# Dechipering the diverse role of Gal4 transcription factor in the regulation of central carbon metabolism of *Saccharomycetaceae* yeasts

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**Abstract:**

The Crabtree effect is defined as the ability of certain yeasts to ferment high concentrations of sugars into ethanol and carbon dioxide under aerobic conditions. This multifactorial phenotype established in the *Saccharomycetaceae* clade around 100 million years ago, appearing at different branches of the phylogenetic tree following the loss of the respiratory complex I, the horizontal gene transfer of *URA1* or whole genome duplication (WGD).

A recent study revealed that aerobic fermentation can emerge in the Crabtree-negative yeast *Komagataella phaffii* in response to the overexpression of *CRA1*, encoding a transcription factor homologous to Gal4. While Gal4 regulates the Leloir galactolytic pathway in the model Crabtree-positive yeast *Saccharomyces cerevisiae*, Cra1 controls glycolytic genes in *K. phaffii*, a species unable to metabolize galactose.

Here, we investigated the regulatory role of Gal4 in *Zygosaccharomyces parabailii*, a mildly Crabtree-positive hybrid yeast of industrial relevance. Based on bioinformatics indication, we hypothesized that its two *GAL4* copies may perform a dual regulatory role, controlling both galactolytic and glycolytic genes, depending on the genomic background. To test this hypothesis, as first we overexpressed *CRA1* and *GAL4* variants derived from *K. phaffii* and *Zygosaccharomyces bailii* (*sensu lato*), respectively, in a *gal4Δ S. cerevisiae* strain, interpreting the findings in the light of the evolutionary path of Gal4 and its role in the central metabolism of *Saccharomycetaceae* yeasts.

Our results support a model in which Gal4 originated as a generalist transcription factor capable of inducing glycolytic gene expression, as observed in *K. phaffii*, and gradually specialized toward activation of the Leloir pathway in *S. cerevisiae*. This evolutionary trajectory highlights that diversification of metabolic strategies in *Saccharomycetaceae* is driven not by Gal4 protein divergence but by rewiring of its genomic binding profile. Together, these observations pinpoint a putative role of *GAL4* as an overlooked factor in the regulation of Crabtree phenotype in *Saccharomycetaceae* yeasts and suggest a possible molecular mechanism for regulating its penetrance.