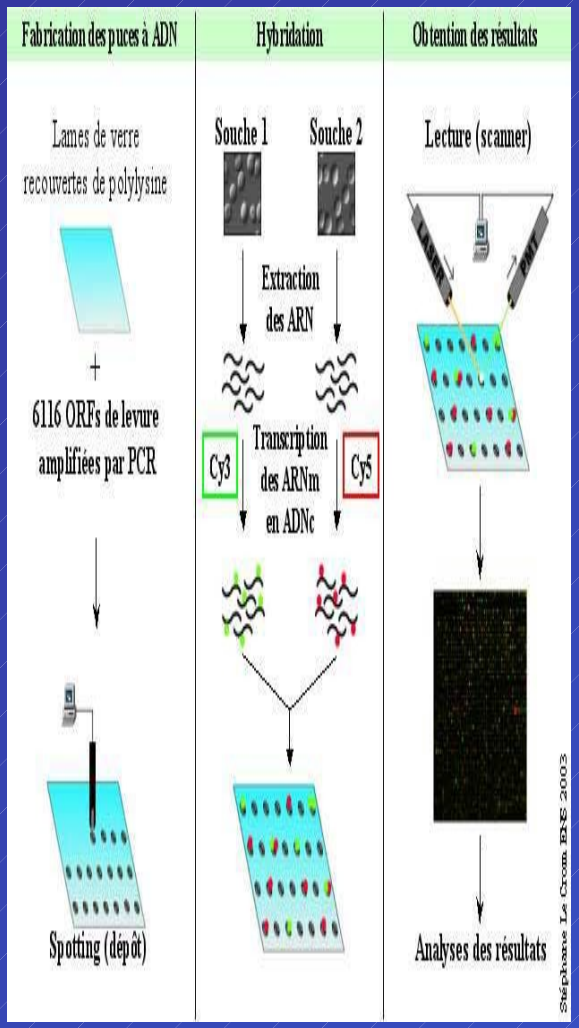


Step1 : synthesis of fluorescent labelled cDNA from each type of cell

genes involved in MCFA resistance

Transcriptome

Step2 : DNA microarray production



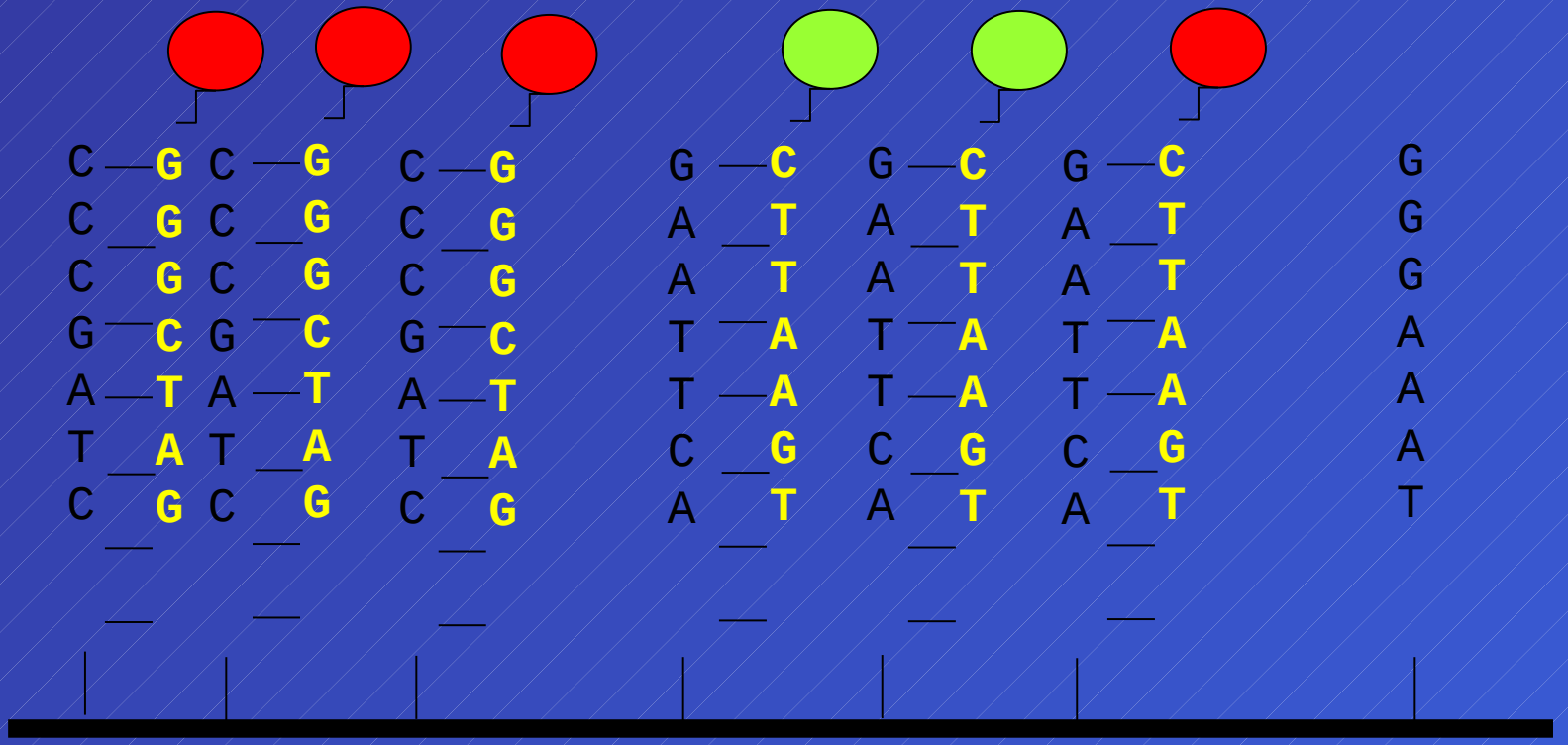
Microarray slide:

commercial array spotted with oligonucleotides (Eurogentec)

genes involved in MCFA resistance

Transcriptome

Step 3 : Hybridization

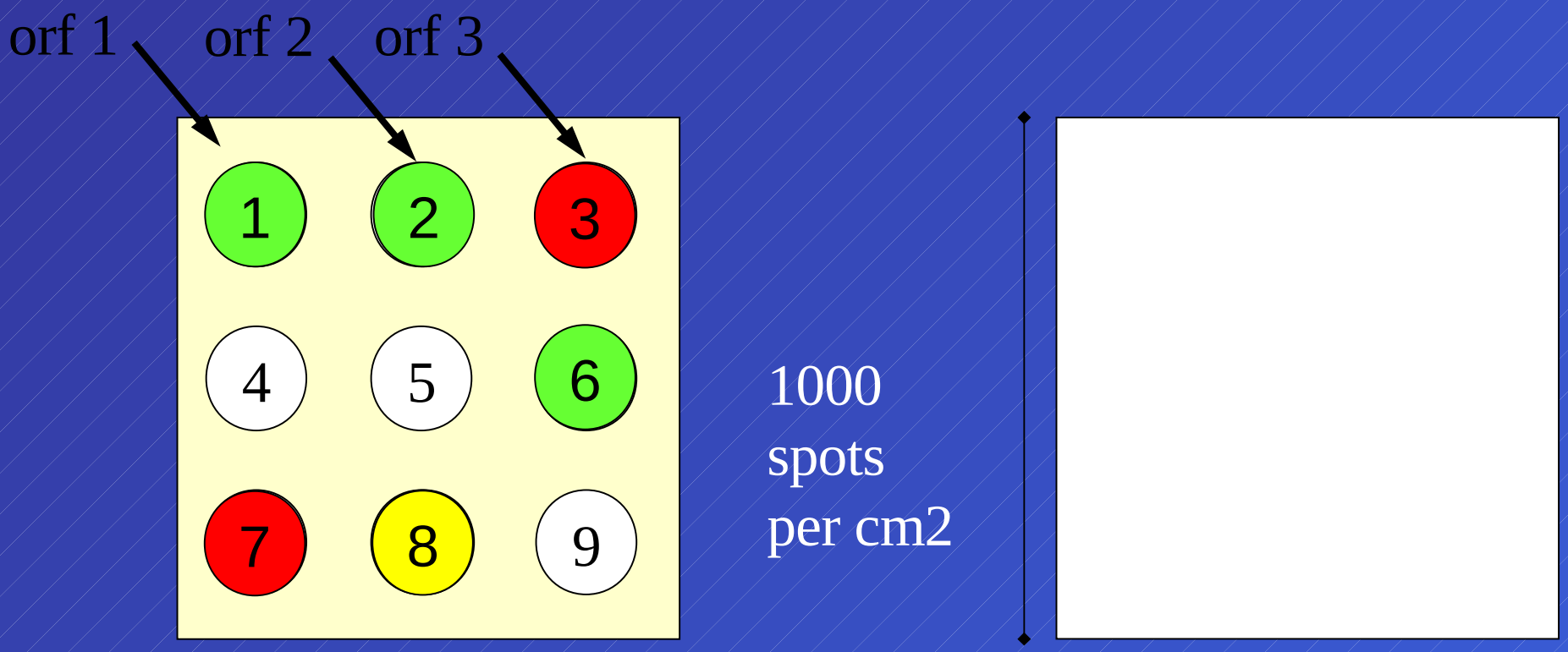


cDNA from the two conditions compete for hybridization on the probes

Genes involved in MCFA resistance

Transcriptome

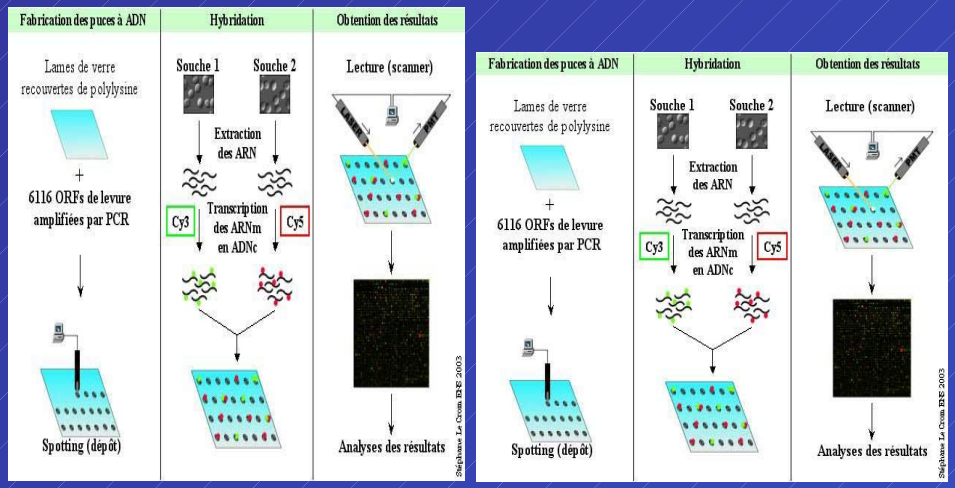
Step 3 : Hybridization

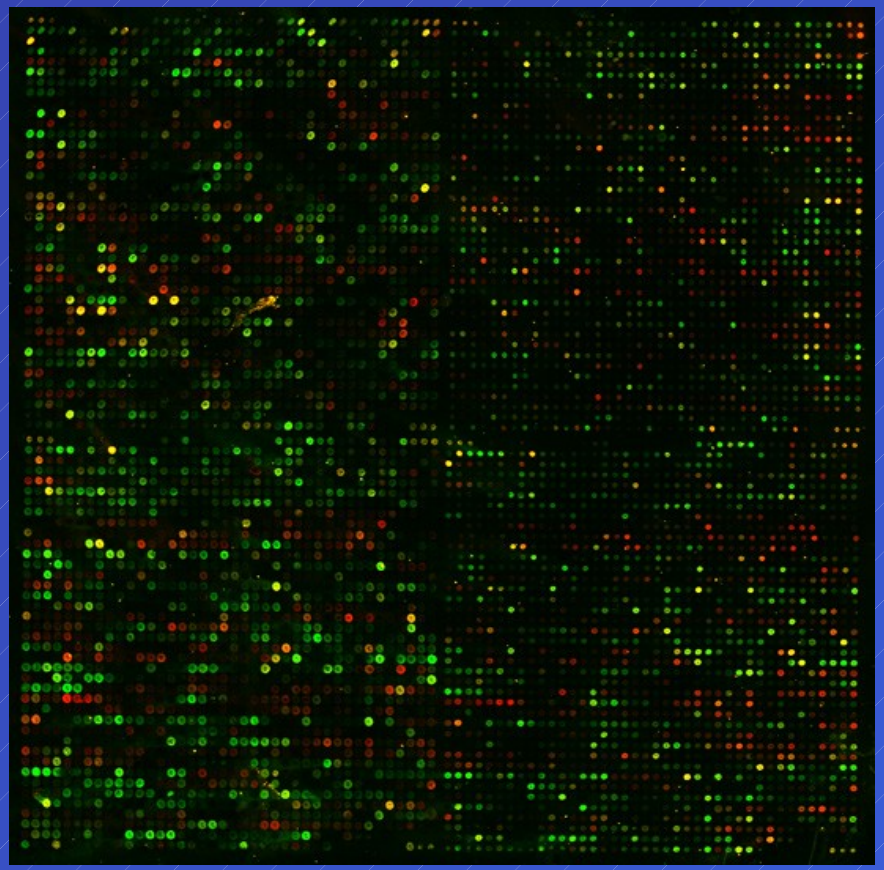


Genes involved in MCFA resistance

Transcriptome

Step 3 : microarray scan





fluorescence measurement for each fluoro phosphorescent compound (excitation at 2 different λ)

⇒2 images one forCy5 the other for Cy3

Genes involved in MCFA resistance

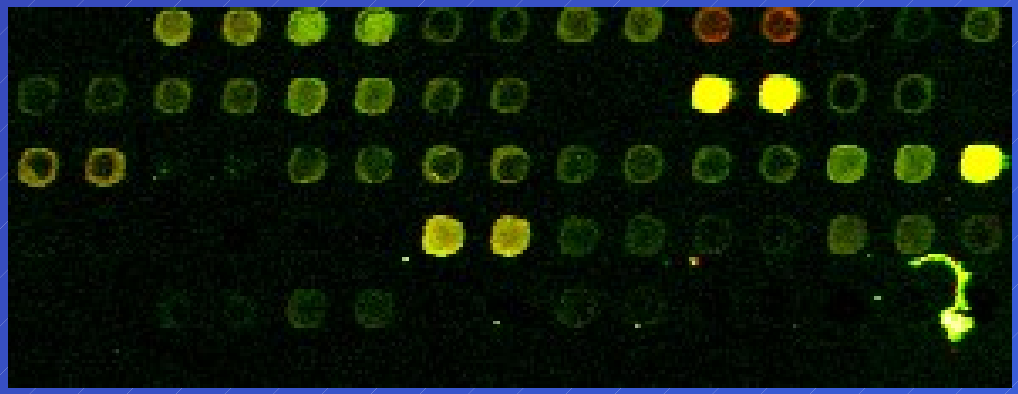
Transcriptome

Step 4 : data analysis

⇒ connect image data and genes iD (grid)

⇒ check for problems (artifacts dust, spot without signal, or saturated spots)

⇒ transformation of scanned image into numerical data

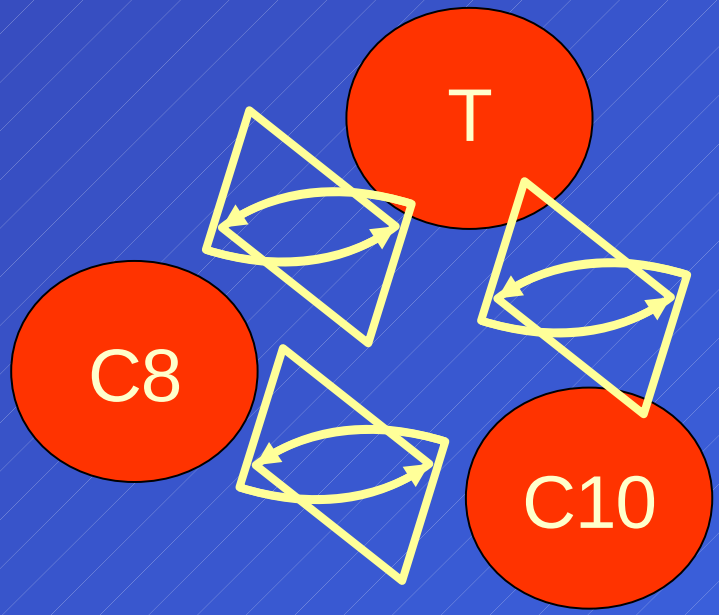


Transcriptome re-modeling after exposure to MCFA

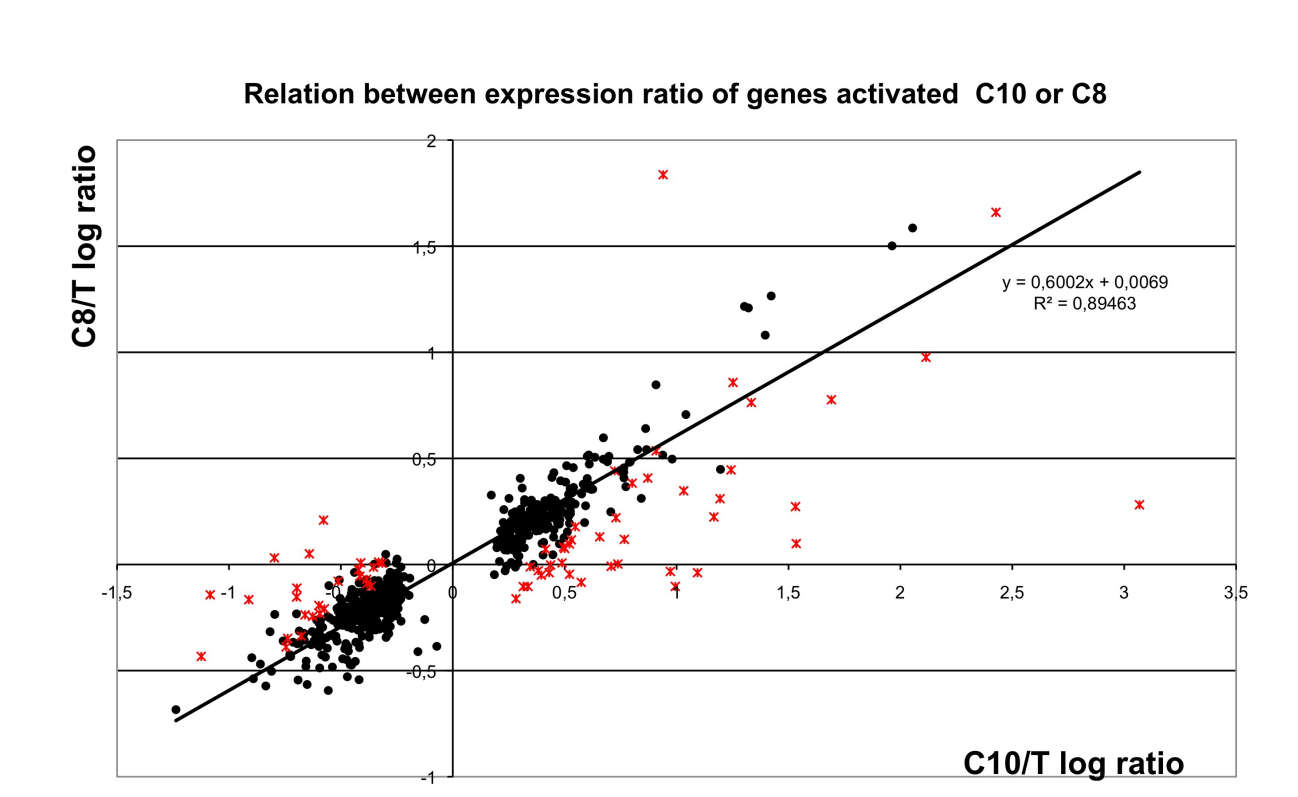
- wine strain U13 resistant to C8 andC10
- 4 independent cultures in three conditions

YPD	+ 0.05 mM C8	+ 0.05 mM C10
Control	C8	C10

- RNA extraction after 20 min
- comparison by pairs
- analysis : LIMA GUI package (R soft)



Transcriptome re-modeling after exposure to MCFA



⇒ Responses with different intensities

⇒ log ratio : different threshold for each compound

- 0.3 pour C8
- 0.5 pour C10 to C8

81 genes activated by C8

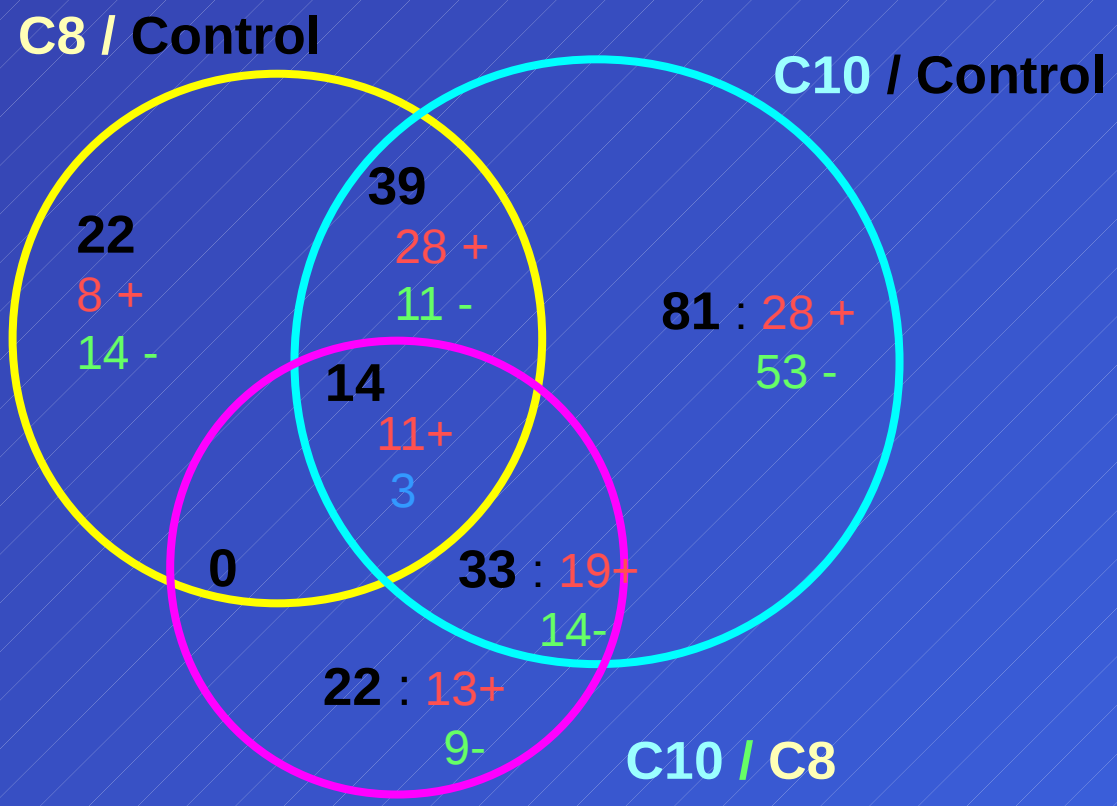
620 genes by C10

71 genes by C10 to C8

(P-value 0,05 corrected FDR)

Transcriptome re-modeling after exposure to MCFA

210 are activated in at least one condition



Functional categories of genes modulated MCFA (funspec website)

⇒ C8 and C10 activate genes shared by the same categories and C10 response involve more genes involved in RNA processing.

Comparison with other stress

Stress	Percentage of genes shared between the different responses											
number of genes involved in each response	75	165	137	548	342	621	463	132	69	172	267	936
C8 acid	100											
C10 acid	71	100										
Sorbic acid	29	19	100									
POELE 1mM	43	31	43	100								
DCP 3mM	19	22	20	28	100							
Octanol 1%	0	23	18	14	19	100						
SDS 1%	45	39	26	21	20	21	100					
Fluphenazin	12	17	12	26	27	17	37	100				
Benomyl 7mM	12	14	12	17	25	16	23	13	100			
2,4 Dichlorophenoxyacetic acid 3mM	16	14	17	20	13	15	21	11	6	100		
Oleic acid	7	6	8	8	10	6	17	16	12	5	100	
H202 3mM	52	47	42	27	35	23	40	38	41	30	22	100

Both responses share many genes with oxidative stress response, and with sorbic acid and SDS stress.

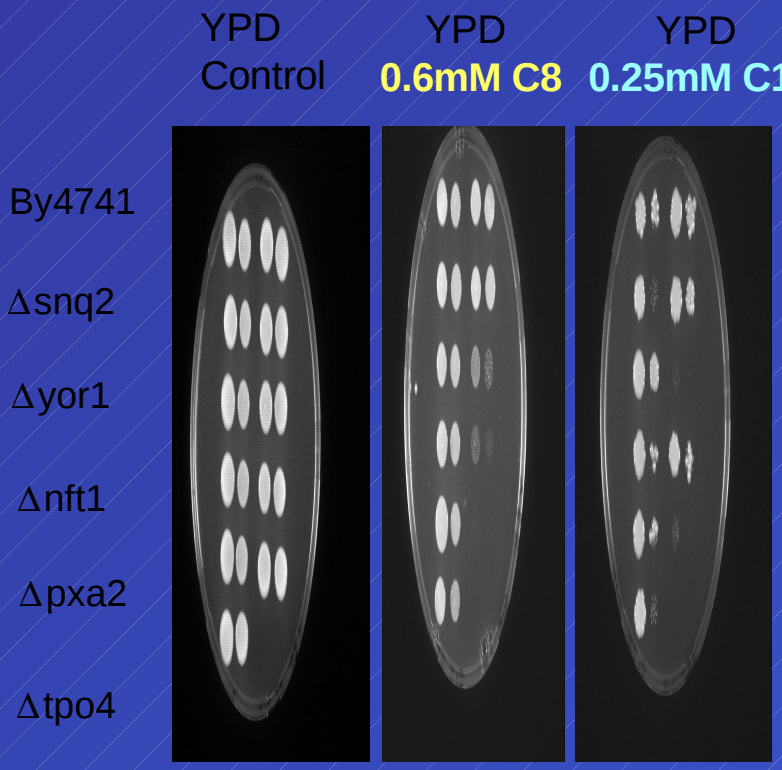
Genes involved in each response

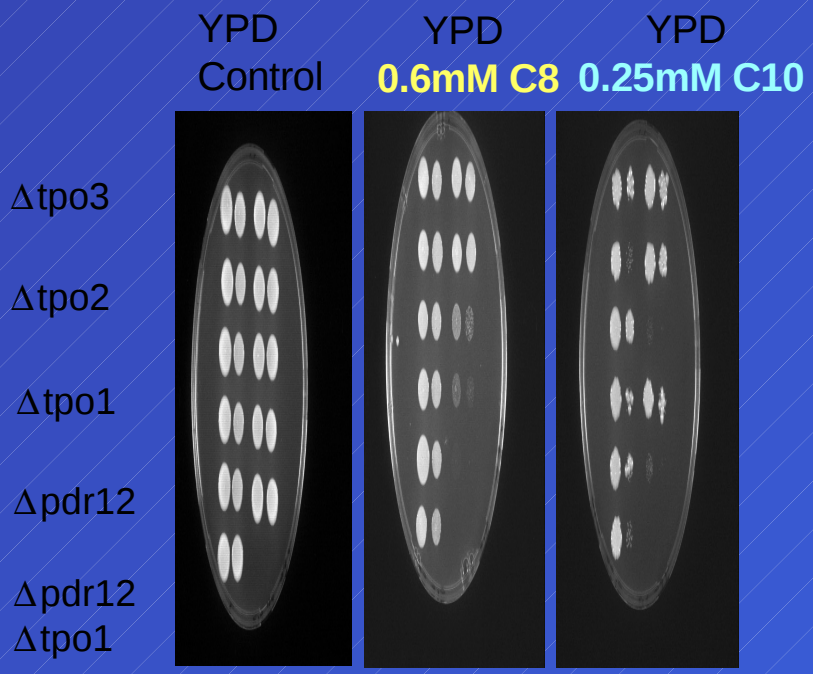
PDR12 transporter has the highest induction level for C8 (* 3,5)

The transporter TPO4 is highly activated (* 3,2). Others transporters are induced : TPO1 , PDR12.

Activation of ethyl ester synthesis EEB1, FAA1 , ELO1

Phenotypic evaluation of candidate genes





Conclusions

- PDR12 and TPO1 are responsible for cell resistance to C8 and C10 respectively
- Other transporters can increase C10 resistance but in a lower extent
- Ethyl ester synthesis is activated and contributes to MCFA resistance

Transcription factors involved in MCFA adaptation and resistance

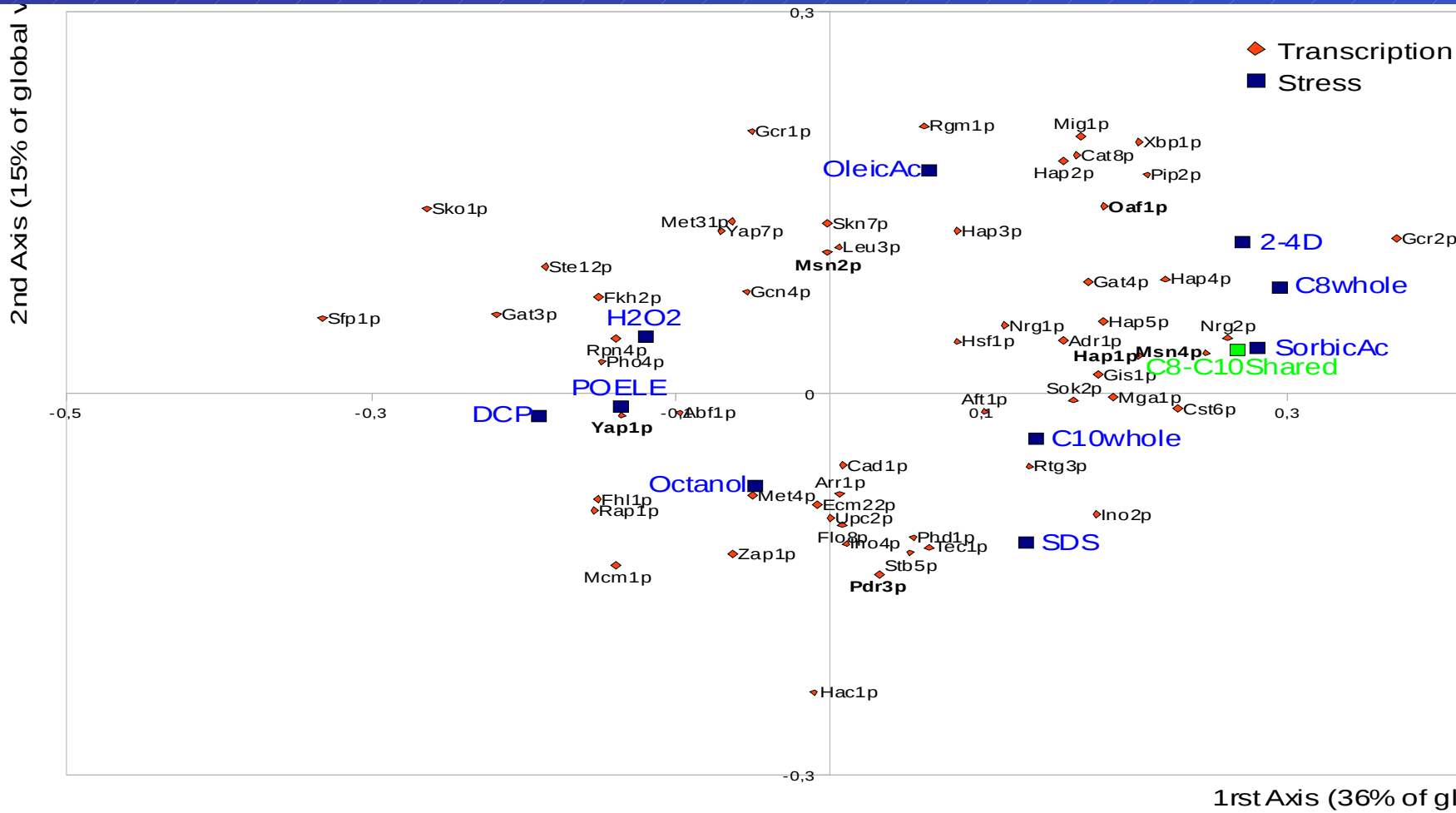
Transcription factors involved in MCFA response

Specific C8 (22 genes)		**Commune C8-C10 (53 genes)**		**Specifique C10 (80 genes)**	
Facteur de Transcription	% gènes de la réponse	Facteur de Transcription	% gènes de la réponse	Facteur de Transcription	% gènes de la réponse
Yap1p	69.6 %	**Msn4p**	**48.6**	Yap1p	44.4 %
Met4p	39.1 %	**Msn2p**	**48.6**	Sok2p	37.0 %
Sok2p	30.4 %	Pdr3p	45.9	Rap1p	31.5 %
Hap5p	26.1 %	Yap1p	45.9	Met4p	31.5 %
Rpn4p	26.1 %	Aft1p	40.5	Cin5p	24.1 %
Cin5p	21.7 %	Sok2p	40.5	Rpn4p	24.1 %
Fhl1p	21.7 %	Met4p	37.8	Sfp1p	24.1 %
Sfp1p	21.7 %	Pdr1p	32.4	Gcn4p	24.1 %
Arr1p	21.7 %	Rpn4p	29.7	Pdr1p	20.4 %
Pdr1p	17.4 %	Hsf1p	27.0	Nrg1p	20.4 %
Msn2p	17.4 %	Hap4p	27.0	Pdr3p	20.4 %

http://www.yeastract.com/index.php

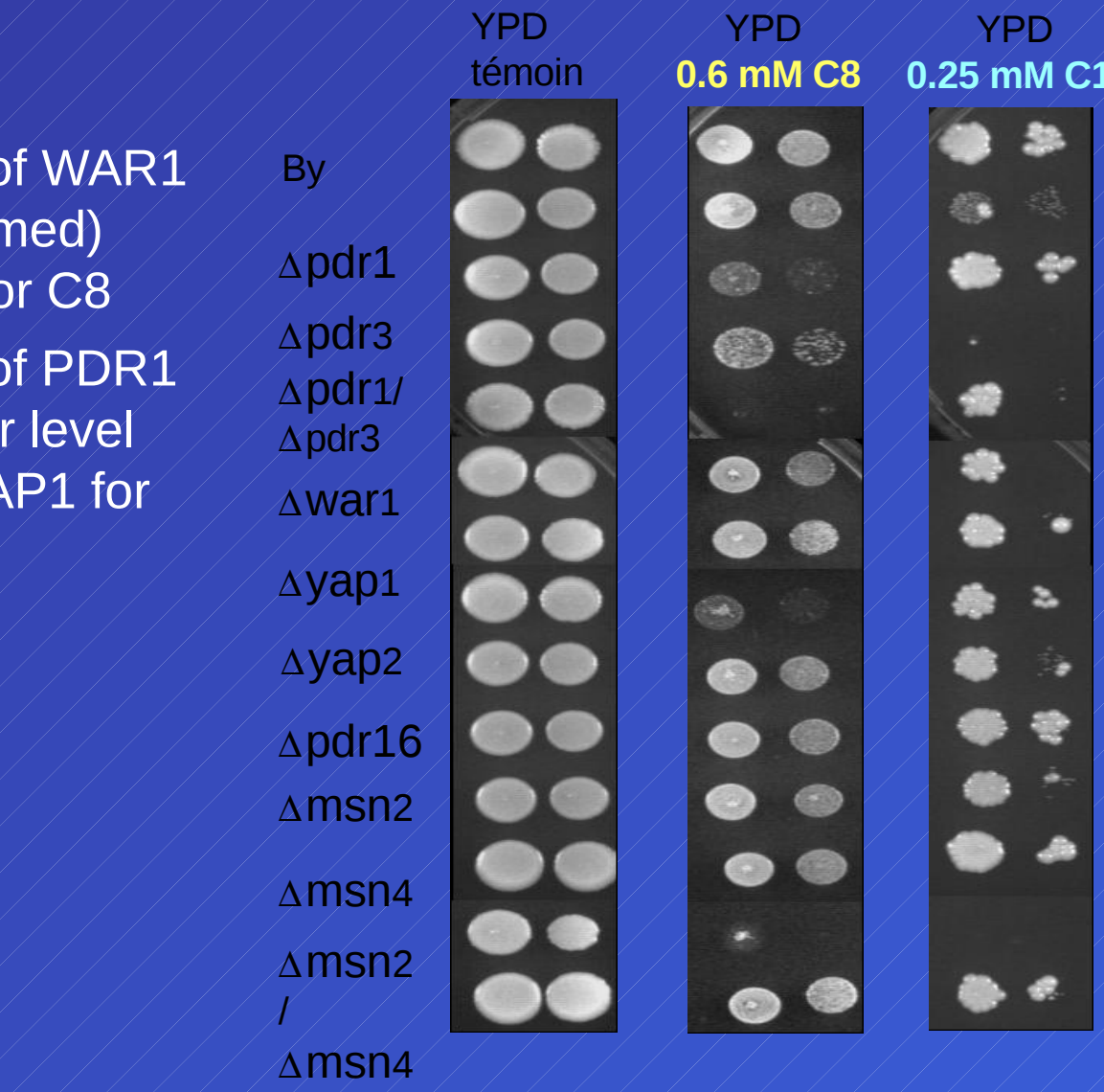
Transcription factors involved in MCFA response

From Yeast

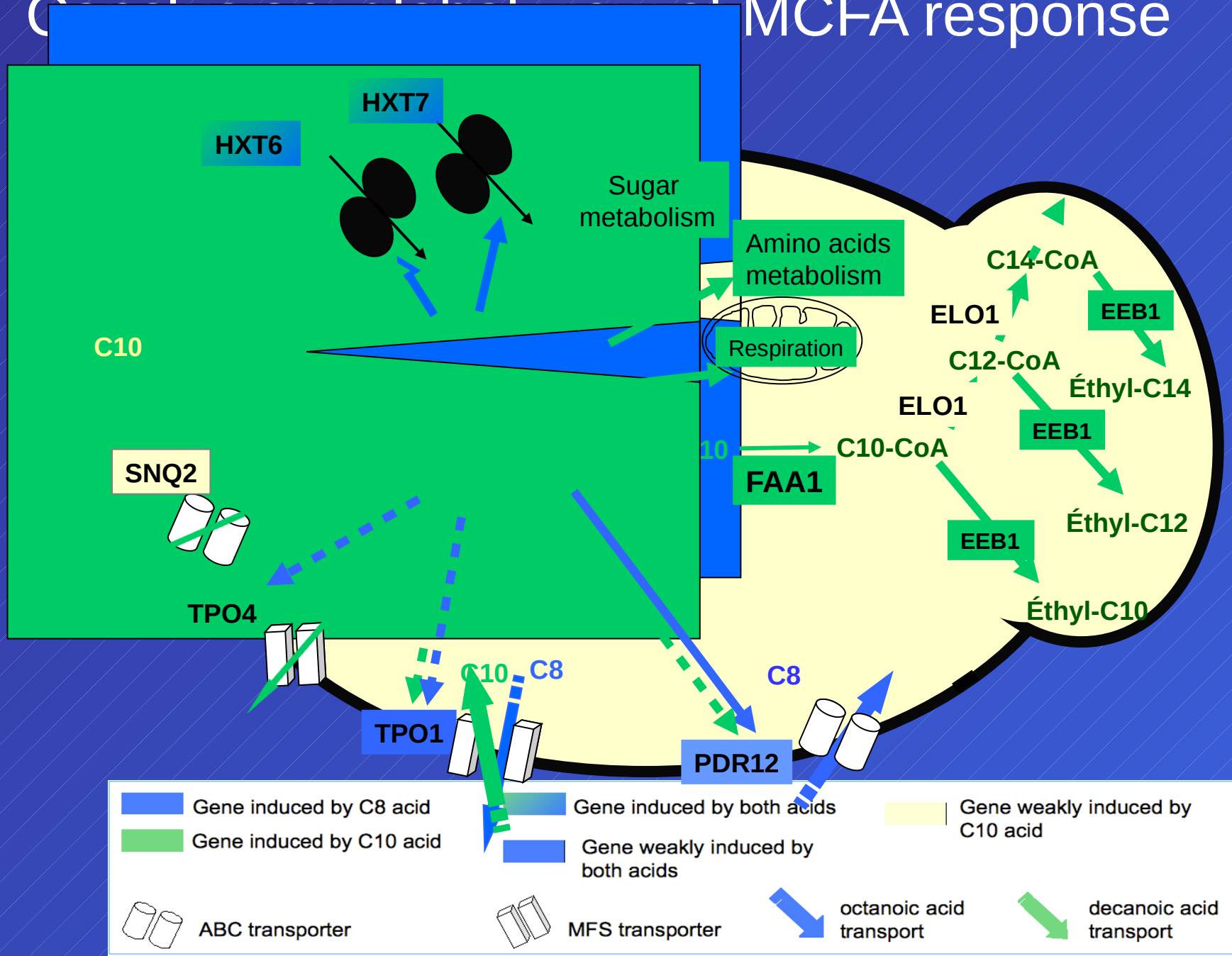


Phenotypic evaluation of TF regulating the responses

- Involvement of WAR1 PDR3 (confirmed) and PDR16 for C8
- Involvement of PDR1 and at a lower level MSN2 and YAP1 for C10



Conclusion and hypothesis of MCFA response



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Deletion strains

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Multumesc !!!