How CCTα puts a leash on phospholipid synthesis

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The proportion of phosphatidylcholine (PC) in the membrane is controlled by CTP:phosphocholine cytidylyltrasferase α (CCTα), which is known to be regulated by a dual auto-inhibitory and membrane-binding domain. However, the detailed mechanism by which this domain regulates CCTα activity is not clear. Ramezanpour et al. use a combined computational and biochemical approach to define new details of this mechanism, providing an elegant illustration of how the lipid-sensing domain of a phospholipid biosynthetic enzyme controls membrane homeostasis.

Phosphatidylcholine (PC) is the major phospholipid component of mammalian cell membranes, lung surfactant, and lipoproteins. PC-rich membranes have unique surface charge and packing properties that are essential for organelle biogenesis, signaling cascades, cell division, and secretion. As such, it is important to precisely maintain PC composition to facilitate cellular health. Although PC can be assembled by several routes, cellular demand for PC is fulfilled by high-capacity de novo synthesis via the CDP-choline or Kennedy pathway, which is regulated by the dimeric rate-limiting enzyme CTP:phosphocholine cytidylyltransferase (CCT) (1). The ubiquitously expressed CCTα isoform is expressed in the nucleoplasm of cells but, depending on the cell type and lipid stimulus, is translocated to the nuclear envelope or exported to the endoplasmic reticulum (ER) or lipid droplets (Fig. 1) (2). There, it reversibly associates with membranes, mediated by an extended amphipathic helix called domain M that inserts into membranes that are enriched in anionic lipids (i.e. fatty acids) or nonbilayer lipids (i.e. diacylglycerol) or are otherwise PC-deficient. This conformational change in turn relieves auto-inhibition of the catalytic domain by an auto-inhibitory (AI) helix, and CDP-choline synthesis increases dramatically (Fig. 1).

How exactly does domain M auto-inhibit CCTα catalysis? Answers to this question have been slow in coming due to the absence of complete structures of either the soluble or membrane-associated forms of CCTα. Clues were obtained from the crystal structure of a catalytically silenced form of CCTα, which revealed that domain M is composed of a disordered “leash” followed by the AI helix that docks against a linking helix (the αE helix) and at the active site to induce a nonproductive conformation (3). However, which if any of these contacts are functionally relevant, and thus the fundamental details of this regulatory mechanism, were not clear. In this issue, Cornell and colleagues utilize molecular dynamics (MD) simulations, mutagenesis, and cross-linking analysis to demonstrate that catalytic silencing by the AI helix involves constraints on catalytic residue Lys122 and the αE helix at the C terminus of the catalytic domain (4). When the domain M leash associates with membranes, the AI helix is also displaced to the membrane surface, and CCTα inhibition is relieved.

The potential importance of the αE helix and Lys122 in CCTα regulation was initially suggested by comparison with the structurally homologous non–lipid-regulated cytidylyltransferases. In contrast to those enzymes, CCTα has a glycine adjacent to Lys122, which should increase flexibility around the catalytic residue, and its αE helix is longer and predicted to be interrupted in the middle by a disordered flexible hinge. Thus, Ramezanpour et al. (4) suspected that interactions with the AI helix could produce a nonproductive conformation by restricting the movement of the Lys122 loop and the αE helix. To test this, the authors initially show that substitution of alanine or proline for the glycine adjacent to Lys122 inhibited activation by lipids, indicating that flexibility of the Lys122 loop is essential. They then ran a series of forty 1-μs MD simulations in which the protein was modeled alone or in combination with the CTP substrate and/or AI. Inclusion of the AI helix significantly constrained the N terminus and central hinge of the αE helix and changed the hydrogen-bonding frequency of the Lys122 loop from interacting with CTP to interacting with other carbonyl groups, notably in the C terminus of the AI helix. As a result, the AI helix was able to steer Lys122 away from a productive complex with its substrate CTP. The presence of Gly123 was critical to allow close access of these groups, in agreement with the biochemical data.

During MD simulations carried out with the AI helix, the authors noticed that the 4-helix AI-αE bundle of the CCTα dimer was stable, there was minimal backbone fluctuation of the αE helix, and its hinge region was constrained. However, when the AI helix was removed from the MD simulations, anticipating the conformational change that would occur when domain M binds membranes, there was a remarkable unwinding of the αE hinge into a splayed, bent configuration, and stable contacts formed between the Lys122 loop and the C terminus of...
the αE helix. To test this experimentally, the authors created a CCTα construct containing Cys217 in the C terminus of the αE helix that could be cross-linked to its dimeric counterpart. This cross-linking was reduced in the presence of phospholipid vesicles, indicating that enzyme binding to membranes due to splaying of the αE helices, but it’s not clear what specific role lipids might play in these AI/αE-mediated interactions. Second, CCTα is known to associate with organelle membranes and monolayers of different composition and structure (Fig. 1). Does the activation mechanism described here apply at these different organelle surfaces, and what is the biological significance of CCTα activation at these different sites? One can envision the domain M leash scanning the surface of different organelle membranes until it identifies negative charge or surface imperfections that allow insertion, but further investigations are needed to know how this leash is being taken on a walk.

References
1. Pelech, S. L., Pritchard, P. H., Brindley, D. N., and Vance, D. E. (1983) Fatty acids promote translocation of CTP:phosphocholine cytidylyltransferase to the endoplasmic reticulum and stimulate rat hepatic phosphatidylcholine synthesis. J. Biol. Chem. 258, 6782–6788 Medline
2. Cornell, R. B., and Ridgway, N. D. (2015) CTP:phosphocholine cytidylyltransferase: Function, regulation, and structure of an amphitropic enzyme required for membrane biogenesis. Prog. Lipid Res. 59, 147–171 CrossRef Medline
3. Lee, J., Taneva, S. G., Holland, B. W., Tieleman, D. P., and Cornell, R. B. (2014) Structural basis for auto-inhibition of CTP:phosphocholine cytidylyltransferase (CCT), the regulatory enzyme in phosphatidylcholine synthesis, by its membrane-binding amphipathic helix. J. Biol. Chem. 289, 1742–1755 CrossRef Medline
4. Ramezanpour, M., Lee, J., Taneva, S. G., Tieleman, D. P., and Cornell, R. B. (2018) An auto-inhibitory helix in CTP:phosphocholine cytidylyltransferase hijacks the catalytic residue and constrains a pliable, domain-bridging helix pair. J. Biol. Chem. 293, 7070–7084 CrossRef Medline
5. Krahmer, N., Guo, Y., Willing, F., Hilger, M., Lingrell, S., Heger, K., Newman, H. W., Schmidt-Supprian, M., Vance, D. E., Mann, M., Farese, R. V., Jr, and Walther, T. C. (2011) Phosphatidylcholine synthesis for lipid droplet expansion is mediated by localized activation of CTP:phosphocholine cytidylyltransferase. Cell Metab. 14, 504–515 CrossRef Medline