Title:	Evaluating a yeast model for 5-Oxoproline toxicity using heterologous expression of a mammalian 5-Oxoproline transporter
Authors:	B.S, Vaishnavi
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Abstract:	5-Oxoproline or pyroglutamic acid (5-OP) is a cyclic lactam of glutamic acid. It is metabolized into glutamate with the help of 5-oxoprolinase enzyme (OPLAH or OXP1). The knockdown of 5-oxoprolinase enzyme in mammals was found to result in heart failure and is thought to be a consequence of oxidative stress (Van der pol et al., 2017). However, this is surprising since 5-oxoproline is a relatively inert compound. The broad goal of this project is to analyze the consequences of 5-oxoproline accumulation in yeast, where its natural transport is limited. In this study we initially attempted to identify the endogenous 5-OP transporter but were unsuccessful. We next explored heterologous expression of a known mammalian 5-OP transporter, MCT1, along with its accessory protein CD147, in yeast cells lacking the OXP1 gene (responsible for hydrolysis of 5-OP to glutamate). This approach successfully enhanced 5-OP toxicity and accumulation into yeast cells even at low external concentrations (1 mM) and the accumulated intracellular 5-OP, due to the OXP1 deletion, resulted in growth defects. Further investigation revealed that 5-OP accumulation triggered oxidative stress within the yeast cells. We observed elevated levels of oxidized glutathione, a marker of oxidative stress, alongside decreased levels of reduced glutathione, an important antioxidant. Hits from gene expression profiling in an alternate yeast model for 5-OP accumulation (Dubay and Bachhawat, unpublished) which did not use the transporter overexpression were also evaluated in the model by qPCR and were found to be upregulated. In conclusion, this study demonstrates that human MCT1 can facilitate 5-OP transport in yeast which leads to 5-OP accumulation within the cell causing oxidative stress, ultimately resulting in growth defects.
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