Role of the DHH1 Gene in the Regulation of Monocarboxylic Acids Transporters Expression in Saccharomyces cerevisiae

Sandra Mota1,2,3*, Neide Vieira1, Sónia Barbosa1, Thierry Delaveau3,4, Claire Torchet5,6, Agnès Le Saux7, Mathilde Garcia3,4, Ana Pereira1, Sophie Lemoine8,9,10, Fanny Couplier8,9,10, Xavier Darzacq8,9,10, Lionel Benard5,6, Margarida Casal1, Frédéric Devaux3,4,6, Sandra Paiva1

1 Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal, 2 Centre of Health and Environmental Research (CISA), School of Allied Health Sciences, Polytechnic Institute of Porto, Vila Nova de Gaia, Portugal, 3 Sorbonne Universités, Université Pierre et Marie Curie, UMR7238, Laboratoire de Biologie computationnelle et quantitative, Paris, France, 4 CNRS, UMR8226, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, Paris, France, 5 Sorbonne Universités, Université Pierre et Marie Curie UPMC, UMR8226, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, Paris, France, 6 Sorbonne Universités, Université Pierre et Marie Curie UPMC, UMR8226, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, Paris, France, 7 CNRS, FRE3630, Laboratoire de l’Expression Génétique Microbienne, Institut de Biologie Physico-Chimique, Paris, France, 8 École normale supérieure, Institut de Biologie de l’ENS, IBENS, Paris, France, 9 Inserm, U1024, Paris, France, 10 CNRS, UMR 8197, Paris, France

Abstract

Previous experiments revealed that DHH1, a RNA helicase involved in the regulation of mRNA stability and translation, complemented the phenotype of a Saccharomyces cerevisiae mutant affected in the expression of genes coding for monocarboxylic-acids transporters, JEN1 and ADY2 (Paiva S, Althoff S, Casal M, Leao C. FEBS Microbiol Lett, 1999, 170:301–306). In wild type cells, JEN1 expression had been shown to be undetectable in the presence of glucose or formic acid, and induced in the presence of lactate. In this work, we show that JEN1 mRNA accumulates in a dhh1 mutant, when formic acid was used as sole carbon source. Dhh1 interacts with the decapping activator Dcp1 and with the deadenylase complex. This led to the hypothesis that JEN1 expression is post-transcriptionally regulated by Dhh1 in formic acid. Analyses of JEN1 mRNAs decay in wild-type and dhh1 mutant strains confirmed this hypothesis. In these conditions, the stabilized JEN1 mRNA was associated to polysomes but no Jen1 protein could be detected, either by measurable lactate carrier activity, Jen1-GFP fluorescence detection or western blots. These results revealed the complexity of the expression regulation of JEN1 in S. cerevisiae and evidenced the importance of DHH1 in this process. Additionally, microarray analyses of dhh1 mutant indicated that Dhh1 plays a large role in metabolic adaptation, suggesting that carbon source changes triggers a complex interplay between transcriptional and post-transcriptional effects.

Citation: Mota S, Vieira N, Barbosa S, Delaveau T, Torchet C, et al. (2014) Role of the DHH1 Gene in the Regulation of Monocarboxylic Acids Transporters Expression in Saccharomyces cerevisiae. PLoS ONE 9(11): e111589. doi:10.1371/journal.pone.0111589

Editor: Marie-Joëlle Viroille, Université Paris South, France

Received July 21, 2014; Accepted September 26, 2014; Published November 3, 2014

Copyright: © 2014 Mota et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files. The S. cerevisiae microarrays used are fully described in Array express (www.ebi.ac.uk/microarray-as/aer/entry; accession number A-MEXP-337).

Funding: This study was supported by the Portuguese grant POCI/BIA-BCM/57812/2004 (Eixo 2, Medida 2.3, QCAIII – FEDER). N.V. received a FCT PhD fellowship (SFRH/BD/74790/2010). F.D.’s work is supported by a grant from the Agence pour la Recherche contre le Cancer (ARC). Support to C.B.M.A. by FEDER through POFC-COMPETE and by Portuguese funds from FCT through the project Pest-OE/BI/UF4050/2014 is also acknowledged. The authors thank Josette Banroques and Kyle Tanner for their advice regarding polysome gradients. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

These authors contributed equally to this work.

Introduction

The cellular metabolism of most yeasts, including Saccharomyces cerevisiae, is set to run essentially on glucose. When this yeast encounters harsh conditions in niches deprived from glucose, the ability to transport and metabolize non-fermentable carbon sources is crucial for its survival. In this manner, the uptake of short-chain carboxylic acids across the plasma membrane plays a defining role in the metabolism of yeast cells and in its pH-stasis [1]. Physiological studies, carried out in this baker’s yeast, identified two distinct monocarboxylate proton symporters, strongly repressed by glucose, with different specificities and regulation. A permease involved in the uptake of lactate-pyruvate-acetate and propionate was identified in lactic or pyruvic acid-S. cerevisiae grown cells [2,3], being encoded by JEN1 [4], whereas, an acetate-propionate-formate permease was found in ethanol or acetic acid grown cells, with no obvious gene candidate at that time [5,6]. Later, ADY2 was identified as the acetate permease encoding gene in S. cerevisiae [7].

In an early attempt to identify the genes involved in acetate-propionate-formate transport, classical genetic studies were carried out. The strain S. cerevisiae W303-1A was subjected to
UV mutagenesis, in order to obtain mutants affected in the ability to utilize acetic acid, but unaffected on the capacity to grow in ethanol, as the sole carbon and energy source [6]. According to this strategy, it was hypothesised that mutants specifically affected in monocarboxylate permease(s) activity could be found. A mutant clone, exhibiting growth on ethanol, but with pronounced growth defect in a medium with acetic acid, as the sole carbon and energy source, was isolated (Ace8 strain) [8]. Further genotypic characterisation of the Ace8 mutant led to the identification of the DHH1 gene as a most likely candidate for explaining the Ace8 phenotype. Indeed, the transformation of Ace8 cells with a genomic fragment containing DHH1 restored their capacity to grow on acetate and the deletion of DHH1 presented slower growth rates than the isogenic wild-type on acetic acid (Paiva, S. 2002 PhD thesis, Fig. S1 in File S1).

**DHH1** encodes a RNA helicase of the DEAD-box subfamily [9,10]. Several homologs have been described in a broad range of organisms, namely in: S. pombe, STE13 [11], in Xenopus laevis, XP54 [12], in Drosophila melanogaster, ME31B [13], in mouse, DDX6 [14,15], in humans, DDX6/P54/RCK (encoding for an oncoprotein) [14] and in Caenorhabditis elegans CGH1 [16]. Dhh1 has been involved in the formation of specific dynamic cytoplasmic loci, the Processing Bodies (P-bodies). P-bodies have been observed in yeasts, insect cells, nematodes and mammalian cells as cytoplasmic foci accumulating translationally silent mRNAs and containing proteins involved in mRNA decay and translation inhibition, including the decapping enzymes (Dcp1/Dcp2), as well as general activators of decapping, like Dhh1, Pat1, Lsm1-7 complex, Edc3, the 5'-3' exonuclease Xrn1, the Non sense mediated mRNA Decay (NMD) regulator Nam7/Upf1, components of the deadenylase machinery, other translational inhibitors, but also translational elongators and ribosomal subunits [17–21] (reviewed in [22]). P-bodies were implicated in mRNA decay, mRNA storage and translation repression [23–25], mRNA-mediated repression [26,27], nonsense-mediated decay [28,29] and viral packaging [30]. In consequence, P-bodies have been proposed to be important players of the cytoplasmic “mRNA cycle”, where normal or aberrant mRNAs having reduced translational rates and enhanced decapping and deadenylation activities are targeted and where they can either be degraded or stored for further translation [31]. However, it should be noted that their role as loci where cytoplasmic mRNA decay actually occurs is still controversial [31]. Namely, many studies have shown that the formation of microscopically detectable P-bodies does not seem to be required for most mRNA degradation pathways and that the mRNAs stored in P-bodies are more stable than the mRNAs found free in the cytoplasm (reviewed in [32]).

Dhh1 plays a fundamental role in regulating the balance between active translation, accumulation in P-bodies and cytoplasmic 5'-3' decay of mRNAs [33]. Dhh1 physically interacts with the decapping enzyme activator Dcp1, the decapping enhancers Pat1, Lsm1, Edc3 and the Ccr4-Pop2-Not deadenylase [34–39]. Mutants deleted for DHH1, showed a deficient mRNA decay, longer half-life times of several mRNAs and accumulated capped deadenylated transcripts, indicating that Dhh1 acts as an activator of decapping [33,40]. More precisely, Dhh1 has been proposed to act on mRNA translation rates [24,41,42], based on the reported competition between mRNA decapping and translation initiation [23,36]. Former experiments suggested that Dhh1 uses its ATPase activity to release eIF4F complex from the mRNP, or somehow destabilize this eIF4F-mRNA cap complex, and in this manner repress translation initiation with concomitant decapping stimulation [36]. However, this model has been recently challenged by data indicating that Dhh1 could promote decapping by slowing translation elongation downstream to the initiation step [43] and that its ATPase activity is required for regulating P-bodies dynamics but not translation inhibition [33]. Hence, Dhh1 plays a role in the regulation of several cellular processes [44] including mating [45], filamentous growth [46] and iron deficiency [47]. Moreover, Dhh1 controls the turn-over of the mRNA encoding the decapping enhancer Edc1 [44] and DHH1 is epistatic on the DCS1 gene, encoding a decapping enzyme scavenger [48]. Furthermore, DHH1 has also been involved in mRNA and tRNA nuclear export [41,49–51]. Dhh1 is required for the efficient retrotransposition of Ty1 elements in yeast [52]. Finally, overexpression of DHH1 also suppresses defects of the mitochondrial Rnase P subunit Rpm2 [53].

In order to elucidate the involvement of this Dead-box RNA helicase in monocarboxylate transport and in the regulation of non-fermentable carbon sources utilization in *S. cerevisiae*, we studied the role of Dhh1 in the expression of the JEN1 gene. We showed that, in the presence of formic acid as sole carbon source, JEN1 expression is negatively controlled at the post-transcriptional level by a Dhh1-dependent mechanism. The deletion of DHH1 led to the accumulation of JEN1 mRNAs which were associated to polysomes but for which no Jen1 protein could be detected, questioning the fact that they are eventually translated. Furthermore, analyses of the wild-type and dhh1 mutant cell transcriptomes evidenced the broad involvement of this RNA helicase in the control of various cellular pathways.

**Materials and Methods**

**Yeast strains, plasmids and growth conditions**

*S. cerevisiae* strains used in this work are listed in table 1 and the plasmids in table 2. The cultures were maintained on plates of yeast extract (1%, w/v), peptone (1%, w/v), glucose (2%, w/v) and agar (2%, w/v). Yeast cells were grown in YNB glucose 2.0% (w/v), supplemented with adequate requirements for prototrophic growth. Carbon sources were glucose (2%, w/v), lactic acid (0.5%, v/v), acetic acid (0.5%, v/v), pH 5.0, acetic acid (0.5%, v/v), pH 6.0, formic acid (0.5%, w/v), pH 5.0 and propionic acid (0.5%, v/v), pH 5.0. Solid media were prepared adding agar (2%, w/v) to the respective liquid media. Growth was carried out at 30°C, both in solid or liquid media. The cells were also directly grown in rich media, YP lactic acid 0.5% pH 5.0, or YP acetic acid 0.5% pH 6.0. YNB glucose-containing media was used for growth of yeast cells under repression conditions. For derepression conditions glucose-grown cells were harvested during the exponential phase of growth, centrifuged, washed twice in ice-cold deionised water and cultivated into fresh YNB medium supplemented with a carbon source of choice.

**Strains construction**

The yeast strains, the plasmids and the primers used in this work are listed respectively in Tables 1, 2 and 3. The mutant strain, dhh1, carrying a dhh1::kanMX4 locus, was transformed with the hygromycin resistance gene hphMX4 resulting in a marker switch producing the dhh1::hphMX4 locus [54]. The same procedure was made for the strain W303-1A: JEN1::GFP-kanMX4 resulting in the strain W303-1A: JEN1::GFP-hphMX4. The *S. cerevisiae* strain BLC 491-U2, was used to amplify the genetic chimaera, JEN1::GFP-kanMX4, using the primers W303-1A forward and MX4 reverse [55]. The dhh1::hphMX4 strain was subsequently transformed with the 2.8 Kb JEN1::GFP-kanMX4 PCR product resulting in strain NV2. Transformed cells were grown in...
spread on YPD plates, containing 200 mg L\(^{-1}\) of Hygromycin (Hygromycin B from Invitrogen). The obtained transformants were confirmed by analytical PCR, with primers A1 and GFP rev [56].

To obtain the 6sm1, pat1, nam7 and ski7 mutants in the W303-1A background, strains BY 1 to 4 were used to amplify the corresponding deletion cassettes, using the respective A and D primers. The strains W303-1A and W303-1A: JEN1::GFP-kanMX4 were then transformed with this PCR product. The transformed cells were grown in YPD media for 4 hours and spread on YPD plates, containing 200 mg L\(^{-1}\) of Geneticin and/or 300 mg L\(^{-1}\) of Hygromycin.

Cloning and PCR amplification analyses were performed as previously described [57].

Transport assays

YNB glucose-containing media was used for growth of yeast cells under repression conditions. For derepression conditions glucose-grown cells were harvested during the exponential phase of growth, centrifuged, washed twice in ice-cold deionized water and cultivated into fresh YNB medium supplemented with a carbon source of choice. Cells were harvested by centrifugation, washed twice and resuspended in ice-cold deionized water to a final concentration of 20–40 mg dry weight/ml. Conical centrifuge tubes containing 30 μl of 0.1 M KH\(_2\)PO\(_4\) buffer at pH 5.0 and 10 μl of the yeast suspension were incubated for 2 min at 26°C. The reaction was started by the addition of 10 μl of an 2 mM aqueous solution (saturation concentration) of 4000 d.p.m./nmol of radiolabeled [\(^{14}\)C] lactic acid (sodium salt; GE Healthcare) and the filters were washed with 10 ml of ice-cold water. The reaction mixtures were filtered immediately through GF/C membranes (GE Healthcare) and the filters were washed with 10 ml of ice-cold water and transferred to scintillation fluid (Opti-Phase HiSafe II; Pharmacia LKB). Radioactivity was measured in a liquid scintillation spectrophotometer (Tri-Carb 2200 CA; Packard Instrument Co.) equipped with a d.p.m. correction facility. For nonspecific adsorption of [\(^{14}\)C] lactic acid was added at time zero after the cold water. All experiments were repeated at least three times, and the data reported represents average values. Data obtained is represented as the mean ± SD of triplicate measurements.

| Strains | Genotype | Reference |
|---------|----------|-----------|
| W303-1A | MATa ade2-1; leu2-3, 112; his3-11, 15; trp1A; ura3-1; can 1–100 | [90] |
| ACE 147 | W303-1A: dhh1Δ::kanMX4 | Paiva S., 2002 PhD thesis |
| jen1 | W303-1A; jen1Δ::HIS3 | (Casal et al. 1999) |
| BLC 491-U2 | MATa ura3-52 JEN1::GFP-kanMX4 | [55] |
| NV1 | ACE 147; dhh1Δ::hphMX4 | This work |
| NV2 | NV1; JEN1::GFP-kanMX4 | This work |
| ACE 145 | W303-1A: JEN1::GFP-kanMX4 | Paiva S., 2002 PhD thesis |
| BY 1 (YJL124c) | BY4742; MAT alpha; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; YJL124c::kanMX4 | Euroscarf |
| BY 2 (YCR077c) | BY4741; MAT a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YCR077c::kanMX4 | Euroscarf |
| BY 3 (YMR080c) | BY4741; MAT a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YMR080c::kanMX4 | Euroscarf |
| BY 4 (YOR076c) | BY4741; MAT a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YOR076c::kanMX4 | Euroscarf |
| MAR 5 | W303-1A: JEN1::GFP-hphMX4 | This work |
| MAR 6 | MAR 5; 6sm1Δ | This work |
| MAR 7 | MAR 5; pat1Δ | This work |
| MAR 8 | MAR 5; nam7Δ | This work |
| MAR 9 | MAR 5; ski7Δ | This work |
| MAR 14 | W303-1A: JEN1::GFP-hphMX4 | This work |
| MAR 15 | W303-1A: pat1Δ | This work |
| MAR 16 | W303-1A: nam7Δ | This work |
| MAR 17 | W303-1A; ski7Δ | This work |

Table 1. S. cerevisiae strains used in this work.

Table 2. Plasmids used in this study.

| Plasmids | Source or references |
|----------|---------------------|
| pT12 | [4] |
| pPDA1 | Andrade, R. (This work) |
| pAG32 | [54] |
Table 3. Primers used in this work.

| Primers | Sequence |
|---------|----------|
| W303-1A forward | GATTTGTCCCTCTGTTATGAAG |
| W303-1A reverse | ATCTTGCTAGTGTTAACGGCTGTTA |
| A1 | GGCCTATCCAAGGATGCTGTC |
| GFP_rev | AACATCACCATCTAATTCAAC |
| A LSM1 | ACCGTATGGGTCTTTGATACACTTA |
| D LSM1 | GGTCTACTGAGCTTACAATAGCAGC |
| A PAT1 | CATTATAATGGAGAATTGTCCTGG |
| D PAT1 | TCAAATGTCGGTCTCCTCAAGTTC |
| A NAM7 | TTTAGTATCATCATGTTTCCTTTTCG |
| D NAM7 | TGAATAAACGGACGTCTTTCAATTTTC |
| A SKI7 | GTGATTTCCTACAATCAAACCC |
| D SKI/ | GAAATCTCAATGGCCTTTTACGA |
| K2 | CGATAGATTGTGCCACCTG |
| K3 | CCATCCTATGGAACTGCCTC |

doi:10.1371/journal.pone.0111589.t003

Figure 1. JEN1 expression profile. A- Transcription analyses of JEN1 in S. cerevisiae W303-1A wild-type and dhh1 cells. Total RNA was isolated from YNB Glucose 2% (w/v) grown cells, collected at mid exponential phase, and after induction for 4 or 6 hours in different non-fermentable carbon sources: G – glucose; L – lactic acid (4 hours); E – ethanol (4 hours); Py – pyruvic acid (4 hours); A – Acetic acid (4 hours); F – formic acid (4 hours); F* – formic acid (6 hours); Pr – propionic acid (4 hours); Pr* – propionic acid (6 hours); Gly – glycerol (4 hours). An internal JEN1 fragment was used as probe. PDA1 was used as a reference, for relatively constant transcription. B- RT-QPCR analyses of JEN1 mRNA expression levels in wild type and dhh1 cells grown on glucose, lactic acid, acetic acid or formic acid. The JEN1 expression levels indicated here are relative to SCR1, a RNA pol III transcript which is not supposed to be sensitive to the deletion of DHH1. A different control was used for Q-PCR (SCR1) and northern blot (PDA1) analyses to ensure that the measured effects were not a bias coming from the control. The levels of JEN1 mRNAs measured in wild-type cells in lactic acid were used as a reference and arbitrarily set up at a value of 1. The experiments were performed three times on biologically independent samples. doi:10.1371/journal.pone.0111589.g001
Microscopy

*S. cerevisiae* living cells were examined with a Leica Microsystems DM-5000B epifluorescence microscope with appropriate filter settings. Images were acquired with a Leica DCF350FX digital camera and processed with LAS AF Leica Microsystems software.

RNA analysis

Total RNA was isolated using the standard hot acidic phenol protocol. In a 1.5% (w/v) agarose/MOPS/formaldehyde gel, samples of 20 μg RNA were electrophorised and blotted onto a Hybond- N+ membrane [58]. An internal fragment of 844 bp, obtained by the digestion of the pT12 plasmid (Table 2) with the restriction enzymes NcoI and PstI, was 32P-labelled and used as a JEN1 probe. As internal control RNA of *PDA1* was also used. For mRNA relative half-life times (t_{1/2} mRNA) determination, inhibition of transcription was accomplished by the addition of 1,10-phenantroline (0.1 mg/ml) for 0, 4, 10 and 20 minutes [59]. Real time quantitative RT-PCR experiments were conducted as previously described [Garcia et al., MBC 2007] using primer pairs specific of JEN1 (sequences), ACT1 (sequence) or SCR1 (sequences). Relative half-life times were determined by measuring the ratio between amounts of JEN1 mRNA and those of SCR1 at each time point and applying, a linear regression equation to the Log (JEN1/SCR1) = f(t) function. The calculation of the slope directly gave access to the relative half-life of JEN1 in the mutant and the wild-type strain. The reported half-life times represent a mean value obtained from three experiments performed on independent biological samples.

Polysome gradients

dhh1 mutant cells were grown in YNB glucose media until they reached an optical density of 0.8. Then, they were washed twice in sterile water and transferred into an equivalent volume of either YNB lactic acid or YNB formic acid media. After 5 hours, cycloheximide was added to the cells at a final concentration of 100 μg/ml. After 5 minutes of incubation on ice, the cultures were centrifuged 5 minutes at 3000 g, washed once with sterile water and a second time with lysis buffer (Tris-HCl pH 7.4 20 mM, NaCl 50 mM, MgCl2 5 mM, DTT 1 mM, cycloheximide 100 μg/ml). After the last centrifugation, the cell pellet was resuspended in 800 μl of lysis buffer including 1X protease inhibitor cocktail (Roche). An equivalent volume of glass beads were added and the cells were disrupted by vortexing 6 times 30 seconds, at 4°C. The supernatant was collected and centrifuged 5 minutes at 5000 g (4°C). The supernatant was cleared by two rounds of centrifugation 10 minutes at 12000 g (4°C). The final supernatant was stored at –80°C after adding 10% glycerol. About 600 μl of this solution was loaded on a 10%–50% sucrose gradient, centrifuged at 39000 g for 3 hours and 0.5 ml fractions were loaded on a 10%–50% sucrose gradient, centrifuged at 39000 g for 3 hours and 0.5 ml fractions were analyzed by real time quantitative RT-PCR.
were collected in 2 ml tubes using a Retriever 500 (ISCO) fraction collector and a Type 11 Optical unit (ISCO) with 254 nm filters. The RNAs contained in the fractions were precipitated by added 50 ml of ammonium acetate 3 M pH 5.3 and 1.2 ml of absolute ethanol. The mixtures were stored overnight at −20°C, and then they were centrifuged for 20 minutes at 15000 g (4°C). The pellets corresponding to the polysomes fractions were resuspended with the same 100 ml of water. The pellets corresponding to the other fractions were pooled in another 100 ml of water. The RNA was then purified and cleaned up using the RNA easy midi kit (Qiagen), following the supplier’s recommendations. The RNA was quantified by spectrometry and 0.5 mg (for lactic acid) or 1.5 mg (for formic acid) were used for reverse transcription and real-time quantitative PCR analyses using the JEN1 and ACT1 primer probes as described above.

Figure 3. Real time quantitative RT-PCR analyses of JEN1 mRNA stability in YP lactic acid-grown wild-type, nam7 and dhh1 mutant cells. Mutant and wild-type cells were grown in YP lactic acid and collected immediately before (time zero) or 4, 10 or 20 minutes after the addition of 1,10-phenantroline (0.1 mg/ml). The expression levels are expressed relatively to the SCR1 expression level used as a control. These relative expression levels were set to one at time zero for both strains, in order to normalize differences in mRNA levels between the two strains at the beginning of the experiment. Squares represent the values obtained for the dhh1 mutant, triangles represent the values obtained for the nam7 mutant and diamonds represent the values obtained for the wild-type. The experiments were performed three times on biologically independent samples. doi:10.1371/journal.pone.0111589.g003

Figure 4. Transport activity of lactic acid in S. cerevisiae W303-1A strains: wild-type, dhh1, jen1 (A), lsm1, ski7, pat1 and nam7 (B). The results are percentages of initial activities of 2 mM [14C] lactic acid uptake, pH 5.0. Cells were grown in YNB glucose and derepressed in YNB lactic acid or YNB formic acid. Wild-type and dhh1 YNB lactic acid derepressed cells were used as a control. doi:10.1371/journal.pone.0111589.g004
Microarray analysis

Detailed protocols are described at http://www.transcriptome.ens.fr/sgdb/protocols/. The S. cerevisiae microarrays used are fully described in Array express (www.ebi.ac.uk/microarray-as/aer/entry; accession number A-MEXP-337). The microarray experiments were conducted as previously described [60]. Raw data were normalized using global lowess followed by print-tip median methods, with background removal, as implemented in Goulphar [61]. Experiments were carried out 2 times, with dye swapping. The microarray data are available in Table S2 and fully available at the GEO database (accession number: GSE60983). The statistical significance of the expression variations measured was addressed by using the TMEV version of SAM with a FDR of 5%, a S0 calculated by the Tusher method and using the exact number of permutation [62–64]. Only genes that passed the SAM filter and had an average log2 of ratio above 0.9 in glucose or in formic acid were considered as significantly changing their expression in the mutant compared with the wild type. These

Figure 5. Jen1 protein expression. A - Subcellular localization of Jen1::GFP in S. cerevisiae living cells. Wild-type, W303-1A, dhh1, lsm1, pat1, nam7 and ski7 mutant cells harboring Jen1::GFP were used to follow Jen1 expression after growth in YNB glucose, and derepression for 4 hours in YNB lactic 0.5%, pH 5.0, or YNB formic acid 0.5%, pH 5.0. B – Total protein extracts from wild type and dhh1 mutant cells harboring Jen1::GFP were used to follow Jen1 expression after growth in YNB glucose, and derepression for 4 hours in YNB lactic 0.5%, pH 5.0, or YNB formic acid 0.5%, pH 5.0 and immunoblotted with the indicated antibodies. We add a sample of extracts from lactic acid 50 fold diluted.

doi:10.1371/journal.pone.0111589.g005
Figure 6. Polysome gradient analyses of *dhh1* mutant cells grown in lactic or formic acids. A: Absorbance profiles along a polysomes gradient obtained with cells grown in lactic acid. The fractions corresponding to the polysomes are indicated. B: the polysomes fractions obtained in acetic and lactic acid were pooled and the percentage of *JEN1* mRNA contained in this fractions compared to the rest of the gradient was quantified by RT-QPCR. In contrast to previous Q-PCR experiments, SCR1 could not be used as a reference because it is not translated. Hence, ACT1 was used as a control for an mRNA which was actively transcribed in both lactic and formic acids. The experiments were performed three times on biologically independent samples. The difference of *JEN1* abundance in polysomal fractions between lactic and formic acid was not significant, according to a Student test.

doi:10.1371/journal.pone.0111589.g006

Figure 7. Transcriptome analyses of Dhh1 impact on gene expression. Venn diagram representing the overlap of down (left) and up (right) regulation effects in a dhh1 mutant, grown either in glucose or in formic acid. The main functional categories enriched in each group are indicated. They were determined using the FUNSPEC web tool (funspec.med.utoronto.ca/). The complete set of genes in each category, together with their functional annotation, can be found in Table S1.

doi:10.1371/journal.pone.0111589.g007
Figure 8. ADY2 expression profile. A- Transcription analyses of ADY2 in S. cerevisiae W303-1A and dhh1 cells. Total RNA was isolated from YNB Glucose 2%-grown cells, collected at mid exponential phase, and after induction for 4 or 6 hours in different non-fermentable carbon sources: G – glucose; L – lactic acid (4 hours); E – ethanol (4 hours); Py – pyruvic acid (4 hours); A – Acetic acid (4 hours); F – formic acid (4 hours); F* – formic acid (6 hours); Pr – propionic acid (4 hours); Pr* – propionic acid (6 hours); Gly – glycerol (4 hours). An internal ADY2 fragment was used as probe. PDA1 was used as a reference, for relatively constant transcription. B- Densiometry analysis of ADY2 Northern blots was performed on scanned films using ImageJ gel analysis tool (public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Absolute intensities were calculated for both ADY2 and the PDA1 control. Relative intensities were calculated for each experimental band by normalizing the absolute intensity to the corresponding control intensity.

Figure 9. Transport activity of Ady2 in S. cerevisiae W303-1A strains: wild-type, dhh1, lsm1, ski7, pat1 and nam7. The results are percentages of initial activities of 2 mM [14C] acetic acid uptake, pH 5.0. Cells were grown in YNB glucose and derepressed in YNB acetic acid or YNB formic acid. Wild-type and dhh1 YNB acetic acid derepressed cells were used as a control.
genes were listed in Table S1. Global functional analyses were performed using FUNSPEC [65].

Results

JEN1 expression profile

W303-1A and dhh1 cells were grown in different carbon and energy sources, in an effort to clarify the mechanisms involved in the expression regulation of JEN1 by DHH1. The expression pattern of JEN1 was studied by Northern blot analyses (Fig. 1A). In the wild-type strain, JEN1 was highly expressed in lactate, acetate and glycerol, poorly expressed in ethanol and almost totally absent in glucose, formic acid and propionic acid. In the dhh1 strain, the JEN1 expression profile was similar to the wild-type with the notable exceptions of pyruvic, acetic, formic and propionic acids. In the presence of formate and propionate, the JEN1 mRNA largely accumulated in the mutant, indicating either a derepression of JEN1 transcription or a stabilization of JEN1 mRNA in this strain as compared with the wild-type. In the presence of pyruvate and acetate, an opposite behavior was observed: the JEN1 mRNA was less abundant in the dhh1 strain than in the wild-type, JEN1 expression was also quantified by real-time quantitative PCR in wild-type and dhh1 cells grown in glucose, lactic acid, acetic acid or formic acid, which confirmed the conclusions taken from the northern blots (Fig. 1B). These results show that DHH1 regulates the expression of JEN1 at the mRNA level, pointing to an involvement of this RNA helicase in the regulation of monocarboxylic acids utilization, in S. cerevisiae.

Involvement of other players in the mRNA degradation pathways

To further investigate the mechanisms by which Dhh1 controls JEN1 expression, we measured the JEN1 mRNA levels in cells

Figure 10. Model for JEN1 expression regulation. This model is inferred from the data presented in this work. Briefly, in glucose, JEN1 is transcriptionally silent. In lactate or acetate, JEN1 is transcriptionally activated by Cat8 and JEN1 mRNA are actively translated, which results in the accumulation of Jen1 in the plasma membrane and in an active transport of carboxylic acids. In formic acid, JEN1 is transcriptionally active, but the JEN1 mRNAs are targeted to degradation in P-bodies and therefore barely detectable by northern blots. In the absence of Dhh1 or Pat1, JEN1 mRNA are no more degraded but still the Jen1 protein was not detectable, which result in an accumulation of mRNA with no or very few Jen1 protein in the membrane and no or very few active transport of carboxylic acids. Our data cannot tell if this low protein level is due to the low RNA level or to actual translation inhibition.

doi:10.1371/journal.pone.0111589.g010
mutated for genes involved in various aspects of cytoplasmic mRNA degradation: decapping activation (pat1 and lsm1), 3'5'-exonuclease-mediated degradation and the non-stop decay (skl7) and nonsense mediated decay (nam7). The inactivation of PAT1 or Nam7 led to an accumulation of JEN1 mRNA in formic acid of 6 and 4 fold, respectively, as compared with the wild-type. This effect was similar to what was observed in the dhh1 strain (Fig. 2, left panel). The lsm1 strain exhibited an increase in JEN1 mRNA, which was significant, but lower for the one observed for pat1 and nam7 strains (about 2 fold). In contrast, the deletion of SKL7 had no effect on JEN1 expression in these growth conditions. With acetic acid as the sole carbon source, the deletion of DHH1 or PAT1 led to a significant decrease of Jen1 activity, and 4 fold, respectively (Fig. 2, right panel). The other mutants examined showed no significant differences as compared with the wild-type. Finally, in lactic acid, the mutations tested had few effects on JEN1 mRNA levels (Fig. 2, lower panel). These results suggested contrasted roles of Dhh1, Pat1 and Nam7 on JEN1 expression, which largely vary depending on the carbon source.

We further investigated the regulation of JEN1 in formic acid.

Decay of JEN1 mRNA in the S. cerevisiae dhh1 and nam7 strains

The relative stability of JEN1 mRNA was analysed in the S. cerevisiae wild-type strain and in a dhh1 or nam7 genetic background. A pulse of 1,10-phenanthroline (0.1 mg/ml) was added to YP lactic acid-grown cells (JEN1 inducing conditions) to stop transcription. RNA samples were prepared 0, 4, 10 and 20 minutes after the pulse. JEN1 mRNA levels were measured using real-time quantitative PCR. The results were normalized to the signals obtained for the SCR1 mRNA (a stable RNA pol III transcript) and time zero was used as a reference to normalize for RNA steady state differences between wild-type and dhh1 strains before the phenanthroline pulse (Fig. 3). The relative half-live times (1/2 mRNA) of JEN1 mRNA were calculated in each strain. This relative half-life increased by two fold in the dhh1 mutant as compared with the wild-type and nam7 mutants (15+/−1.05 minutes in dhh1 mutant, 8.3+/−0.9 and 8.7+/−1.2 minutes in the wild type and nam7 mutant, respectively) (Fig. 3). These results suggest that dhh1 actively participates to the regulation of the stability of JEN1 mRNA in formic acid. In contrast, the increase of JEN1 mRNA seen in the nam7 mutant is not related to a higher stability, suggesting that Dhh1 and Nam7 act at different levels in the regulation of JEN1.

Activity of monocarboxylic acids transporters in S. cerevisiae dhh1 strain

In order to determine whether JEN1 mRNAs detected in dhh1, pat1 and nam7 mutant strains grown in formic acid was being translated to a functional protein, the uptake of 2 mM of labelled lactic acid pH 5.0, was assessed in wild-type and mutant cells grown on glucose and shifted to YNB formic acid 0.5%, pH 5.0, for 4 hours (Fig. 4B). As a control, wild-type and dhh1 mutant cells were grown in glucose and shifted to YNB lactic acid 0.5%, for 4 hours and no significant differences were observed in the uptake of labelled lactic acid (Fig. 4A), in contrast to what was found for jen1 mutant, where no active transport was observed. In formic acid, no lactate transporter activity was observed in the wild-type cells, which is in accordance with the fact that no mRNA expression was found in these conditions. In dhh1, pat1 and nam7 mutants, although the JEN1 mRNA was accumulating, no active lactate transport could be detected (Fig. 4B), suggesting that the accumulation of JEN1 mRNAs in the absence of DHH1 does not lead to detectable Jen1 activity.

Jen1 protein is undetectable in the dhh1, pat1 and nam7 mutant strains grown in formic acid

To try to detect Jen1 protein in formic acid, cells of the wild-type and the dhh1, nam7, pat1, lsm1 and skl7 (Table 1) mutant strains, harboring a gene encoding a Jen1::GFP chimera in their genome, were grown in YNB formic acid or in YNB lactic acid (positive control) for 4 hours. Cells were harvested and equal volumes of cell suspension were resuspended in low-melt agarose (1.0%, w/v), and observed by epifluorescence microscopy. In lactic acid, the fluorescence was unambiguously localized to the plasma membrane in all tested cells as previously described for the wild-type strain [55] (Fig. 5A). The same experiments were conducted with cells grown in formic acid as sole carbon and energy source for 4 and 6 hours. In these conditions, there was no fluorescence of Jen1::GFP in any of the strains tested (Fig. 5A). Again, this result was expected for the wild-type, in which almost no JEN1 mRNA is present, but not for dhh1, pat1 or nam7 mutants, in which significant amounts of JEN1 mRNA were detected by Q-PCR and northern blots. However, this may just be a problem of detection sensitivity, because, even in the dhh1, pat1 and nam7 mutants, the JEN1 mRNA levels in formic acid are still 50 fold lower than the one measured in lactic acid. Hence, we used a more sensitive technique than GFP fluorescence to try to detect Jen1. We performed western blots, using anti-GFP antibodies, in wild type and dhh1 mutant grown in glucose, lactic acid or formic acid (Fig. 5B). As expected, the Jen1-GFP protein was detected in no strain in glucose and in all strains in lactic acid. No signal was detected in formic acid, even in the dhh1 strain, which supported the fact that the jen1 protein was not produced in these conditions (Fig. 5B). Still, these experiments did not exclude the possibility that Jen1 is actually produced but below the detection sensitivity of the method. Indeed, with a simple 50 fold dilution of the lactic acid sample (which roughly mimics the amount of protein that may be expected in the formic acid grown mutant cells, based on the mRNA levels measured in Fig. 1B), the protein became barely detectable (Fig. 5B).

JEN1 mRNA is associated with polysomes in the dhh1 mutant grown in formic acid

To clarify the translational status of the JEN1 mRNA, we performed polysome gradients in formic acid grown dhh1 mutant cells (Fig. 6A). Similar experiments were conducted in lactic acid grown dhh1 cells, as a control for a condition in which JEN1 mRNA are actively translated. ACT1 was used as a control for an mRNA which is actively translated in both formic and lactic acid grown cells. The mRNA levels were estimated using real-time quantitative PCR. Because the JEN1 mRNA levels were very low in formic acid, we had to pool all the fractions corresponding to polysomes on one hand and all the other fractions on the other hand, and make a rough estimate of the percentage of ACT1 or JEN1 mRNAs present in each of the two categories of fractions (Fig. 6B). As expected, JEN1 mRNAs were enriched in the polysome fractions in cells grown in lactic acid. Interestingly, it was also clearly enriched in the polysomes fractions in cells grown in formic acid. These experiments indicated that, although we could not detect any Jen1 activity, the JEN1 mRNAs, which accumulate in formic acid in the absence of Dhh1, are associated with polysomes.
Genome-wide analyses of the *dhh1* role in metabolic adaptation

To highlight the role of Dhh1 in the gene regulation associated with carboxylic acids and non fermentative growth conditions, we performed DNA microarray analyses of the transcriptome of yeast wild-type and *dhh1* mutant cells, grown in glucose or shifted from glucose to formic acid 0.5%, pH 5.0, for 4 hours. About 920 genes were identified as being significantly up or down regulated in the *dhh1* mutant compared with the wild-type, in at least one of the two tested conditions (Fig. 7). The mRNAs which amounts increased in the mutant were mostly involved in proteasomal and vacuolar proteolysis, respiration, oxidative and general stress responses and carbohydrate metabolism. The mRNA which steady-state decreased in the mutant were involved in ammonia and amino acid metabolism (including most of the corresponding transcriptional regulators), DNA topology and the maintenance and silencing of telomeres, aminoaeyl-tRNA synthesis, translational elongation and mating (Fig. 7). About 75% of these effects were independent of the carbon source, i.e. they were found both in glucose and formic acid. Among the genes that accumulated independently of the carbon source, we found the previously identified targets of Dhh1: *EDC1*, *COX17* [25,44] and *SDH4* [47]. Also, the decrease in expression of genes involved in mating and in RNA metabolism is reminiscent of the roles of Dhh1 in Ste12 induction [46] and tRNA maturation [51], respectively. Interestingly, several genes involved in mRNA decay were up- (*EDC1*, *EDC2*, *DCS1*, *DCS2*, *PUF3*, *PUF2*) or down- (*POP1* and the subunits of the CCR4-NOT complex *CAF16*, *CAF4* and *NOT3*) regulated in the mutant, suggesting the existence of feedback controls between the activity of Dhh1 and the components of the mRNA degradation pathways. Moreover, some translation regulators exhibited increased (*SR09*, *PET122*, *PPQ1*, *SU11*, *CBP6*) or decreased (TPA1, *RPS31*, *GCN1*, *RBB2*, *MDM38*, *GCN3*, *ECM32*) expression in the *dhh1* mutant. Intriguingly enough, many genes encoding RNA helicases (*SLH1*, *BRR2*, *DED1*, *DBP1*) and telomeric DNA helicases (Table S1) showed significant expression changes in the mutant.

Finally, microarray results confirmed the accumulation of the *JEN1* mRNA in the mutant strain, only in the presence of formic acid, as previously that was described in this work. Then, we focused our attention on the genes which, like *JEN1*, are repressed in the presence of glucose and induced by acetate [7] (Table S1). We found that 32 of these genes behaved like *JEN1* in the *dhh1* mutant. This is for instance the case of the other carboxylic acid transporter, *ADY2*, of *CAT8*, the transcriptional regulator of *JEN1*, and of the positive regulator of respiratory gene expression, *HAP4*. Northern blot analyses confirmed that the *ADY2* mRNA indeed accumulated in the *dhh1* mutant only in the presence of formic acid (Fig. 8). Additionally, transport activity experiments in formic acid derepressed cells showed that the accumulation of *ADY2* mRNA did not produce detectable amounts of Ady2 protein, nor in *dhh1* mutant nor in the other mutants tested in this work, similar to what was found for Jen1 (Fig. 9). These results suggest that the post-transcriptional regulation that we characterized for *JEN1* is shared by several other genes involved in carbon source metabolism. Surprisingly, several genes that were known to be similarly subjected to glucose repression already accumulated in the *dhh1* mutant in the presence of glucose (Table S1). This is for instance the case of *ADR1*, a transcription factor which collaborates with *CAT8* under non fermentative growth conditions. Noteworthy, *ADR1* mRNA had been already shown to be post-transcriptionally down-regulated by the non-sense mediated mRNA decay machinery and the decapping enzyme Dcp1 in presence of glucose [66]. This raises the interesting hypothesis that the balance between transcriptional and post-transcriptional regulations ensuring glucose catabolic repression may largely differ from one gene to another.

**Discussion**

Jen1 is localized at the plasma membrane of *S. cerevisiae* cells and it is involved in the transport of lactic, pyruvic, acetic and propionic acids. This permease is induced in the presence of non-fermentable carbon and energy sources, like lactic and pyruvic acids and its expression is undetectable in the presence of glucose, formic or propionic acids [4,67]. The disruption of the RNA helicase encoding gene *DHH1* attenuated growth on acetic acid. *Dhh1* was known to participate in the mRNA cycle [25] controlling, together with the Pat1-Lsm complex, the balance between translation and mRNA degradation by inhibiting translation initiation, targeting mRNAs to the P-bodies and contributing to the recruitment of the decapping machinery [68]. In this work, we showed that Dhh1 in particular, and the decapping complex in general, have roles in the post-transcriptional regulation of *JEN1* expression, which depend on carbon source. In the absence of *Dhh1*, *Pat1* or *Lsm1*, *JEN1* mRNAs accumulated in formic acid and associated with polysomes, although we could not detect the translated functional protein. Hence, the translational status of *JEN1* mRNAs in these conditions remains an open question. The same phenomena occurred in a mutant for Nam7/Upf1, which is an important actor of the NMD pathway. Additionally, we confirmed that the half-lives of the *JEN1* mRNA actually increased in the absence of *Dhh1*, but not in the *nam7* mutant. In contrast, in acetic acid, the inactivation of *Pat1* or *Dhh1* had a negative effect on *JEN1* mRNA expression. Our microarray experiments suggest that other key genes of metabolic adaptation, like the transcription factor encoding gene *CAT8* or the acetate transporter encoding gene *ADY2* (Fig. 8), may encounter similar regulations. Hence, the model that we can draw from our results and from previous studies is the following (Fig. 10). In glucose, *JEN1* is transcriptionally silent, as described previously. In lactic, its transcription is induced by Cat8 and Adr1, which results in high levels of Jen1 protein. In formic acid, the glucose transcriptional repression is also released, but *JEN1* mRNAs are rapidly degraded. This degradation requires Dhh1, Pat1 and Lsm1, which are known to collaborate in the activation of decapping and 5’-3’ mRNA decay, but not Ski7, which is involved in the 3’-5’ degradation of cytoplasmic mRNA by the exosome. Notably, the stability of the *JEN1* mRNA increase in the *dhh1* mutant was only two fold, when its accumulation was about 6 fold, suggesting additional levels of controls of Dhh1 on *JEN1* mRNA steady-state. This accumulation of *JEN1* mRNA in formic acid is also dependent on the presence of Nam7, but Nam7 does not act at the level of *JEN1* mRNA stability. *Nam7/Ufp1* is involved in the NMD pathway which degrades aberrant mRNAs exhibiting a premature stop codon and “normal” mRNAs which present particular features (long 3’UTRs, alternative translation initiation sites, upstream ORFs) [69], reviewed in [31]. However, our results suggest that *JEN1* mRNA is not a target of Nam7. One possibility is that Nam7 acts indirectly on *JEN1* expression by regulating the levels of a transcriptional regulator of *JEN1* in formic acid.

In acetic acid, the regulation of *JEN1* seems to be totally different. In the wild type, the *JEN1* mRNA is highly expressed. Mutations of *DHH1* or *PAT1* decreased this expression level (Fig. 1 and 2). GFP-fusion experiments showed that the *JEN1* mRNAs are translated in the *dhh1* mutant but that this lower level of mRNA expression resulted in a lower permease activity, as
measured by lactate transport assays (Fig. S2 in File S1). These observations may explain the slow-growth phenotype of the \textit{ddh1} mutant in acetic acid. This effect on the mRNA levels of \textit{JEN1} in acetic acid was independent from Nam7 (Fig. 2). The fact that the inactivation of a degradation pathway can lead to a decrease in gene expression may seem counter-intuitive. It was shown recently that the inactivation of the cytoplasmic 5'-3' exonuclease Xrn1 or of the decapping enzyme Dcp2 leads to accumulation of long non-coding RNAs (lncRNAs), some of which being located in the promoter or in antisense position of coding genes [70,71]. In some cases, this accumulation can lead to the transcriptional silencing of the overlapping genes. This system seems to preferentially target inducible genes, as for instance the GAL system [70,72]. The \textit{JEN1} genomic region has been shown to be able to produce two stable unannotated transcripts (SUTs) [73] in sense and antisense positions (www.yeastgenome.org). Moreover, it overlaps with one large Xrn1 sensitive lncRNA (XUT) antisense to the \textit{JEN1} mRNA sequence [71] (www.yeastgenome.org). Therefore, it was tempting to speculate that the negative effects of Dhh1 and Pat1 deletion on \textit{JEN1} expression in acetic acid could be mediated by an accumulation of one or several of these intergenic or antisense lncRNAs. Northern blot analyses of the three non-coding RNAs overlapping the \textit{JEN1} locus could not show any difference of expression between the wild type and the \textit{ddh1} mutant grown in acetic acid (data not shown). This suggested that Dhh1 does not act on \textit{JEN1} expression in acetic acid by degrading overlapping transcripts. This is consistent with previous observations that Dhh1 and Pat1 had no role in the transcriptional silencing by the accumulation of lncRNAs [70].

More generally, we pointed out about 900 potential targets for Dhh1, which are involved in many, different cellular pathways. These results emphasized the large role of Dhh1 in gene expression regulation. Still, this number (about 15% of the genes) is relatively small, considering that Dhh1 participates to a general mRNA degradation pathway. Interestingly, in trypanosomes, microarray analyses of \textit{ddh1} mutants suggested that it controls the expression of only 1% of the genes, several of them being involved specifically in developmental processes [74,75]. More recently, CLIP-seq experiments have shown that Dhh1 was able to bind about 300 mRNAs in standard growth conditions [76]. Our microarray results and the model of \textit{JEN1} regulation discussed above support the idea that, besides its general role in the global cytoplasmic mRNA decay, Dhh1, like Xrn1 or Dcp2 [70–72] may have more specific roles in the post-transcriptional and/or transcriptional regulation of some genes, in response to environmental stimuli. Moreover, our list of genes whose expression is affected in the \textit{ddh1} deletion strain provides explanations for the various phenotypes reported for \textit{DHH1} mutations, including defects in G1/S checkpoint recovery, filamentous growth, stress responses, membrane asymmetry, sporulation, ion homeostasis, apoptosis, vacuolar trafficking, ethanol, 2-deoxyglucose and zinc resistance [41,46,77–96]. However, the interpretation of these mRNA steady-state measurements in terms of direct and indirect effects is not straightforward, since Dhh1 impacts on the expression of a large number of transcriptional and post-transcriptional regulators and of their target genes (Table S1). For instance, the expression of the transcription factor encoding gene \textit{WAR1} (involved in weak acid resistance) and of its main target gene \textit{PDR12} decreased in the \textit{ddh1} mutant. Noteworthy, the level of expression of \textit{DHH1} increased in a \textit{WAR1} gain of function mutant [87]. Similarly, Dhh1 apparently controls the level of expression of several proteins regulating mRNA stability, including for instance \textit{PUF2} and \textit{PUF3}. Some PUF proteins have been shown to promote mRNA decay depending on Dhh1 [88,89]. This suggests a complex interplay between transcriptional and post-transcriptional effects, with regulatory feedbacks between them. Clearly, further genome-wide mRNA stability and proteome studies of the \textit{ddh1} mutant will be required to decipher the global regulatory roles of Dhh1.

In conclusion, this study revealed that regulation of \textit{JEN1}, \textit{ADY2} and possibly many other mRNAs in carboxylic acids is much more complex than a simple relieve of glucose repression, and that the mechanisms which control this expression considerably vary from one carbon source to another.

**Supporting Information**

**File S1** **Figures S1 and S2.** Figure S1. Representative growth curves of wild-type and \textit{ddh1} cells grown in YNB glucose 2% (A) and in YP acetic acid 0.5% (B) media. Figure S2. Transport activity and subcellular localization of \textit{jen1}:GFP in \textit{S. cerevisiae} W303-1A strains. A – Percentages of initial activities of 2 mM lactic acid uptake, at pH 5.0, in cells grown in YNB glucose and derepressed in YNB acetic acid 0.5%, pH 6.0. B – Wild-type and \textit{ddh1} cells harboring \textit{Jen1}-GFP were used to monitor \textit{Jen1} expression after growth in YNB glucose and derepression in YNB acetic acid 0.5% pH 6.0 for 6 hours or YNB lactic acid 0.5% pH 5.0 for 4 hours.

**Table S1 Lists of genes with significant expression variations.** The criteria used to select these genes can be found in the material and methods. There are three sheets corresponding to the following categories: gene changing expression 1- only in formic acid, 2- only in glucose or 3- in both glucose and formic acid. Column 1: ORF ID, column 2: fold change in acetate compared with glucose (data from Paiva et al., Yeast 2004); Column 3: average log of fold change (mutant/wild-type) in glucose, Column 4: average log of fold change (mutant/wild-type) in formic acid, Column 5: gene name, Column 6: functional annotation taken from the SGD.

**Table S2 Complete microarray results.** Column 1: ORF ID. Column 2 to 4: Log2 of normalized fluorescence ratios (\textit{ddh1} mutant/wild-type) for the 4 experiments (two growth conditions in duplicate).

**Acknowledgments**

We thank Josette Banroques and Kyle Tanner for their advices regarding polysome gradients.

**Author Contributions**

Conceived and designed the experiments: NV MG SP FD. Performed the experiments: SM NV SB TD CT AS MG AP SL FC LB. Analyzed the data: NV SM MG SP FD. Contributed reagents/materials/analysis tools: XD. Contributed to the writing of the manuscript: NV SM MG SP FD.

**References**

1. Casal M, Paiva S, Queiroz O, Soares-Silva I (2008) Transport of carboxylic acids in yeasts. FEMS Microbiol Rev 32: 974–994.

2. Cassio F, Leao C, van Uden N (1987) Transport of lactate and other short-chain monocarboxylates in the yeast Saccharomyces cerevisiae. Appl Environ Microbiol 53: 509–513.
34. Hata H, Mitsui H, Liu H, Bai Y, Denis CL, et al. (1998) Dhh1p, a putative RNA helicase, associates with the general translation factors Pob2p and Ccr4p from Saccharomyces cerevisiae. Genetics 148: 571–579.

35. Coller JM, Tucker M, Sheer U, Valencia-Sanchez MA, Parker R (2001) The Ddh1 box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. RNA 7: 1717–1727.

36. Frankis TM, Lykke-Andersen J (2008) The control of mRNA decapping and P-body formation. Mol Cell 30: 568–577.

37. Maitett Y, Collart MA (2002) Interaction between Notlp, a component of the Ccr4-Not complex, a global regulator of translation, and Dhh1p, a putative RNA helicase. J Biol Chem 277: 2083–2092.

38. Khurtsagar M, Parker R (2004) Identification of Edc3p as an enhancer of mRNA decapping in Saccharomyces cerevisiae. Genetics 166: 729–739.

39. Shari H, Oguz S, Sharma K, Basquin C, Uralba H, et al. (2013) Structural analysis of the yeast Dhh1-Pab1 complex reveals how Dhh1 engages Pab1, Edc3 and RNA interactions. Nucleic Acids Res. 41:

40. Fischer N, Wris K (2002) The DEAD box protein Dhh1 stimulates the decapping enzyme Dcp1. EMBO J 21: 2788–2797.

41. Tseng-Rojenski SS, Chong JL, Thomas CB, Enamoto S, Berman J, et al. (2003) Functional conservation of Dhh1p, a cytoplasmic DEAD/H-box protein present in large complexes. Nucleic Acids Res 31: 4995–5002.

42. Wyers F, Minit M, Dufour ME, Vo LT, Lacroute F (2000) Deletion of the PAB1 gene affects translation initiation and suppresses a PAB1 gene deletion in yeast. Mol Cell Biol 20: 3530–3549.

43. Sweet T, Kovalak C, Coller J (2012) The DEAD-box protein Dhh1 promotes decapping by slowing ribosome movement. PLoS Biol 10: e1001342.

44. Mulholland D, Parker R (2003) The yeast EDC1 mRNA undergoes deadenylate-independent decapping stimulated by Not2p, Not4p, and Not5p. EMBO J 22: 10331–10345.

45. Ka M, Park YU, Kim J (2008) The DEAD box RNA helicase, Dhh1, functions in regulating m6A1 translation by regulating Ste12 translation in Saccharomyces cerevisiae. Biochem Biophys Res Commun 367: 262–269.

46. Park YU, Hur H, Ka M, Kim J (2006) Identification of translational regulation target genes during filamentous growth in Saccharomyces cerevisiae: regulatory role of Cas20 and Dhh1. Eukaryot Cell 5: 2120–2127.

47. Pedraza-Zegarra E, Vargas S, Ballesteros R, Parker R, Thiele DJ, et al. (2008) The C2H2-ABE-binding protein recruits the Dhh1 helicase to promote the decay of succinate dehydrogenase SDH1 mRNA in response to iron deficiency. J Biol Chem 283: 26827–26835.

48. Liu H, Kiledjian M (2003) Synergistic mRNA decay activity facilitates 5’-mRNA decay. Mol Cell Biol 25: 9764–9772.

49. Tseng SS, Weaver PL, Liu Y, Hitomi M, Tartakoff AM, et al. (1998) Dhp5p, a cytoplasmic RNA helicase, is required for poly(A)-RNAs export. EMBO J 17: 2623–2632.

50. Snay-Hodge CA, Colot HV, Goldstein AL, Cole CN (1998) Dhp5p/Ratlp is a yeast nuclear pore-associated DEAD-box protein essential for RNA export. EMBO J 17: 8653–8667.

51. Hurto RL, Hopper AK (2011) P-body components, Dhh1 and Pat1, are involved in RNA nuclear-cytoplasmic dynamics. RNA 17: 912–924.

52. Checkley MA, Nagashima K, Lockett SJ, Nyanzeri KM, Garrifken DJ (2010) P-body components are required for Ty1 retrotransposition during assembly of transposition-competent virus-like particles. Mol Cell Biol 30: 382–398.

53. Strubinski V, Ramos KS (2007) Rpm2p, a protein subunit of mitochondrial RNase P, physically and genetically interacts with cytoplasmic processing bodies. Nucleic Acids Res 35: 1301–1311.

54. Goldstein AL, McCusker JH (1999) Three new dominant drug resistance cassettes for studying the expression and turnover of the monocarboxylate permease. J Biol Chem 274: 2651–2662.

55. Tseng SS, Weaver PL, Liu Y, Hitomi M, Tartakoff AM, et al. (1998) Dhp5p, a cytoplasmic RNA helicase, is required for poly(A)-RNAs export. EMBO J 17: 2623–2632.

56. Snay-Hodge CA, Colot HV, Goldstein AL, Cole CN (1998) Dhp5p/Ratlp is a yeast nuclear pore-associated DEAD-box protein essential for RNA export. EMBO J 17: 8653–8667.

57. Lemoine S, Combes F, Servant N, Le Crom S (2006) Goulphar: rapid access to microarray data analyses of the Saccharomyces cerevisiae role on gene expression in yeast. PLoS ONE 1:393 (Pt 3). doi:10.1371/journal.pone.000393.
Robinson MD, Griegull J, Mohammad N, Hughes TR (2002) FunSpec: a web-based cluster interpreter for yeast. BMC Bioinformatics 3: 35.

Taylor R, Kebaara BW, Nazarenus T, Jones A, Yamanaka R, et al. (2005) Gene set coregulated by the Saccharomyces cerevisiae nonsense-mediated mRNA decay pathway. Eukaryot Cell 4: 2066–2077.

Andrade RP, Casal M (2001) Expression of the lactate permease gene JEN1 from the yeast Saccharomyces cerevisiae. Fungal Genet Biol 32: 103–111.

Nissan T, Rajayaguru P, She M, Song H, Parker R (2010) Decapping activators in Saccharomyces cerevisiae act by multiple mechanisms. Mol Cell 39: 775–783.

Guo Z, Zheng W, Tang S, Liu X, Zinkel RA, et al. (2006) Impact of nonsense-mediated mRNA decay on the global expression profile of budding yeast. PLoS Genet 2: e203.

Geider S, Lojek L, Khalil AM, Baker KE, Coller J (2012) Decapping of long noncoding RNAs regulates inducible genes. Mol Cell 45: 279–291.

van Dijk EL, Chen CL, d’Aubenton-Carafa Y, Gourvennec S, Kwapisz M, et al. (2011) XI:Ts are a class of Xrn1-sensitive antisense regulatory non-coding RNA in yeast. Nature 475: 114–117.

Hoosey J, Rabb L, Grünstein M, Tolliver D, Vogelauer M (2008) A mRNA modulates histone modification and mRNA induction in the yeast GAL gene cluster. Mol Cell 32: 603–695.

Xu Z, Wei W, Gagneur J, Perocchi F, Clauder-Munster S, et al. (2009) Bidirectional promoters generate pervasive transcription in yeast. Nature 457: 1033–1037.

Kramer S, Queiroz R, Ellis L, Hoehn JD, Clayton G, et al. (2010) The RNA helicase DHH1 is central to the correct expression of many developmentally regulated mRNAs in trypanosomes. J Cell Sci 123: 699–711.

Holstein FB, Alves LR, Probst CM, Dallagiovanna B, Marchini FK, et al. (2010) Protein and mRNA content of TcDHH1-containing mRNPs in Trypanosoma cruzi. FEBS J 277: 3415–3426.

Mitchell SF, Jain S, She M, Parker R (2013) Global analysis of yeast mRNPs. Nat Struct Mol Biol 20: 127–133.

Erez O, Kahana Y, Mazzoni C, Mancini P, Verdone L, Madeo F, Serafini A, et al. (2003) A truncated form of KlLsm4p and the absence of factors involved in mRNA decapping trigger apoptosis in yeast. Mol Biol Cell 14: 721–729.

Kushner N, Zhang D, Touzjian N, Esse M, Lieberman J, et al. (2003) A fragment of anthrax lethal factor delivers proteins to the cytosol without requiring protective antigen. Proc Natl Acad Sci U S A 100: 6652–6657.

Kihara A, Igarashi Y (2004) Cross talk between sphingolipids and glycerophospholipids in the establishment of plasma membrane asymmetry. Mol Biol Cell 15: 4949–4959.

Fujita K, Matsuyama A, Kobayashi Y, Iwashashi H (2006) The genome-wide screening of yeast deletion mutants to identify the genes required for tolerance to ethanol and other alcohols. FEMS Yeast Res 6: 744–750.

Pagani MA, Casamayor A, Serrano R, Atrian S, Arino J (2007) Disruption of iron homeostasis in Saccharomyces cerevisiae by high zinc levels: a genome-wide study. Mol Microbiol 65: 521–537.

Raher M, Wameling MM, Struyns EA, Joppich G, Krobutsch S, et al. (2008) A catalytic block does not sufficiently explain how 2-deoxy-D-glucose inhibits cell growth. Proc Natl Acad Sci U S A 105: 17807–17811.

Benners MG, Moreno DE, Olson DK, Nguyen Q, Ricarte F, et al. (2010) Genomic analysis of severe hypersensitivity to hygromycin B reveals linkages to vacuolar defects and new vacuolar gene functions in Saccharomyces cerevisiae. Curr Genet 56: 121–137.

Gregori C, Schuller C, Frohmer IE, Ammerer G, Kuchler K (2008) Weak organic acids trigger conformational changes of the yeast transcription factor War1 in vivo to elicit stress adaptation. J Biol Chem 283: 25752–25764.

Blewett NH, Goldstrohm AC (2012) A eukaryotic translation initiation factor 4E-binding protein promotes mRNA decapping and is required for PUF repression. Mol Cell Biol 32: 4181–4194.

Goldstrohm AC, Hook BA, Scay DJ, Wickens M (2006) PUF proteins bind Pop2p to regulate messenger RNAs. Nat Struct Mol Biol 13: 535–539.

Thomas BJ, Rothstein R (1989) Elevated recombination rates in transcriptionally active DNA. Cell 56: 619–630.