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A Novel Mechanism of Responding to High Salt Concentration by Yeast Hkr1p and Molecular Breeding of Highly Salt-Tolerant Yeast.

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Summary

HKR1 was originally isolated from the genome of *Saccharomyces cerevisiae* as a gene that confers resistance to HM-1 killer toxin produced by the killer yeast *Lindnera mrakii* (synonym *Hansenula mrakii*). *HKR1* is an intronless gene with a 5.4 kb ORF encoding a mucin-like multidomain transmembrane protein, Hkr1p. Hkr1p contains a consensus sequence of EF hand, a calcium-binding motif and the DNA-binding leucine zipper motif in its cytoplasmic tail, and has actually been known as an osmosensor of the HOG MAP kinase complex. We recently found that *HKR1* has another cryptic promoter in its exon and is transcribed not only from the promoter in the 5' upstream region but also from the region around the 3330th nucleotide (nt. $^{\#}$3330) from the translation initiation site. In addition, it has been confirmed that the transcriptional activity of the exonic promoter is silenced by its upstream sequence within the exon of *HKR1*. In this study, we investigated whether the suppressed transcription is restored by external conditions such as osmotic pressure by using reporter assay systems. Plasmids containing various length of the exonic promoter region were constructed and a fluorescence protein gene or the *lacZ* gene of *Escherichia coli* was ligated to each promoter sequence, introduced into *S. cerevisiae* cells, then expression levels were evaluated by measuring the fluorescence intensity or β-galactosidase activity. The maximum transcriptional activity was observed when the reporter genes were ligated to the 410 bp-long region of *HKR1* starting at the nt. $^{\#}$3000 through the ATG (^{3409}ATG) which corresponds to the internal translation initiation site (^{1137}Met). A significantly lower transcriptional activity was detected when the reporter genes were ligated to the region between the nt. $^{\#}$2600 and ^{3409}ATG. These results suggest that the region between the nt. $^{\#}$3000 and the nt. $^{\#}$3330 is the core of the *HKR1* exonic promoter and its upstream sequence functions as a silencer-like regulator. Interestingly, the suppressed transcription was partially restored when the transformants were cultured under high osmotic pressure conditions. These observations suggest a novel mechanism of eukaryotic gene regulation with multiple promoters, one of which is even located within an exon and is conditionally activated to express a latter portion of the protein. It could be a remarkable example of how a limited number of genes can generate more complex biological phenomena.