Thiamine in *Schizosaccharomyces pombe*: dephosphorylation, intracellular pool, biosynthesis and transport

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Summary. We have investigated the thiamine metabolism in *Schizosaccharomyces pombe* and shown that: (1) Thiamine-repressible acid phosphate, coded for by the gene pho4, dephosphorylates thiamine phosphates indicating that the enzyme acts as a thiamine phosphate phosphatase. (2) In vivo synthesized thiamine is present intracellularly mainly as thiamine diphosphate. Starving cells for glucose decreases the intracellular thiamine pool. (3) The genes thi2, thi3 and thi4 control thiamine biosynthesis and probably code for thiamine biosynthetic enzymes. Thi3, which is involved in the synthesis of the pyrimidine moiety of the thiamine molecule, is allelic to the thiamine repressible gene nmt1. (4) Thiamine uptake is a thiamine regulated process, probably occurs by active transport and is controlled by the gene ptr1.

Key words: Thiamine repressible acid phosphatase – Thiamine biosynthesis – Thiamine transport – *Schizosaccharomyces pombe*

Introduction

Thiamine (vitamin B1) regulates gene expression in yeast. Schweingruber et al. (1986) showed that it represses the synthesis of thiamine-repressible acid phosphatase; in *S. pombe* it is coded for by the gene pho4 and in *Saccharomyces cerevisiae* by pho3. Recent results of Maundrell (1990) demonstrate that in *S. pombe* thiamine also represses the expression of a gene, nmt1, which is probably involved in thiamine biosynthesis. Regulation of *pho4* and *nmt1* by thiamine occurs at the level of transcription initiation. Thiamine-regulatable expression systems can be developed by the *pho4* and *nmt1* promoters. It has further been documented that thiamine acts as a specific inhibitor of the mating of haploid cells of opposite mating type in *S. pombe* (Schweingruber and Edenharter 1990). This phenomenon is not understood yet but probably also involves thiamine-regulated gene expression (Schweingruber, unpublished).

It has long been known that thiamine, in the form of its diphosphate, acts as cofactor of enzymes mainly involved in carbohydrate metabolism. These include pyruvate decarboxylase, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and transketolase (for review see Friedrich 1987). The role of thiamine as regulator of gene expression has, however, not yet been explored. To understand it in fission yeast some basic data about thiamine metabolism need to be known for this organism. In addition, mutants blocked at defined steps in thiamine metabolism have to be available.

In this communication we show that thiamine-repressible acid phosphatase acts in vivo as thiamine phosphate phosphatase; we also describe intracellular thiamine pools in *S. pombe* cells and report on the isolation and characterization of mutants defective in thiamine biosynthesis and transport.

Materials and methods

Strains and media. *S. pombe* wild-type strains and acid phosphatase-deficient (pho-)mutants are from our collection; pho1-44 has the entire *pho1* gene coding for phosphate-repressible acid phosphatase deleted and *pho4-4* carries an unknown mutation in the structural gene coding for thiamine-repressible acid phosphatase (Elliot et al. 1986; Yang and Schweingruber 1990). Strains were grown in supplemented or unsupplemented liquid or solid minimal medium (MM) as described by Schweingruber and Edenharter (1990).

Isolation and mapping of thi and ptr mutants. Equal amounts of cells from *pho4-44* h- and *pho1-44* h- were mixed and mutagenized with nitro-nitrosoguanidine (NNG) as described by Dhamija et al. (1987). Cells were sporulated and the spore suspension was used as source to select thi (thiamine auxotrophic) and ptr (pyrithiamine resistant) mutants. Thi mutants were selected by replica plating colonies grown on MM containing 50 nmoles thiamine/1 in two successive steps on MM. The thiamine auxotrophic phenotype of thi mutants became apparent only after the second plating. Thi mutants were tested for allelism by crossing and sporulating them on

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malt extract agar and plating 300–500 spores on MM and MM supplemented with thiamine. If no wild-type recombinants occurred between the two mutants crossed with each other we concluded that the two were allelic. Ptr mutants were isolated by plating 4 000–
16 000 spores per plate of the mutagenized stock on MM containing 5 μmoles pyrithiamine (Sigma) and by selecting growing colonies. Allelism of the mutants was examined by counting wild-type recom-
binants of progeny spores from crosses with the different ptr mu-
tants. Standard genetical methods used for the procedure men-
tioned above have been described by Gutz et al. (1974).

**Growth experiments with thi mutants.** Growth of thi mutants on thiamine, or on intermediates of thiamine biosynthesis, was tested as follows. Cells pregrown in two successive precultures containing 40 nmol thiamine/l were inoculated in 10 ml MM (supplemented or unsupplemented) and cultured at 30°C on a rotary lab shaker. 4-amino-5-hydroxymethyl-2-methylpyrimidine and 5-(2-hydroxy-
ethyl)-4-methylthiazole (later referred to as pyrimidine and thiazole
moieties) were kindly supplied by Dr. G. Moine from Hoffmann-
La Roche and Co. AG (Basel).

**Extraction of thiamine.** Thiamine was extracted either by sodium
acetate at 85°C, as described by Iwashima et al. (1973), or by HCl
according to a modified protocol of White and Spencer (1979). Cells
were grown in 50 ml Erlenmeyer flasks containing 10 ml MM at
39°C, harvested and washed in 0.1 M sodium acetate pH 4.0. For
extraction they were resuspended in 0.5 ml 0.1 M sodium acetate,
heated to 85°C (about 7 min) and left at this temperature for
20 min, or alternatively they were resuspended in 0.5 ml 0.6 M HCl
and extracted for 5 min at 25°C. Extracted cells were pelleted and
the supernatant was filtered through a Millex GV filter (Millipore).
If not immediately analyzed, extracts were stored at −20°C.

**Determination of thiamine and thiamine phosphates by HPLC.** Thia-
mine and thiamine phosphates were derivatized to thiochromes and
chromatographed by HPLC essentially as described by Ben-
tempsky et al. (1984). To this end 160 μl extract and 100 μl 0.02% KFe
(CN) in 15% NaOH were mixed and 20 μl of this mixture was
injected by a Rheonde 7010 injection valve equipped with a Tefzel
rotorseal. Thiamine and its phosphorylated derivatives were sepa-
rated on a PRP-1 precolumn (Hamilton) and a PRP-1 main column
(250×4.1 mm, O 10μ). Elution was achieved with solvent A
(8.5 mM sodium phosphate buffer pH 8.5 prepared with Milli-Q-
water) and solvent B (methanol, HPLC-grade, Merck). Thiamine
and its phosphorylated derivatives were separated on a PRP-1 pre-
column (Hamilton) and a PRP-1 main column (250×4.1 mm, O 10μ). Elution was achieved with solvent A
(8.5 mM sodium phosphate buffer pH 8.5 prepared with Milli-Q-
water) and solvent B (methanol, HPLC-grade, Merck). Thiamine
phosphates elute in the order triphosphate, diphosphate and
monophosphate at 10% B; following a steep gradient, thiamine
elutes at 50% B. Detection occurred in a Kontron spectroflu-
ophotometer SFM23 equipped with a 20 μl flow cell at an excita-
tion of 365 nm and emission of 430 nm. A Shimadzu integrator
recorded and integrated eluted peaks. The system was automatized

**Table 1.** Recovery of thiamine from wild-type and pho- mutants
after extraction with sodium acetate or HCl. Cells were grown in
MM up to an optical density OD530 of 2.5 and thiamine was ex-
tracted with sodium acetate or HCl as described in Materials and
methods.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Extraction with NaAc</th>
<th>Extraction with HCl</th>
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|        | TDP b, TMP b, Thia-
mine b | TDP b, TMP b, Thia-
mine b |
| pho1 + | nd c, nd | 5.8 | 5.2 | 0.12 | 0.26 |
| pho4 + | nd c, nd | 6.2 | 6.2 | 0.20 | 0.31 |
| pho1-44 pho4-4 | 5.4 | 0.97 | 0.19 | 5.2 | 0.93 | 0.24 |
| pho1-44 pho4-4 | 5.2 | 0.93 | 0.24 | 7.0 | 0.36 | 0.26 |

a Sodium acetate  

b Given as pmole/10^7 cells  
c Not detectable

**Results**

**Thiamine repressible acid phosphatase can dephosphorylate thiamine phosphates in situ**

To determine the intracellular thiamine content of *S. pombe*, cells were extracted either with 0.1 M sodium acetate at 85°C or with 0.6 N HCl at 25°C as described in Materials and methods. Both methods have been used to extract thiamine from *S. cerevisiae*. The two extraction methods gave different results for *S. pombe*. Extracting with sodium acetate yielded only unphosphorylated thiamine; extraction with HCl, however, gave mainly TDP. The total amount of extracted thiamine was roughly the same for both methods. The results for wild-type cells grown in MM are shown in Table 1. It is well known that at least a substantial fraction of vitamin B1 exists inside the cell in its active form as diphosphate. We, therefore, had to assume the occurrence of a thiamine dephosphorylating activity which acts in 0.1 M sodium acetate but not in 0.6 N HCl. Earlier we described two acid phosphatases which are located in the cell wall (for review see Schweingruber 1987). The two enzymes have a broad, but differ-
ent, substrate specificity in vitro. Their pH optimum is around 4 and they are coded for by the genes *pho1* and *pho4*, respectively. The *pho1*-coded acid phosphatase is phosphate-repressible whereas the enzyme coded for by *pho4* is thiamine-repressible. To test the possibility that the latter enzyme is in vivo a thiamine phosphate phosphatase, and responsible for the hydrolysis of extracted TMP and TDP, we tested different mutants defective in the activity of one or both of the acid phosphatase activities. As shown in Table 1 mutants exhibiting no thiamine-repressible acid phosphatase activity yield mainly TDP by both extraction methods. As tested by free spore and tetrad analyses the absence of phosphorylated thia-
mine in sodium acetate extracts always cosegregates with a functional *pho4* gene; in addition, when cells are grown in the presence of thiamine, which represses the activity of *pho4*-coded acid phosphatase activity, phospho-
phylated thiamine could be extracted with sodium acetate (data not shown). These observations strongly sug-
gest that thiamine-repressible acid phosphatase is specifi-
cally responsible for the dephosphorylation of TMP and
TDP in sodium acetate. The sodium acetate extraction