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Multiple transcripts regulate glucose-triggered mRNA decay of the lactate transporter *JEN1* from *Saccharomyces cerevisiae* ☆

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Abstract

The *Saccharomyces cerevisiae JEN1* gene encoding the lactate transporter undergoes strong catabolic repression at both transcriptional and post-transcriptional levels. *JEN1* mRNA decay is greatly accelerated upon the addition of a pulse of glucose, fructose or mannose to induced cell cultures. Mapping of the 5′UTRs and 3′UTRs of *JEN1* transcripts revealed multiple transcription start-sites located at position −51, +391 or +972, depending on the cell culture conditions. The presence of the *JEN1*(+391) transcript correlated with rapid glucose-triggered mRNA degradation of the *JEN1*(−51) transcript, whereas when the small transcript started at position +972, the *JEN1*(−51) mRNA turnover rate was unaffected. Overexpressed *JEN1*(+391) transcript accelerated *JEN1*(−51) mRNA decay in all conditions tested but was not translated. We propose that the *JEN1*(+391) transcript may have a "sensor-like" function, regulating glucose-triggered degradation of *JEN1*(−51) protein-coding mRNA.

Keywords: Yeast; mRNA degradation; Glucose repression; *JEN1*

Saccharomyces cerevisiae employs more than half the genome in response to various environmental changes [1]. Microarray analysis of mRNA expression in yeast showed that nearly 20% of all yeast mRNAs are down-regulated and that nearly 14% are up-regulated at least twofold upon entering the diauxic shift [2]. When glucose, or another rapidly fermenting sugar, is abundant, genes involved in mitochondrial biogenesis and oxidative phosphorylation as well as genes involved in transport and metabolism of non-fermentable carbon sources are shut off, while genes involved in glycolysis/fermentation are active [3–8].

An increase in mRNA degradation in the presence of glucose has been reported [9], but its mechanism has not yet been uncovered. To date, only few cases of glucose-accelerated mRNA degradation have been demonstrated in *S. cerevisiae*. These include the *SDH2* mRNA, which encodes the iron-protein subunit (Ip) of succinate dehydrogenase [10], the functionally related *SDH1* mRNA, coding for the flavoprotein subunit (Fp) [11], the *SUC2* mRNA encoding invertase [12], meiotic mRNAs such as *SPO13* [13], and the gluconeogenic *PCK1* mRNA [14]. Very low glucose concentrations (<0.02%, w/v) have been reported to accelerate mRNA turnover for a limited number of genes, namely *PCK1*, *FBP1* [14,15], and *HSP12* [16]. In the latter case, however, only the transcription is affected, while the mRNA degradation rate is unaltered.

The mRNA 5′UTR seems to be a major element regulating glucose-stimulated mRNA decay. This is the case for the *SDH2* [11], *SDH1*, and *SUC2* mRNAs [17]. There appears to be no common properties associated with these 5′UTRs: no shared consensus sequences

☆ *Abbreviations:* 3′UTR/5′UTR, 3′/5′ untranslated regions.

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for protein binding have been identified, nor were characteristic secondary structures, overall length of the 5′UTR neither context of the start codon [9,17] found to be involved. Instead, competition between the decapping machinery and the assembly of the translation initiation complex for the 5′cap region has been proposed [17,18]. Since decapping is a prerequisite for glucose-induced turnover [19], it is postulated that the addition of glucose causes the 5′cap of the mRNA to become accessible to the decapping enzyme. Regulatory sequences located at the 3′UTRs that respond to environmental cues have also been reported [20].

In *S. cerevisiae*, active lactate transport across plasma membrane is strictly dependent on the expression of *JEN1* [21]. Upon the addition of 1,10-phenanthroline, *JEN1* mRNA half-life is 15 min, while the addition of glucose greatly accelerates mRNA decay [22]. Although this gene was reported to have two transcripts [23], the present work shows that *JEN1* encodes three different transcripts starting at positions −51, +391 or +972. We also provide evidences indicating that the *JEN1* small transcript, starting at +391, senses glucose availability and regulates the degradation of the large *JEN1*(−51) mRNA.

Materials and methods

Strains and growth conditions. *Saccharomyces cerevisiae* strains used and their genotypes are listed in Table 1. YP (yeast extract 1.0%, w/v, peptone 1.0%, w/v) and YNB (yeast nitrogen base 0.67%, w/v) media were supplemented with the required carbon sources: DL-lactic acid (0.5%, v/v, pH 5.0), ethanol (3.0%, w/v), acetic acid (0.5%, v/v, pH 6.0), glucose (2.0%, w/v), mannose (2.0%, w/v) or fructose (2.0%, w/v). YNB-glucose and YNB-lactic acid without methionine (−MET) were employed when indicated. Growth was carried out with shaking (160 rpm) at 28 °C.

Plasmids. The plasmid pT12 [21] contains the *JEN1* gene starting at position −435 bp from the translation start site; pDS1 [24] contains the *JEN1* ORF, including 51 bp upstream of the ATG under the control of the constitutive *GPD* promoter in the p416GPD plasmid [25]. To obtain the YEpXJ4 construct, *JEN1* promoter was amplified from *S. cerevisiae* W303-1A genomic DNA using the primers JEN1-*Xho*I (5′-TTTTCT CGAGGTATAAACGCACAGTATG-3′) and J4*Pst*I (5′-TTCTGCA GTAATTGACGACGACATATTTTCAG-3′). The resulting fragment, containing 1037 bp of the upstream region and the first 6 codons of *JEN1*, was inserted into the *lacZ* expression vector YEp366 [26]. All constructions were confirmed upon sequencing.

RNA analysis. RNA analysis was performed as previously described [22]. An internal 720 bp fragment of *FBP1* and the 1021 bp *Eco*RV–*Pvu*II inner fragment of *lacZ* were used as probes for the expression of these genes. For mRNA half-life time determination, transcription was efficiently inhibited by the addition of 1,10-phenanthroline (0.1 mg ml^{-1}) [27]. Transcript half-life times ($t_{1/2}$ mRNA) were calculated by applying a non-linear regression equation to the values fitting the initial slope of the mRNA decay semi-log plot and calculating the value correspondent to the time point where 50% of the initial mRNA levels were present. The half-life times reported represent the mean value obtained from at least three independent experiments.

Rapid amplification of cDNA ends experiments. Rapid amplification of cDNA ends (RACE) experiments were performed using the First-Choice RLM-RACE Kit (Ambion, Austin, TX). Primers used were as follows: JRACEouter (5′-CCATAGCAATACCGGTTATCC-3′); JRACEinner (5′-GTAATGTACGGCCACTTTC-3′); JA (5′-GTCTTT GGTGTCTGGGGTATC-3′); JC (5′-GATACCCCAGACACCAAA GAC-3′); JD (5′-GATAAACCGGCAACCAAAGC-3′); and JE (5′-C GTGATTTGTCCTCTCCTGTTATG-3′).

JEN1 in silico analysis. The search for putative TATA boxes in *JEN1* was performed with the RSA-tools program (http://www.ucmb.ulb.ac.be/bioinformatics/rsa-tools/), using the consensus sequence TATAWAW, obtained from SCPD—The Promoter Database of *S. cerevisiae* (http://cgsigma.cshl.org/jian/). The *JEN1* 3′-processing site element predictions were made using the DSM (HMM) method [28], available online at http://bmerc-www.bu.edu/polyA/, and according to the literature [29–31].

Measurement of transport activity. The measurement of labelled lactic acid uptake was performed as described previously [22].

Results

JEN1 sugar-triggered mRNA degradation

Saccharomyces cerevisiae W303-1A grown in YP-lactic acid presents a half-life time of 15 min for *JEN1* mRNA decay [22]. In the present work, *JEN1* mRNA was significantly destabilized by glucose at all concentra-

Table 1
Saccharomyces cerevisiae strains

Lab collection	Strain; relevant genotype	Source or reference
BLC001	W303-1A; *a ade2 leu2 his3 trp1 ura3*	[38]
BLC002	W303-1B; *α ade2 leu2 his3 trp1 ura3*	[38]
BLC203	W303-1A *jen1*Δ::*HIS3*	[21]
BLC503	YSH 434; W303-1A *mig1*Δ::*LEU2*	S. Hohmann
BLC504	YSH 310; W303-1A *hxk2*Δ::*LEU2*	S. Hohmann
BLC470	CEN.PK2-1C; *a leu2 trp1 ura3 his3 MAL2-8^c SUC2*	K.-D. Entian
BLC475	CEN.PK2-1D; *α leu2 trp1 ura3 his3 MAL2-8^c SUC2*	K.-D. Entian
BLC495	CEN.PK656-2D; *a leu2 trp1 ura3 his3 MAL2-8^c SUC2 jen1(1,398)::loxP-Kan-loxP-TPI1p*	This work
BLC497	CEN.PK277-2D; *a leu2 trp1 ura3 his3 MAL2-8^c SUC2 jen1(41,1810)::loxP-Kan-loxP*	This work
BLC496	CEN.PK657-3B; *a leu2 trp1 ura3 his3 MAL2-8^c SUC2 jen1(1,398)::loxP-Kan-loxP-MET25p*	This work
BLC500	W303*lcr1*; W303-1A *cyr1*^{met1876}	[32]
BLC528	W303-1B × CEN.PK656-2D	This work
BLC527	CEN.PK2-1D × CEN.PK656-2D	This work
BLC540	W303-1A × CEN.PK2-1D	This work
BLC573	CEN.PK2-1D × CEN.PK657-3B	This work