Carnitine Palmitoyltransfersase-1c and 5-oxoprolinase interact in the mouse brain.

Michael J Wolfgang
Biological Chemistry, Johns Hopkins University School of Medicine

Abstract
Carnitine palmitoyltransferase-1c, Cpt1c, is an enigmatic neuron-specific protein with no definitive function although it retains high sequence similarity to other known carnitine acyltransferases. To gain insight into the function of Cpt1c in neurons, an immunoprecipitation mass spectrometry screen was performed to identify new binding partners to inform cellular pathways relevant to Cpt1c. Therefore, lysate from the brains of transgenic mice that express an epitope-tagged Cpt1c where immunoprecipitated and binding partners eluted and identified by mass spectrometry. 5-oxoprolinase, Oplah, was identified as a Cpt1c-binding partner in vivo. These data support a possible role of Cpt1c in cellular redox metabolism.

Introduction
Carnitine acyltransferases are a class of enzymes that are important for the regulated transport of acyl groups across membranes to enable inter-organelle transport. Carnitine Palmitoyltransferase 1 isoenzymes (Cpt1a and Cpt1b) are positioned at the outer mitochondrial membrane and are critical for the transfer of long-chain fatty acids across the mitochondrial inner membrane. Therefore, they represent a rate determining step for mitochondrial long-chain fatty acid β-oxidation and are coordinately regulated by the rate determining step in de novo fatty acid synthesis, malonyl-CoA. A third member of this family, Cpt1c, has been identified via its close sequence homology that is encoded by an independent gene [1]. Interestingly, Cpt1c is expressed almost exclusively in neurons, a cell type that has a limited, if any, capacity for mitochondrial long-chain fatty acid β-oxidation. Cpt1c acyltransferase activity could not be measured for a broad range of fatty acids in vitro and Cpt1c does not support fatty acid oxidation in vivo or in heterologous systems [1] [2] [3]. In fact, it has been demonstrated that Cpt1c is not associated with mitochondria but rather is localized to the Endoplasmic Reticulum [4]. Therefore, the enzymatic role for Cpt1c, if any exists, remains unknown [5]. Consistent with its localization to a diverse array of neuron subtypes, Cpt1c knockout mice exhibit broad behavioral deficits associated with disparate brain regions [6] [7] [2] [3]. Cpt1c has been identified in the AMPA receptor-trafficking complex in multiple unbiased protein-protein interaction screens [8] [9]. It has also been shown to affect AMPA receptor transport and signaling at the synapse [10] [11]. Humans with missense mutations in Cpt1c exhibit hereditary spastic paraplegia (HSP) [12] although the human missense mutations in Cpt1c more closely resemble the phenotype of mice over-expressing Cpt1c rather than the KO mice [13]. To gain insight into the biochemical and biological mechanisms of Cpt1c, mice with an epitope-tagged Cpt1c were used to identify novel Cpt1c interacting proteins in vivo in an unbiased manner. Here, Cpt1c was shown to interact with 5-oxoprolinase (Oplah) in vivo in the mouse brain. Interestingly, the most abundant metabolic alterations in Cpt1c KO brains were those in glutathione and 5-oxoprolin metabolism [14]. Given that these two completely orthogonal unbiased screens (metabolomics and protein-protein interactions) identified this association, these data support a role of Cpt1c in neuronal redox metabolism.

Objective
To determine binding partners for Carnitine Palmitoyltransferase-1c to gain insight into its biological function.
Carnitine Palmitoyltransferase-1c and Oplah interact in the mouse brain.

Results & Discussion

To gain insight into potential binding partners of Cpt1c, interacting partners were sought that could co-immunoprecipitate with Cpt1c from mouse brains in vivo. This strategy has worked well to elucidate functional pathway interactions for other proteins using similar methodology [15]. Mice were generated with a conditional Cre-inducible transgene encoding a C-terminal FLAG-tagged mouse Cpt1c (Cpt1cTg). The epitope-tagged Cpt1c is expressed only when Cpt1cTg mice are bred to mice that express Cre recombinase under various tissue-specific promoters [13]. Here mice that expressed Cpt1c specifically in the brain were generated by crossing the Cpt1cTg to Nestin-Cre transgenic mice. Then Cpt1c-Flag was immunoprecipitated from the brains of Cpt1cTg mice fed a high fat diet. The immunoprecipitates were able to pull down the epitope-tagged Cpt1c while the endogenous enzyme was present in the flow through (Fig. 1A). Because epitope-tagged Cpt1c could be purified, it was next determined if there were any proteins that eluted in a complex with Cpt1cTg. Therefore, Cpt1cTg was eluted from the column with an excess of Flag peptide and ran the concentrated eluate on SDS-PAGE. Proteins that were differentially eluted from control and Cpt1cTg brains were identified by Coomassie brilliant blue staining.

Figure Legend

Figure 1. Cpt1c and Oplah interact in mouse brain.
(A) Cpt1c immunoprecipitation with anti-FLAG (M2) resin from transgenic mice (Cpt1cTg; Nestin-cre) expressing Cpt1c-Flag fed either a high or low fat diet.
(B) Coomassie stained gel of Cpt1c-binding partners immunoprecipitated from WT (control) or TG (Cpt1cTg; Nestin-cre) and eluted with FLAG peptide and run on SDS-PAGE in duplicate.
(C) Expression of Oplah in Cpt1c KO, Tg (Cpt1cTg; Nestin-cre) and WT mice fed a high or low fat diet.
(D) Confirmation of co-immunoprecipitation with Oplah-specific antibodies.
Several bands were identified that were differentially eluted from Cpt1cTg but not control brains (Fig. 1B). These bands were then submitted to the Johns Hopkins Proteomic Core for identification by MS/MS. 5-Oxoprolinase (Oplah) was identified as a potential Cpt1cTg interacting protein. To determine if Oplah was differentially regulated by Cpt1c or diet brain lysates from WT control, Cpt1cKO and Cpt1cTg mice fed a low or high fat diet were subjected to SDS-PAGE followed by Western blotting for Oplah. Oplah was not significantly altered under these conditions demonstrating that Oplah abundance was not affected by either diet or Cpt1c (Fig. 1C). Finally, Oplah was confirmed to be present in a complex with Cpt1c in Cpt1cTg brains. Fractions of flow through or eluate from the columns were subjected to SDS-PAGE followed by Western blotting specifically for Oplah, consistent with the mass spectrometry data. Oplah was bound to Cpt1c only in Cpt1cTg brains (Fig. 1D). Diet composition had little impact on this interaction. This data shows that Cpt1c and Oplah interact in the mouse brain. These data are of particular interests because previous data has shown that Cpt1c KO brains had significant changes in glutathione and 5-oxoproline metabolism, the metabolic pathway that Oplah regulates [14]. Although the biochemical reaction mediated by Cpt1c is enigmatic, Cpt1c plays a critical role in neuron function in rodents and humans. The loss of Cpt1c in mice results in clear behavioral deficits [3] [4] [7] [8] and mutations in human Cpt1c causes Hereditary Spastic Paraplegia [12]. Oplah, like Cpt1c, is enriched in the nervous system. Missense mutations in Oplah cause 5-oxoprolinuria (OMIM #260005); however, unlike other inborn errors in glutathione metabolism that result in 5-oxoprolinuria, the clinical manifestations of Oplah mutations have been questioned [16] [17]. Deletion of Oplah in mice has been shown to result in an increased startle response and decreased heart rate (http://www.mousephenotype.org). Finally, Cpt1c is rather specific to neurons under normal conditions, but is up-regulated in transformed cells including primary human tumors. It has been suggested that Cpt1c enables tumors to combat metabolic stress, however, via an undefined mechanism [18]. Because oxidative stress has been shown as an important contributor to cancer progression, the regulation of Oplah and GSH metabolism via Cpt1c could be a unifying mechanism underlying the role of Cpt1c in tumors.

Conclusions
Using an unbiased in vivo protein interaction screen, it was found that Carnitine Palmitoyltransfersase-1c and 5-oxoprolinase interact in the mouse brain.

Limitations
Although Cpt1c and Oplah interact, it cannot be concluded that they interact directly or are merely present in a larger protein complex.

Additional Information

Methods and Supplementary Material
Please see https://sciencematters.io/articles/201609000009.

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Ethics Statement
All procedures were performed in accordance with the NIH’s Guide for the Care and Use of Laboratory Animals and under the approval of the Johns Hopkins Medical School Animal Care and Use Committee.