the thiopeptide biosynthesis regulation, such as those described for GE2270 (Flinspach et al., 2014) or cyclothiazomycin (Zhang et al., 2014). In all cases, a single transcriptional regulator encoded within the biosynthetic gene cluster seems to be involved, suggesting similarity in the regulatory patterns and the presence of a single master switch in each case. Now, in the case of nosiheptide regulation, NosP interaction with promotor DNA was studied, and interactors have been identified for the first time. Interestingly, both "small-molecule" biosynthesis products as well as leader-peptide-derived compounds regulate nosiheptide biosynthesis. These data suggest not only a feedback mechanism for regulation by product sensing but also specific modifications that may allow manipulation of biotechnological nosiheptide production. The new study thus paves the way for exciting future research on how the molecular recognition of the regulatory peptides and small molecules work in detail and how other, external factors are involved in regulating thiopeptide production and secretion.

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Insights into an Ancient Atypical Kinase Essential for Biosynthesis of Coenzyme Q

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COQ8 proteins are homologs of atypical protein kinases required for the biosynthesis of coenzyme Q (CoQ). In this issue of Cell Chemical Biology, Reidenbach et al. (2018) show that COQ8 has an ATPase activity, required for CoQ biosynthesis, that is strongly activated by cardiolipin and small molecule mimics of early CoQ intermediates.
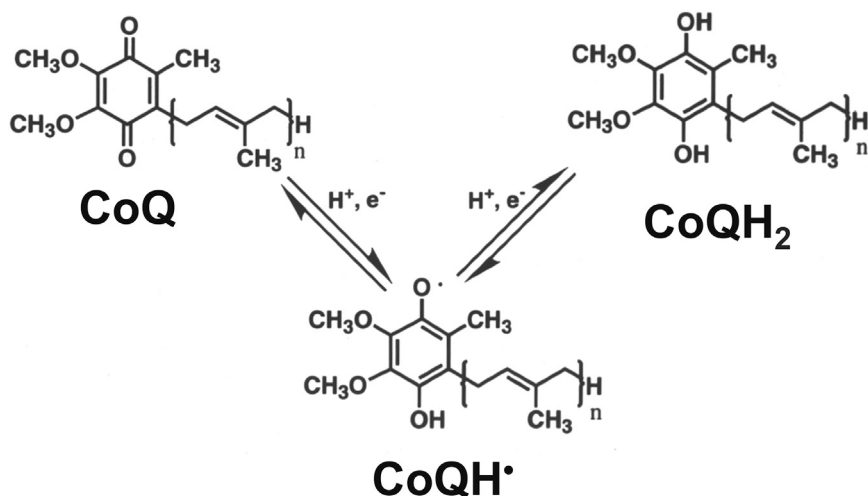
Coenzyme Q (ubiquinone or CoQ) is a ubiquitous lipid quinone that plays essential roles in the respiratory electron transport chain, a central pathway of energy metabolism. CoQ contains a long polyisoprene tail that anchors the molecule at the midplane of the membrane bilayer. The tail of CoQ is attached to a fully substituted benzoquinone ring that im-

parts reversible redox chemistry enabling CoQ to accept and donate electrons and protons in central metabolic pathways (Figure 1). Other functions include an antioxidant activity similar to vitamin E.

In this issue of Cell Chemical Biology, Reidenbach and co-authors (Reidenbach et al., 2018) identify new attributes of the COQ8 homologs: an atypical protein ki-

nase family required for the biosynthesis of CoQ. The authors show that COQ8 homologs from Escherichia coli (UbiB), Saccharomyces cerevisiae (Coq8p), and Homo sapiens (COQ8A) each co-purify with early CoQ biosynthetic intermediates. Additionally, the COQ8 proteins possess ATPase activity that is strongly activated by interaction with liposomes
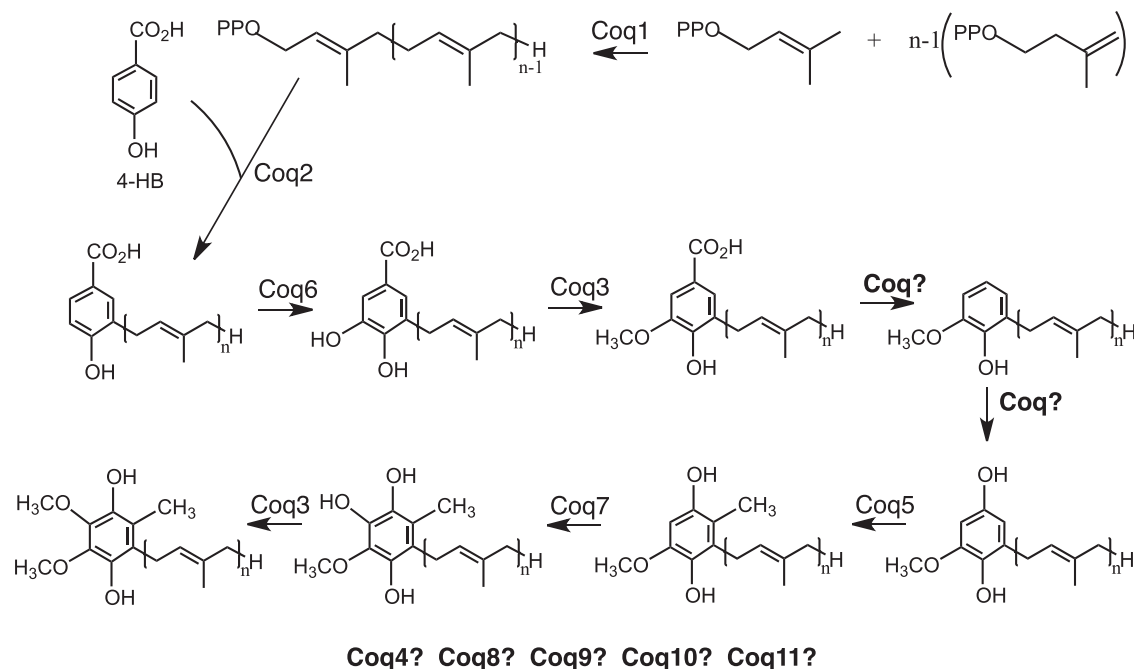
Figure 1. Coenzyme Q Is a Two-Part Molecule
The oxidized form of CoQ (CoQ the quinone) may be reduced via two steps to form the semiquinone radical (CoQH·) and the hydroquinone (CoQH$_2$). The long polyisoprenyl tail contains variable numbers of isoprene units (*S. cerevisiae*, n = 6; *E. coli*, n = 8; *H. sapiens*, n = 10).

containing cardiolipin and by small molecule phenols that mimic the structure of early CoQ intermediates. The authors also prepared an ATP analog-sensitive mutant of the yeast Coq8p (Coq8p-AS). This variant protein is active in CoQ synthesis in vivo, but inhibited by a cell-permeable compound that uniquely accesses the roomier mutant active site, enabling fast-acting specific covalent modification and inhibition of the ATPase activity of Coq8p-AS. Their studies also show that the inhibition of the ATPase activity of Coq8p-AS halts *de novo* biosynthesis of CoQ and results in the build-up of the earliest yeast polyisoprenylated intermediate, PPHB (3-polyisoprene-4-hydroxy benzoic acid). Their findings suggest that the COQ8 ATPase activity plays an essential role in CoQ biosynthesis via binding of the protein to cardiolipin-containing membranes and to early hydrophobic CoQ-intermediates. The resulting enhanced ATPase activity thus appears to be necessary for de novo CoQ biosynthesis. The mechanism connecting ATPase activity and CoQ biosynthesis, however, remains to be determined.

Elucidating the biosynthesis of CoQ has proved to be a surprisingly sticky problem (Stefely and Pagliarini, 2017). The membrane-bound Coq polypeptides are unstable and difficult to assay in isolation, and the polyprenylated CoQ intermediates are extremely hydrophobic and not available commercially. Figure 2 depicts an overview of the biosynthesis of CoQ in yeast. Coq1p is required for the biosynthesis of the polyisoprenyl diphosphate tail, and Coq2p attaches the tail to the aromatic ring precursor 4-hydroxybenzoic acid. Coq3p and Coq5p catalyze *O*-methylation and *C*-methylation steps, respectively. Coq6p is required for the first hydroxylation step, and Coq7p performs the last hydroxylation step. Many

Figure 2. The Biosynthetic Pathway of CoQ in *S. cerevisiae* Is Mediated by the Coq Polypeptides
Steps labeled as "Coq?" are catalyzed by yet-to-be characterized proteins, and the Coq polypeptides identified in the lower part of the panel have not been assigned specific enzymatic functions.

outstanding questions remain. The enzyme(s) mediating the decarboxylation and the hydroxylation steps required to form the para-hydroxy group of the hydroquinone have yet to be identified. In addition to the unknown specific role of COQ8, functional roles for Coq4p, Coq9p, Coq10p, and Coq11p remain to be characterized.

In yeast, the Coq polypeptides are associated with the matrix side of the mitochondrial inner membrane. The biosynthesis of CoQ depends on a membrane-associated high-molecular-mass multi-subunit complex termed the "CoQ synthome," which is comprised of at least eight distinct Coq polypeptides (Coq3p-Coq9p and Coq11p) that co-purify with CoQ and CoQ-intermediates (Allan et al., 2015). An analogous multi-subunit complex, or "Complex Q," is needed for the biosynthesis of CoQ in mouse and human cells (Lohman et al., 2014; Floyd et al., 2016). In yeast *coq* null mutants, the multi-subunit complex is unstable and only PPHB, an early CoQ-intermediate produced by the action of Coq1 and Coq2, is detected in lipid extracts. Curiously, overexpression of Coq8p in certain of the *coq* null mutants allowed for stabilization of Coq polypeptides and sub-complexes and enabled the isolation of several late-stage CoQ intermediates (Xie et al., 2012; He et al., 2014).

Thus, the Coq8p atypical kinase is implicated in forming or stabilizing the CoQ synthome. How might Coq8p accomplish this? Phosphorylation and de-phosphorylation of the yeast Coq7 polypeptide modulates CoQ biosynthesis, and Ptc7p was identified as the phosphatase involved in this regulation (Martín-Montalvo et al., 2013). Indeed, regulated splicing of the

phosphatase encoded by *PTC7* acts to target the spliced isoform of Ptc7p to the mitochondria, where it stimulates CoQ biosynthesis (Awad et al., 2017). If Ptc7p serves as a phosphatase, Coq8p may act as a protein kinase. However, the studies reported here by Reidenbach et al. (2018) suggest a different model. The authors posit that it is the stimulation of the ATPase activity of Coq8p that facilitates the assembly of the CoQ synthome and *de novo* biosynthesis of CoQ. The activation of Coq8p ATPase activity by cardiolipin (enriched in the inner mitochondrial membrane) is postulated to localize and anchor Coq8p where it can then intercept early CoQ-intermediates imbedded in the midplane of the membrane bilayer. In this capacity, Coq8p is modeled as a chaperone with ATPase activity that is involved in forming or stabilizing the CoQ synthome. Could this Coq8p chaperone recruit partner proteins that either facilitate or possess protein kinase activity? Based on interactions of pseudo-kinases that modulate kinases in other systems (Dhawan et al., 2016), it is tempting to speculate this may be the case. If so, it will be important to identify other potential partner proteins that might interact with Coq8.

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