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A tale of two Hippo pathway modules

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The Hippo pathway is an evolutionarily conserved pathway with crucial roles in development, organ size control, tissue homeostasis and cancer. Over two decades of research have elucidated the core Hippo pathway kinase cascade, but its precise organization has not been fully understood. In this issue of The EMBO Journal, Qi *et al* (2023) report a new model of two modules for the Hippo kinase cascade, providing new insights into this long-standing question.

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See also: **S Qi *et al*** (June 2023)

The core kinase cascade of the mammalian Hippo pathway comprises kinases LATS1/2 and MST1/2, their respective adaptors MOB1A/B and SAV1, and recently discovered MAP4K-family of kinases (MAP4K1–7 or MAP4Ks). MST1/2 and MAP4Ks act in parallel to phosphorylate and activate LATS1/2, which in turn phosphorylate two Hippo downstream effectors YAP and TAZ, resulting in their cytoplasmic retention and inhibition (Fig 1A). In addition, studies from different model systems have pinpointed NF2 and WWC1-3 as Hippo pathway key components controlling YAP/TAZ via its core kinase cascade (Ma *et al*, 2019; Zheng & Pan, 2019). It is known that NF2 binds and translocates LATS1/2 onto plasma membrane, thus facilitating their phosphorylation and activation by MST1/2 (Yin *et al*, 2013), while WWC1-3

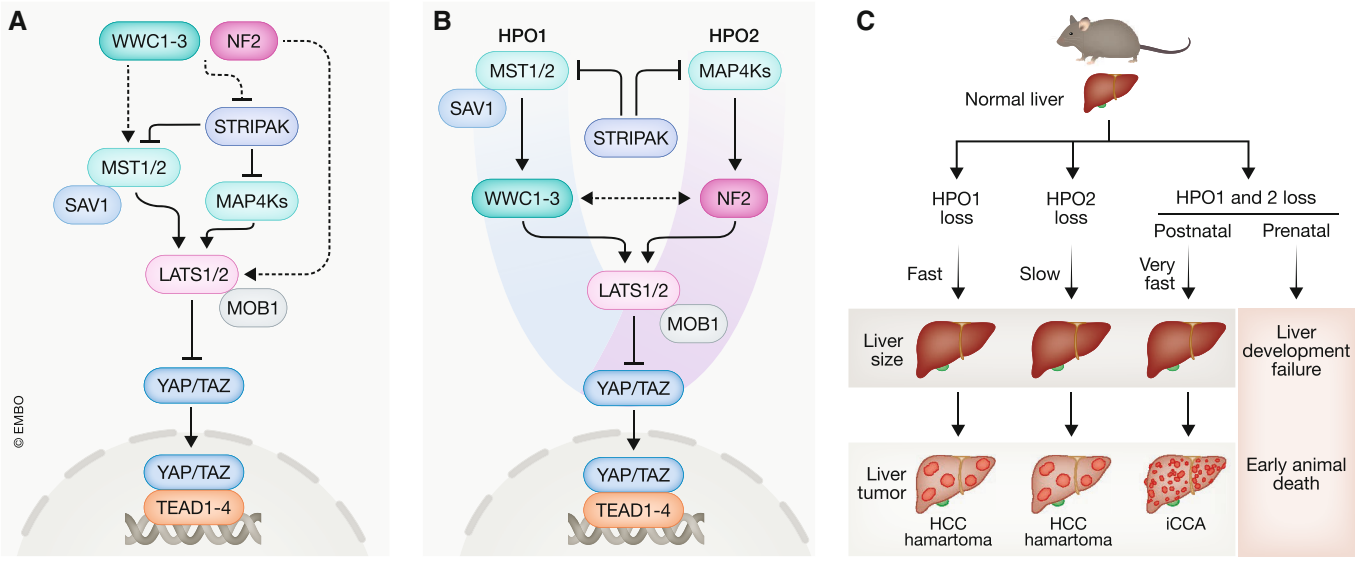

Figure 1. The two kinase modules of the Hippo kinase cascade.
(A) In the traditional Hippo pathway model, NF2 and WWC1-3 function as upstream regulators of the Hippo kinase cascade. WWC1-3 act together with NF2 to activate MST1/2. These two Hippo components also inhibit the STRIPAK PP2A complex and release its inhibition of MST1/2 and MAP4Ks. In addition, NF2 binds and moves LATS1/2 onto plasma membrane, where LATS1/2 are phosphorylated and activated by MST1/2. (B) In this issue, Qi *et al* (2023) propose two independent Hippo pathway modules, HPO1 and HPO2, within the Hippo kinase cascade. In the HPO1 module, WWC1-3 act as "adaptor" proteins bridging the MST1/2-SAV1 complex to bind and phosphorylate LATS1/2. In the HPO2 module, NF2 functions downstream of MAP4Ks and is required for MAP4K-mediated LATS1/2 phosphorylation. (C) The HPO1 and HPO2 modules play different roles in control of mouse liver size and cancer development. Loss of both HPO1 and HPO2 phenocopies Lats1/2 deficiency in mouse livers. HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma.

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function together with NF2 to activate MST1/2 and LATS1/2 (Baumgartner *et al*, 2010; Genevet *et al*, 2010; Yu *et al*, 2010; Fig 1A). In addition, NF2 and WWC1-3 target STRIPAK, a phosphatase 2A (PP2A) complex, to release its inhibitory effect on MST1/2 and MAP4Ks (Chen *et al*, 2019; Fig 1A). Based on these findings, NF2 and WWC1-3 have been placed upstream of the Hippo core kinase cascade.

In this issue, Qi *et al* (2023) re-examined the roles of NF2 and WWC1-3 in the Hippo pathway and proposed a new working model for the Hippo core kinase cascade. According to this study, NF2 and WWC1-3 function as "adaptor" proteins downstream of MAP4Ks and MST1/2, respectively, mediating MAP4K- and MST1/2-induced LATS1/2 phosphorylation. Molecular evidence supporting this model includes (i) loss of NF2 and WWC1 attenuates LATS1/2 activities but does not affect that of MAP4Ks and MST1/2; (ii) NF2 and WWC1-3 both strongly interact with LATS1/2; (iii) WWC1-3 bind SAV1 to recruit MST1/2 to phosphorylate and activate LATS1/2 independently of NF2 and MAP4Ks (Qi *et al*, 2022); and (iv) NF2 binds MAP4Ks and mediates the MAP4K-induced LATS1/2 phosphorylation and activation independently of WWC1-3, SAV1 and MST1/2. Based on these findings, Qi *et al* (2023) suggest that two signaling modules HPO1 (MST1/2–SAV1–WWC1-3–LATS1/2) and HPO2 (MAP4Ks–NF2–LATS1/2) independently activate LATS1/2 in the Hippo core kinase cascade (Fig 1B). Echoing on this model, their genetic mouse liver data further show that the HPO1 and HPO2 modules differently control mouse liver size and cancer development, while their concurrent loss phenocopies Lats1/2 deficiency in mouse livers (Fig 1C). Collectively, the observations by Qi *et al* (2023) suggest an interesting model of how WWC1-3 and NF2 fit into the Hippo core kinase cascade, providing a glimpse into the precise molecular organization of the Hippo pathway.

Despite the intriguing discoveries, key questions about this two-module model for the Hippo kinase cascade remain to be addressed in future. First, regarding the roles of WWC1-3 and NF2 in the Hippo pathway within this new model, it will be important to elucidate their discrepancies with the findings from previous studies produced by different labs. Second, although the provided biochemistry and genetic evidence supports the existence of the proposed HPO1 and HPO2 modules, it would be interesting to determine how these two pathway modules are spatiotemporally coordinated to regulate LATS1/2 in the Hippo-related signaling contexts. Third, since all the HPO1 and HPO2 components are conserved in *Drosophila* and can be even traced to unicellular organisms (Sebe-Pedros *et al*, 2012; Chen *et al*, 2020), whether such two-module model for the Hippo kinase cascade also exists in other species deserves further investigation.

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Disclosure and competing interests statement

The authors declare that they have no conflict of interest.

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