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JB Commentary

What is the Hippo pathway? Is the Hippo pathway conserved in *Caenorhabditis elegans?*

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The Hippo pathway was originally identified in *Drosophila* as the signalling pathway that governs organ size. The core of the pathway harbours two protein kinases (Hippo and Warts). Hippo phosphorylates and activates Warts, which in turn phosphorylates and inactivates the transcriptional co-activator Yorkie. As Yorkie mediates cell cycle-promoting and anti-apoptotic gene transcriptions, the Hippo pathway suppresses cell cycle progression and induces apoptosis. The pathway was named after Hippo, which was regarded as a key component. The pathway was initially considered to be well conserved in mammals. Indeed the mammalian homologues of Hippo, and Warts negatively regulate Yorkie homologue and function as the tumour suppressors. However, the researchers have identified numerous additional components both in *Drosophila* and mammals and the significant interspecies diversity is now evident. To make things more complicated, the regulation of the pathway does not necessarily depend on Hippo homologues. In this commentary, we reconsider what is essential for the Hippo pathway and try to sort out the controversial arguments in the discussion of the evolutionary root of the pathway.

Keywords: *Caenorhabditis elegans*/evolution/Hippo pathway/tumour suppressor/Yes-associated protein.

Abbreviations: *C. elegans*, *Caenorhabditis elegans*; *C. owczarzali*, *Capsaspora owczarzali*; GPCR, G protein-coupled receptor; LATS, large tumour suppressor; MST, mammalian Ste20-like.

The Hippo pathway is a relatively new signalling pathway and attracts overwhelming interest of researchers in cancer and stem cell biology fields. The pathway is well conserved from fly to mammals. Very recently the paper entitled 'Activation of the Yeast Hippo pathway by Phosphorylation-Dependent Assembly of Signaling Complexes' has appeared (*1*). Does yeast have the Hippo pathway? The answer is yes and no. It depends on how we define the Hippo pathway. The Wnt pathway is the signalling pathway that is triggered by the Wnt ligands. It does not matter which signalling molecules are activated downstream of the Wnt ligands. The ligands may activate the canonical or the non-canonical pathway. Likewise, if the Hedgehog ligands or the Notch receptors mediate the signals, such signals are regarded as parts of the Hedgehog pathway and the Notch pathway. The circumstances are different for the Hippo pathway.

Most reviews of the Hippo pathway start with the functional and the constitutional definitions of the pathway (*2*–*8*). They designate the Hippo pathway the signalling that governs organ size through the balance of cell proliferation and apoptosis, and subsequently list the components of *Drosophila* Hippo pathway in chronological order. Warts was identified as a tumour suppressor long before the Hippo pathway. The discovery of Salvador, a gene that promotes cell cycle exit and apoptosis, opened the history of the Hippo pathway. Hippo was shortly afterwards reported as a key regulator that links Salvador and Warts. Further later Mats was shown to interact with and activate Warts. Given with the physical and the genetic interactions, the researchers started to regard Salvador, Hippo, Warts and Mats as a distinct complex of negative growth regulators. In most reviews, these four gene products are classified as the core components of the Hippo pathway. The term 'Hippo pathway' did not explicitly appear on papers until the downstream target, Yorkie, was identified. Yorkie does not have a DNA-binding domain but works with Scalloped, a transcriptional factor of TEAD family, to enhance cell cycle-promoting and anti-apoptotic gene transcriptions. When the Hippo pathway is activated, Yorkie is phosphorylated and recruited from the nucleus to the cytoplasm, so that Yorkie-dependent gene transcriptions are shut off. Tissue overgrowth, the hallmark of the Hippo pathway dysfunction, is explained by the hyperactivity of Yorkie. Therefore, Yorkie is occasionally included as one of the core components. That is to say, if we naively define the Hippo pathway, it is the signalling pathway that is composed of Salvador, Hippo, Mats and Warts and that negatively regulates Yorkie (Fig. 1, left). As frequently emphasized, these molecules are well conserved in mammals. WW45 (or Sav1), mammalian Ste20-like kinases (MST1 and MST2), MOB1, large tumour suppressor kinases (LATS1 and LATS2) and YAP are homologues of Salvador, Hippo, Mats, Warts and Yorkie, respectively, and the physical interactions among them are also conserved. Therefore, we can define the mammalian Hippo pathway in a similar manner (Fig. 1, left).

The architecture of the pathway initially looked simple. However, the old good days did not last long. Untiring efforts of researchers have revealed numerous molecules as additional components and have gradually made the whole blue print ambiguous. Upstream regulators are most problematic.

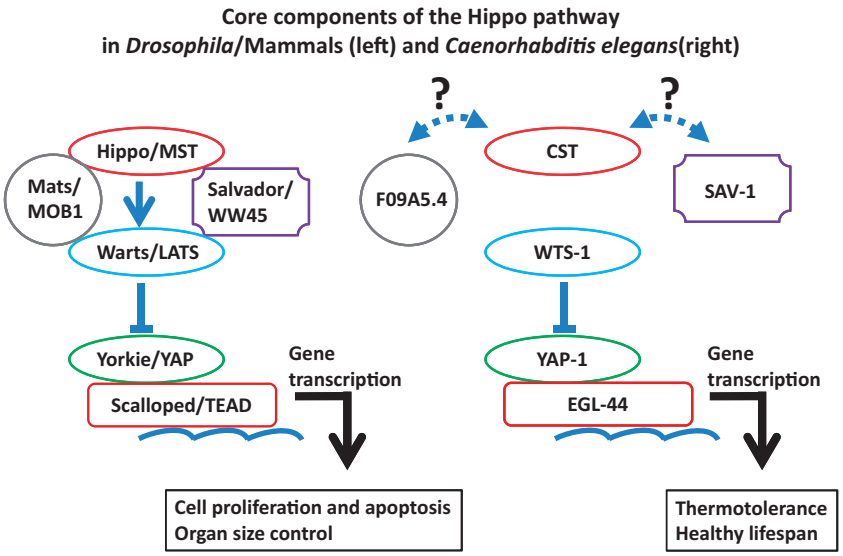

Fig. 1 Core components of the Hippo pathway in *Drosophila*/mammals (left) and *C. elegans* (right). In *Drosophila* and mammals, Hippo/MST co-operate with Mats/MOB1 and Salvador/WW45 to activate Warts/LATS, which negatively regulates Yorkie/YAP. Yorkie/YAP interact with Scalloped/TEAD to promote gene transcriptions and control organ size through the balance between cell proliferation and apoptosis. In *C. elegans*, there is no evidence that CST functions upstream of YAP-1. Putative Mats homologue (F09A5.4) and Salvador homologue (SAV-1) have not yet been analysed. WTS-1–YAP-1–EGL-44 axis is conserved and regulates thermotolerance and healthy lifespan. If we consider that Warts–Yorkie–Scalloped or LATS–YAP–TEAD axis is the essence of the pathway, the Hippo pathway is conserved in *C. elegans*.

In *Drosophila*, three upstream inputs, Fat/Dachs, Crumbs/Kibra/Merlin/Expanded and Lgl/Scribble/Dlg/aPKC, regulate the Hippo pathway. These components are conserved in mammals, but the mammalian homologues are not necessarily linked to the Hippo pathway. Furthermore, some molecules bypass Hippo to regulate the pathway. Among various examples, we mention here mammalian angiomotin and angiomotin-like proteins that directly interact with YAP and negatively regulate it. Some researchers describe angiomotin as a novel component of the Hippo pathway, whereas other researchers say that angiomotin restricts YAP in the Hippo pathway-independent way (*9*, *10*). G protein-coupled receptors (GPCRs) are other important regulators of YAP in mammals (*8*). Gs-coupled receptor activates protein kinase A, which activates LATS1 to inhibit YAP (*11*, *12*). Other GPCRs modulate actin cytoskeleton and activate YAP. Thus, the researchers are now studying the Hippo pathway that is independent of Hippo. The Wnt pathway without Wnt, the Notch pathway without Notch and the Hedgehog pathway without Hedgehog are contradictory. But the Hippo pathway works without Hippo in some cases. Hippo reigns, but does not govern. Should we rename the pathway? It is non-realistic. The name has already permeated the researchers so well. The researchers including us frequently illustrate hippopotamus in the powerpoint slides to symbolize the signalling pathway, whose dysfunction results in the organ hypergrowth. We must deal with the Hippo pathway without Hippo.

Although this discussion may sound much ado about nothing, it takes on a more serious aspect when we start to trace the evolutionary origin of the pathway. The components of the Hippo pathway are conserved not only in metazoan. The mitotic exit network and the separation initiation network in yeast may represent the roots of the pathway. The mitotic exit network is a kinase cascade that regulates exit from mitosis in yeast. It contains two serine–threonine kinases (Cdc15 and Dbf2) and an adaptor protein, Mob1. Although Cdc15 is remote from *Drosophila* Hippo and mammalian MST kinases in the size and in the structure (it lacks the SARAH domain, which mediates important protein–protein interactions in *Drosophila* and mammals), it uses Mob1 as an adaptor and phosphorylates Dbf2. This mode of action is reminiscent of that of Hippo and MST, which co-operate with Mob1-like proteins (Mats and MOB1) to phosphorylate and activate nuclear Dbf2-like kinases (Warts and LATS). This similarity allows Cdc15 to be described as the Hippo-like kinase. Yeast also has the TEAD homologue, Tec1, which activates G1 cyclin transcription and is required for adhesion, but has no YAP homologue. From the standpoint that YAP is an essential component, the pathway should not date back to before the first appearance of YAP homologue. Hilman and Gat detected proteins with YAP-like molecular structures in non-metazoan but concluded that they are not YAP homologues (*13*). Sébé-Pedrós *et al*. later expressed *Capsaspora owczarzali* (*C. owczarzali*) YAP and TEAD homologues in *Drosophila* to demonstrate that they promote tissue overgrowth and discussed the possibility that the Hippo pathway evolved in non-metazoan (*14*).

Caenorhabditis elegans genome has homologues of almost all components of the Hippo pathway (Fig. 1, right). We can reason that the Hippo pathway is conserved in *C. elegans*. However, the key component, the homologue of YAP, had not been studied. We have recently characterized *C. elegans* F13E6.4 gene as YAP homologue and named it *yap-1* (*15*). YAP-1 shows sequence similarities to YAP in the N-terminal TEAD-binding domain and in the WW domain. We confirmed that it interacts with Warts homologue (WTS-1) and

TEAD homologue (EGL-44) and that it can work with EGL-44 to activate gene transcription in heterologous cells. We also showed that the subcellular localization of YAP-1 is affected by WTS-1. More importantly, the phenotype induced by *wts-1* knockdown (reduced thermotolerance) is canceled by *yap-1* knockdown, while the phenotype of YAP-1 overexpression (increased thermotolerance) is attenuated by *egl-44* knockdown. These findings indicate that EGL-44 functions downstream of YAP-1, which functions downstream of WTS-1. Although the data concerning to endogenous protein are limited, our findings support that *C. elegans* has a functional YAP homologue. Nevertheless, we should not jump to the conclusion without deliberations that the Hippo pathway is conserved in *C. elegans*. The knockdowns of the putative upstream regulator homologues including the Hippo homologues, CST-1 and CST-2, had no effect on the subcellular localization of YAP-1. CST-1 overexpression increases lifespan via FOXO homologue, DAF-16 (*16*). The suppression of YAP-1 improves swimming locomotory capacity in the middle age (healthy lifespan) but does not change lifespan at the end point (*15*). Moreover, this effect does not depend on DAF-16. In short we could not find out the evidence that YAP-1 functions downstream of CST-1 and CST-2. However, as mentioned above, species-specific diversification is obvious in the upstream regulators. The systematic analysis by Bossuyt *et al.* uncovered that the upstream regulatory mechanism undergoes a fundamental shift during evolution (*17*). For instance, Fat controls growth through the Hippo pathway in *Drosophila*, but not in vertebrates. Instead angiomotin is lost in *Drosophila*. Therefore, even if YAP-1 does not work downstream of Hippo homologue, we do not need to renounce the Hippo pathway in *C. elegans*. The central part of the pathway, Warts–Yorkie–Scalloped or LATS–YAP–TEAD, is likely to be conserved. We speculate that if *C. elegans* YAP-1 and EGL-44 are expressed in *Drosophila*, they may induce tissue overgrowth as well as *C. owczarzali* YAP and TEAD homologues. If we tolerate the Hippo pathway without Hippo, we can conclude that the Hippo pathway is conserved in *C. elegans*.

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Conflict of Interest

None declared.

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