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Review

Regulation of the Hippo pathway by cell architecture and mechanical signals

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ABSTRACT

The Hippo signaling pathway is an evolutionarily conserved mediator of growth control, cell fate decisions and stem cell identity. At the heart of the pathway is a kinase cascade that is reminiscent of other signaling pathways, but recent studies indicate that the Hippo pathway is unique in that it is regulated by cellular architecture and the mechanical properties of the environment. The Hippo pathway may thus serve as a sensor of tissue structure and mechanical tension, integrating information regarding the size and shape of an organ into cellular behavior, such as whether or not to proliferate. In this review we summarize recent discoveries regarding the regulation of the Hippo pathway by cellular polarity, cell junctions, and the cytoskeleton and discuss how these data inform the study of development and disease.

Contents

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1. Introduction

Cells are exposed to many kinds of extracellular signals which modulate a cell's behavior. Past studies focused mainly on understanding chemical signals, especially in the context of cell–cell interactions. These studies were highly fruitful and identified a handful of signaling pathways that regulate cell fate, cell proliferation, and cell morphology during development and homeostasis [1]. Among these signaling pathways is the Hippo pathway, a relatively recently discovered conserved signal transduction pathway [2–4]. The Hippo pathway is a key regulator of tissue growth, stem cell pluripotency, and cell fate decisions, and de-regulation of Hippo signaling has been implicated in cancer [2,3,5,6]. Because Hippo signaling is a key regulator of growth, a great deal of interest and work is invested in identifying upstream regulators of the pathway. Interestingly, recent work showed that the Hippo pathway is regulated by cell morphology, the cytoskeleton, and mechanical signals. An understanding of how the Hippo pathway is regulated may thus provide information regarding how tissues monitor their integrity and size, and convey that information to cells to regulate growth and patterning.

The Hippo pathway was discovered through genetic screens in *Drosophila* intended to identify genes required for the regulation of organ size, specifically adult structures derived from imaginal discs [2,3,6]. These screens identified mutations that resulted in dramatic overgrowth of the mutant tissues due to hyper-proliferation and decreased apoptosis of mutant cells. The first mutations identified from these screens disrupted three genes, named *warts* (*wts*), *salvador* (*sav*), and *hippo* (*hpo*) [7–15]. Since then, many additional components of the Hippo pathway have been identified and a complex signaling network has emerged (Fig. 1). Most components of the Hippo pathway are conserved between flies and vertebrates, although some differences exist [2,4,5]. At the core of the pathway is a kinase cascade comprised of the Hpo kinase (MST1/2 in vertebrates) that, along with its co-factor Sav (SAV or WW45 in vertebrates), binds and phosphorylates the serine threonine kinase Wts (LATS1/2 in vertebrates) [7–15]. Activated Wts operates with its co-factor Mats (Mob as tumor suppressor; MOBKL1A/B in vertebrates) to phosphorylate the transcriptional co-activator Yorkie (Yki; YAP and TAZ in vertebrates) [16–22]. When Yki, YAP, or TAZ are phosphorylated, they are inactive and retained in the cytoplasm. Un-phosphorylated Yki, YAP, or TAZ enter the nucleus and interact with transcription factors, including Scalloped (Sd, TEAD1-4 in vertebrates), Homothorax, Teashirt, and Mothers against dpp (Mad), to regulate transcription of downstream target genes [23–31]. Thus, when the Hpo/MST1/2 and Wts/LATS1/2 kinases are active, they suppress growth by inhibiting the activity of Yki/YAP/TAZ.

As upstream components of the Hippo pathway were identified, many had something unexpected in common: roles in maintenance of cellular architecture, such as cell polarity and the cytoskeleton [3,32]. Notably, YAP/TAZ activity is also affected by cell geometry and matrix rigidity [33,34]. Together, these findings suggest that Hippo signaling is responsive to mechanical signals and the presence of neighboring cells. Such cues may take the form of mechanical forces transmitted through cell junctions, either by the cytoskeleton or other mechanisms. This unconventional regulation of a signal transduction pathway, the dramatic phenotypes of pathway mutants, as well as the relationship to organ growth control and cancer, have made investigation of the Hippo pathway a major area of current research. In this article we first discuss how determinants of apical-basal polarity affect Hippo signaling, examining the role that different domains of the cell membrane play in organizing and regulating pathway components. Then we describe data that identify mechanical forces and the cytoskeleton as regulators of Hippo pathway activity. We discuss a role for Hippo signaling as a transducer of information regarding the physical environment and mechanical conformation of cells. Finally, we integrate this information into a model for the role of the Hippo pathway as a sensor for cellular integrity, social cues, and tissue status.

2. Apical-basal cell polarity complexes and the Hippo pathway

Recent studies showed that cell polarity is a major regulator of the Hippo pathway, with multiple inputs into Hippo signaling. In this section we describe the three major conserved signaling modules that regulate apical-basal polarity and their interactions with the Hippo pathway.

The three major polarity modules: the Crumbs complex, the atypical protein kinase C (aPKC) complex, and the Scribble module, establish the formation of apical-basal polarity in epithelial cells through antagonistic interactions with one another [35]. The Crumbs complex localizes to the apical region of cells, just above the adherens junction (AJ) [35]. Crumbs (Crb), a large transmembrane protein with a small intracellular domain, has three vertebrate homologs (CRB1-3), and forms a complex with the adaptor proteins Stardust (PALS1,2 proteins in mammals) and PALS1 associated tight junction protein (Patj) [35–37]. The aPKC complex, which is also apically localized, includes the Par3 and Par6 PDZ-binding proteins (Bazooka and Par6 in *Drosophila*) in addition to aPKC [35–37]. The apical complexes antagonize the activity of the Scribble polarity module, which consists of the Scribble (Scrib), Discs large (Dlg), and Lethal giant larvae (Lgl) scaffold proteins localized to the basolateral domain [35–37]. Thus, subdivision of the plasma membrane into distinct domains occurs through cross-regulatory interactions between three conserved polarity modules, although the precise molecular mechanisms of these interactions are poorly understood [35–37].

In the following paragraphs we discuss how the apical domain of epithelial cells may be a key location where Hippo pathway components are organized into active complexes, review the data linking components of the three cell polarity modules to the Hippo pathway, and discuss a feedback loop whereby the Hippo pathway regulates apical domain size.

2.1. Hippo pathway components assemble at the apical membrane

The concept of Hippo signaling has shifted from a model of a simple kinase cascade to a "super-complex" with multiple protein–protein interactions that may occur between pathway components to regulate YAP/TAZ and Yki activity in a network-like fashion. In *Drosophila*, four apically localized transmembrane proteins regulate the Hippo pathway: the atypical cadherins Fat and Dachsous, the apical polarity determinant Crb, and the cell adhesion molecule Echinoid (Ed) [38–43] (Fig. 1). In addition to these transmembrane proteins, other proteins that signal to the core of the Hippo pathway are also localized to the apical membrane. The WW-domain containing protein Kibra (Kib) and the FERM-domain containing adaptor proteins Merlin and Expanded (Ex) localize to the apical membrane [44–47], as does the atypical myosin Dachs, which transduces signals from Fat to Wts [48]. Apical localization of the Hpo kinase and the Rassf and Mats components has also been reported [49,50]. Kib, Mer, Ex, Hpo, and Sav are present in a complex [11,13–15,44–46,51], and Ed complexes with Kib, Ex, Mer, Sav, and Yki [38]. Yki can directly bind to Ex, Hpo, and Wts, which results in the sequestration of Yki in the cytoplasm, a mechanism of Yki regulation that does not rely upon phosphorylation by Wts [52,53]. In mammals, the Hippo pathway also contains apically localized