Stochastic gene expression, phenotypic variability and adaptation of budding yeast to environmental stresses

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INTRODUCTION AND HYPOTHESIS

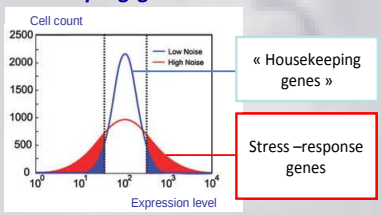
Development of single cell analysis by flux cytometry has led to the discovery of large fluctuations in gene expression levels among individual cells in isogenic populations (Elowitz et al. 2002; Blake et al. 2003; Raser and O'Shea 2004).

"Noise" in gene expression is the stochastic variation in the expression level of a gene under constant environmental conditions (Raser and O'Shea 2005).

In unicellular organisms, variability allowing heterogeneous phenotypes even in clonal populations could be an advantage favouring emergence of adapted cells when environment is fluctuating and when stress appears (Fraser and Kaern 2009).

As noise could be advantageous in regard to environmental fluctuations, are genes related to stress responses noisier than housekeeping genes ?

Protein-specific differences in noise strongly correlate with a protein's mode of transcription and function (Newman et al. 2006).
Noise in protein levels seem to have been selected to reflect the costs and potential benefits of this variation.

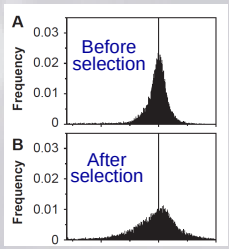


Technological and industrial yeast strains which have evolved in stressful conditions are more tolerant to stress and fluctuating environments.

The genetic basis of the technological properties of industrial yeasts, *e.g.* resistance to high sugar content (140-180 g/L), high alcohol content, low pH, high temperature..., is largely unknown.

Stochastic gene expression favours the appearance of resistant sub-populations in challenging environments (Blake et al. 2006).It is a major source of phenotypic diversification and can be modulated to change the population dynamics in response to stress.

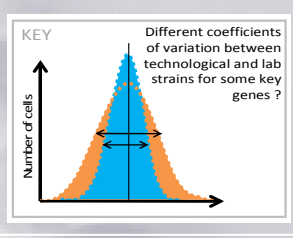
Recent works show how selection influences phenotypic fluctuations in evolutionary experiments.
For example, mutants with larger degrees of phenotypic variation emerge under strong fluctuating selection pressure (study on *E. coli, by* Ito et al. 2009).



⇨ ***Increase in phenotypic fluctuation through noise in gene expression is clearly a relevant evolutionary strategy***

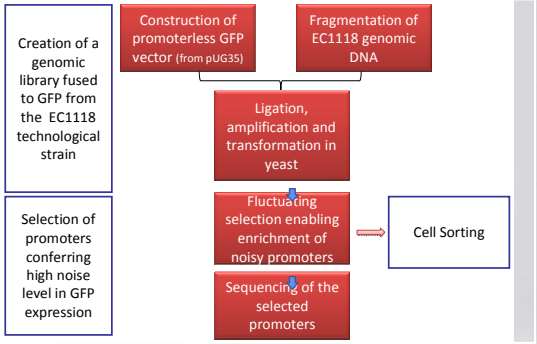
Our central hypothesis is that technological strains exhibit higher noise level in the expression of key genes that are important for survival in face of the stressful and fluctuating environments they have to deal with.

Such a phenotypic variability could have been selected thanks to the conferred benefit in stressful fluctuating environments.

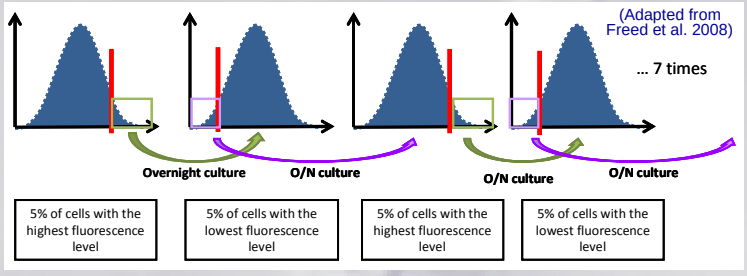


STRATEGY

Screening for the « noisiest » promoters in a technological strain (EC1118, recently sequenced (Novo et al, 2009)) to compare their noise levels to the ones of their lab strain counterparts. Study their potential impact on tolerance and population dynamics in stressful conditions.



Fluctuating cell sorting of yeast (DO = 2, optimal growth conditions) by FACS



⇨ Enrichment in promoters allowing rapid switches from high to low and low to high fluorescence level (⇨ noisiest promoters)

RESULTS

Following the selection of yeast cells displaying the most variable GFP expresssion, we extracted their plasmid DNA. After transformation of *E. coli*, **480 independant clones were isolated and amplified. 310 genomic fragments of more than 100bp were sequenced and analysed. They identified 295 uniquely mapped regions, among which 22 were found to contain genetic variations compared to their counterparts in the sequenced laboratory strain S228C.** We selected for further investigations 4 of these fragments corresponding to promoter regions of selected genes, because we hypothesize that these variations are possibly involved in differences of noise levels.

PERSPECTIVES

We isolated genomic fragments of the technolgical EC1118 strain that confer highly variable expression of the GFP reporter, and identifed those corresponding to promoters regions presenting genetic differences from their counterparts in the S288c lab strain. Using GFP-fusions and promoter replacement methods, we will determine if these genetic differences effectively generate differences in noise levels in the same genetic background. The impact of these noise level differences on tolerance to various stresses will be studied, and experiments will be performed to determine if an increased noise level in the expression of some genes confers an adaptive advantage in fermentation conditions.

This study explores an original evolutionary strategy to identify genetic determinants of yeast tolerance to stress.

References : **Elowitz** et al., Science, 2002; **Blake** et al., Nature, 2003; **Raser and O'Shea**, Science, 2004; **Raser and O'Shea**, Science, 2005; **Newman** et al., Nature, 2006; **Blake** et al., Mol Cell, 2006; **Freed** et al., PloS Genetics, 2008; **Fraser and Kaern**, Mol Microbiol, 2009; **Ito** et al., Mol Sys Biol, 2009; **Novo** et al., PNAS, 2009;