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Note

Mitochondrial NADP$^+$-isocitrate dehydrogenase Idp1 involves CoQ biosynthesis in parallel with NAD kinase Pos5

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Abstract

Mitochondrial NADPH is synthesized by isocitrate dehydrogenase (Idp1) from NADP$^+$ and NAD kinase (Pos5) from NADH. Coenzyme Q levels in *Schizosaccharomyces pombe* were decreased by deletion of *idp1* and further lowered by deletion of *pos5*. The NADP$^+$/NADPH ratio of the Δ*idp1* strain shifted to an oxidized state. The mitochondrial NADPH pool and its redox state are critical for CoQ biosynthesis.

Keywords: CoQ, NADP, mitochondria, fission yeast

Coenzyme Q (CoQ) is an essential component in the electron transport chain of mitochondria. CoQ_{10}, which has 10 units of isoprene in its side chain, is a commercially available product. Fission yeast *Schizosaccharomyces pombe* produces a human utilizing type CoQ_{10} (Kawamukai *et al.* 2016). Therefore, understanding the biosynthesis of CoQ_{10} in *S. pombe* is important in both biotechnological and metabolic aspects (Moriyama *et al.* 2015). The genes responsible for CoQ_{10} biosynthesis have been mostly identified in *S. pombe*, but its regulatory mechanism remains to be elucidated (Hayashi *et al.* 2014; Kawamukai *et al.* 2016; Nishida *et al.* 2019; Nishida *et al.* 2023). Recently, we showed that mitochondrial NAD kinase Pos5 is crucial for CoQ biosynthesis in *S. pombe* (Nishihara *et al.* 2026). Deletion of *pos5* resulted in 20% CoQ_{10} level of the wild-type strain. However, how mitochondrial NADP(H) redox affect CoQ biosynthesis is not clear. Mitochondrial NADPH is maintained by at least two ways; synthesis from NADH by mitochondrial NAD kinase Pos5 and reduction of NADP$^+$ by mitochondrial NADP$^+$ reductase. In *S. pombe*, Idp1 is the only known mitochondrial NADP$^+$ reductase which catalyzes the oxidation of isocitrate to α-ketoglutarate. By contrast, four mitochondrial NADP$^+$ reductases Idp1p, Ald4p, Ald5p, and Mae1p have been found in *Saccharomyces cerevisiae* (Haselbeck *et al.* 1991; Boles, de Jong-Gubbels and Pronk 1998; Saint-Prix, Bönquist and Dequin 2004). *S. pombe* enzymes Atd1 and Mae2 that are homologous to *S. cerevisiae* Ald4p, Ald5p, and Mae1p localize to cytosol (Matsuyama *et al.* 2006). Therefore, investigating the role of mitochondrial NADP$^+$ reductase Idp1 is a key subject to understand the impact of mitochondrial NADPH on CoQ_{10} biosynthesis.

Schizosaccharomyces pombe strains used in this study are listed in Table 1. Yeast standard media and genetic manipulation methods were described previously (Moreno, Klar and Nurse 1991). The experimental methods are provided in supplementary materials.

We first analyzed the NADP$^+$/NADPH ratio of whole cells in the *S. pombe* Δ*idp1* strain. Compared to the wild-type strain, NADP$^+$ content was increased in the Δ*idp1* strain, while NADPH content was concurrently decreased (Figure 1a). Consequently, the NADP$^+$/NADPH ratio was markedly increased (2.2-fold) in the Δ*idp1* strain (Figure 1b), indicating Idp1 is important for NADP$^+$/NADPH redox balance. We next measured the CoQ content in the Δ*idp1* strain and found it was decreased to about 30% compared to that of the wild-type strain in both per cells and per volume (Figure 1c and d). The Δ*idp1* strain grew normally on the minimal media and on YEGES in which respiration activity is required to grow, while a CoQ-deficient Δ*ppt1* strain failed to grow in both media (Figure 1e) due to the deficiency of oxidative phosphorylation, elevated production of reactive oxygen species, and cysteine requirement as previously reported (Suzuki *et al.* 1997; Uchida *et al.* 2000; Hayashi *et al.* 2014). The result that the Δ*idp1* strain grew well on YEGES indicates that Idp1 does not affect respiration. We then examined the Idp1 localization in the Idp1–GFP strain, and clearly observed the GFP signal overlapped with the Mito–Tracker Red CMX signal (Figure 1f), confirming mitochondrial localization of Idp1.

To clarify the relationship between Idp1 and Pos5 in CoQ biosynthesis, we constructed the Δ*pos5* Δ*idp1* double mutant and measured its CoQ level. The Δ*pos5* Δ*idp1* strain exhibited an additive effect on decreasing CoQ content compared to the Δ*pos5* single mutant. While CoQ levels of the Δ*pos5* and Δ*idp1* single mutants were about 20% and 70% of the wild-type strain level, respectively, it was about 10% in the Δ*pos5* Δ*idp1* double mutant (Figure 2a and b).

The Δ*pos5* strain exhibits low NADP(H) levels and disrupted NADP(H) redox homeostasis (Nishihara *et al.* 2026). Therefore, we next assessed the effect of Idp1 overexpression in the Δ*pos5* background. To test this, we constructed the strain (NSP62) which

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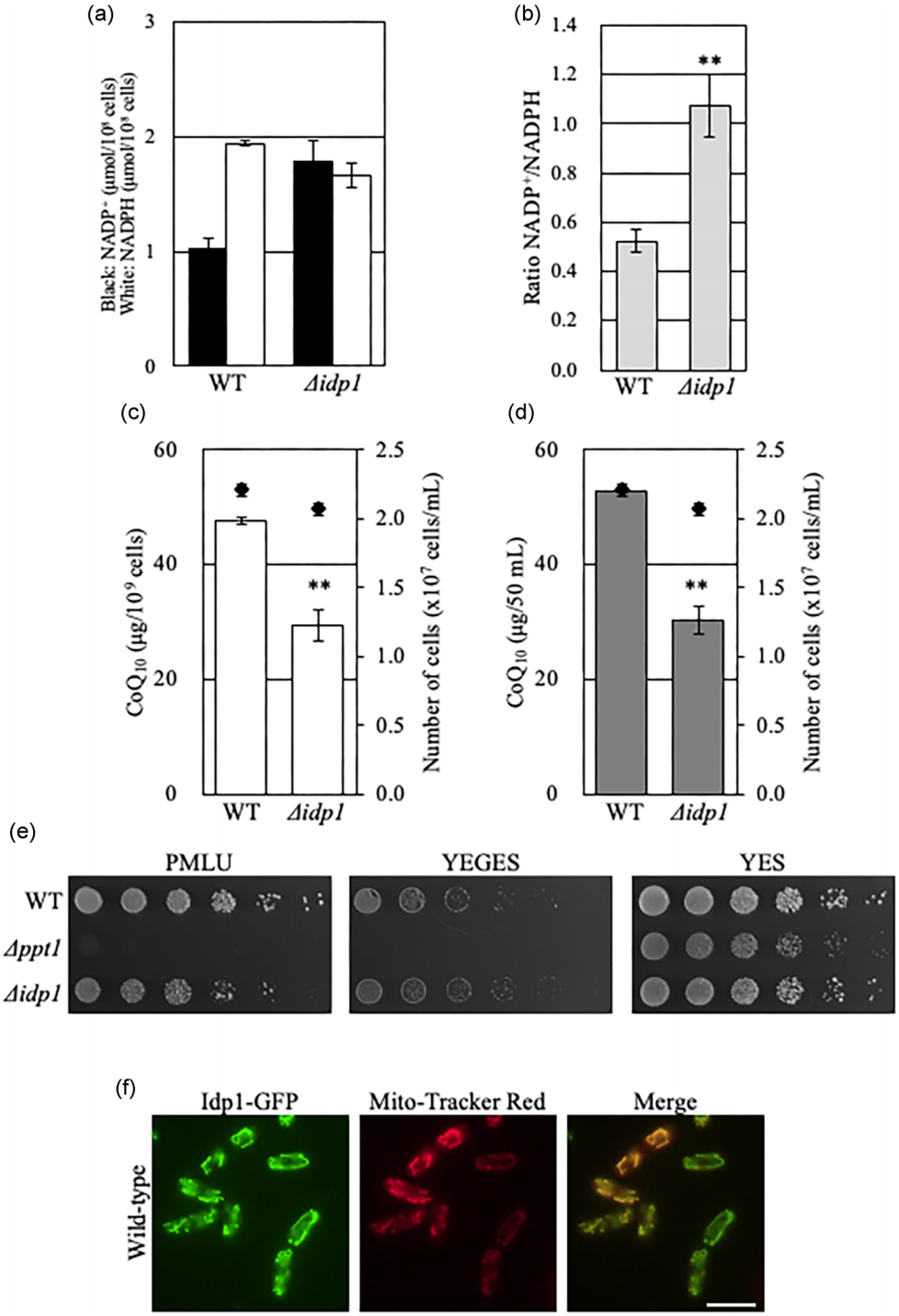

Figure 1 NADP(H) and CoQ_{10} quantitation of the *idp1* deletion strain, and localization of Idp1. (a) NADP(H) quantification in wild-type and Δ*idp1* strains. Wild-type strain (PR110) and the Δ*idp1* strain (NSP50) were grown at 30 °C in 55 mL YES medium for 18 h, starting from 2.5×10^5 cells/mL. 1×10^8 cells were harvested, and $NADP^+$/NADPH were extracted by multibeads shocker. After homogenization, NADP(H) concentrations were quantified by enzyme reaction method using glucose-6-phosphate dehydrogenase (Nishihara *et al.* 2026). Black bar shows $NADP^+$, and white bar shows NADPH. S.D. means the standard deviation of three measurements. (b) The ratio of $NADP^+$/NADPH. $^{**}P < .01$ versus wild-type strain by Student's *t*-test. (c, d) CoQ_{10} quantitation of the Δ*idp1* strain. Wild-type and Δ*idp1* strains were grown at 30 °C in 55 mL YES medium, starting from 1×10^5 cells/mL. Cells were collected at 48 h. The amount of CoQ_{10} was measured by HPLC. S.D. means the standard deviation of three measurements. $^{**}P < .01$ versus wild-type by Student's *t*-test. Diamonds (♦) show cell number. Bars indicate CoQ_{10} content per cell (c) and per volume (d). (e) Growth assay of the Δ*idp1* strain on minimal media and nonfermentable carbon source media. Cells were serially diluted (1:5) from 1×10^7 cells/mL, were spotted to grow on PMLU (for 4 days), YEGES (for 6 days), and YES (for 3 days) at 30 °C. The Δ*ppt1* strain (QDP2) was used as a negative control. (f) Localization analysis of Idp1–GFP. The Idp1–GFP strain (NSP68) was collected at mid-log phase and stained with Mito–Tracker Red for 1 h. After washing, the sample was observed under fluorescent microscopy. The scale bar; 10 μm.

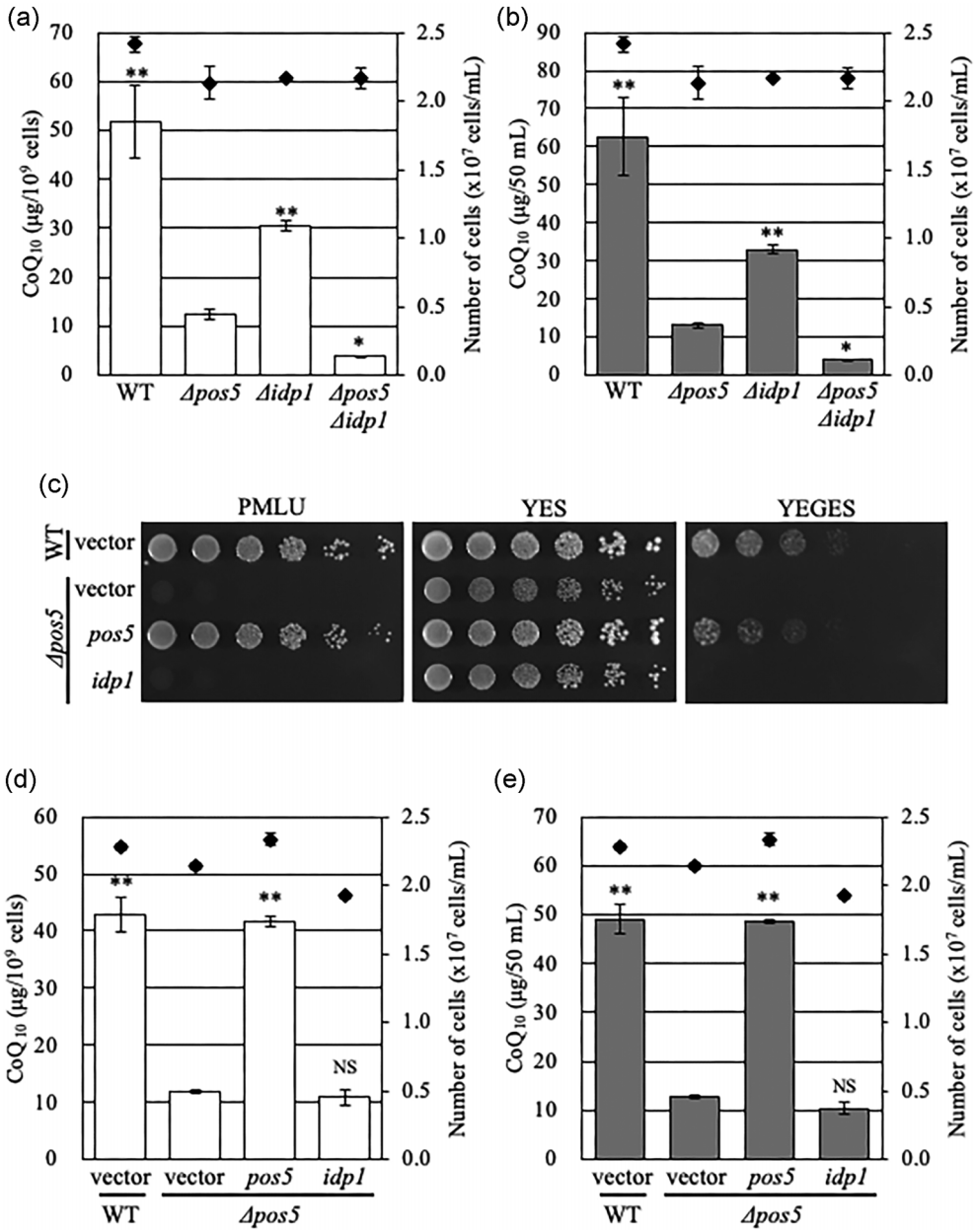

Figure 2 Idp1 affected CoQ biosynthesis in parallel with Pos5. (a, b) CoQ_{10} quantitation of the Δ*pos5* Δ*idp1* strain. Wild-type (PR110), Δ*pos5* (NSP19), Δ*idp1* (NSP50), and Δ*pos5* Δ*idp1* (NSP69) strains were grown at 30 °C in 55 mL YES medium, starting from 1 × 10^5 cells/mL. Cells were collected at 48 h. The amount of CoQ_{10} was measured by HPLC. S.D. means the standard deviation of three measurements. $^{**}P < .01$, $^{*}P < .05$ versus the Δ*pos5* strain by Student's *t*-test. Diamonds (♦) show cell number. Bars indicate CoQ_{10} content per cell (a) and per volume (b). (c) Growth assay of the Δ*pos5* strain with chromosomally expressed *idp1* on minimal media and nonfermentable carbon source media. Cells were serially diluted (1:5) from 1 × 10^7 cells/mL and were spotted to grow on PMLU (for 4 days), YEGES (for 11 days), and YES (for 4 days) at 30 °C. (d, e) CoQ_{10} quantitative analysis of the Δ*pos5* strain expressing *idp1*. Wild-type with the vector (NSP23) or the Δ*pos5* background with the vector (NSP26), chromosomally expressed *pos5* (NSP11), or chromosomally expressed *idp1* (NSP62) strains were grown at 30 °C in 55 mL YES medium, starting from 1 × 10^5 cells/mL. Cells were collected at 48 h. The amount of CoQ_{10} was measured by HPLC. S.D. means the standard deviation of three measurements. $^{**}P < .01$, $^{*}P < .05$ versus Δ*pos5* with vector strain by Student's *t*-test. NS: no significant difference versus Δ*pos5* harboring vector strain. Diamonds (♦) show cell number. Bars indicate CoQ_{10} content per cell (d) per volume (e).

overexpresses the *idp1* gene driven by the *nmt1* promoter on the chromosome as well as its negative control strain (NSP26) and the positive control strain which expresses *pos5* (NSP11). Obtained results showed no suppressive effect of *idp1* overexpression on the growth delay or respiratory deficiency observed in the Δ*pos5* strain (Figure 2c), while positive control strain clearly restored respiration-dependent growth on YEGES. In addition, Idp1 overexpression failed to restore the CoQ level in the Δ*pos5* strain (Figure 2d and e), while positive control strain clearly restored the CoQ level. These results suggest that Pos5 and Idp1 are involved in the CoQ biosynthesis via independent mechanisms.

In this study, we demonstrated that the mitochondrial NADP pool and its redox status independently and coordinately contribute to CoQ biosynthesis in *S. pombe*. The mitochondrial NADP pool is controlled by NAD kinase Pos5 (Nishihara *et al.* 2026), in-

Table 1 Strain list

Strain	Genotype	Source
PR109	*h⁻ leu1-32 ura4-D18*	Lab stock
PR110	*h⁺ leu1-32 ura4-D18*	Lab stock
KH2 (OG1)	*h⁺ leu1-32 ura4-D18 ppt1::kanMX6*	Hayashi *et al.* (2014)
NSP11	*h⁺ leu1-32:leu1-pJK148P41nmt1-pos5 ura4-D18 pos5::kanMX6*	Nishihara *et al.* (2026)
NSP19	*h⁻ leu1-32 ura4-D18 pos5::kanMX6*	This study
NSP23	*h⁺ leu1-32: leu1-pJK148Pnmt1 ura4-D18*	Nishihara *et al.* (2026)
NSP26	*h⁺ leu1-32: leu1-pJK148Pnmt1 ura4-D18 pos5::kanMX6*	Nishihara *et al.* (2026)
NSP50	*h⁺ leu1-32 ura4-D18 idp1::hphMX6*	This study
NSP51	*h⁻ leu1-32 ura4-D18 idp1::kanMX6*	This study
NSP62	*h⁺ leu1-32: leu1-pJK148Pnmt1-idp1 ura4-D18 pos5::kanMX6*	This study
NSP68	*h⁻ leu1-32 ura4-D18 idp1-GFP(S65T)-kanMX6*	This study
NSP69	*h⁻ leu1-32 ura4-D18 pos5::kanMX6 idp1::hphMX6*	This study
QDP6	*h⁻ leu1-32 ura4-D18 ppt1::natMX6*	This study
RYP7	*h⁺ leu1-32 ura4-D18 pos5::kanMX6*	Nishihara *et al.* (2026)

dependently from Idp1 which catalyzes the reduction of $NADP^+$ to NADPH. The NADPH redox status is coordinately controlled by both Pos5 and Idp1. Although we can not rule out the possible involvement of Idp1 in the NADP pool, based on the reaction catalyzed by Idp1, it is unlikely. Our current characterization of Idp1 confirmed its role as a key regulator of mitochondrial NADP redox homeostasis. The mitochondrial localization of Idp1 and the significant shift toward an oxidized state (higher $NADP^+$/NADPH ratio) in the Δ*idp1* strain (Figure 1) support its function as a mitochondrial NADP-dependent isocitrate dehydrogenase. While the loss of *idp1* did not cause respiratory growth defects, it resulted in a significant decrease in CoQ levels. Notably, the whole-cell $NADP^+$/NADPH ratio in wild-type strain used in this study was consistent with previous reports (Corkins *et al*. 2017).

We showed that the NADP pool size regulated by the NAD kinase Pos5, and the redox state maintained by Idp1, are critical for CoQ_{10} synthesis, as the Δ*pos5* Δ*idp1* strain exhibited an additive decrease in the CoQ content compared to the single mutant (Figure 2). This is consistent with findings that *S. cerevisiae* Δ*pos5* Δ*idp1* mutant showed an additive decline in respiratory chain complex activities and severe phenotypes on arginine auxotrophy (Miyagi, Kawai and Murata 2009; Shi *et al*.2011). These comparative phenotypes support that Pos5 and Idp1 function independently to maintain mitochondrial NADP(H) homeostasis.

A hypothesis to explain NADPH requirement in CoQ biosynthesis is related to the Coq6-catalyzed hydroxylation (Ozeir *et al*.2015), because Coq6 requires ferredoxin reductase Arh1, which utilizes NADPH as an electron donor as well as NADH (Ewen *et al*. 2008). Our findings suggest that a decrease in mitochondrial NADPH levels may partially limit the Coq6 reaction via the Arh1-mediated electron transport system. Alternatively, given that the *S. cerevisiae pos5* deletion strain exhibits lower activities of respiratory complexes (Miyagi *et al*. 2009), and that CoQ content in *S. pombe* is influenced by the integrity of the electron transport chain (ETC) (Nishihara *et al*. 2026), the decreased CoQ level in *pos5* deletion strain might be a consequence of partially impaired ETC function.

In conclusion, our results indicate that both the total size of the mitochondrial NADP pool and its redox state are critical for optimal CoQ biosynthesis. This study clarified the distinct metabolic roles of Pos5 and Idp1, and provides a new insight into the regulation of CoQ metabolism through mitochondrial pyridine nucleotide homeostasis.

Supplementary material

Supplementary material is available at *Bioscience, Biotechnology, and Biochemistry* online.

Data availability

Data sets are available from corresponding authors upon request.

Author contribution

S. N. carried out experiments; S. N., Y. M., and M. K. conceptualization; M. K. funding acquisition; S. N., Methodology; Y. M. and M. K. supervision, S. N. and M. K., writing original draft; S. N., Y. M., and M. K., reviewing and editing.

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Disclosure statement

The authors declare that they have no conflicts of interest with the contents of this article.

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