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Poster Presentation

A sensor of the Unfolded Protein Response to study the stress induced in *Yarrowia lipolytica* strains by the production of heterologous proteins

Catherine Madzak* and Jean-Marie Beckerich

Address: UMR1238 Microbiologie et Génétique Moléculaire, INRA/CNRS/INA-PG, CBAI, 78850 Thiverval-Grignon, France
* Corresponding author

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Background

A critical step in the production of heterologous proteins in yeasts seems to be the efficiency of protein folding and assembly in the endoplasmic reticulum (ER). Saturation of the machinery involved in these steps leads to an accumulation of unfolded and misfolded proteins in the ER, activating the unfolded protein response (UPR). The UPR pathway controls the expression of genes for ER-resident chaperones and foldases, together with genes encoding diverse functions such as translocation, protein secretion and processing, and protein degradation. It is generally admitted that the UPR pathway is triggered by the lowering of free Kar2/Bip level in the ER, due to the binding of this chaperone to misfolded proteins. The titration of Kar2/Bip liberates Ire1p kinase, which auto-activates, becoming able to maturate the intron from *HAC1* mRNA. This processing enables the translation of the transcriptional activator Hac1p, which enhances the expression of the UPR pathway genes via the recognition of an UPR element in their promoter.

Results

In order to analyse the UPR in the non-conventional yeast *Yarrowia lipolytica*, especially in relation with the production of heterologous proteins, we designed an UPR-sensor carried on an integrative vector (pINA1300). This sensor comprises the promoter from *KAR2* gene (containing 3 putative UPR elements) directing the expression of the *lacZ* reporter gene (see Figure 1). The induction of UPR in *Yarrowia* strains carrying the sensor will result in beta-galactosidase production, easily detectable and measurable using coloured reactions.

The UPR-sensor pINA1300 vector was used to transform several *Yarrowia* strains sharing the same genetic background: a non-producing control strain (Po1g [1]), and two strains producing different heterologous proteins. We first performed validation experiments on the UPR-sensor control strain (Po1g + pINA1300), in order to check if growth conditions known to induce UPR were able to increase beta-galactosidase production above the background level. The results are displayed in the Figure 2.

Validation of the UPR-sensor: exponential phase cultures of UPR-sensor control strain (Po1g + pINA1300) were treated or not with either 20 micrograms per mL tunicamycin, or 20 micromolar dithiothreitol, for 3 hours, and beta- galactosidase production in the cells was measured as previously described [1]. The treatment, beta-galactosidase activity, and increase above the background level are indicated under each bar.

We performed similar validation experiments on the two UPR-sensor strains carrying heterologous gene expression cassettes and obtained similar results (data not shown – note that in the conditions of the validation experiment, the heterologous genes were not, or very poorly, expressed due to the growth-dependent characteristics of the promoter used [1]). Thus, our validation experiments showed that beta-galactosidase activity reflects the induction of UPR in *Yarrowia* strains carrying the UPR-sensor.

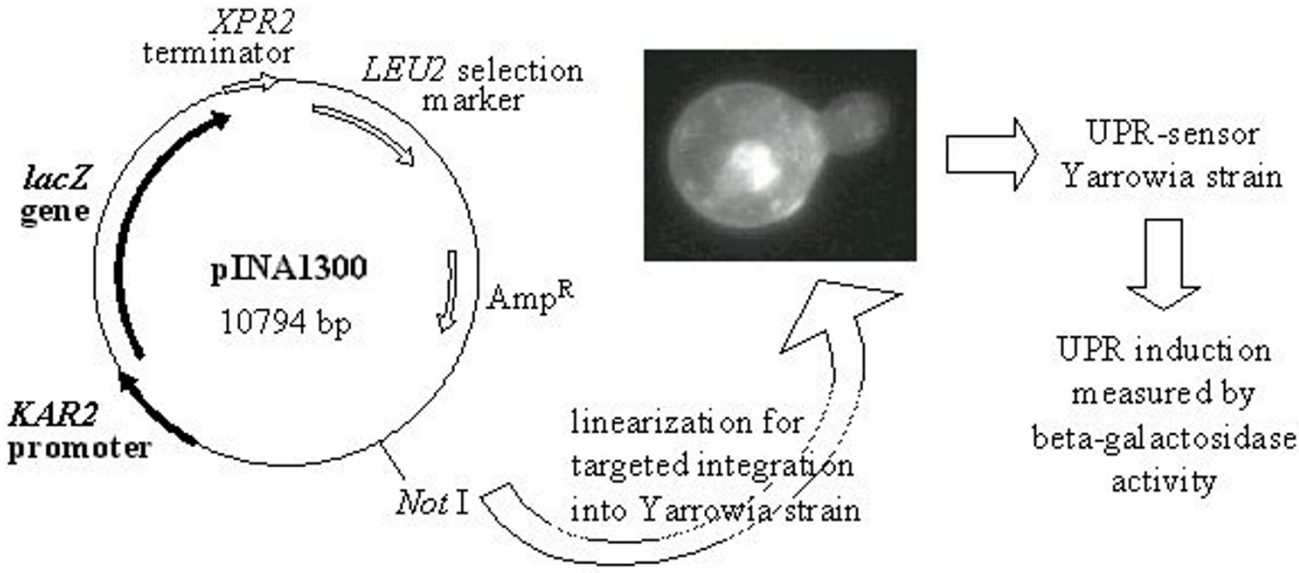

Figure 1
Map of pINA1300 vector and scheme of the experiments.

Conclusion

The UPR is extensively studied in *Saccharomyces cerevisiae*, but we found it interesting to analyse it in *Yarrowia lipolytica*, in which the secretion system is co-translational, as in mammalian cells. Moreover, an efficient expression/secretion system has been developed in this yeast [2], and the data obtained could contribute to improve further its performances. The use of the pINA1300 vector will allow us to detect UPR in *Yarrowia* strains, and to measure its level of induction. This UPR-sensor will be used to analyse UPR induction in various conditions, such as the stress imposed on *Yarrowia* strains by the production of different heterologous proteins.

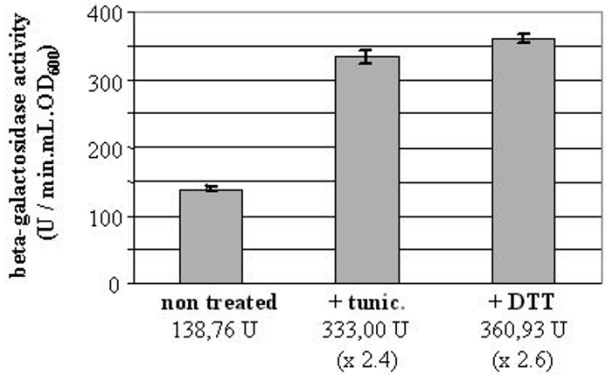

Figure 2

References

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2. Madzak C, Gaillardin C, Beckerich JM: **Heterologous protein expression and secretion in the non-conventional yeast *Yarrowia lipolytica* : a review.** *J Biotechnol* 2004, **109:**63-81.