Correspondence

Transcription of the yeast *TNA1* gene is not only regulated by nicotinate but also by *p*-aminobenzoate

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In a recent paper published in FEBS Letters, Llorente and Dujon presented results on the transcriptional regulation of the *YLR004c* gene and the *YGR260w* gene of *Saccharomyces cerevisiae* [1]. Both genes represent members of the *DAL5* gene family [2]. Two members of this gene family had previously been shown to encode plasma membrane-localised transporters for the vitamins biotin (the *VHT1* gene [3]) and panthotenate (the *FEN2* gene [4]). It had been published that the expression levels of *VHT1* [3] and *THI10*, the yeast thiamine permease [5], which is not related to the *DAL5* family, are enhanced at low extracellular substrate concentrations. Based on these findings, Llorente and Dujon postulated that the expression of the five other, so far uncharacterised members of the *DAL5* gene family (*YLR004c*, *YLL055w*, *YGR260w*, *YIL166c* and *YAL067c*) might also be modulated by decreased substrate concentrations.

Screening for changes in the transcript levels of *YLR044c* and *YGR260w*, Llorente and Dujon were able to show that a decrease in the extracellular concentration of thiamine increased the expression of *YLR044c* and a decrease in the concentration of nicotinate increased the expression of *YGR260w* [1]. Whereas the function of *YLR044c* remained unclear, *YGR260w* could clearly be characterised as a transport protein for nicotinate (vitamin B3) and the gene was named *TNA1* [1].

In our laboratory, we had used the same approach to study this question and we obtained identical results, showing enhanced expression of *YLR044c* at low thiamine and of *YGR260w* at low nicotinate. As Llorente and Dujon, we were able to describe the protein encoded by *YGR260w* as a transporter for nicotinate with a K_M of 2 μM. In this correspondence, we would like to add some results that were obtained during our attempts to analyse *TNA1* transcription and the function of Tna1p.

In contrast to the work of Llorente and Dujon, who analysed possible changes in *TNA1* transcription in the presence of thiamine, pantothenate, pyridoxine, *myo*-inositol and biotin [1], we examined also the effect of folate, riboflavin and *para*-aminobenzoate (PABA). Whereas reduced concentrations of riboflavin and folate had no effect on the transcriptional regulation, *TNA1* mRNA levels increased strongly at reduced extracellular concentrations of both, nicotinate and PABA (Fig. 1A). A consequent comparison of the structures of nicotinate and PABA (Fig. 1B) revealed the close similarity of these molecules and suggested that Tna1p might be a transporter for both compounds. Therefore, we generated yeast strains overexpressing the *TNA1* gene under the control of the *PMA1* promotor or missing an intact *TNA1* gene due to the insertion of the *Schizosaccharomyces pombe HIS5* gene. Analyses of *TNA1* mRNA levels in both mutant strains (Fig. 1C) confirmed these mutations. Using the *TNA1* wild type strain, the *TNA1*-overexpressing strain and the Δ*tna1* deletion mutant, we analysed the transport of radiolabelled [^{14}C]nicotinate (Fig. 1D) and [^{14}C]PABA (Fig. 1E). Unexpectedly, only the measurements with [^{14}C]nicotinate yielded increased transport rates in the overexpressing strain and an almost total lack of uptake activity in the Δ*tna1* knock-out mutant. In contrast, transport of [^{14}C]PABA was not influenced by the overexpression or deletion of *TNA1*.

These results showed that the structurally closely related compounds, nicotinate and PABA, can modulate the expression of the *S. cerevisiae TNA1* gene in the same way. However, only one of these molecules, nicotinate, is a substrate for the Tna1p transporter. In fact, Tna1p seems to be highly

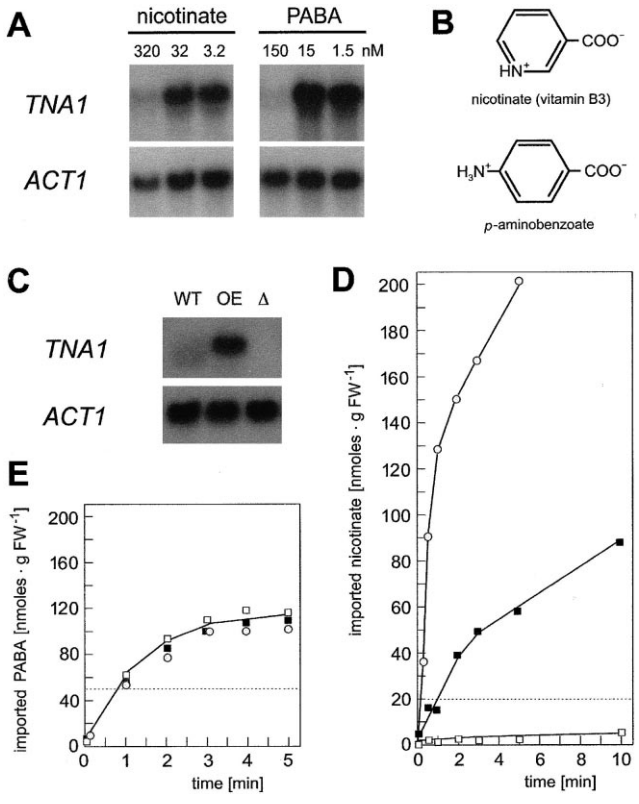

Fig. 1. Transcriptional regulation of *TNA1* by nicotinate and PABA and transport properties of Tna1p. A: Northern blot analysis of *YGR260w* mRNA levels at varying concentrations of nicotinate or PABA. Cells were grown on synthetic dextrose medium with the indicated concentrations of the two compounds. Northern blots were probed with a radiolabelled *TNA1* probe or with a radiolabelled *ACT1* probe. B: Nicotinate and PABA represent highly similar molecules. C: A *TNA1* wild type strain (WT) and strains overexpressing (OE) or missing (Δ) the *TNA1* gene show low, increased or no *TNA1* mRNA levels, respectively. Analyses of the transport properties of these strains for [^{14}C]nicotinate (in D) or [^{14}C]PABA (in E) revealed increased [^{14}C]nicotinate uptake in the *TNA1*-overexpressing strain and a lack of [^{14}C]nicotinate transport in the Δ*tna1* mutant. In contrast, uptake of [^{14}C]PABA was not influenced (wild type strain: closed squares; disruptant: open squares; overexpressing strain: open circles. The dotted lines depict the concentration equilibria for imported nicotinate or PABA).

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specific for nicotinate, because uptake of [^{14}C]nicotinate is inhibited only by excess concentrations of unlabelled nicotinate. No inhibition was observed when a 100-fold excess of unlabelled PABA, nicotinamide or iso-nicotinate was added (data not shown). Also the presence of 50 μM CCCP or 50 μM DNP, two uncouplers of transmembrane proton gradients, had no effect on the transport and accumulation of nicotinate by Tna1p (data not shown). This was unexpected, because transport of biotin by Vht1p [3] and of pantothenate by Fen2p [4] was strongly inhibited by these uncoupler concentrations. Moreover, in the uptake experiments presented in Fig. 1D, [^{14}C]nicotinate seemed to be accumulated to concentrations above the concentration equilibrium, suggesting an active transport mechanism for Tna1p. However, it cannot be excluded that this apparent accumulation of nicotinate results from rapid metabolism in the cytoplasm or from partitioning into intracellular compartments. Besides the unspecific intestinal H^+/monocarboxylate symporter, MCT1, from mammals [6], which accepts nicotinate as one of numerous substrates in vitro, Tna1p is the only so far described transporter for vitamin B3.

The observation that the reduction of each of the two compounds was able to enhance *TNA1* transcription alone (data not shown), even if the other compound was still present at non-inducing concentrations, suggests two independent sensing and/or signal transduction mechanisms for nicotinate and PABA. The physiological relevance of this PABA-driven induction/derepression of *TNA1* is unclear and suggests a so far unknown intracellular cross-talk between nicotinate on one hand and PABA, a biosynthetic precursor of PABA or their biosynthetic products NAD^+, $NADP^+$ and tetrahydrofolate on the other. Further analyses of the mechanisms involved in nicotinate and PABA sensing and in the transcriptional activation of the *TNA1* gene are on the way.

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