View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Universidade do Minho: RepositoriUM

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochemical and Biophysical Research Communications 332 (2005) 254–262

BBRC

www.elsevier.com/locate/ybbrc

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

R.P. Andrade [a,c], P. Kötter [b], K.-D. Entian [b], M. Casal [a,*]

[a] *Centro de Biologia, Departamento de Biologia, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

[b] *Institut für Mikrobiologie, Johann Wolfgang Goethe-Universität Frankfurt, Marie-Curie-Straße 9, 60439 Frankfurt, Germany*

[c] *Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

Received 18 April 2005

Available online 30 April 2005

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.
© 2005 Elsevier Inc. All rights reserved.

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.

* Corresponding author. Fax: +351 253 678980.
E-mail address: mcasal@bio.uminho.pt (M. Casal).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.04.119

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

tions tested (2.0%, 0.2%, and 0.02%, w/v), presenting half-life values of 3–4 min (Table 2). This was independent of the transcription factor Mig1p since *JEN1* mRNA half-life values of 4 and 5 min were obtained in the W303 *mig*1Δ strain (Table 2). An isogenic strain carrying the hexokinase-deleted *hxk*2Δ allele was also studied and a slight decrease of *JEN1* mRNA decay could be observed. This could be due to an alleviation of transcriptional repression, rather than mRNA stabilization. In the wild-type, *mig*1Δ, and *hxk*2Δ strains, *JEN1* mRNA degradation was equally stimulated upon the addition of a pulse of mannose or fructose (2.0%, w/v) (Table 2), whereas the addition of a pulse of ethanol did not accelerate *JEN1* mRNA degradation (not shown).

JEN1 mRNA decay presented a very different behaviour in *S. cerevisiae* YP-lactic acid-grown CEN.PK2-1C cells. *JEN1* mRNA retained a half-life of 17–20 min independently of the addition of glucose to the cell culture (Fig. 1A). This was specific for the gene *JEN1* since *FBP1* mRNA decay was accelerated by glucose (Fig. 1A), as previously described [15]. The diploid W303-1A × CEN.PK2-1D, strain BLC 540, presented a *JEN1* mRNA half-life of 3 min in the presence of glucose (2.0%) (Table 2, Fig. 1A), indicating that the absence of glucose-triggered *JEN1* mRNA degradation in the CEN.PK2-1C strain was a recessive phenotype. The CEN.PK strains lack the cAMP peak upon addition of glucose to derepressed cells, which is due to a mutation within the *CYR1* gene encoding adenylate cyclase [32]. Introduction of the $cyr1^{met1876}$ mutant allele in a W303 strain (strain W303-1A $lcr1^{-}$—for lack of cAMP responses) did not change the *JEN1* mRNA half-life time observed (Table 2). This was indicative of a cAMP-independent pathway mediating glucose-triggered *JEN1* mRNA decay.

When *S. cerevisiae* CEN.PK2-1C cells were grown in media containing a carbon source other than lactic acid, such as YP-ethanol, the *JEN1* mRNA half-life was reduced from 26 to 7 min upon the addition of glucose to the culture (Fig. 1A). The physiological conditions under which the cells grew greatly influenced the effect of glucose on *JEN1* mRNA decay. The *FBP1* mRNA half-life times were comparable to those previously obtained in lactic acid-grown cells (48 min vs. 18 min), indicating that this observation was specific for the gene *JEN1*.

The role of transcriptional regulation in glucose-triggered mRNA decay

Cells of *S. cerevisiae* W303 *jen1*Δ and CEN.PK *jen*1Δ strains were transformed with the pT12 plasmid containing the *JEN1* gene [21]. A pulse of 1,10-phenanthroline without or with glucose (2.0%, w/v) was added to the transformed cells induced in YNB-lactic acid for 4 h. Glu-

Table 2
JEN1 mRNA half-lives determined in YP-lactic acid-grown cells of distinct *S. cerevisiae* strains upon the addition of a pulse of 1,10-phenanthroline with or without different sugars

	$t_{1/2}$ mRNA (min) Strain					
	W303-1A	W303*mig*1Δ	W303*hxk*2Δ	W303lcr^{-}	CEN.PK2-1C	BLC540
Glucose 2.0%	3	5	8	5	19	3
Glucose 0.2%	4	4	6	4	17	—
Glucose 0.02%	4	4	7	4	20	—
Fructose 2.0%	5	6	7	—	—	—
Mannose 2.0%	4	5	6	—	—	—
1,10-Phenanthroline	15	15	13	15	20	12

The mRNAs were monitored by Northern-blot analysis using *PDA1* gene as internal standard.
—, not determined.

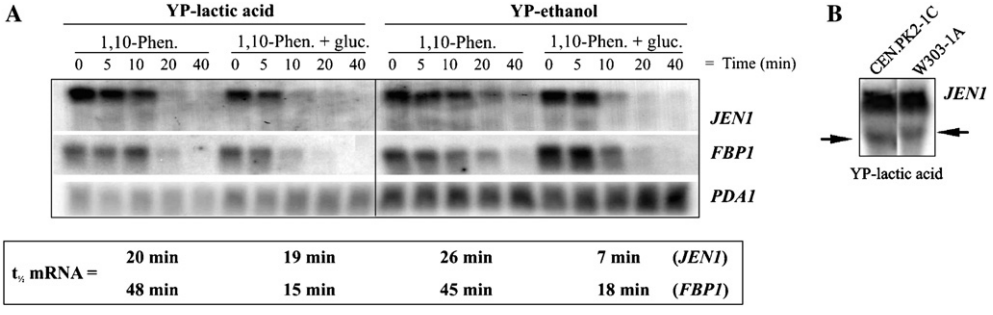

Fig. 1. (A) Northern-blot analyses of *JEN1* and *FBP1* mRNA decay in YP-lactic acid or YP-ethanol-grown *S. cerevisiae* CEN.PK2-1C cells. The *JEN1* mRNA half-life values shown correspond to the *JEN1*(−51) transcript. Time zero corresponds to cells collected immediately before the addition of a pulse of 1,10-phenanthroline with or without glucose (2.0%, w/v). (B) *JEN1* transcripts observed in *S. cerevisiae* W303-1A and CEN.PK2-1C strains exponentially growing in YP-lactic acid.

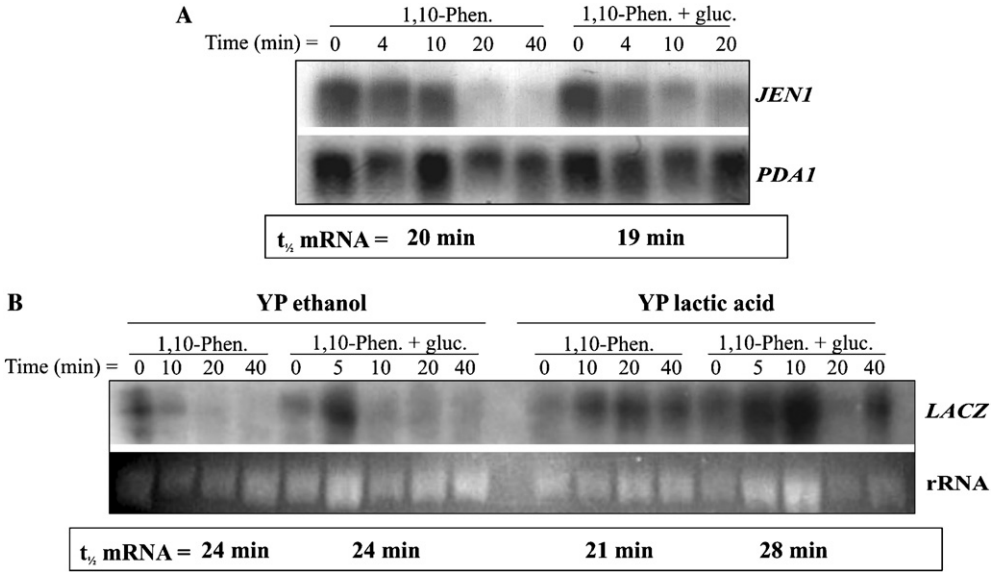

Fig. 2. Northern-blot analyses and determination of mRNA half-life times: (A) *GPDp::JEN1* mRNA decay in YNB-acetic acid-grown *S. cerevisiae* W303 *jen1*Δ(pDS1) cells. The mRNA half-life values correspond to the *JEN1*(−51) transcript; (B) *JEN1p::lacZ* mRNA degradation in *S. cerevisiae* W303 (YEpXJ4) cells grown in YNB-glucose and incubated 4 h in YNB-lactic acid. Time zero corresponds to cells collected immediately before the addition of a pulse of 1,10-phenanthroline with or without glucose (2.0%, w/v).

cose-triggered *JEN1* mRNA decay was more pronounced in W303-1A (mRNA half-life of 20 min without glucose vs. 7 min with glucose) than in CEN.PK2-1C cells (18 min vs. 14 min) (not shown). This result was similar to what was observed for the wild-type cells. Accordingly, sequencing of the *JEN1* ORF and 1000 bp from its promoter showed that this genomic region was identical in W303-1A and CEN.PK2-1C *S. cerevisiae* strains.

A similar experiment was performed with the W303 *jen*1Δ(pDS1) strain, containing the *JEN1* ORF plus the full 5′UTR driven by the GPD promoter, grown in the conditions described by Soares-Silva et al. [24]. In these conditions, we observed that glucose was no longer capable of accelerating mRNA degradation (Fig. 2A).

Finally, *S. cerevisiae* W303-1A and CEN.PK2-1C cells transformed with a *JEN1p::lacZ* fusion (YepXJ4 plasmid) were grown in YNB-glucose and derepressed 4 h in YNB-lactic acid or in YNB-ethanol. *lacZ* mRNA half-life was determined and, in all the conditions tested, the values found were of the same order of magnitude of the value reported in the literature for *lacZ* mRNA half-life in yeast, approximately 26 min [33] (Fig. 2B shows representative results). These results show that neither the *JEN1* promoter (−1037 to +18) nor the 5′UTR (−51 to +1) per se is capable of conferring glucose-sensitive mRNA decay, contrarily to what has been described for other systems [17].

Mapping of JEN1 mRNA 5′UTR and 3′UTR

JEN1 presents two transcripts detectable in standard Northern-blot experiments (Fig. 1B). With the purpose of characterizing the 5′UTRs of these transcripts, 5′RACE experiments were performed with total RNA extracted from *S. cerevisiae* W303-1A and CEN.PK2-1C cells growing exponentially in YP-lactic acid medium. Using the nested primer pair JRACEouter/JRACEinner, two PCR products were obtained in *S. cerevisiae* W303-1A (Fig. 3, lane 1) matching with the presence of two transcripts. Although diverse PCR conditions were employed (not shown), only one PCR fragment could be found in CEN.PK2-1C cells, corresponding to the *JEN1*(−51) transcript (Fig. 3, lane 2). A second pair of nested primers (JC/JD) was employed and a fragment of approximately 500 bp was observed (Fig. 3, lane 3). When using JD as the outer primer, and both JRACEinner and JC as internal primers, both 700 and 500 bp bands were obtained, supporting the previous results (Fig. 3, lane 4). Sequencing of the 5′ RACE products revealed that the larger transcripts from both strains were identical, beginning at position −51. In the growth conditions utilized, the smaller transcript started at position +391 in *S. cerevisiae* W303-1A cells and at position +972 in CEN.PK2-1C. In fact, a difference in the size of the smaller *JEN1* transcripts was already visible in Northern-blot experiments (Fig. 1A, arrows). Analogous 5′RACE experiments were performed with total RNA extracted from *S. cerevisiae* CEN.PK2-1C cells grown in YP-ethanol. The 5′ ends of the two fragments obtained started at positions −51 and +391 (Fig. 3, lane 6), corresponding to the same transcripts found in *S. cerevisiae* W303-1A cells. In both cases, *JEN1*(−51) mRNA degradation was triggered in the presence of glucose.

5′RACE analysis was further applied to *S. cerevisiae* W303 *jen1*Δ (pDS1) cells grown in YNB-acetic acid

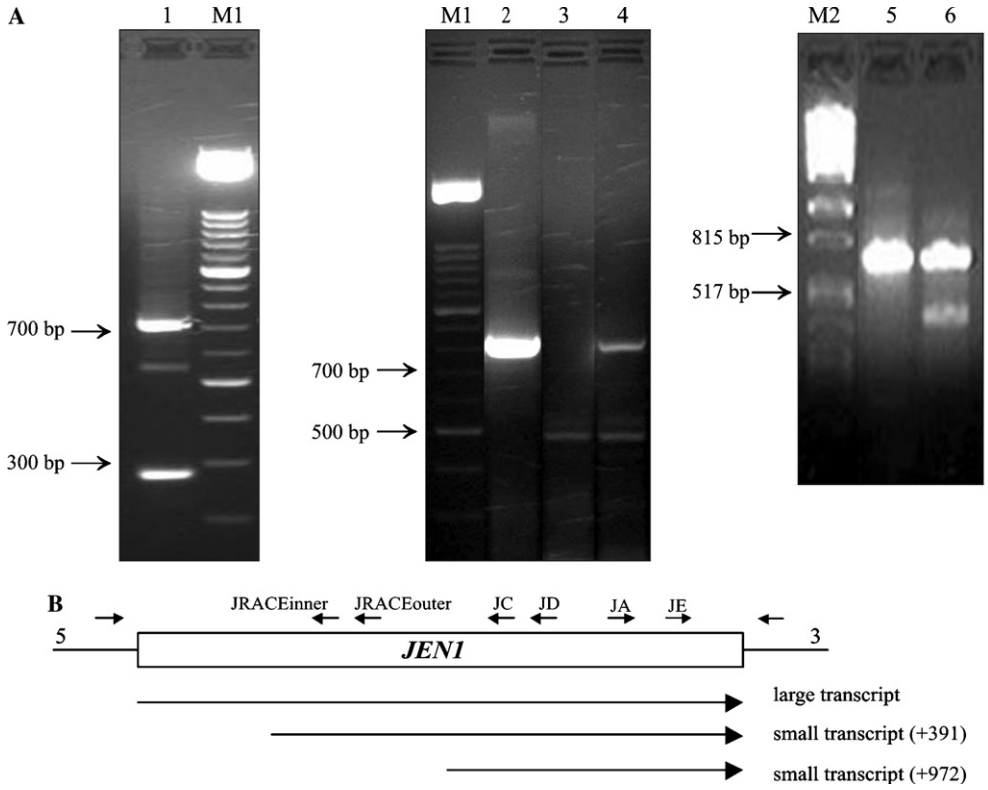

Fig. 3. 5′RACE analyses of *JEN1* mRNA in distinct *S. cerevisiae* strains. (A) Agarose gel electrophoresis of the RT-PCR products using the *JEN1* nested primers: lanes 1, 2, 5, and 6, JRACEouter/JRACEinner; lane 3, JD/JC; and lane 4, JD/JRACEinner and JD/JC. Strains used: *S. cerevisiae* W303-1A grown in YP-lactic acid (lane 1); *S. cerevisiae* CEN.PK2-1C grown in YP-lactic acid (lanes 2–4) or in YP-ethanol (lane 6); and *S. cerevisiae* W303 *jen1*Δ(pDS1) grown in YNB-acetic acid (lane 5). M1, molecular weight marker XIV (Roche Applied Science); M2, λ*Pst*I. (B) Diagram showing the position of the primers used in the RACE assays.

medium and the pattern obtained in agarose gel electrophoresis (Fig. 3, lane 5) was equal to the one found for YP-lactic acid-grown *S. cerevisiae* CEN.PK2-1C cells (Fig. 3, lane 2). In both cases, glucose was incapable of accelerating *JEN1* mRNA decay.

To characterize the 3′UTRs of the *JEN1* transcripts, 3′RACE experiments were performed with total RNA extracted from *S. cerevisiae* W303-1A and CEN.PK2-1C cells grown in YP-lactic acid medium until mid-exponential growth phase. Using the primer JA or JE, PCR products of similar sizes were obtained (not shown). Four individual clones containing PCR fragments obtained from each strain were sequenced. The mRNA 3′UTRs of both W303 and CEN.PK strains were found to be very similar. Polyadenylation started 123–138 bp downstream of the stop codon.

JEN1(+391) small transcript overexpression

Saccharomyces cerevisiae CEN.PK656-2D (*TPI1p*::*jen1*Δ(1,398)) and CEN.PK657-3B (*MET52p*::*jen1*Δ(1,398)) strains were constructed, containing a strong-constitutive (TPI1p) and a weak-repressible promoter (MET25p) fused to the transcription start site of the *JEN1*(+391) transcript, respectively. No measurable lactate permease activity could be found in either strain (not shown), indicating that the *JEN1*(+391) transcript did not have a lactate transport function in the cell. In *S. cerevisiae* CEN.PK656-2D cells grown in YP-lactic acid medium, *JEN1* mRNA half-life was 54 min (not shown). Although the transcript decay rate was slightly affected by the presence of glucose (41 min, not shown), it remained stabilized when compared to the larger transcript in W303-1A cells (Table 2).

Total RNA from exponentially YP-lactic acid-growing cells of the diploid *S. cerevisiae* strains BLC527 [CEN.PK2-1D, wild-type × CEN.PK656-2D, *TPI1p*::*jen1*Δ(1,398)] and BLC528 [W303-1B, wild-type × CEN.PK656-2D, *TPI1p*::*jen1*Δ(1,398)] was analysed on Northern blot. When greatly overexpressed, the *JEN1*(+391) transcript destabilized the *JEN1*(−51) mRNA, regardless of the presence of glucose and of the strain genetic background (Fig. 4). This effect was not due to generalized mechanisms affecting the mRNA degradation machinery, since the control *PDA1* mRNA preserved its stability. A similar analysis was performed with the diploid *S. cerevisiae* strain BLC573 [CEN.PK2-1D, wild-type × CEN.PK657-3B (*MET25p*::*jen1*Δ(1,398))]. In promoter-repression conditions, *JEN1* mRNA half-life in the absence of glucose was similar to that obtained

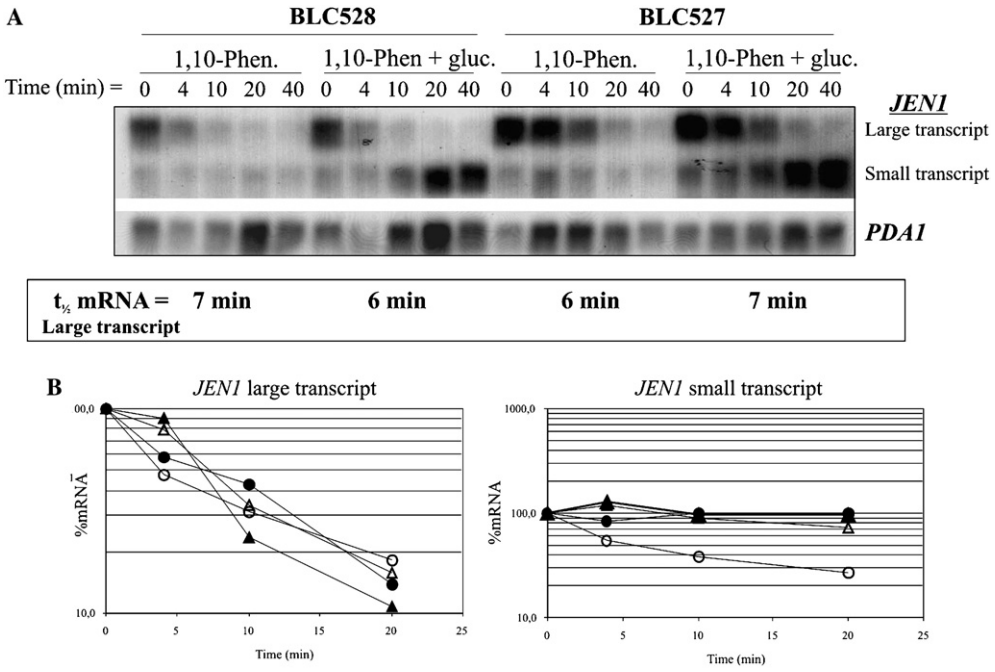

Fig. 4. *JEN1* mRNA half-life determination in *S. cerevisiae* BLC527 [CEN.PK2-1D, wild-type × CEN.PK656-2D, *TPI1p*::*jen1*Δ(1,398)] (●, ○) and BLC528 [W303-1B, wild-type × CEN.PK656-2D, *TPI1p*::*jen1*Δ(1,398)] (▲, △) by Northern-blot analysis. Time zero corresponds to YP-lactic acid-grown cells collected immediately before the addition of a pulse of 1,10-phenanthroline without (open symbol) or with (filled symbol) glucose (2.0%, w/v). (A) mRNA levels monitored by Northern-blot analysis at different times after the pulse. The mRNA half-life values shown correspond to the *JEN1*(−51) transcript. (B) Quantitation of the Northern blot. mRNA levels are expressed as a percentage of the signal at time zero.

in the wild-type CEN.PK2 strain (Fig. 5). When the cells were cultured in the absence of methionine, i.e., promoter-induction conditions, *JEN1* mRNA decay was already accelerated before glucose addition, and was evermore induced when a pulse of glucose was added to the cell culture. This set of experiments clearly illustrates the direct involvement of *JEN1*(+391) transcript in *JEN1*(−51) mRNA decay in response to the presence of glucose in the culture medium.

From the data presented we can infer that the second transcript regulates glucose-triggered *JEN1*(−51) mRNA decay. The effect of the *JEN1*(+391) transcript appears to involve the mRNA molecule directly, since it does not seem to be translated: Western blots per-

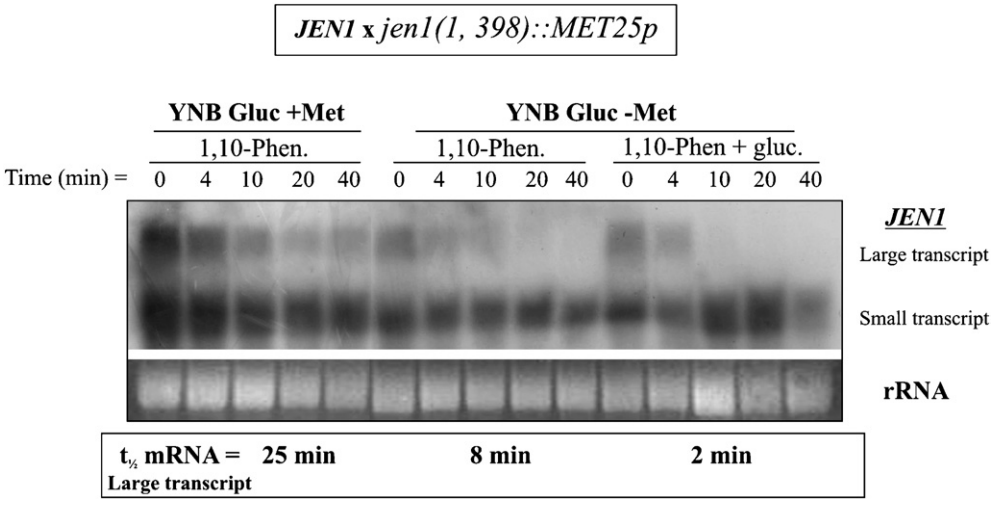

Fig. 5. Northern-blot determination of *JEN1* mRNA half-life in cells of *S. cerevisiae* BLC573 [CEN.PK2-1D, wild-type × CEN.PK657-3B (*MET25p*::*jen1*Δ(1,398))] grown in YNB-glucose and derepressed for 4 h in YNB-lactic acid with (promoter-repression conditions) or without (promoter-induction conditions) methionine. Time zero corresponds to cells collected immediately before the addition of a pulse of 1,10-phenanthroline without or with glucose (2.0%, w/v). The mRNA half-life values shown correspond to the *JEN1*(−51) transcript.

formed with a strain containing the green fluorescent protein (GFP) [34] or the HA epitope fused to the C-terminal region of Jen1p did not detect a second protein derived from *JEN1* (not shown).

Discussion

Glucose triggers JEN1 mRNA decay

JEN1 mRNA decay is greatly accelerated in the presence of glucose in *S. cerevisiae* W303-1A cells. This phenomenon, however, is strain-specific and dependent on the carbon source used for gene expression induction. Contrary to the *S. cerevisiae* W303-1A strain, *JEN1* mRNA turnover in lactic acid-grown cells of CEN.PK2-1C was "glucose-insensitive." However, in ethanol-grown cells the addition of glucose significantly accelerated *JEN1* mRNA decay.

JEN1 mRNA behaves as the gluconeogenic mRNAs, *FBP1* and *PCK1* [14,15], being destabilized by both high and very low glucose concentrations. A Mig1p-dependent pathway was shown to be involved in triggering *PCK1* and *FBP1* glucose-induced mRNA decay in response to high glucose signals (about 1.0%, w/v) [15]. In contrast, *JEN1* mRNA glucose-mediated decay mechanism is independent of the Mig1p pathway in all glucose concentrations tested. Besides its enzymatic activity in the glycolytic pathway, Hexokinase PII (Hxk2p) plays a regulatory function in the transcriptional regulation of different genes by glucose [35–37]. In the *S. cerevisiae hxk2* mutant, an inhibition of glucose-triggered *JEN1* mRNA decay could be observed, but most probably this could be related to a lack of complete transcriptional repression rather than mRNA stabilization.

The present work showed that *JEN1* transcriptional regulation is not the main factor responsible for accelerated *JEN1* mRNA decay in the presence of glucose. A strain containing a *JEN1p::lacZ* fusion was constructed and the *JEN1* promoter sequence was incapable of conferring glucose-induced degradation to *lacZ* mRNA. Work has been reported where the 5′UTR of the mRNA molecule is necessary and sufficient for glucose-triggered mRNA decay [17]. This was not the case for *JEN1*, since *JEN1p::lacZ* mRNAs contained the full *JEN1* 5′UTR.

Second transcripts regulate JEN1 mRNA decay

JEN1 presents two transcripts, as do many yeast genes, and no role for these second transcripts has been found in yeast genome. Computational analysis of the DNA sequence revealed the existence of two alleged TATA-boxes: one at position −134 and the other at +123. In the present work, mapping of the mRNA 5′UTRs was performed for *JEN1*. In all conditions tested, a 5′UTR of the *JEN1* large transcript begins at position −51. However, the place where the second transcript maps was found in two positions (+391) or (+972). In a first observation, the small transcript starts at position +391 in *S. cerevisiae* W303-1A and at +972 in *S. cerevisiae* CEN.PK2-1C cells grown in YP-lactic acid. These results led us to suspect the presence of a correlation with the difference found on the second transcript size. Further analysis revealed that in *S. cerevisiae* CEN.PK2-1C cells grown in YP-ethanol, where glucose had triggered *JEN1* mRNA decay, the transcripts obtained were the same as the ones found in *S. cerevisiae* W303-1A lactic acid-grown cells. Additionally, in *S. cerevisiae* cells containing the *GPDp::JEN1* fusion, the pattern for the second transcript was equal to the one found for lactic acid-grown *S. cerevisiae* CEN.PK2-1C cells. In both cases, glucose had not been able to induce *JEN1* mRNA accelerated degradation. From our study, a clear association between alternative *JEN1* small transcripts and the regulation of mRNA decay was found: the presence of the *JEN1*(+391) transcript consistently correlated with the observation of glucose-triggered mRNA degradation; when the small transcript started at position +972, *JEN1* mRNA turnover rate was unaffected by the addition of glucose to the medium. Regarding the analysis of 3′UTRs of the transcripts, no significant differences were found and putative poly(A) site-specific elements could be identified. Polyadenylation started 123–138 bp downstream of the STOP codon.

To further clarify these observations, strains overexpressing the *JEN1*(+391) transcript were constructed using weak-repressible (*MET25p*::*jen1*Δ(1,398)) and a strong-constitutive (*TPI1p*::*jen1*Δ(1,398)) promoters. Regardless of the conditions used, once the *JEN1*(+391) transcript was overexpressed, *JEN1*(−51) mRNA half-life was always reduced, both in the absence and in the presence of glucose.

De la Cruz et al. [17] observed that changes in mRNA stability correlated with changes in translational efficiency of the respective transcripts. They proposed a model for glucose-dependent mRNA decay in yeast, suggesting a competition between translation and degradation machinery for the 5′cap, which is affected by a signal from the carbon source present in the medium. Based on this model, a working model for *JEN1* mRNA glucose-triggered degradation was constructed (Fig. 6). We propose that the *JEN1*(+391) transcript may exist in two different interconvertible molecular "states" (Inactive vs. Active), regarding the capability to accelerate *JEN1*(−51) mRNA decay. In the absence of glucose, the main form present is the *JEN1* (+391) inactive state—translation of Jen1p occurs and only basal mRNA degradation is observed. Upon sensing even very low amounts of glucose in the medium, *JEN1*(+391) is converted to the active form, inhibiting translation and accelerating *JEN1*(−51) mRNA decay.

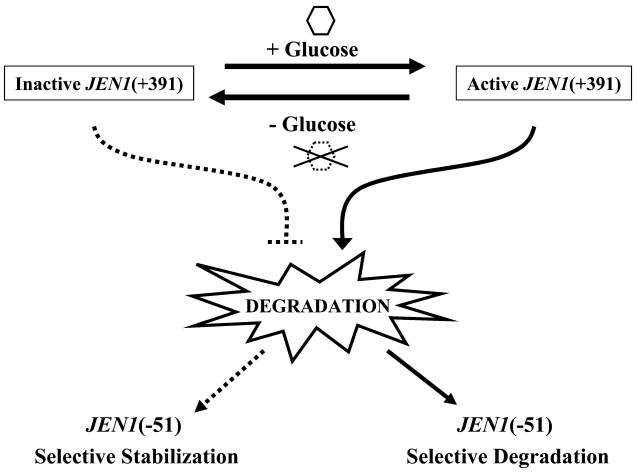

Fig. 6. Working model for *JEN1* mRNA glucose-triggered degradation, based on the model of selective translation and degradation of glucose-repressed mRNAs proposed by De la Cruz et al. [17]. The *JEN1*(+391) transcript may exist in two different interconvertible molecular "states" in the cell (inactive vs. active). In the absence of glucose, the main form present is the *JEN1*(+391) inactive state—only basal mRNA degradation is observed. Upon sensing even very low amounts of glucose in the medium, *JEN1*(+391) is converted to the active form, accelerating *JEN1*(−51) mRNA decay.

In the conditions where *JEN1*(+391) mRNA is overexpressed, the raised levels of *JEN1*(+391) active form would be sufficient to induce degradation regardless of the presence of glucose. Differential proportions of both forms in the cell would modulate the overall *JEN1* mRNA degradation observed.

Our findings indicate, for the first time, that an alternative small transcript may function as a sensor of sugar metabolism in yeast. Studies aiming at the clarification of the molecular mechanisms involved are underway.

Acknowledgments

We are grateful to Dr. Stefan Hohmann for providing strains. This study was supported by the Portuguese Grant POCTI/BIO/38106/2001 (Eixo 2, Medida 2.3, QCAIII-FEDER), by the Deutsche Forschungsgemeinschaft (SFB 579) and the Fonds der Chemischen Indistrie. R.P.A. was supported by the Portuguese Government (BD/15737/98; SFRH/BPD/9432/2002).

References

[1] H.C. Causton, B. Ren, S.S. Koh, C.T. Harbison, E. Kanin, E.G. Jennings, T.I. Lee, H.L. True, E.S. Lander, R.A. Young, Remodeling of yeast genome expression in response to environmental changes, Mol. Biol. Cell 12 (2001) 323–337.

[2] J.L. DeRisi, V.R. Iyer, P.O. Brown, Exploring the metabolic and genetic control of gene expression on a genomic scale, Science 278 (1997) 680–686.

[3] J.M. Gancedo, Yeast carbon catabolite repression, Microbiol. Mol. Biol. Rev. 62 (1998) 334–361.

[4] M. Carlson, Glucose repression in yeast, Curr. Opin. Microbiol. 2 (1999) 202–207.

[5] M. Johnston, Feasting, fasting and fermenting. Glucose sensing in yeast and other cells, Trends Genet. 15 (1999) 29–33.

[6] F. Rolland, J. Winderickx, J.M. Thevelein, Glucose-sensing and -signalling mechanisms in yeast, FEMS Yeast Res. 2 (2002) 183–201.

[7] Z. Yin, S. Wilson, N.C. Hauser, H. Tournu, J.D. Hoheisel, A.J.P. Brown, Glucose triggers different global responses in yeast, depending on the strength of the signal, and transiently stabilizes ribosomal protein mRNAs, Mol. Microbiol. 48 (2003) 713–724.

[8] R. De Caterina, R. Madonna, J. Hassan, A.D. Propicio, Nutrients and gene expression, World Rev. Nutr. Diet. 89 (2001) 23–52.

[9] I.E. Scheffler, B.J. de la Cruz, S. Prieto, Control of mRNA turnover as a mechanism of glucose repression in *Saccharomyces cerevisiae*, Int. J. Biochem. Cell Biol. 30 (1998) 1175–1193.

[10] A. Lombardo, G.P. Cereghino, I.E. Scheffler, Control of mRNA turnover as a mechanism of glucose repression in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 12 (1992) 2941–2948.

[11] G.P. Cereghino, D.P. Atencio, M. Saghbini, J. Beiner, I.E. Scheffler, Glucose-dependent turnover of the mRNAs encoding succinate dehydrogenase peptides in *Saccharomyces cerevisiae*: sequence elements in the 5′ untranslated region of the Ip mRNA play a dominant role, Mol. Biol. Cell 6 (1995) 1125–1143.

[12] G.P. Cereghino, I.E. Scheffler, Genetic analysis of glucose regulation in *Saccharomyces cerevisiae*: control of transcription versus mRNA turnover, EMBO J. 15 (1996) 363–374.

[13] R.T. Surosky, R. Strich, R.E. Esposito, The yeast *UME5* gene regulates the stability of meiotic mRNAs in response to glucose, Mol. Cell. Biol. 14 (1994) 3446–3458.

[14] J.J. Mercado, R. Smith, F.A. Sagliocco, A.J.P. Brown, J. Gancedo, The levels of yeast gluconeogenic mRNAs respond to environmental factors, Eur. J. Biochem. 224 (1994) 473–481.

[15] Z. Yin, R.J. Smith, A.J.P. Brown, Multiple signalling pathways trigger the exquisite sensitivity of yeast gluconeogenic mRNAs to glucose, Mol. Microbiol. 20 (1996) 751–764.

[16] E. De Groot, J.-P. Bebelman, W.H. Mager, R.J. Planta, Very low amounts of glucose cause repression of the stress-responsive gene *HSP1* in *Saccharomyces cerevisiae*, Microbiology 146 (2000) 367–375.

[17] B.J. De la Cruz, S. Prieto, I.E. Scheffler, The role of the 5′ untranslated region (UTR) in glucose-dependent mRNA decay, Yeast 19 (2002) 887–902.

[18] D.C. Schwartz, R. Parker, Mutations in translation initiation factors lead to increased rates of deadenylation and decapping in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 19 (1999) 5247–5256.

[19] S. Prieto, B.J. de la Cruz, I.E. Scheffler, Glucose-regulated turnover of mRNA and the influence of poly(A) tail length on half-life, J. Biol. Chem. 275 (2000) 14155–14166.

[20] A. Spicher, O.M. Guicherit, L. Duret, A. Aslanian, E.M. Sanjines, N.C. Denko, A.J. Giaccia, H.M. Blau, Highly conserved RNA sequences that are sensors of environmental stress, Mol. Cell. Biol. 18 (1998) 7371–7382.

[21] M. Casal, S. Paiva, R.P. Andrade, C. Gancedo, C. Leão, The lactate-proton symport of *Saccharomyces cerevisiae* is encoded by *JEN1*, J. Bacteriol. 181 (1999) 2620–2623.

[22] R.P. Andrade, M. Casal, Expression of the lactate permease gene *JEN1* from the yeast *Saccharomyces cerevisiae*, Fungal Genet. Biol. 32 (2001) 105–111.

[23] G.-F. Richard, C. Fairhead, B. Dujon, Complete transcriptional map of yeast chromosome XI in different life conditions, J. Mol. Biol. 268 (1997) 303–321.

[24] I. Soares-Silva, D. Schuller, R.P. Andrade, F. Baltazar, F. Cássio, M. Casal, Functional expression of the lactate permease Jen1p of *Saccharomyces cerevisiae* in *Pichia pastoris*, Biochem. J. 376 (2003) 781–787.

[25] D. Mumberg, R. Müller, M. Funk, Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds, Gene 156 (1995) 119–122.
[26] A.M. Myers, A. Tzagoloff, D.M. Kinney, C.J. Lusty, Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions, Gene 45 (1986) 299–310.
[27] R. Parker, D. Herrick, S.W. Peltz, A. Jacobson, Measurement of mRNA decay rates in *Saccharomyces cerevisiae*, in: C. Guthrie, G.R. Fink (Eds.), Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, vol. 194, Academic Press, London, 1991, pp. 415–423.
[28] J.H. Graber, G.D. McAllister, T.F. Smith, Probabilistic prediction of *Saccharomyces cerevisiae* mRNA 3′-processing sites, Nucleic Acids Res. 30 (2002) 1851–1858.
[29] Z. Guo, F. Sherman, Signals sufficient for 3′-end formation of yeast mRNA, Mol. Cell. Biol. 16 (1996) 2772–2776.
[30] E. Wahle, U. Rüegsegger, 3′-end processing of pre-mRNA in eukaryotes, FEMS Microbiol. Rev. 23 (1999) 277–295.
[31] J. van Helden, M. del Olmo, J.E. Pérez-Ortín, Statistical analysis of yeast genomic downstream sequences reveals putative polyadenylation signals, Nucleic Acids Res. 28 (2000) 1000–1010.
[32] M. Vanhalewyn, F. Dumortier, G. Debast, S. Colombo, P. Ma, J. Winderickx, P. van Dijck, J.M. Thevelein, A mutation in *Saccharomyces cerevisiae* adenylate cyclase, $Cyr1^{K1876M}$, specifically affects glucose- and acidification-induced cAMP signalling and not the basal cAMP level, Mol. Microbiol. 33 (1999) 363–376.
[33] I.J. Purvis, L. Loughlin, A.J.E. Bettany, A.J. Brown, Translation and stability of an *Escherichia coli* β-galactosidase mRNA expressed under the control of pyruvate kinase sequences in *Saccharomyces cerevisiae*, Nucleic Acids Res. 15 (1987) 7963–7974.
[34] S. Paiva, A.L. Kruckeberg, M. Casal, Utilization of green fluorescent protein as a marker for studying the expression and turnover of the monocarboxylate permease Jen1p of *Saccharomyces cerevisiae*, Biochem. J. 363 (2002) 737–744.
[35] K.-D. Entian, Genetic and biochemical evidence for hexokinase PII as a key enzyme involved in carbon catabolite repression in yeast, Mol. Gen. Genet. 178 (1980) 633–637.
[36] F. Randez-Gil, P. Sanz, K.-D. Entian, J.A. Prieto, Carbon source-dependent phosphorylation of hexokinase PII and its role in the glucose-signalling response in yeast, Mol. Cell. Biol. 18 (1998) 2940–2948.
[37] S. Hohmann, J. Winderickx, J.H. de Winde, D. Valckx, P. Cobbaert, K. Luyten, C. de Meirsman, J. Ramos, J.M. Thevelein, Novel alleles of yeast hexokinase PII with distinct effects on catalytic activity and catabolite repression of SUC2, Microbiology 145 (1999) 703–714.
[38] B.J. Thomas, R. Rothstein, Elevated recombination rates in transcriptionally active DNA, Cell 58 (1989) 819–830.