TRANSCRIPTIONAL CONTROL OF THE XPR2 PROMOTER IN THE YEAST *Yarrowia lipolytica*

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The yeast *Yarrowia lipolytica* has been used for the large scale production of single cell proteins and organic acids like citric acid, for fatty acids bioconversion and, more recently, for the synthesis and secretion of heterologous proteins valuable for the pharmaceutical or agrofood industries. Improving some of these processes may obviously benefit from a better knowledge of the basic biology of this organism, for example protein secretion or control of gene expression. This talk will focus on recent developments in gene expression control.

For both historical and practical reasons, the promoter of the XPR2 gene encoding an extracellular alkaline protease (AEP) has been widely used to direct heterologous protein production in *Y. lipolytica*. The XPR2 promoter is silent in early exponential phase and strongly induced in late exponential phase where it directs the synthesis of 20 f total mRNAs. Its regulation is however complex and poorly understood, a feature directly limiting its utilization: XPR2 is induced in a growth phase dependent manner (when the yeast-mycelium transition occurs), in a specific pH window (pH6 to 8), and its full induction requires high amounts of peptones in the growth medium (which complicates further purification of secreted proteins). We decided to construct variants of this promoter, or strains with improved genetic backgrounds, in order to get rid of some of these limitations without affecting the overall efficiency of this promoter.

Deletion analysis of the promoter evidenced two major UAS, one close to the TATA box and the other 700 bp upstream, both of which are permanently bound by proteins *in vivo*. The role of each UAS was investigated using the LEU2 TATA box as a reporter basal element. In this system, the upstream UAS drives a constitutive expression, while the downstream UAS seems to respond to several environmental factors, including pH, carbon and nitrogen availability. When several copies of the upstream UAS are tandemly inserted upstream from the LEU2 TATA box, a strong constitutive expression is observed. This hybrid promoter is at least as efficient as the wild type one, but can now be used in a simple synthetic medium.

Trans-acting factors involved in transcriptional control of XPR2 have been looked for sing several genetic screens. Recessive mutations preventing XPR2 derepression identified four unlinked genetic loci, PAL1 to PAL4. PALI1, -2 and -3 are XPR2 specific, and do not affect the expression of the acid extracellular protease (AXP), the regulation of which mimicks that of XPR2 but for the pH (AXP is induced at pH lower than 5.5). PAL4 mutants however express AXP irrespective of the pH of the growth medium, and never express AEP. A dominant multicopy suppressor of all four PAL mutations was isolated from a replicative library. It identifies a homologue of the Zn-domain containing transcriptional factors Rim1p (controlling entry into meiosis in *S. cerevisiae*) and PacCp (controlling expression of pH sensitive genes in *A. nidulans*). The Y1RIM1 suppressor form encodes actually a C-terminally truncated version of Y1Rim1p, which apparently renders XPR2 expression independent of all PAL genes identified so far. Deletion of Y1RIM1 abolishes XPR2 expression, but has no effect on AXP expression at pH4. In a second screen, mutants able to express XPR2 under acidic conditions were searched for. A monogenic dominant mutation PAL5d was identified, which promotes AEP expression and AXP shut down irrespective of the pH. Although the mutants behave as if they were always growing under alkaline conditions, they are not detectably affected in the ability to regulate cytoplasmic pH in response to extracellular pH changes. XPR2 expression in this context is still dependent on all other environmental conditions, showing that pH is an independent signal for XPR2 regulation. The PAL5 gene has been cloned and identifies a new protein, with a possible transmembrane domain and a calmoduline dependent-kinase target. Epistasis studies suggest that PAL5 acts downstream from PAL4 and upstream from RIM1, PAL1,-2 and -3. Deletion of PAL5 results in an intermediate activation of both XPR2 and AXP expression irrespective of the pH.

Strains carrying the PAL5d allele may be useful for XPR2-driven expression of foreign proteins irrespective of the pH.

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