OSMOTIC STRESS

Is CoQ a membrane stabilizer?

Coenzyme Q_{10} is an essential lipid in aerobic respiratory metabolism and a membrane antioxidant. A new function is revealed for CoQ: as a membrane-stabilizing agent that enhances resistance of bacterial cell and liposome membranes to salt stress.

Catherine F Clarke, Amy C Rowat & James W Gober

Coenzyme Q functions as an electron and proton carrier in aerobic respiration and has an additional crucial role as a chain-breaking antioxidant[1]. The long polyisoprenyl tail of CoQ_n functions to anchor this lipid in the membranes of cells (*n* designates the number of five carbon isoprene units; for instance six, eight or ten in *Saccharomyces cerevisiae* CoQ_6, *Escherichia coli* CoQ_8 or human CoQ_{10}, respectively). In this issue, Sevin and Sauer[2] make the surprising discovery that *E. coli* cells have 100-fold higher levels of CoQ_8 when they are plunged into high-salt solutions. The authors found that the rescuing attribute of high CoQ_8 was independent of its role in respiration or its action as a radical scavenger. Importantly, they showed that artificial liposomes prepared with long polyisoprenol tail compounds were more stable under high-salt stress.

Enhanced salt tolerance is a sought-after trait in nutritionally important crop plants and an important adaptive mechanism of microbes[3]. *E. coli* tolerance of salt stress is mediated by flux control of water across the cell membrane, adjustments of intracellular potassium levels, synthesis of the disaccharide trehalose and/or transport of small-molecule osmoprotectants. Sevin and Sauer[2] demonstrate that increased levels of polyisoprene lipids may also contribute to osmoprotection by increasing resistance to high-salt conditions in the cytoplasmic membrane of *E. coli* and in the membrane bilayers of liposomes. This unexpected increase in *E. coli* CoQ_8 levels was detected with an MS-based high-throughput screen for metabolites that changed in response to sustained high-salt stress. The hypersensitivity to high-salt was rescued by supplementation with CoQ_8 or CoQ_{10}; even the addition of just the long polyisoprenol tail component (solanesol; $n = 9$) partially restored cell volume in high salt.

Their discovery shifts the focus from the redox-active benzoquinone ring of CoQ as the functional unit of this lipid to include the polyisoprene tail. The authors suggest that lipid membranes that contain CoQ_n with $n > 8$ isoprene units may have enhanced mechanical stabilization. However, the physical properties of the membranes and the molecular mechanisms resulting in their enhanced mechanical stability remain unresolved. Is the presence of an increased proportion of CoQ conferring resistance to high salt or osmotic stress? Indeed, it has been previously suggested that such polyisoprene lipids may have a role in decreasing permeability of the membrane to proton and sodium leaks[4]. Future experiments comparing the effects of impermeable osmolytes (i.e., PEG or dextran) to that of high salt should provide insights into the protective effects of these lipids. Additionally, it will be fascinating to define how CoQ isoforms affect membrane mechanical properties through biophysical measurements of membrane bending rigidity, area expansion modulus and lysis tension.

Importantly, it has been shown that CoQ_n isoforms with $n \geq 6$ reside at the membrane bilayer midplane[5]. Sevin and Sauer[2] noted that CoQ_8 in salt-stressed *E. coli* accounted for 1 mol% of the lipids and that 5 mol% was

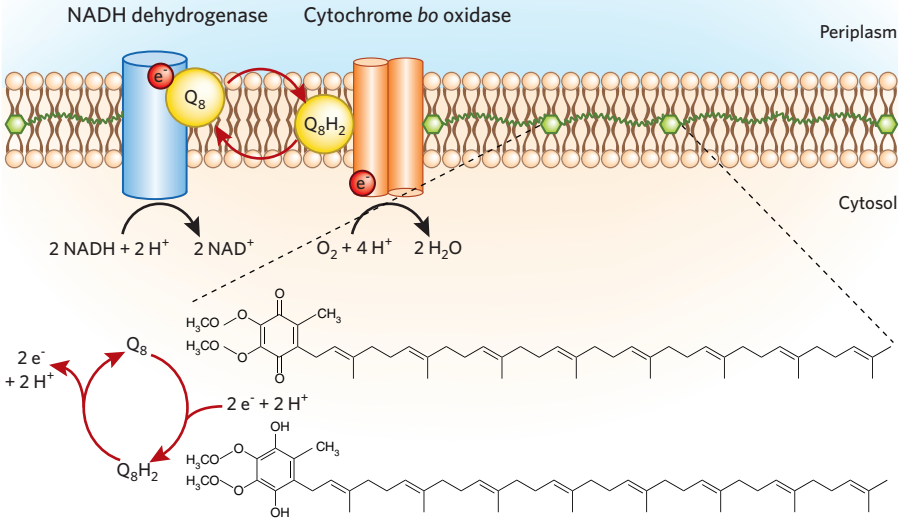

Figure 1 | Osmotic stress response and membrane stability. A new function for coenzyme Q_8 in *E. coli*. Aerobic respiration in *E. coli* occurs in the cytoplasmic membrane. Examples of two steps in electron transport are shown: NADH dehydrogenase (blue) oxidizes NADH and transfers electrons and protons to Q_8 forming Q_8H_2; cytochrome *bo* oxidase (orange) then accepts electrons and protons from Q_8H_2 to reduce oxygen and reform Q_8. The yellow dot depicts Q_8/Q_8H_2 as it is typically represented as a mobile carrier of electrons and protons between respiratory protein complexes. However, Q_n with polyisoprene tails of $n \geq 6$ isoprene units are known to lie at the midplane of the membrane bilayer; Q_8 with an octaprenyl tail is shown here that provides coverage for about 12 phospholipid molecules (six on each side of the bilayer). Sevin and Sauer[2] show that it is this 'tail' feature of the Q_n molecule that functions to enhance membrane stability in response to high-salt stress.

sufficient to stabilize liposomes. Modeling predicts that each isoprene unit occupies 0.4–0.5 nm^2 when oriented parallel to the bilayer plane[6]. Assuming that CoQ_{10} occupies about 5 nm^2, it has been estimated that it would cover an area occupied by 16 phospholipid molecules (8 in each leaflet), and hence 6 mol% of CoQ_{10} is sufficient to provide full coverage[6]. (The area that CoQ_8 would occupy at the midplane is depicted in **Fig. 1**.) Membrane proteins may also influence the 'coverage' needed. In addition, the reduced or hydroquinone form of $CoQH_2$ is considerably more polar and shows a greater tendency to penetrate the aqueous interface of fluid phospholipid bilayers[4]. It will be important to determine the ratio of CoQ_8/CoQ_8H_2 in response to salt stress.

CoQ is present in all membranes of animal cells, with the highest content (per protein basis) in the mitochondrial inner membrane and Golgi[1]. Dedicated pools of CoQ exist in mitochondria, yet in some tissues, such as muscle, a substantial fraction of the mitochondrial CoQ pool is unavailable for respiratory electron transport[7]. Indeed, the surplus of CoQ at the midplane may need a chaperone to be assimilated into respiratory protein complexes or to mediate efficient CoQ biosynthesis as part of the CoQ synthome[8]. It is generally assumed that this excess CoQ serves an important antioxidant function. Might this new function of the polyisoprenyl tail of CoQ_n help explain why the longer CoQ_n isoforms are more potent antioxidants as compared to shorter forms? Could 'inactive' CoQ at the midplane have a role in membrane stabilization? Patients and mice with defects in CoQ biosynthesis display a bewildering array of symptoms, including nephrotic syndrome[9,10]. Could CoQ at midplane contribute to the kidney filtration barrier mediated by the membrane foot processes of podocytes either through membrane stability or by influencing ion permeability? More broadly, understanding the impact of membrane stability and breakdown could provide insight into how such isoprenoid molecules could be effectively removed from microorganisms, which remains a challenge in realizing practical application of synthetic biology.

Catherine F. Clarke and James W. Gober are in the Department of Chemistry and Biochemistry and the Molecular Biology Institute. Amy C. Rowat is in the Department of Integrative Biology and Physiology and the Molecular Biology Institute. All authors are at the University of California–Los Angeles, Los Angeles, California, USA.
e-mail: cathy@chem.ucla.edu

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Competing financial interests

The authors declare no competing financial interests.

DNA DAMAGE

Walking the edge

An inhibitor of the deubiquitinase complex USP1-UAF1 highlights the requirement for reversible ubiquitination in DNA repair pathways that are critical for human development and disease.

Melissa N Locke & Michael Rape

Genomic stability is vital for all eukaryotes. To safeguard the integrity of their genome, eukaryotic cells activate specialized pathways that recognize and repair DNA damage[1]. Although mutations in genes encoding DNA repair proteins can lead to tumorigenesis, genotoxic compounds that induce massive DNA damage and overburden these repair systems are used to eliminate rapidly dividing cancer cells. Underscoring this delicate balancing act, the efficacy of chemotherapeutics in eradicating cancer cells can be limited by the same DNA repair pathways that ensure survival of untransformed cells. In this issue, Liang *et al.*[2] report a specific inhibitor of the deubiquitinase (DUB) complex USP1–UAF1, an important regulator of the DNA damage response, that highlights the tight relationship between DNA repair and chemotherapeutic sensitivity.

Post-translational modification with a single ubiquitin, or monoubiquitination, can regulate protein interactions and has a pivotal role in DNA repair[3]. During interstrand crosslink DNA repair, a complex of eight proteins mutated in the cancer predisposition syndrome Fanconi anemia monoubiquitinates FANCD2 and FANCI, which in turn locate to sites of DNA damage and recruit a set of downstream repair factors[4]. In a similar manner, bulky DNA adducts that block the progression of high-fidelity DNA polymerases during replication promote the monoubiquitination of the DNA polymerase processivity factor PCNA[5]. Monoubiquitinated PCNA attracts low-fidelity translesion synthesis DNA polymerases, which can plow through the site of DNA damage to resume replication at a stalled fork[6]. Intriguingly, proper DNA repair also requires timely removal of the ubiquitin mark, with the ubiquitin-specific protease 1 (USP1) being a key mediator of this activity. When in complex with UAF1, USP1 has been shown to deubiquitinate FANCD2, FANCI, PCNA and inhibitors of DNA binding transcriptional repressors[7,8]. Accordingly, ablation of the *USP1* locus in mouse or chicken cells not only increased the steady-state levels of ubiquitinated FANCD2 and PCNA but also led to phenotypes that were reminiscent of Fanconi anemia, such as defects in homologous recombination or higher sensitivity to DNA crosslinking agents[9].

These observations suggested that inhibition of USP1 could sensitize cancer cells to DNA crosslinking agents, such as the chemotherapeutic cisplatin, and might also be of therapeutic benefit in skin carcinoma and osteosarcoma.

Using a medicinal chemistry approach starting from a low-potency compound, Liang *et al.*[2] identified ML323 as a potent and reversible inhibitor of USP1. Illustrating the high specificity of this molecule, ML323 targets the USP1–UAF1 complex but has no activity against other DUBs, including the highly related USP46–UAF1, and the effects of ML323 in cells were eliminated by depletion