Transcriptional regulation of the *Saccharomyces cerevisiae DAL5* gene family and identification of the high affinity nicotinic acid permease *TNA1* (*YGR260w*)

Bertrand Llorente*, Bernard Dujon

Unité de Génétique Moléculaire des Levures (URA 2171 CNRS, UFR927 Univ. P. and M. Curie), Département des Biotechnologies, Institut Pasteur, 25 rue du Dr Roux, F-75724 Paris Cedex 15, France

Received 9 May 2000

Edited by Horst Feldmann

Abstract We have studied the transcript levels of *YGR260w* and *YLR004c*, two genes encoding members of the yeast Dal5p subfamily of the major facilitator family, and we show that they increase when extracellular nicotinic acid and thiamine, respectively, are absent. The deletion of *YGR260w* in a *bna1* auxotrophic mutant for nicotinic acid prevents growth at low nicotinic acid concentration. This suggests that *YGR260w* is necessary for nicotinic acid import into the cell. The direct measurement of nicotinic acid uptake on whole cells demonstrates that *YGR260w* encodes the yeast high affinity nicotinic acid permease. Its apparent K_m of 1.7 μM is low enough to allow the uptake of the low concentrations of nicotinic acid normally secreted by wild type cells.

Key words: Vitamin; Niacin; Thiamine; Genetic redundancy; Yeast; Transporter

1. Introduction

In *Saccharomyces cerevisiae*, the *DAL5* gene family is composed of the eight following genes *YJR152w* (*DAL5*), *YLR004c*, *YLL055w*, *YGR260w*, *YIL166c*, *YAL067c* (*SEO1*), *YGR065c* (*VHT1*), *YCR028c* (*FEN2*), and of two contiguous ORFs, *YOL163w* and *YOL162w*, constituting a putative frame-shifted pseudogene [1]. This subfamily belongs to the anion:cation symporter (ACS) family (TC number 2.A.1.14) from the major facilitator superfamily (MFS) [2]. All of its yeast members encode putative weak acid permeases, and, before this work, their exact substrates were identified for only three of them. *DAL5* encodes an allantoate and ureidosuccinate permease subjected to nitrogen catabolite repression [3,4]; *FEN2* encodes a pantothenate permease [5] and *VHT1* encodes a high affinity biotin permease [6]. *VHT1* transcripts specifically accumulate when the extracellular biotin concentration is low. Accumulation of the transcript of a transporter gene induced by low concentrations of its substrate has also been observed in yeast in the case of *THI10* (*YLR237w*), the thiamine permease encoding gene [7].

Such transcriptional regulation suggested to us that other transporter-encoding genes could undergo the same kind of regulation. We thus searched such transporter-encoding genes by analyzing the transcript accumulation of all the members of the *DAL5* gene subfamily at low external concentrations of various vitamins. We found that transcripts of *YGR260w* and *YLR004c* accumulate when cells are grown in low concentrations of nicotinic acid and thiamine, respectively. By a genetical approach, we show that *YGR260w* is necessary for the import of nicotinic acid into the cell, and by direct measurement of nicotinic acid uptake, that it encodes a high affinity nicotinic acid permease. We propose to call this gene *TNA1*, this being the first, and only one so far, transporter of nicotinic acid encoding gene identified in a non-mammalian organism.

2. Materials and methods

2.1. Yeast strains and gene deletions

All yeast strains used in this study (Table 1) are direct isogenic derivatives of the sequenced strain S288C [8].

Single gene deletions were made according to [9] using *TRP1* as the selection cassette in the case of *YJR025c* and *YGR260w*, and *HIS3* in the case of *YLR004c*. Table 2 lists the oligonucleotides used to perform the deletions. Each deletion eliminates the entire ORF. All deletion strains were verified by genomic Southern blot analysis using the transformation cassette as a probe. The double deletion strains for both *YJR025c* and *YGR260w* were obtained by crossing FYBL243 and FYBL1-22B/BL238 deleted for *YJR025c* and *YGR260w*, respectively, and sporulating the resulting diploid strain FYBL244. Haploid double deletions are the [TRP^+] ascospores isolated from non-parental ditypes.

2.2. Growth media and molecular methods

Standard DNA and RNA manipulations as well as standard yeast growth media are as described in [10]. The synthetic complete growth medium used (SC) corresponds to the G0 minimal medium with sulfate [11] but lacking $(NH_4)H_2PO_4$ and containing various concentrations of vitamins as indicated. For solid media, both Bacto-agar (DIFCO) and HGT agarose (SeaKem) were used, and no growth difference was observed.

2.3. Measurement of nicotinic acid uptake

Nicotinic acid uptake was measured at 30°C on exponentially growing cells ($1–3\times10^7$ cells per ml) in SC medium without nicotinic acid (SC-Nic) following the protocol described in [12]. 1 ml of yeast culture was added to 2–20 μl of a solution containing 10^{-3}M non-radiolabelled nicotinic acid plus 1.6×10^{-4}M (1739 Bq/nmol) nicotinic acid-carboxy-^{14}C (Sigma) and incubated for 15 s, then quickly filtered through Whatman GF/C filters, which were washed twice with 5 ml ice-cold water and counted for radioactivity. After 15 s of incubation, nicotinic acid uptake is still a linear function of time (data not shown).

*Corresponding author. Fax: (33)-1-40 61 34 56.
E-mail: llorente@pasteur.fr

PII: S0014-5793(00)01698-7

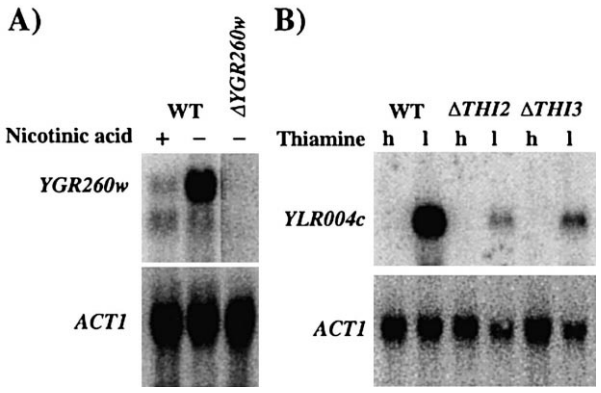

Fig. 1. Transcripts of *YGR260w* and *YLR004c* visualized by Northern blot experiments. A: Transcript of *YGR260w*. 10 μg of total RNA from the wild type strain FYBL1-8B and the *YGR260w*-deleted strain FYBL1-22B/BL239 grown in the presence of 4 μM nicotinic acid (lane +) and in the absence of nicotinic acid (lane −) were run on an 1.5% agarose gel containing 0.65% formaldehyde, transferred onto a Hybond N+ membrane (Amersham) and then hybridized, first, with the entire sequence of the PCR amplified *YGR260w* ORF (see Table 2 for the primers used) and, second, with *ACT1*, used as an internal control. The specificity of the *YGR260w* hybridization signal is demonstrated by the absence of signal in the lane containing FYBL1-22B/BL239 transcripts hybridized with *YGR260w*. B: Transcript of *YLR004c*. 10 μg of total RNA from FYBL1-8B, FYBL138 (Δ*THI2*) and FYBL142 (Δ*THI3*) [10] grown in the presence of 1 μM of thiamine (lane h for high concentration) and in the presence of 10 nM of thiamine (lane l for low concentration) were treated as in A.

3. Results and discussion

3.1. YGR260w and YLR004c transcript levels are regulated by extracellular nicotinic acid or thiamine, respectively

By analogy to the transcript regulation of the yeast biotin permease encoding gene *VHT1* [6] and the yeast thiamine transporter gene [7], we have tested the influence of the absence of various vitamins and growth factors on the transcript levels of all the genes of the *DAL5* yeast permease gene subfamily. As the known substrates of the *DAL5* family are weak acids, nicotinic acid was worth testing. We also decided to test the influence of other vitamins and growth factors, namely, thiamine, pantothenate, pyridoxine, *myo*-inositol and biotin. Fig. 1 shows that the transcript levels of *YGR260w* and *YLR004c* increase when extracellular concentrations of nicotinic acid or thiamine, respectively, are low. We confirmed that the *VHT1* transcript level is regulated by extracellular biotin (data not shown). No other gene of the *DAL5* family undergoes a similar transcriptional regulation by any of the other vitamins and growth factors tested (data not shown).

YGR260w is the first yeast gene known to be regulated by nicotinic acid. We thus hypothesized that *YGR260w* could encode a nicotinic acid permease. In the case of *YLR004c*, the thiamine transporter was already known [7], but *YLR004c* could encode a permease of a metabolite involved in the thiamine biosynthesis pathway (see below).

3.2. The DAL5 gene subfamily and the nicotinic acid uptake

S. cerevisiae is a nicotinic acid prototrophic species [13]. *YJR025c* (*BNA1*) encodes a 3-hydroxyanthranilic acid dioxygenase (EC 1.13.11.6) and is the only gene demonstrated to be involved in this biosynthetic pathway [14], that leads to the formation of the two essential cofactors NAD and NADP. Deletion of *BNA1* (*YJR025c*) leads to an almost complete absence of growth in the absence of nicotinic acid into the medium, while no distinctive growth phenotype is detected when 4 μM nicotinic acid is present [14]. This suggests that this compound can cross the yeast plasma membrane. As *YGR260w* is part of the *DAL5* permease encoding gene family, and that its transcripts accumulate when extracellular concentration of nicotinic acid is low (Fig. 1A), we hypothesized that *YGR260w* could encode a nicotinic acid permease.

To test this hypothesis, we constructed the strains FYBL241, FYBL1-22B/BL238 and FYBL244-3A, deleted respectively for *YJR025c* (*BNA1*), *YGR260w*, and for both. These strains were tested for growth in the presence of various amounts of nicotinic acid (Fig. 2). We confirmed that the deletion of *YJR025c* leads to an almost complete absence of growth at low nicotinic acid concentration. We observed that the deletion of *YGR260w* alone did not alter growth either in the presence or in the absence of nicotinic acid (data not shown). Phenotypic analysis of FYBL244-3A did not reveal a growth defect in the presence of high nicotinic acid concen-

Table 1
Strains used in this study

Strain	Genotype	Origin
FYBL1-8B	MATa ura3-Δ851 leu2-Δ1 his3-Δ200 lys2-Δ202	our collection
FYC2-6A/BL2	MATα ura3-Δ851 leu2-Δ1 trp1-Δ63 his3-Δ200	our collection
FYBL1-22B	MATa ura3-Δ851 trp1-Δ63 his3-Δ200 lys2-Δ202	our collection
FYBL241	MATα ura3-Δ851 leu2-Δ1 trp1-Δ63 his3-Δ200 *YJR025c::TRP1*	FYC2-6A/BL2 transformant
FYBL243	MATα ura3-Δ851 leu2-Δ1 trp1-Δ63 his3-Δ200 *YJR025c::TRP1*	FYC2-6A/BL2 transformant
FYBL1-22B/BL238	MATa ura3-Δ851 trp1-Δ63 his3-Δ200 lys2-Δ202 *YGR260w::TRP1*	FYBL1-22B transformant
FYBL1-22B/BL239	MATa ura3-Δ851 trp1-Δ63 his3-Δ200 lys2-Δ202 *YGR260w::TRP1*	FYBL1-22B transformant
FYBL244	MATa/MATα ura3-Δ851/ura3-Δ851 leu2-Δ1/+ his3-Δ200/his3-Δ200 trp1-Δ63/trp1-Δ63 lys2-Δ202/+ *YJR025c::TRP1*/+ *YGR260w::TRP1*/+	cross of FYBL1-22B/238
FYBL244-3A	MATα ura3-Δ851 his3-Δ200 lys2-Δ202 trp1-Δ63 *YJR025c::TRP1 YGR260w::TRP1*	FYBL244 ascospore
FYBL244-6C	MATα ura3-Δ851 his3-Δ200 lys2-Δ202 trp1-Δ63 *YJR025c::TRP1 YGR260w::TRP1*	FYBL244 ascospore
FYBL1-8B/BL194	MATa ura3-Δ851 leu2-Δ1 his3-Δ200 lys2-Δ202 *YLR004c::HIS3*	FYBL1-8B transformant

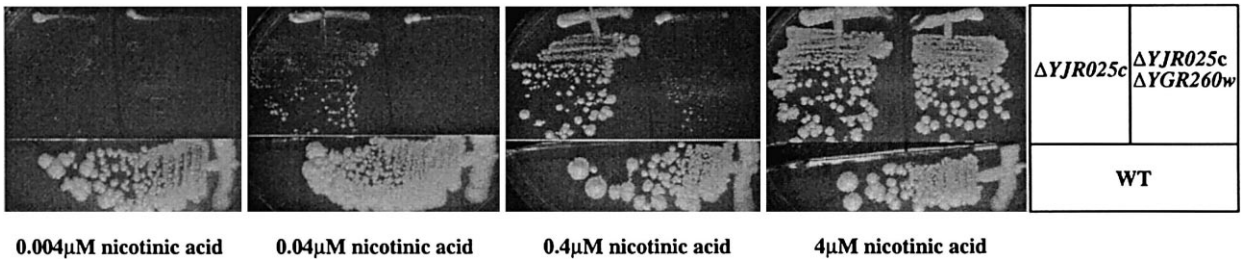

Fig. 2. Plate growth assays performed on FYBL241 (Δ*YJR025c*) and FYBL244-3A (Δ*YJR025c* Δ*YGR260w*). Cells were streacked on SC media containing various concentrations of nicotinic acid. FYBL244-3A growth is abolished at 0.04 μM nicotinic acid, while residual growth of FYBL241 is observed at 0.004 μM nicotinic acid and even in the absence of nicotinic acid (data not shown). Note that the three strains were streacked onto the same plates but that a strip of agar was removed between the wild type FYBL1-8B strain and the two other ones in order to avoid possible diffusion of secreted nicotinic acid (see Fig. 4).

tration (4 μM). Growth defects appear at lower nicotinic acid concentrations. When the nicotinic acid concentration is lower than 0.04 μM, the doubly deleted strain FYBL244-3A cannot grow while the singly deleted strain FYBL241, can. This double deleted strain is thus a real auxotrophic strain for nicotinic acid, while the strain deleted only for *YJR025c* is not. Those results are consistent with the hypothesis that *YGR260w* could encode a high affinity nicotinic acid permease, and that another system is responsible for the import of this compound when it is present at high concentration into the medium. This second system could either be passive diffusion as was proposed for biotin [6], or the existence of a low affinity permease. At this stage of our analysis, we could not, however, exclude the possibility that Ygr260p just acts as a sensor of the external nicotinic acid concentration that would activate an import system operating when the concentration of nicotinic acid is low, as is the case for glucose [15]. In contrast to sensor proteins, Ygr260p does not possess an amino or carboxy terminal long cytoplasmic extension compared to the other members of the *DAL5* family.

In order to directly demonstrate that *YGR260w* encodes the high affinity yeast nicotinic acid permease, we performed nicotinic acid uptake experiments (Fig. 3). In the wild type strain FYC2-6A/BL2, nicotinic acid uptake is compatible with a Michaelis–Menten reaction, with an apparent K_m of 1.7 μM of nicotinic acid. The same experiments performed in the *YGR260w*-deleted strain FYBL1-22B/BL238 (Fig. 3) shows an almost complete absence of uptake. The results of all experiments being measured after only a 15 s incubation of the cells with the ^{14}C-labelled nicotinic acid, it is very unlikely that Ygr260p just acts as a sensor of the external nicotinic acid concentration triggering a dependent reaction. Rather, we believe that Ygr260p is the high affinity nicotinic acid permease itself.

Fig. 4 shows a 'cross-feeding' experiment that illustrates that nicotinic acid is released from prototrophic cells into the medium as are many other metabolites such as thiamine [16]. The concentration of the released nicotinic acid is high enough to allow its uptake by cells containing *YGR260w* and lacking *YJR025c*, but too low to allow the growth of cells deleted for both *YGR260w* and *YJR025c*.

3.3. The DAL5 gene subfamily and the thiamine metabolism

S. cerevisiae is a thiamine prototrophic species that, nevertheless, imports and metabolizes exogenous thiamine when available [17]. The thiamine transporter gene *THI10* (*YLR237w*) [7] is part of the nucleobase:cation symporter-1 (NCS1) family (TC number 2.A.39) [2]. It is essential for thiamine uptake. Its transcript, like those of other genes involved in the yeast thiamine biosynthesis pathway [10], accumulates in the presence of low extracellular thiamine concentration. Transcriptional activation of these genes is under the positive control of the two genes *THI2* and *THI3*.

Fig. 1B shows that transcript from *YLR004c* also accumulates in the absence of exogenous thiamine, and that this accumulation is also under the positive control of *THI2* and *THI3*. Given the data concerning *THI10*, it is excluded that *YLR004c* encodes another plasma membrane thiamine transporter. It could however encode a transporter of a metabolite involved in the yeast thiamine biosynthesis pathway. We thus performed the deletion of *YLR004c* and searched for a growth phenotype in the absence of exogenous thiamine. The strain

Table 2
Oligonucleotides used in this study

	Oligonucleotide nomenclature	Sequence (5′ to 3′)
Deleted ORF		
YJR025c	D_yjr025c lo	GTACAACTAACAACTCTTCTAATACTTAATTAGATTGAGGGCGTGCGTAGgaattcgagctcgtttaaac
	D_yjr025c up	ATGTTTAATACTACACCAATTAATATCGACAAATGGTTGAAGGAGAACGAcggatccccgggttaattaa
YGR260w	D_ygr260_lo	AGTGGAGGGTGAAGAAAGTCAAGCCTAATACATGTACTTAAACTCAGGGTgaattcgagctcgtttaaac
	D_ygr260_up	GAAATATTTCTTCACTTTCGAGCATTGAACTATTGTCATTACCTCTAGcggatcccgggttaattaa
YLR004c	upper delta ylr004c	TGAATATTACAGACTAAAAATATTATATATTGTAAATTAAAAGTTGATTActcttggcctcctctag
	lower delta ylr004c BIS	TTTACTTTGTTGTTGCTGAGAAGTAGTGAGAGTGTCAGCAAATCAATACAtcgttcagaatgacacg
Amplified ORF		
YGR260w	GR260A	ATGAGCAACAAATTTACAAT
	GR260T	CTAATACATGTACTTAAACT
YLR004c	ylr004c-A	CGGGATCCATGAAAAACATGTCACAACG
	ylr004c-T	AATATGCGGCCGCCGTAAATGTACCTGAAATTT

TRP1 and HIS3 homologous sequences are in lower cases.

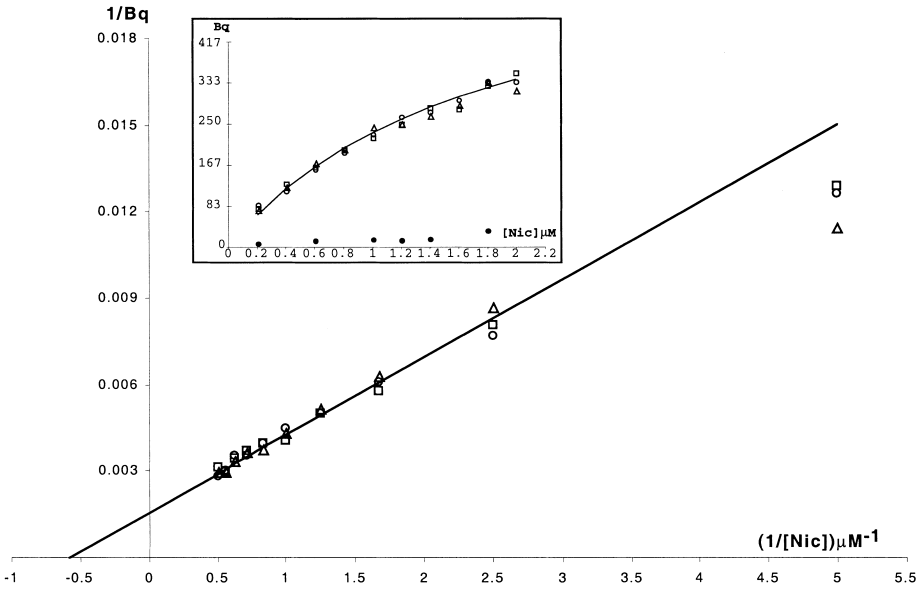

Fig. 3. Nicotinic acid uptake measurements. Nicotinic acid uptake (Bq) of the wild type strain FYC2/6A-BL2 (open symbols) and the *YGR260w* deleted strain FYBL1-22B/BL238 (black circles, upper panel) were determined at different nicotinic acid concentrations as described in Section 2. Three independent measurements were performed on the wild type FYC2/6A-BL2 strain (open triangles, open squares and open circles). Fitting the experimental data to the equation $Bq = Bq_{max} \times [Nic]/(K_m + [Nic])$ was performed by minimizing the sum of the χ^2 calculated for each experimental point. The constants obtained for the fitting curve (black) are $K_m = 1.7$ μM and $Bq_{max} = 632$ Bq. The release of nicotinic acid out of the cells has not been taken into consideration for the K_m calculation.

FYBL1-8B/BL194 deleted for *YLR004c* is able to grow as well as the wild type strain FYBL1-8B in the absence of exogenous thiamine (data not shown). This demonstrates either that *YLR004c* is not essential for the transport of any essential component involved in the yeast thiamine biosynthesis pathway, or that another functionally equivalent transporter exists.

4. Conclusion

At the beginning of our work, only three of the eight members of the Dal5p subfamily [1] were known to be high affinity permeases. Interestingly, their substrates, biotin, pantothenate, allantoate and ureidosuccinate (for Ygr065p, Ycr028p and Yjr152p, respectively, see Fig. 5), contain a carboxylic group suggesting that this radical might be the substrate-recognition determinant of the entire family [5]. In addition, the expression of *YGR065c* is highly induced when the external concentration of biotin is low [6], while that of *YJR152w* is subjected to nitrogen catabolite repression [3].

We have now demonstrated that two other members of the family, *YGR260w* and *YLR004c*, also exhibit specific transcriptional induction by limited concentrations of nicotinic acid and thiamine, respectively. Interestingly, nicotinic acid, which also contains a carboxylic group, appears to be the substrate of Ygr260p, demonstrating that this gene product

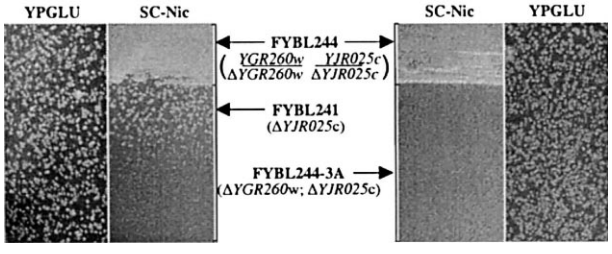

Fig. 4. Cross-feeding experiment. Approximately 3000 cells of the FYBL241 and FYBL244-3A strains were plated homogeneously on SC medium lacking nicotinic acid and on YPGLU. The same quantity of cells from the prototrophic diploid strain FYBL244 were put on a small region of the plates containing SC medium without nicotinic acid. Pictures were taken after a 2 days incubation at 30°C. Growth rate of FYBL241 was enhanced at the vicinity of FYBL244, while absolutely no growth of FYBL244-3A was observed.

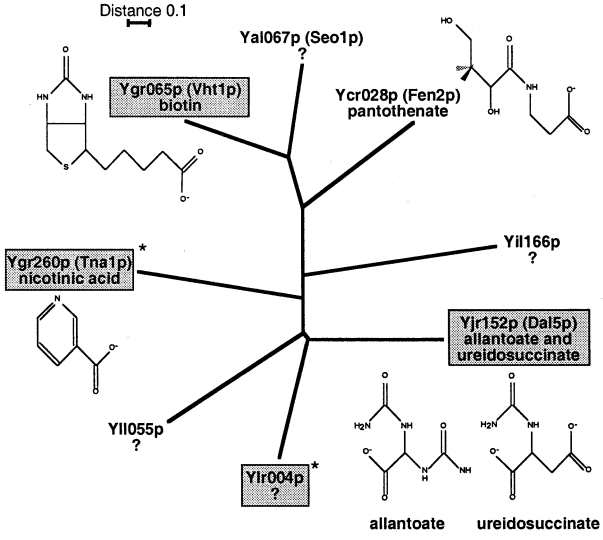

Fig. 5. Phylogenetic tree of the yeast Dal5p transporter subfamily adapted from [1] showing the known substrates of its members. Shadowed boxes identify the genes for which transcriptional regulation is known. Asterisks indicate results from this work.

is another high affinity permease of the Dal5p subfamily with yet another vitamin substrate. Conversely, thiamine, which is devoid of the carboxylic group is not the substrate of the product of *YLR004c*, despite the transcriptional regulation of this gene.

In the new era of transcription experiments, the remaining transporter encoding genes undergoing the same kinds of transcriptional regulation should rapidly be identified, and the substrates of most of them should rapidly be discovered.

Acknowledgements: We are very grateful to Christiane Volland and Rosine Haguenauer-Tsapis to have allowed us to perform the nicotinic acid uptake experiments. We thank André Goffeau and Cécile Fairhead for critical reading of the manuscript and our colleagues of the Unité de Génétique Moléculaire des Levures for advice and discussions. B. Dujon is a member of Institut Universitaire de France. This work was supported by Grant BIOCT97-2294 (EUROFAN2) from the European Commission (DGXII).

References

[1] Nelissen, B., De Wachter, R. and Goffeau, A. (1997) FEMS Microbiol. Rev. 21, 113–134.
[2] Paulsen, I.T., Sliwinski, M.K., Nelissen, B., Goffeau, A. and Saier Jr., M.H. (1998) FEBS Lett. 430, 116–125.
[3] Rai, R., Genbauffe, F., Lea, H.Z. and Cooper, T.G. (1987) J. Bacteriol. 169, 3521–3524.
[4] Rai, R., Genbauffe, F.S. and Cooper, T.G. (1988) J. Bacteriol. 170, 266–271.
[5] Stolz, J. and Sauer, N. (1999) J. Biol. Chem. 274, 18747–18752.
[6] Stolz, J., Hoja, U., Meier, S., Sauer, N. and Schweizer, E. (1999) J. Biol. Chem. 274, 18741–18746.
[7] Enjo, F., Nosaka, K., Ogata, M., Iwashima, A. and Nishimura, H. (1997) J. Biol. Chem. 272, 19165–19170.
[8] Winston, F., Dollard, C. and Ricupero-Hovasse, S.L. (1995) Yeast 11, 53–55.
[9] Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F. and Cullin, C. (1993) Nucleic Acids Res. 21, 3329–3330.
[10] Llorente, B., Fairhead, C. and Dujon, B. (1999) Mol. Microbiol. 32, 1140–1152.
[11] Hauswirth, W.W., Lim, L.O., Dujon, B. and Turner, G. (1987) in: Mitochondria, a Practical Approach, (Darley-Usmar, V.M., D.R.a.M.T.W., Eds.), chapter 7, pp. 171–244.
[12] Jund, R., Chevallier, M.R. and Lacroute, F. (1977) J. Membr. Biol. 36, 233–251.
[13] Ahmad, F. and Moat, A.G. (1966) J. Biol. Chem. 241, 775–780.
[14] Kucharczyk, R., Zagulski, M., Rytka, J. and Herbert, C.J. (1998) FEBS Lett. 424, 127–130.
[15] Ozcan, S., Dover, J. and Johnston, M. (1998) EMBO J. 17, 2566–2573.
[16] Haj-Ahmad, Y., Bilinski, C.A., Russell, I. and Stewart, G.G. (1992) Can. J. Microbiol. 38, 1156–1161.
[17] Iwashima, A., Nishino, H. and Nose, Y. (1973) Biochim. Biophys. Acta 330, 222–234.