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# *PDA1* mRNA: a standard for quantitation of mRNA in *Saccharomyces cerevisiae* superior to *ACT1* mRNA

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The expression of many genes is regulated at the transcriptional level. Information about transcriptional regulation of a gene may provide insight into its function and mechanism by which transcription is regulated. A straightforward technique to analyze the transcription level of a gene is to determine its mRNA concentration in cells grown under the conditions of interest by Northern analysis (1). This technique usually involves separation of RNA molecules by gel electrophoresis, blotting onto membrane filters and subsequent hybridization to a suitable probe. However, due to practical problems, the amount of mRNA detected is often not representative for the actual mRNA concentration in the cell. This problem is partially overcome by determining the amount of the mRNA of interest relative to an internal standard, usually mRNA from a gene that is constitutively transcribed.

Actin mRNA encoded by the *ACT1* gene in *S.cerevisiae* is commonly used as such a standard (2). *ACT1* is constitutively transcribed during most conditions tested. We found however, that the concentration of *ACT1* mRNA decreased when cultures of *S.cerevisiae* reached or proceeded into stationary phase (Fig. 1). Hence, under these conditions the level of *ACT1* mRNA is not constant thus jeopardizing its function as an internal standard. Since actin is a component of the cytoskeleton which is mainly required during cell division, it might be questioned if transcription *ACT1* is constitutive at other than the logarithmic growth stage. This could be a serious hindrance in experiments with batch cultures where the growth stage is usually inadequately defined or when samples from stationary phase cultures have to be analyzed.

We have investigated the transcriptional regulation of the *PDA1* gene which encodes the E1α subunit of the pyruvate dehydrogenase complex from *S.cerevisiae* (3). *PDA1* mRNA of about 1300 nt is present in approximately one third of the concentration of *ACT1* mRNA in the exponential growth phase as determined by dot-blot hybridizations and easily detected in total RNA samples from *S.cerevisiae* (4). The level of *PDA1* mRNA is almost constant under all conditions tested. These include various growth stages in batch cultures on glucose. We also compared mRNA levels during growth on glucose, galactose, pyruvate and ethanol in rich media in batch cultures and during growth on glucose and ethanol and under anaerobic conditions in continuous cultures in defined media. In all cases the same amount of *PDA1* mRNA relative to the total amount of RNA is found (5). In contrast to *ACT1*, transcription of *PDA1* is also stable in stationary phase cultures (Fig. 1). Quantitation of the transcripts showed that the *ACT1* mRNA decreased more than 100-fold whereas the *PDA1* mRNA decreased only 3-fold (Table 1).

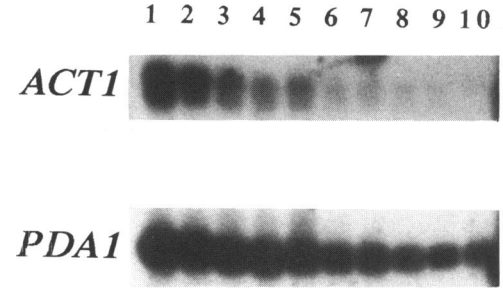


**Figure 1.** Northern hybridization of total RNA isolated from samples taken at different timepoints from a fermentation of *S.cerevisiae* strain MPY3 (6). The laboratory-scale fermentation was done in 15% w/w sugar wort, initially $O_2$ saturated, at 9°C in a Biostat E fermenter (Braun, Melsungen, Germany) with a working volume of 2.5 l, $N_2$ in the headspace and mechanically stirred at 200 r.p.m. Total RNA was isolated using the method of Schmitt *et al.* (7) and quantified by measuring the A260 in a LKB Ultraspec II spectrophotometer. RNA molecules were separated by electrophoresis in 2.2 M formaldehyde gels (1% agarose) in MOPS-buffer (0.2 M MOPS, 0.03 M $NaC_2H_3O_2$, 0.01 M EDTA, pH 7.0). Prior to loading, 2 µl sample was incubated 10 min at 65°C with 7 µl sample-buffer (100 µl formamide, 35 µl formaldehyde 36%, 20 µl 10 × MOPS-buffer). Before loading, 2 µl loading buffer (30% glycerol, 0.25% BromoPhenol Blue) was added. After electrophoresis the RNA was blotted onto Gene Screen Plus Nylon membranes (NEN research products, DuPont, Boston, USA) using the LKB 2016 VacuGene vacuum blotting system with 20× SSC (175.3 g/l NaCl, 88.2 g/l $Na_3C_6H_5O_7 \cdot 2H_2O$ pH 7.0 for 2 h). Subsequently the membrane was washed once with 6 × SSC and the RNA was fixed to the membrane by irradiation with UV. Hybridizations were done in hybridization buffer [2 × SSC, 1% SDS, 0,1% $Na_4P_2O_7$ and 1% blocking reagent (Boehringer Mannheim GmbH, Germany)] at 68°C. Washing was done in 2 × SSC + 1% SDS once for 5 min at RT and twice for at least 30 min at 68°C. Probes (DNA) were radioactively labelled using the oligolabelling kit (Pharmacia, Sollentuna, Sweden) and [α-$^{32}$P]dCTP (3000 Ci/mmol, Amersham). Kodak XAR-5 films were used for autoradiography. Amounts of mRNA were determined by densitometry of the autoradiographs on a Shimadzu Dual wavelength TLC scanner CS 930. Different exposures were scanned to ensure linearity. Before the second hybridization the first probe (*PDA1*) was entirely removed by a 30 min incubation in 0.1 × SSC + 0.1% SDS at 95°C. Equal amounts (~10 µg) of total RNA were loaded in each lane. Timepoints (h): 1 = 26.5; 2 = 51; 3 = 74.5; 4 = 98; 5 = 121.5; 6 = 145.5; 7 = 172.5; 8 = 214.5; 9 = 261.5; 10 = 362.5.

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**Table 1.** *PDA1* and *ACT1* mRNA levels in stationary phase culture

| | Time (h) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 26.5 | 51 | 74.5 | 98 | 121.5 | 145.5 | 172.5 | 214.5 | 261.5 | 362.5 |
| *PDA1*[a] | 100 | 76 | 60 | 102 | 64 | 63 | 48 | 34 | 32 | 35 |
| *ACT1*[a] | 100 | 82 | 106 | 43 | 20 | 12 | 3 | 0.5 | 0.8 | 0.2 |

[a]The amount of mRNA hybridizing to the *PDA1* or the *ACT1* probe is given as percentage of the initial amount at 26 h.

In summary, transcription of *PDA1* is constitutive under all conditions tested and the *PDA1* mRNA is easily detected in total RNA samples making it an excellent standard for mRNA quantitation.

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