



Identification of the thiamin salvage enzyme thiazole kinase in *Arabidopsis* and maize

Mohammad Yazdani^{a,1}, Rémi Zallot^{b,1}, Meral Tunc-Ozdemir^a, Valérie de Crécy-Lagard^b, David K. Shintani^{a,*}, Andrew D. Hanson^{c,*}

^a Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV 89557, United States

^b Microbiology and Cell Science Department, University of Florida, Gainesville, FL 32611, United States

^c Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, United States

ARTICLE INFO

Article history:

Received 10 April 2013

Received in revised form 23 May 2013

Available online 28 June 2013

Keywords:

Arabidopsis thaliana

Brassicaceae

Zea mays

Poaceae

Thiamin

Thiazole

Salvage

Comparative genomics

ABSTRACT

The breakdown of thiamin (vitamin B₁) and its phosphates releases a thiazole moiety, 4-methyl-5-(2-hydroxyethyl)thiazole (THZ), that microorganisms and plants are able to salvage for re-use in thiamin synthesis. The salvage process starts with the ATP-dependent phosphorylation of THZ, which in bacteria is mediated by ThiM. The *Arabidopsis* and maize genomes encode homologs of ThiM (At3g24030 and GRMZM2G094558, respectively). Plasmid-driven expression of either plant homolog restored the ability of THZ to rescue *Escherichia coli* thiM⁻ mutant strains, showing that the plant proteins have ThiM activity in vivo. Enzymatic assays with purified recombinant proteins confirmed the presence of THZ kinase activity. Furthermore, ablating the *Arabidopsis* At3g24030 gene in a thiazole synthesis mutant severely impaired rescue by THZ. Collectively, these results show that ThiM homologs are the main source of THZ kinase activity in plants and are consequently crucial for thiamin salvage.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Thiamin (8) (vitamin B₁), as its active diphosphate form, is an essential cofactor for various enzymes that make or break C–C bonds (Müller et al., 2009b). Plants and most microorganisms can synthesize thiamin (8) de novo, but animals cannot and thus require it in the diet (Jurgenson et al., 2009). The de novo biosynthesis pathway and most of the enzymes involved are known in microorganisms and plants (Jurgenson et al., 2009; Goyer, 2010). In this pathway, the thiazole and pyrimidine moieties of thiamin are made separately and coupled together to form thiamin phosphate, (7), which is then converted to the diphosphate (9). The plant thiamin (8) biosynthesis pathway is shown in Fig. 1.

Thiamin (8) and thiamin diphosphate (9) are chemically and enzymatically labile (McCourt et al., 2006; Goyer, 2010; Fitzpatrick et al., 2012), and microorganisms and plants have the capacity to re-use the thiazole and pyrimidine fragments from thiamin (8) breakdown for thiamin synthesis (Li and Rédei, 1969; Jurgenson et al., 2009). For the thiazole moiety, 4-methyl-5-(2-hydroxy-

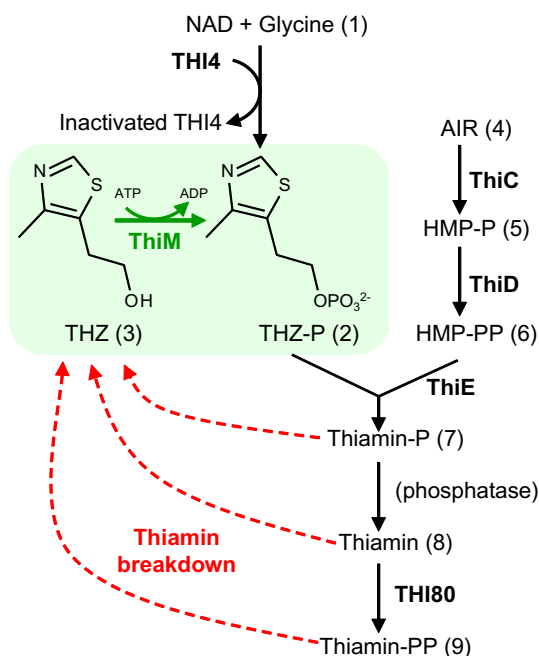
ethyl)thiazole (THZ) (3), the key salvage step is phosphorylation to give 4-methyl-5-(2-phosphonoxyethyl)thiazole (2) (THZ-P) (Fig. 1). The THZ kinase (EC 2.7.1.50) responsible for this step is encoded by thiM in *Escherichia coli* and other bacteria, and by the 3' region of THI6 in *Saccharomyces cerevisiae* (Mizote and Nakayama, 1989; Nosaka et al., 1994; Jurgenson et al., 2009; Paul et al., 2010). Nothing is yet known, however, about the THZ kinase enzyme in plants and the plant THZ kinase gene has not been identified (Goyer, 2010). Identifying this gene has become particularly worthwhile in light of the recent realization that THZ (3) salvage in fungi and plants is highly energetically beneficial (Chatterjee et al., 2011; Gerdes et al., 2012). The THZ synthesis protein THI4 (Fig. 1) is a single-turnover enzyme from which a cysteine residue provides the THZ sulfur atom (Chatterjee et al., 2011), so that producing a single THZ molecule irreversibly inactivates a THI4 polypeptide, comprised of ~350 amino acids. Therefore each THZ (3) molecule salvaged in effect saves the energy cost of re-synthesizing a whole 350-residue protein.

Here, the *Arabidopsis* and maize (*Zea mays*) THZ kinase genes were identified by demonstrating that ThiM homologs from these plants can functionally replace *E. coli* ThiM, that the recombinant plant proteins have THZ kinase activity, and that ablating the *Arabidopsis* gene results in severe loss of ability to salvage THZ (3) from the medium.

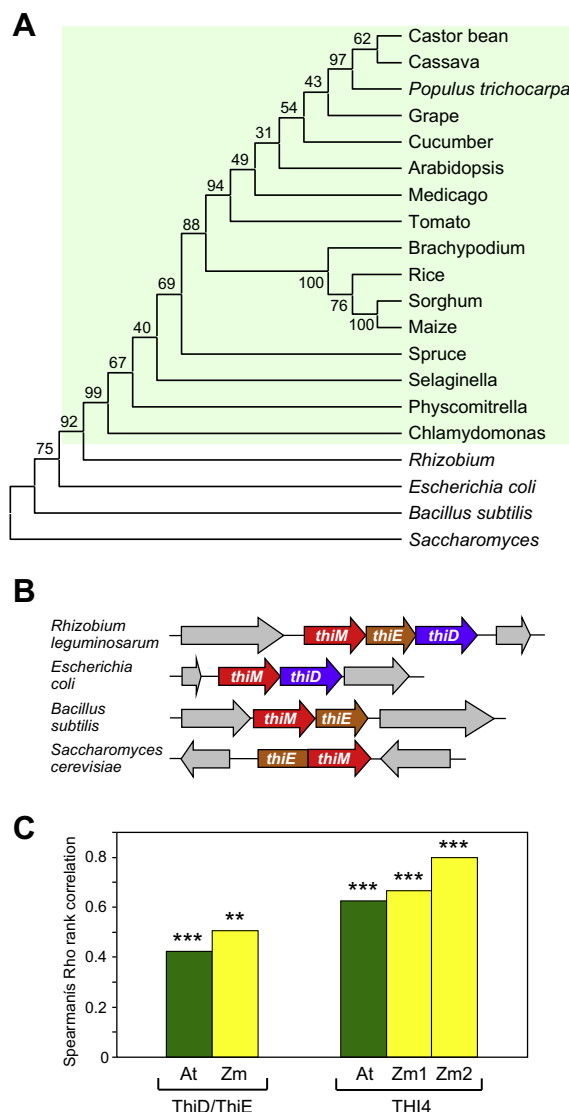
* Corresponding authors. Tel.: +1 775 784 4631; fax: +1 775 784 1650 (D.K. Shintani), tel.: +1 352 273 4856; fax: +1 352 392 5653 (A.D. Hanson).

E-mail addresses: shintani@unr.edu (D.K. Shintani), adha@ufl.edu (A.D. Hanson).

¹ These authors contributed equally to this work.



Apart from experimental evidence (Mizote and Nakayama, 1989; Nosaka et al., 1994; Zhang et al., 1997; Karunakaran et al., 2006), comparative genomic evidence robustly links microbial *thiM* genes with thiamin (8), based on chromosomal clustering, or fusion, of *thiM* with the thiamin synthesis/salvage genes *thiD* or *thiE* (Fig. 2B). The clustering of these genes in operonic structures, or their fusion, results in co-expression. There is analogous evidence for co-expression of the plant ThiM homologs with the same thiamin genes. Thus, expression of the *Arabidopsis* homolog is strongly positively correlated ($P < 0.001$) with that of the bifunctional ThiD-ThiE gene, and there is a similar correlation in maize (Fig. 2C). Moreover, the expression of the *Arabidopsis* ThiM homolog is highly correlated with that of the de novo THZ synthesis gene TH14 (Fig. 2C), and there are comparable correlations in maize



A functional complementation assay for ThiM (thiazole kinase) activity was developed in *E. coli* by deleting either of two de novo THZ synthesis genes, *thiG* or *thiH* (Leonardi et al., 2003), as well as *thiM*. The resulting double deletant strains can neither synthesize

Download English Version:

<https://daneshyari.com/en/article/5165080>

Download Persian Version:

<https://daneshyari.com/article/5165080>

[Daneshyari.com](https://daneshyari.com)