

vidence that the phosphatase Sit4 is involved in Art2-dependent Thi7 endocytosis upon thiamine and CHX treatment. However, our data do not support an inhibitory role of Npr1 in thiamine-induced pathway. Therefore, additional work will be required to describe in detail all the facets of Npr1-dependent regulation of α -arrestins and thiamine transporters.

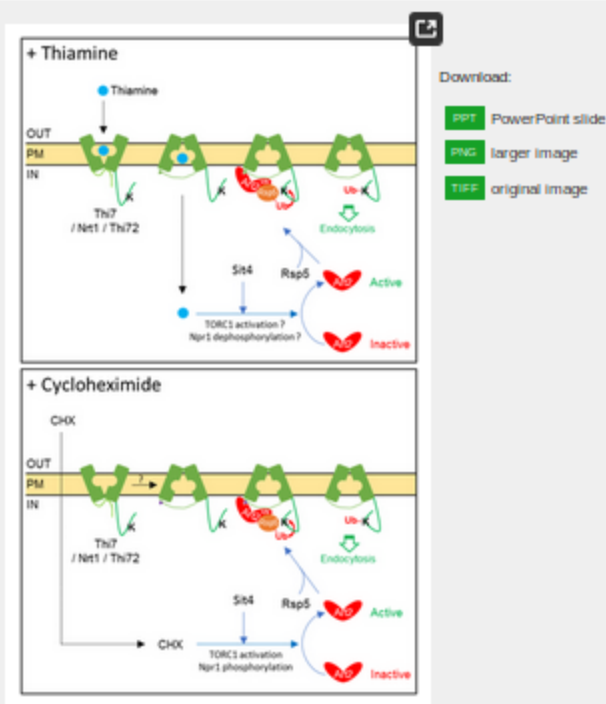


Fig 9. Model of Art2-dependent ubiquitylation and endocytosis of Thi7, Nrt1, and Thi72.

(Top panel) In the absence of thiamine, Art2 is inactive. Thiamine uptake through Thi7, Nrt1, or Thi72 induces conformational changes, leading to the exposure of an Art2-binding domain. Thiamine accumulation within the cytosol leads to Art2-mediated ubiquitylation of thiamine transporters. This process requires the Sit4 phosphatase, and our results also suggest an active TORC1 and a poorly phosphorylated version of Npr1. (Bottom panel) Alternatively, CHX diffuses through the cell membrane and stimulates TORC1. CHX-activated TORC1 leads to hyperphosphorylation and inactivation of Npr1. CHX treatment induces Art2-dependent endocytosis of thiamine transporters, which also depends on the Sit4 phosphatase. In the absence of thiamine transport, no conformational change occurs on the transporter. CHX, cycloheximide; K, Rsp5-targeted lysine residue(s); Nrt1, nicotinamide riboside transporter 1; PM, plasma membrane; TORC1, target of rapamycin complex 1; Ub, ubiquitin.

<https://doi.org/10.1371/journal.pbio.3000512.g009>

Materials and methods

Yeast strains and growth conditions

Yeast strains used in this study (S3 Table) are derivatives of *Saccharomyces cerevisiae* auxotrophic strain BY4742. The *npi1-1* (*rsp5*) mutant strain, obtained by insertion of a selective marker in the *RSP5* promoter and resulting in an attenuated *RSP5* gene expression by a factor of 10, was provided by Pr. Bruno André (ULB, Belgium). The *art2Δart9Δ* strain was obtained by yeast crossing and tetrad dissection. The *end3Δart2Δ* strain was generated by homologous recombination using a *natNT2* deletion cassette on an *end3Δ* background. The strains lacking genes encoding the thiamine transporters Thi7, Nrt1, and Thi72 and the gene encoding the thiazole synthase Thi4 were received from Pr. Jürgen Stolz (TUM, Germany). For proteomic analysis, yeast strains were grown in YD medium (2% glucose and 2% KAT yeast extract). For Thi7 endocytosis experiments, transformed yeast strains were grown in selective thiamine-free