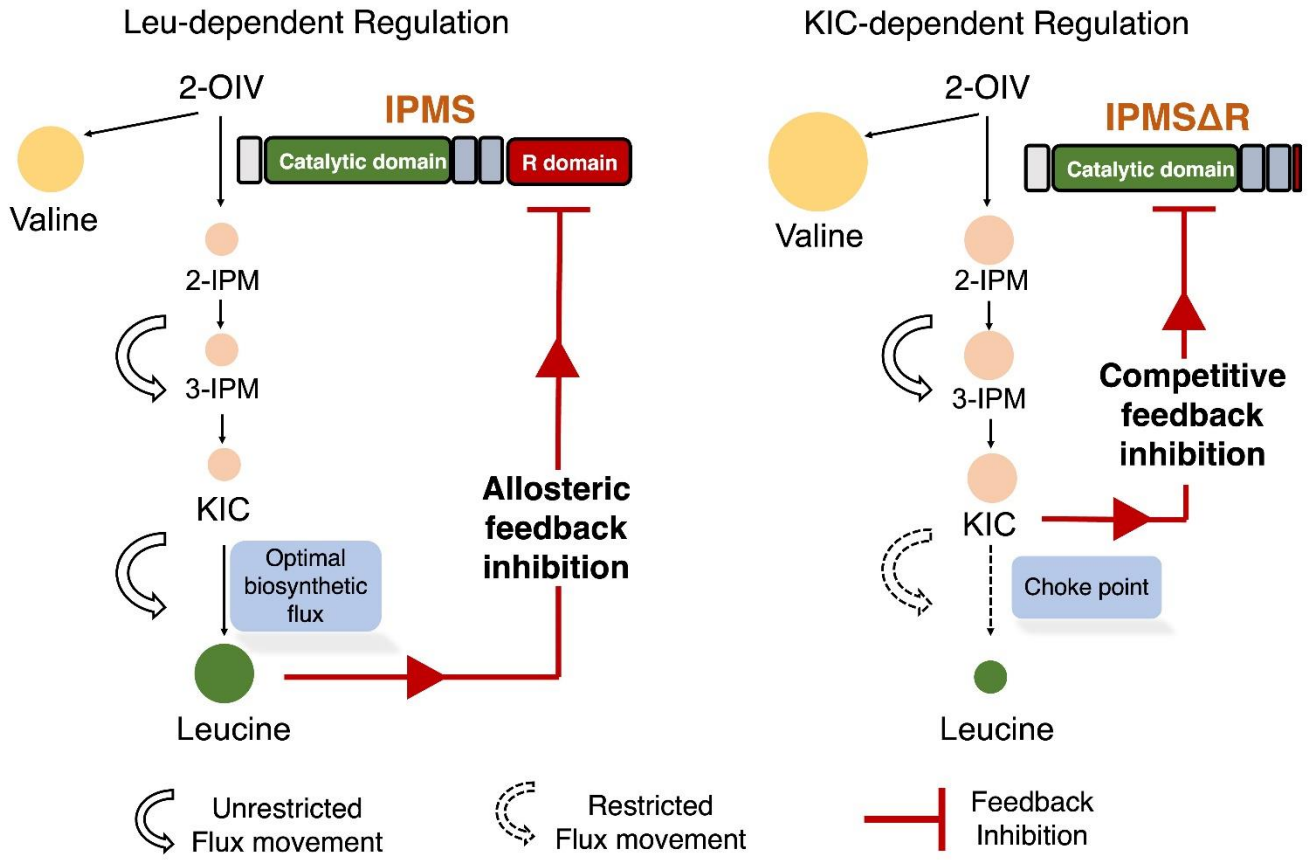
Research highlights

Title of story: Isopropylmalate synthase regulatory domain removal abolishes feedback regulation at the expense of leucine homeostasis in plants



Brief Story: Feedback inhibition loops are frequent in amino acid biosynthetic pathways to tightly regulate the accumulation of the end products. Greater knowledge of these mechanisms is important for understanding metabolic networks and should find application with plant breeders, for example, to avoid limitations on the production of amino acids essential for humans in crop plants.

In Leu biosynthesis, it is well known that the amino acid end product inhibits the rate-limiting pathway enzyme isopropylmalate synthase (IPMS) by binding to its C-terminal regulatory domain. Previous *in vitro* and structural findings have showed that the loss of C-terminal regulatory domain from plant IPMS has facilitated the neofunctionalism of IPMS to secondary metabolite (glucosinolate) pathway enzymes like methylthioalkylmalate synthase (MAMS) in Brassicaceae. Further unlike in bacteria, the *in vitro* removal of the C-terminal regulatory domain maintains plant IPMS enzyme activity without the feedback inhibition by Leu. However, we still know very little about what would happen to Leu pathway flux *in*

vivo upon removal of the IPMS C-terminal domain. Exploring this question may provide a much more realistic understanding of how the regulatory mechanism works to control Leu biosynthesis in plants.

When we introduced an edited IPMS without its C-terminal regulatory domain into Arabidopsis and the oilseed crop, Indian mustard, we were surprised to find no increase in Leu accumulation in intact plants. In fact, Leu levels were even reduced, while flux through the pathway increased. Extensive metabolomic and biochemical analyses revealed that an intermediate of the Leu pathway was a competitive inhibitor of IPMS without its regulatory domain. Our detailed biochemical analysis showed that in the absence of the C-terminal regulatory domain, a Leu pathway intermediate (α-ketoisocaproate) could compete with the native IPMS substrate (2-oxoisovalerate) for the active site. Thus, we found for the first time that the Leu-feedback regulatory domain of IPMS functions not only to regulate enzyme activity according to cellular Leu concentration, but also bypasses competitive inhibition by a pathway intermediate that could severely limit pathway end product. These results give new insight into the regulation of a critical primary metabolic pathway and suggest novel approaches for manipulating Leu homeostasis.

Article link: https://academic.oup.com/plphys/article/197/2/kiaf041/7984316?login=true