The Hippo Pathway in Heart Development, Regeneration, and Diseases

Qi Zhou, Li Li, Bin Zhao, Kun-Liang Guan

Abstract: The heart is the first organ formed during mammalian development. A properly sized and functional heart is vital throughout the entire lifespan. Loss of cardiomyocytes because of injury or diseases leads to heart failure, which is a major cause of human morbidity and mortality. Unfortunately, regenerative potential of the adult heart is limited. The Hippo pathway is a recently identified signaling cascade that plays an evolutionarily conserved role in organ size control by inhibiting cell proliferation, promoting apoptosis, regulating fates of stem/progenitor cells, and in some circumstances, limiting cell size. Interestingly, research indicates a key role of this pathway in regulation of cardiomyocyte proliferation and heart size. Inactivation of the Hippo pathway or activation of its downstream effector, the Yes-associated protein transcription coactivator, improves cardiac regeneration. Several known upstream signals of the Hippo pathway such as mechanical stress, G-protein–coupled receptor signaling, and oxidative stress are known to play critical roles in cardiac physiology. In addition, Yes-associated protein has been shown to regulate cardiomyocyte fate through multiple transcriptional mechanisms. In this review, we summarize and discuss current findings on the roles and mechanisms of the Hippo pathway in heart development, injury, and regeneration. (Circ Res. 2015;116:1431-1447. DOI: 10.1161/CIRCRESAHA.116.303311.)

Key Words: cardiomegaly ■ stem cells ■ Yes-associated protein

In mammals, organ size is relatively constant under regulation by both organ-intrinsic mechanisms and extrinsic physical and chemical cues, including mechanical stress and circulating factors. Heart size is also tightly controlled to ensure proper blood circulation. A small-sized heart will not be able to generate sufficient cardiac output to sustain physiological activities. However, increased myocardium mass could shrink cavity size and obstruct cardiac outflow. Alternatively, heart enlargement could result in heart failure as that in pathological cardiac hypertrophy. Mechanistically, the enlargement of heart size during development could be grossly divided into 2 phases. Fetal heart growth is mainly achieved by cardiomyocyte proliferation. Soon after birth, heart growth switches to increase of cardiomyocyte size, which is also called physiological hypertrophy. The molecular mechanism underlying this switch is unclear. Although it has been demonstrated that adult cardiomyocytes still maintain some proliferation ability, the large loss of mitotic potential in cardiomyocytes is a key barrier for cardiac regeneration after heart injury.
Proliferation of cardiomyocytes during development is regulated by various growth factors such as insulin-like growth factors, bone morphogenetic proteins (BMPs), Wnts, and neuregulins. However, the cell intrinsic signaling pathways regulating cardiomyocyte proliferation are not well understood. It was recently demonstrated that the Hippo signaling pathway is critical for cardiomyocyte proliferation, heart size control, and cardiac regeneration. The Hippo pathway is a signaling cascade that plays an evolutionarily conserved role in organ size control from _Drosophila_ to human by regulating cell proliferation, apoptosis, and stem cell/progenitor cell fate determination. It has also been studied extensively in the context of tumor suppression and cancer in mammals. In this review, we briefly outline current understandings of the basic mechanisms of the Hippo pathway, and then focus on the relevance of these mechanisms in recent findings of the Hippo pathway in cardiac physiology, such as developmental heart size control, heart injury and hypertrophy, and cardiac regeneration.

**Composition of the Hippo Pathway**

Core components of the Hippo pathway were first identified in _Drosophila_ by genetic screens for tissue growth regulators. Mutations of these genes lead to a common phenotype of tissue overgrowth and enlarged organ size in _Drosophila_ eyes and wings. More significant is that core components of the Hippo pathway are highly conserved in mammals (Table 1). As illustrated in Figure 1, serine/threonine kinase 4/3 (MST1/2) homologs of the _Drosophila_ Hippo kinase, are known to be proapoptotic and activated by apoptotic stress. MST1/2 physically interact with an adaptor protein salvador (SAV1). The interaction is mediated by dimerization of Salvador, RASSF, and Hpo homology domains, which is present at the carboxyl terminal regions of both proteins. To date, Salvador, RASSF, and Hpo homology domain is found only in components of the Hippo pathway. Binding to SAV1 activates MST1/2 although the underlying mechanism is not completely understood. MST1/2 phosphorylates several proteins including SAV1, the nuclear Dbf2-related (NDR) family kinases large tumor suppressor kinase 1/2 (LATS1/2), and the LATS1/2-interacting adaptor proteins MOBKL1A/1B (MOB1). These phosphorylations lead to activation of LATS1/2, which in turn phosphorylate the Yes-associated protein (YAP) transcription coactivator on 5 serine residues. YAP could shuttle between cytoplasm and nucleus, where it stimulates gene transcription. Phosphorylation of YAP serine residue 127 leads to 14-3-3 binding and thus cytoplasmic retention and inactivation of YAP. In addition, phosphorylation of YAP serine residue 381 by LATS1/2 results in further phosphorylation of a phosphodegron motif on YAP by casein kinase 1/2 and recruitment of SCFβ-TrCP E3 ligase, thus poly ubiquitination and degradation of YAP. Such a dual-inhibitory mechanism may allow spatial and temporal regulation of YAP activity dependent on strength and duration of Hippo pathway activity. Transcriptional coactivator with PDZ-binding motif, also called WWTR1 (TAZ), the YAP paralog, is inhibited by the Hippo pathway in a similar manner, whereas protein stability plays a more prominent role in regulation of TAZ activity, possibly because of the presence of an additional phosphodegron in TAZ. YAP was also reported to be tyrosine phosphorylated by Src/Yes or c-Abl kinases, which resulted in enhanced interaction with RUNX or p73 transcription factors. The functional significance of YAP tyrosine phosphorylation needs further examination in vivo.

### Table 1. Major Hippo Pathway Components in _Drosophila_ and Mammals

<table>
<thead>
<tr>
<th><em>Drosophila</em> Full Name</th>
<th>Symbol</th>
<th><em>Mammals</em> Full Name</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scalloped</td>
<td>Sd</td>
<td>TEA domain family member</td>
<td>TEAD</td>
</tr>
<tr>
<td>Yorkie</td>
<td>Yki</td>
<td>Yes-associated protein</td>
<td>YAP</td>
</tr>
<tr>
<td>Tondu-domain–containing growth inhibitor</td>
<td>Tgi</td>
<td>Transcription cofactor with PDZ-binding motif</td>
<td>VGLL4</td>
</tr>
<tr>
<td>Warts</td>
<td>Wts</td>
<td>Large tumor suppressor kinase 1/2</td>
<td>LATS1/2</td>
</tr>
<tr>
<td>Mob as tumor suppressor</td>
<td>Mats</td>
<td>Mps one binder kinase activator-like 1A/1B</td>
<td>MOB</td>
</tr>
<tr>
<td>Hippo</td>
<td>Hpo</td>
<td>serine/threonine kinase 4/3</td>
<td>MST1/2</td>
</tr>
<tr>
<td>Salvador</td>
<td>Sav</td>
<td>Salvador</td>
<td>SAV1</td>
</tr>
<tr>
<td>Ras association family member</td>
<td>Rassf</td>
<td>Ras association domain–containing protein 1–6</td>
<td>RASSF1-6</td>
</tr>
<tr>
<td>Merlin</td>
<td>Mer</td>
<td>Neurofibrin 2</td>
<td>NF2</td>
</tr>
<tr>
<td>Expanded</td>
<td>Ex</td>
<td>FERM domain-containing protein 6</td>
<td>FRMD6</td>
</tr>
<tr>
<td>Kibra</td>
<td>Kbra</td>
<td>Kibra</td>
<td>KBR</td>
</tr>
<tr>
<td>Fat</td>
<td>Fat</td>
<td>Angiomotin</td>
<td>AMOT</td>
</tr>
<tr>
<td>Protocadherin Fat1-4</td>
<td></td>
<td></td>
<td>FAT1-4</td>
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</table>
Both YAP and TAZ lack DNA-binding domains and therefore have to cooperate with transcription factors to bind proper DNA elements and to stimulate gene transcription. Most of the known YAP target transcription factors could be broadly divided into 2 groups: the proline-proline-X-tyrosine (PPXY) containing transcription factors and the TEA family domain members (TEADs). The first group contains several proteins such as p73, runt-related transcription factor (RUNX), receptor tyrosine-protein kinase erbB-4 (ERBB4) cytoplasmic domain, and Mothers against decapentaplegic homolog 1 (Drosophila). These transcription factors interact with the WW domains of YAP or TAZ through their PPXY motifs. The TEAD family transcription factors interact with YAP/TAZ via the N-terminal TEAD binding domains in YAP/TAZ. Pairing of YAP and TAZ with different transcription factors could exert different functional outcomes. For example, TAZ may promote osteogenesis by stimulating RUNX target gene expression and YAP may promote pluripotency by mediating p73 target gene expression in embryonic stem cells through interaction with SMAD1. Moreover, YAP may paradoxically promote apoptosis by interacting with and stimulating p73 target genes. These findings from cell culture studies suggest functional roles of the YAP WW domains. Further examination of YAP/TAZ WW domain knockin mouse models, especially in comparison with Yap/Taz knockout mice, would help to clarify the importance of the WW domains.

Both genetic and biochemical studies have convincingly established a critical role of the TEAD family transcription factors in mediating biological functions of YAP in tissue growth. By large, YAP displays much stronger interaction with TEAD family members than other transcription factors described above. This point is confirmed by several recent systematic proteomic interaction studies of the Hippo pathway. Crystal structures of the YAP–TEAD complex have been solved, which revealed several critical interaction surfaces. Of particular interest is the YAP S94–TEAD1 Y406 hydrogen bond. Mutation of TEAD1 Y406 to histidine is found to cause a rare autosomal dominant human genetic disease Sveinsson chondriotypal atrophy. Remarkably, either YAP S94A or TEAD1 Y406H mutation almost completely disrupts YAP–TEAD interaction. This observation highlights the physiological role of YAP–TEAD interaction in tissue homeostasis. In tissue culture, mutation of YAP S94 abolishes the majority of YAP-induced gene expression and cell proliferation, oncogenic transformation, and epithelial–mesenchymal transition. More importantly, knockin of this mutation in mice skin phenocopies YAP knockout, further validating an essential role of TEADs in the biological functions of YAP. Recently, it was demonstrated that VGLL4, another cofactor of TEADs, represses YAP function by competing with YAP for TEAD binding. The discovery of this mechanism adds another layer of complexity to the control of YAP activity. The functional interaction between Yki (the Drosophila YAP homolog) and Scalloped (the Drosophila TEAD homolog) has also been demonstrated by genetic studies in Drosophila. Moreover, YAP regulates transcription likely through interaction with additional transcription regulators. For example, in both Drosophila and mammals, Yki/TAZ were shown to interact with the switch/sucrose nonfermentable nucleosome remodeling complex complex, which modulates chromatin structure and plays an important role in Hippo pathway target gene expression.

Regulation of the Hippo Pathway by Polarity and Junctional Proteins

Signals upstream of the Hippo pathway core kinase cascade have been intensively investigated. It has been shown that neurofibromin 2 (NF2, Merlin), a membrane-localized cytoskeleton-related ERM (Ezrin, Radixin, Moesin) family protein and a human tumor suppressor, is upstream of the Hippo pathway in both Drosophila and mammalian cells. NF2 may function together with FERM domain-containing protein 1 (the Drosophila homolog) and Scrib (the Drosophila Scrib homolog) has also been demonstrated by genetic studies in Drosophila. Moreover, YAP regulates transcription likely through interaction with additional transcription regulators. For example, in both Drosophila and mammals, Yki/TAZ were shown to interact with the switch/sucrose nonfermentable nucleosome remodeling complex complex, which modulates chromatin structure and plays an important role in Hippo pathway target gene expression.
have also been implicated in regulation of the Hippo pathway. The angiomotin complex at tight junction inhibits YAP/TAZ by both direct binding and indirectly activating LATS1/2.94–97 However, it has also been reported that the p130 isoform of angiomotin activates YAP in the context of liver tumorigenesis.98 About 70% of angiomotin knockout mice die around E7.5 and the rest survive normally without cardiac phenotype.99 Northern blot indicates low expression of angiomotin in adult mouse heart. However, the other angiomotin family members, angiomotin like 1 and 2, which could also bind to YAP, express at relatively high levels.100 The cardiac function of angiomotin like 1 and 2 as part of the Hippo pathway would worth further study. α-Catenin (α-CAT) at adherens junction may inhibit YAP by binding to 14-3-3 bound phosphorylated YAP.63,101 The basolateral domain protein scribble may promote the formation of MST–LATS–TAZ complex and thus facilitates TAZ inhibition.102,103 In addition, the basolateral localization of scribble and its function in promoting Hippo pathway activity are under positive regulation by the polarity regulator LKB1.104 In Drosophila, the Hippo pathway is also regulated by signal from a protocadherin, Fat, which plays an important role in planar cell polarity.105–108 Fat4 is the mammalian ortholog of Drosophila Fat. However, whole body or liver-specific ablation of Fat4 does not support a role in regulation of the mammalian Hippo pathway.111,112 Regulation of the Hippo pathway by polarity and junctional proteins has been reviewed in detail elsewhere.113

Interestingly, the Hippo pathway is also regulated by specific junctional structures in cardiomyocytes.114 Intercalated discs are cell–cell adhesion structures joining cardiomyocytes end-to-end and responsible for maintaining mechanical integrity of the heart. Mutations of genes encoding intercalated disc proteins such as PKP2, JUP, and DSG2 cause arrhythmogenic cardiomyopathy (AC), which is characterized by replacement of cardiomyocytes with fibro-adipocytes predominantly in the right ventricle.115 Notably, NF2 also localizes to intercalated discs in cardiomyocytes and is phosphorylated. In human AC hearts, phosphorylated NF2 is lost from intercalated discs and YAP phosphorylation seems to be increased.114 In mouse models of AC by either transgenic expression of Jup or conditional heterozygous knockout of Dsp, NF2 protein level was increased whereas its phosphorylation was dramatically decreased.114 In these mutant cardiomyocytes, strong YAP phosphorylation was also observed. Another study showed repression of CTGF, a direct YAP target gene, in hearts of the same mouse models.116 Thus, pathological abnormalities of cardiac cell junctions in AC may result in inhibition of YAP. YAP/TAZ are known to promote osteogenesis and inhibit adipogenesis in other cell types.57 Consistently, inactivation of the Hippo pathway in Pkp2 knockout cardiomyocytes rescued the characteristic adipogenesis in AC.114 Therefore, deregulation of YAP and the Hippo pathway because of junctional abnormalities may result in YAP inhibition and thus pathogenesis of AC.

**Regulation of the Hippo Pathway by Mechanical Stress**

Mechanical stress is increasingly recognized as a critical regulator of cell behavior and is directly relevant to heart physiology. Remarkably, the Hippo pathway effectors, YAP and TAZ, have been shown to be critical mediators of mechanical stress in several contexts.117–122 For example, mesenchymal stem cells have the ability to differentiate into various lineages depending on matrix stiffness.123 YAP/TAZ, subcellular localization is sensitive to matrix stiffness.117 On stiff matrix, YAP/TAZ localize to cell nuclei and promote osteogenesis.117 On soft matrix, YAP/TAZ translocate to the cytoplasm and mesenchymal stem cells adopt adipogenic fate.117 Interestingly, this mechanosensing mechanism may also exist in cardiac cells. For example, it was noticed that nuclear YAP, which is absent in normal adult cardiomyocytes, appears in infarcted cardiac tissue with stiffer extracellular matrix.124 The regulation and function of YAP in cardiac infarction and regeneration are further discussed below.

Consistent with a central role of the actomyosin cytoskeleton in generation and transduction of mechanical force in cells, response of YAP/TAZ to mechanical stress depends on the actin cytoskeleton.117–120,125 Pharmacological disruption of F-actin or inhibition of Rho GTPase, which plays a critical role in actin polymerization, leads to YAP inactivation. Robust regulation of the Drosophila Hippo pathway effector Yki by F-actin has also been demonstrated in vivo.120,126 The involvement of the Hippo pathway kinase cascade in YAP/TAZ regulation by mechanical stress is under debate. On one hand, mechanical stress clearly regulates LATS1/2 activity and YAP/TAZ phosphorylation,118,119 and on the other hand, knockdown of LATS1/2 is insufficient to rescue YAP/TAZ activity in cells cultured on soft matrix.117,122 It is possible that both LATS1/2-dependent and LATS1/2-independent mechanisms are involved, which need to be further elucidated. To date, the mechanosensor that initiates signal transduction to the Hippo pathway has not been pinpointed. Cell–cell junctional proteins and cell–extracellular matrix adhesion molecules, such as integrins, might be involved. The junctional protein angiomotin complex and α-CAT complex directly localize YAP/TAZ to tight junctions and adherens junctions, which are both associated with actin fibers. Although YAP localization in isolated cells are affected by mechanical stress, which excludes an essential role of cell–cell junction remodeling in mediating mechanical signals to YAP/TAZ, it remains possible that differential subcellular distribution of junctional proteins but not cell junction remodeling per se under various mechanical conditions modulates YAP/TAZ localization and activity. As a biological pump, the heart endures mechanical forces all the time. Pathological mechanical overload could lead to heart hypertrophy, injury, and heart failure. It is tantalizing to speculate that the Hippo pathway in the heart is regulated by mechanical force and modulates heart physiological function and pathological injury and regeneration.

**Regulation of the Hippo Pathway by G-Protein–Coupled Receptor Signaling**

Classical signaling pathways are initiated by extracellular ligands and respective cell surface receptors. Despite the discovery of mechanical stress and physical environment in regulation of the Hippo pathway, a traditional ligand-receptor pair upstream of the Hippo pathway was missing until recently. The first example of such upstream signaling has been...
demonstrated to originate from activation of G-protein–coupled receptors (GPCRs). The serum borne lysophosphatidic acid and sphingosine-1-phosphate are potent mitogens and strongly inhibit the Hippo pathway kinases LATS1/2, leading to activation of YAP/TAZ. These phospholipids act through their respective GPCRs and downstream heterotrimeric G proteins. Activation of Rho and F-actin remodeling are involved in YAP/TAZ activation in response to lysophosphatidic acid and sphingosine-1-phosphate. Other GPCR ligands such as thrombin also stimulate YAP/TAZ activity. Strikingly, epinephrine and glucagon act through their respective GPCRs leading to YAP/TAZ inhibition.

Subsequently, it was realized that GPCRs and heterotrimeric G proteins have broad roles in regulation of the Hippo pathway. YAP/TAZ can be either activated or inhibited depending on the coupled G subunits. For example, activation of Gs/12/13 or Gai/11 induces YAP/TAZ activity, whereas activation of Goai represses YAP/TAZ activity. GPCRs are the largest class of cell surface receptors encoded by the human genome and also the largest class of drug targets. It is estimated that there are >200 GPCRs expressed in the heart. For example, adrenergic receptors are GPCRs targeted by a large number of prescription drugs for cardiovascular diseases. Stimulation of β-adrenergic receptors (β1- and β2-adrenergic receptors) activates Gs proteins and increases intracellular Ca2+ concentration in turn, which ultimately results in cardiac muscle contraction. However, chronic cardiac β1-adrenergic receptor activation is detrimental and proapoptotic in the heart. Mice overexpressing β1-adrenergic receptors developed dilated cardiomyopathy (DCM). Consistently, mice overexpressing Goai also developed DCM associated with myocyte apoptosis. These phenotypes could potentially be explained by YAP inhibition downstream of activation of Goai-coupled GPCRs. However, whether the Hippo pathway and YAP/TAZ are indeed involved in the deleterious cardiac effects of chronic β-adrenergic receptors activation waits to be determined. Modulation of the Hippo pathway as a common outcome of various drugs and conditions targeting cardiac GPCRs is an important topic to be studied.

Hippo Pathway in Regulation of Heart Development

Organ size control is one of the most long-standing mysteries in biology. The most striking phenotype of Hippo pathway dysfunction in Drosophila is the alteration of organ size. In mouse, liver-specific transgenic expression of YAP or knock-out of Mst1/2 leads to enlargement of the liver to as much as one-fourth of the mouse body weight. Remarkably, the size of the liver shrinks back to normal on cessation of YAP expression. Thus, the Hippo pathway plays an evolutionarily conserved role in organ size control. The size of the mammalian heart is precisely controlled throughout development. However, little is known about the intrinsic regulation of heart size. Whether the Hippo pathway also controls heart size is therefore an intriguing question, which has been nicely answered by studying a large collection of genetic mouse models (summarized in Table 2).

Conditional knockout (cKO) of Suv3 by a knockin Nkx2.5 Cre, which drives deletion at E7.5 in the cardiac crescent, leads to substantial cardiomegaly although general organization of the heart is preserved. The mutant mice die postnatally. A similar phenotype is observed in embryos of Mst1/2 and Lats2 cKO mutants. Despite the dramatic change of myocardium thickness and heart size, cardiomyocyte size is unaffected. Instead, cardiomyocyte proliferation is significantly increased. Noteworthy, defects caused by Lats2 cKO are not compensated by Lats1. Differential expression of Lats1 and Lats2, which has not been carefully compared in the heart, could be a reason. Alternatively, despite the presence of highly similar kinase domains, the differential N-terminal sequences of LATS1 and LATS2 could mediate specific regulation or substrate binding. In agreement with increased heart size caused by knockout of Hippo pathway kinase cascade components, conditional ablation of Yap early in development by the same Nkx2.5 Cre or cardiomyocyte-specific Tmnt2 Cre leads to severe myocardium hypoplasia and embryonic lethality. In Yap cKO mice, although hearts are smaller, ectopic apoptosis is not seen in unstressed condition. Nevertheless, cardiomyocyte proliferation is severely reduced.

Wnt signaling pathway also plays critical roles in cardiogenesis. There have been many studies suggesting cross-talks between Wnt and Hippo signaling in various contexts. Noteworthy, cardiac phenotypes of genetic mouse models of the 2 pathways exhibit interesting similarities and differences. Wnt pathway inactivation during heart development had been modeled by conditional deletion of the Wnt effector protein β-CAT at different stages of cardiogenesis using various Cre lines. Conditional inactivation of β-catenin has been done using a transgenic Nkx2.5 Cre line, which is different from the aforementioned knockin Nkx2.5 Cre in that its expression begins from E8 and is throughout ventricular myocardium from E8.5. Developing hearts of these β-catenin cKO mice do not show ectopic apoptosis, but have reduced cell proliferation, significant reduction of ventricular size, thinner compact layer in the ventricular wall, and the embryos decease by E12.5. These phenotypes are similar to that caused by cKO of Yap using the transgenic Nkx2.5 Cre or Tmnt2 Cre although the time point of embryonic death varies by a few days. One interesting finding is that β-catenin inactivation by transgenic Nkx2.5 Cre has a more profound effect in the right ventricle. Developmentally, the 2 ventricles of mouse hearts are derived from distinct populations of progenitor cells. Cells of the first heart field contribute to the left ventricle and progenitors in the second heart field (SHF) form the rightward looping of the cardiac tube, therefore contributing to the right ventricle and inflow and outflow tracts. The differential effects on left and right ventricles suggest that Wnt signaling has specific functions in the SHF. Remarkably, inactivation of β-CAT at an earlier stage in all heart progenitor cells using Mesp1 Cre or more specifically in SHF progenitors by Islet1 Cre or Mef2e-ANF Cre leads to dramatic defects of SHF-derived right ventricle and outflow tract. However, inactivation of YAP or the Hippo pathway components by the knockin Nkx2.5 Cre, which also expresses in both first heart field and SHF, seems to affect both ventricles equally, suggesting that different from Wnt, the Hippo pathway does not specifically function in the SHF. Nevertheless, a more precise comparison of
the Hippo and Wnt function in the SHF progenitors would require examination of phenotypes after deletion of the Hippo pathway genes using SHF-specific Islet1 Cre line or general cardiac progenitor–specific Mesp1 Cre line. Interestingly, in cultured cardiac progenitor cells, YAP/TAZ are expressed and their subcellular localization shifts from cytoplasm to nucleus when matrix is remodeled from soft to stiff. However, in this case, the functional consequence is unclear, and as we discussed above, the roles of YAP/TAZ in cardiac progenitors in vivo would require further evidence. Nevertheless, YAP/TAZ...

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mouse Models</th>
<th>Promoter</th>
<th>Phenotypes</th>
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<tbody>
<tr>
<td>Yap</td>
<td>cKO</td>
<td>Nkx2.5-Cre</td>
<td>EL by E10.5, decreased proliferation, thin myocardium17</td>
</tr>
<tr>
<td></td>
<td>cKO</td>
<td>Tnnt2-Cre</td>
<td>EL by E16.5, hypoplastic ventricles, reduced proliferation, no elevated apoptosis, normal hypertrophy in basal and pathological conditions15</td>
</tr>
<tr>
<td></td>
<td>cKO</td>
<td>α-MHC-Cre</td>
<td>Die by 11 wk, dilated cardiomyopathy, increased apoptosis and fibrosis; worse injury, less proliferation and hypertrophy after chronic MI in cHET142; defective neonatal cardiac regeneration16</td>
</tr>
<tr>
<td></td>
<td>cKO</td>
<td>SM22α-Cre</td>
<td>Perinatal lethality, hypoplastic myocardium, VSD141</td>
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<td></td>
<td>cTG mYap1-S112A</td>
<td>β-MHC</td>
<td>Embryonic hearts have enhanced proliferation, thickened myocardium, expanded trabecular layer; adult heart size normal because of reduced cell size13</td>
</tr>
<tr>
<td></td>
<td>cTG mYap1-S112A</td>
<td>α-MHC</td>
<td>Increased proliferation, myocardium thickness, heart size, and cardiac regeneration16</td>
</tr>
<tr>
<td></td>
<td>Inducible</td>
<td>Tnnt2-Cre</td>
<td>Induction at E8.5 leads to EL by E15.5 with increased proliferation, thickened myocardium, cardiomegaly; induction at P5 increases heart weight and proliferation but not hypertrophy15</td>
</tr>
<tr>
<td></td>
<td>cKI Yap1f6070/84</td>
<td>Tnnt2-Cre</td>
<td>Myocardium hypoplasia comparable with Yap1 cKO15</td>
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<td>Taz</td>
<td>cKO</td>
<td>α-MHC-Cre</td>
<td>Normal heart, but when combined with Yap1 cKO enhances phenotypes including reduced proliferation, increased apoptosis, dilated cardiomyopathy and heart failure16</td>
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<tr>
<td>Tead1</td>
<td>KO</td>
<td>MCK</td>
<td>Myocyte misalignment, wall-thickening, fibrosis, reduced heart output, heart failure within 4 days by pressure overload145</td>
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<tr>
<td>Lats2</td>
<td>KO</td>
<td>…</td>
<td>EL by E12.5, at E10.5 ventricular hypoplasia in 36% of embryos146</td>
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<td></td>
<td>cKO</td>
<td>Nkx2.5-Cre</td>
<td>Myocardial expansion12</td>
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<tr>
<td></td>
<td>cTG</td>
<td>α-MHC</td>
<td>Reduced cardiomyocyte size and ventricle size, basal apoptosis not affected; enhancement of apoptosis in response to pressure overload147</td>
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<td></td>
<td>cTG-DN Lats2-K697A</td>
<td>α-MHC</td>
<td>Ventricular hypertrophy, less cardiomyocyte apoptosis induced by TAC147</td>
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<td>Lats1/2</td>
<td>Inducible cKO</td>
<td>Myh6-CreERT2</td>
<td>Increased renewal of adult cardiomyocytes, better regeneration after apex resection15</td>
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<tr>
<td>Sav1</td>
<td>Inducible cKO</td>
<td>Myh6-CreERT2</td>
<td>Increased renewal of adult cardiomyocytes; increased proliferation and better morphological and functional regeneration after apex resection or MI13</td>
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<td></td>
<td>cKO</td>
<td>Nkx2.5-Cre</td>
<td>Increased proliferation, thickened myocardium, cardiomegaly14</td>
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<tr>
<td></td>
<td>cTG</td>
<td>α-MHC</td>
<td>Premature death, increased cardiomyocyte apoptosis, fibrosis, no hypertrophy, dilated cardiomyopathy144</td>
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<td>cTG-DN Mst1-K59R</td>
<td>α-MHC</td>
<td>Reduced apoptosis after I/R146; reduced apoptosis, fibrosis, cardiac dilation, and dysfunction, but not hypertrophy after MI149</td>
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<tr>
<td>Mst1</td>
<td>cKO</td>
<td>Nkx2.5-Cre</td>
<td>Myocardial expansion12</td>
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<tr>
<td></td>
<td>Inducible KO</td>
<td>CAGG-CreR</td>
<td>Heart enlargement (partial penetrance)140</td>
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<td>Rassf1A</td>
<td>KO</td>
<td>…</td>
<td>No cardiac defects at basal condition; reduced apoptosis, enhanced hypertrophy, fibrosis, and LV chamber dilatation in response to TAC148,151</td>
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<tr>
<td></td>
<td>cKO</td>
<td>α-MHC-Cre</td>
<td>No cardiac defects at basal condition; reduced apoptosis, hypertrophy, and fibrosis after TAC151</td>
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<tr>
<td></td>
<td>cTG</td>
<td>α-MHC</td>
<td>No gross difference in cardiac morphology and function; elevated Mst1 phosphorylation and cardiomyocyte apoptosis; increased apoptosis and fibrosis after TAC151</td>
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<tr>
<td></td>
<td>cTG-DN Rassf1A-L308P</td>
<td>α-MHC</td>
<td>Abrogated Mst1 activation, reduced fibrosis and apoptosis in response to TAC141</td>
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</table>

α-MHC indicates α-myosin heavy chain; cKI, conditional knockin; cKO, conditional knockout; cTG, tissue-specific transgenic expression; EL, embryonic lethal; KO, knockout; MI, myocardial infarction; P, postnatal day; TAC, transverse aortic constriction; and VSD, ventricular septal defect.
as potential mediators of mechanical stress to cardiac progenitors is still an intriguing possibility.

The function of the Hippo pathway in regulation of cardiomyocyte proliferation is further supported by the observed dramatic myocardial overgrowth and cardiomegaly in embryos of active Yap conditional transgenic mice.\textsuperscript{16}\textsuperscript{,}17 When inducible Yap expression is driven by Tmtn2 Cre and induced from E8.5, the trabecular myocardium of fetal hearts seems to be especially affected such that the ventricles are almost obliterated and the fetuses demise by E15.5.\textsuperscript{15} Expression of trabecular myocardium marker Nppa (natriuretic peptide A) is markedly downregulated in Yap transgenic myocardium, suggesting that elevated cardiomyocyte proliferation is associated with impaired differentiation.\textsuperscript{15} In other tissues such as the skin, Sav1 knockout has also been shown to delay cell cycle exit and impair differentiation but does not affect the speed of cell proliferation.\textsuperscript{16}\textsuperscript{0} Thus, it is possible that the Hippo pathway regulates heart size by preventing cardiomyocytes to enter mitosis, albeit the rate of proliferation may not differ once cells are licensed to proliferation.

In another report of Yap conditional transgenic under α-myosin heavy chain (α-MHC) promoter, which mainly expresses postnatally (although expression could be detected as early as E10.5), mice are viable and thickened myocardium is obvious in 4-month-old adult hearts.\textsuperscript{16} Interestingly, when Yap expression is driven by β-MHC promoter, which expresses from E9, adult heart size is normalized because of reduced cardiomyocyte size, although the cell numbers are elevated than normal controls.\textsuperscript{16}\textsuperscript{2} Such a normalization of organ size under conditions of cell overproliferation has been reported for other growth regulators but has not been reported for the Hippo pathway in other organs. The reason for the cross-talk between cell number and cell size to maintain a predetermined heart size under this specific YAP activation condition is unclear but fascinating.

The Hippo pathway also plays a role in early cardiac development. In zebrafish, an activity reporter indicates the expression and activity of YAP/TAZ in cardiac progenitor cells.\textsuperscript{16}\textsuperscript{1} During zebrafish development, cardiac precursors migrate to the midline to form the heart tube.\textsuperscript{16}\textsuperscript{2} Interestingly, when a dominant-negative form of Yap was expressed, the migration of these cells was impaired resulting in cardiac bifida, although formation of the heart was not completely blocked.\textsuperscript{16}\textsuperscript{3} YAP and TAZ are known to promote cell migration in other contexts such as cancer metastasis.\textsuperscript{34}\textsuperscript{,}163 Thus, this observation expands the physiological role of YAP/TAZ-induced cell migration into heart development. More interestingly, sphingosine-1-phosphate is known to be required for midline migration of cardiac progenitor cells in zebrafish.\textsuperscript{16}\textsuperscript{4}\textsuperscript{,}165 Therefore, the finding may provide a physiological niche for GPCR in regulation of the Hippo pathway in the context of heart development as sphingosine-1-phosphate may induce cardiac progenitor cell migration via activation of YAP/TAZ.

**Hippo Pathway in Cardiomyocyte Apoptosis and Myocardium Infarction**

MST1/2 kinases were known to be activated by apoptotic stress even before their role in the Hippo pathway was characterized.\textsuperscript{38} MST1/2 can be activated by caspase-dependent cleavage,\textsuperscript{39} dimerization, and autophosphorylation.\textsuperscript{86} The pro-apoptotic function of MST1/2 is also stimulated by upstream molecule RASSF1A.\textsuperscript{167}\textsuperscript{–}170 One of the most physiologically relevant apoptotic stimuli of MST1/2 is oxidative stress. It has been shown that MST1 mediates neuronal cell death in response to hydrogen peroxide.\textsuperscript{171}\textsuperscript{–}173 Ischemia/reperfusion (I/R) is one of the most common injuries to human hearts. I/R leads to death of cardiomyocytes largely because of the production of reactive oxygen species.\textsuperscript{174} Therefore, the potential regulation of MST1/2 by I/R-induced reactive oxygen species and the role of MST1/2 in myocardium injury have been extensively examined.\textsuperscript{148}\textsuperscript{–}151 The kinase activity of MST1/2 is indeed activated by I/R as indicated by in vitro kinase assay.\textsuperscript{148} Both caspase-dependent cleavage\textsuperscript{148}\textsuperscript{,}149\textsuperscript{,}151 and interaction with RASSF1A\textsuperscript{151} have been shown to be involved in MST1/2 activation by I/R in myocardium. Interestingly, transgenic expression of a dominant-negative forms of MST1 under α-MHC promoter blocks MST1/2 activation and dramatically reduces acute cardiomyocyte apoptosis and the size of myocardial infarction.\textsuperscript{149} In models of long-term myocardium infarction, introduction of dominant-negative MST1 also attenuated endogenous MST1/2 activation, myocardium apoptosis, fibrosis, and cardiac dysfunction.\textsuperscript{149} Consistent with the role of RASSF1A in MST1/2 activation, conditional transgenic expression of MST-binding–deficient form of RASSF1A or cKO of RassflA, both driven by cardiomyocyte-specific α-MHC promoter, largely blocked MST1/2 activation, cardiomyocyte apoptosis, and fibrosis under pressure overload.\textsuperscript{151} Nevertheless, whole body knockout of RassflA leads to worsened heart fibrosis although cardiomyocyte apoptosis was still reduced.\textsuperscript{150}\textsuperscript{,}151 Further in vitro experiments suggest an antiproliferative and anti-inflammatory role of RASSF1A-MST1/2 in cardiac fibroblasts.\textsuperscript{151} Thus, RASSF1A-MST1/2 also plays a role in nonmyocytes of the heart during heart injury. In line with the Hippo pathway in mediating cardiomyocyte apoptosis on pressure overload, LATS2 protein level was significantly elevated on pressure overload, and expression of a dominant-negative LATS2 under α-MHC promoter reduced cardiomyocyte apoptosis induced by transverse aortic constriction.\textsuperscript{147} Furthermore, α-MHC promoter–driven cardiomyocyte-specific conditional heterozygous knockout of Yap significantly increased cardiomyocyte apoptosis and fibrosis after chronic myocardium infarction.\textsuperscript{142} Thus, the MST1/2-LATS1/2 kinase cascade, which is activated by heart damage, may contribute to cardiomyocyte apoptosis and infarction by inhibiting YAP.

However, functions of MST1/2 and LATS1/2 in cardiomyocyte apoptosis are not identical because α-MHC promoter–driven transgenic expression of MST1, but not LATS2, in cardiomyocytes induces apoptosis in basal condition.\textsuperscript{147}\textsuperscript{,}148 This finding suggests that MST1/2 may promote cardiomyocyte apoptosis through additional mechanisms. Interestingly, MST1 was found to inhibit autophagy based on the observation that Mst1 facilitates accumulation of protein aggresomes and p62, which are normally removed by autophagy.\textsuperscript{173} By directly phosphorylating Beclin1, MST1 disrupts the formation of the proautophagic Atg14L–Beclin1–Vps34 complex and promotes Beclin1 interaction with apoptosis regulator Bcl-2 (Bcl-2) and Bcl-2-like protein 1 (Bcl-X (L)) as well as Beclin1 homodimerization.\textsuperscript{173} Autophagy may play a
protective role in cardiomyocytes by alleviating energy loss and recycling damaged organelles and protein aggregates. The role of autophagy inhibition on Hippo pathway activation in mediating cardiac damage still awaits further confirmation in vivo. Nevertheless, the activation of MST1, increase of Beclin1 phosphorylation, and signs of autophagy inhibition such as accumulation of p62 and decreased LC3 cleavage are indeed observed in failing hearts of human patients. The promotion of Beclin1 binding to Bcl-2/Bcl-xL by MST1 releases Bax from these proteins. Although this may provide a LATS1/2-YAP–independent mechanism for MST1/2 to induce apoptosis, the precise function of this mechanism in MST1/2-induced cardiomyocyte apoptosis also needs to be carefully examined in vivo.

**Hippo Pathway in Cardiac Hypertrophy and DCM**

Hypertrophic growth is a necessary phase of cardiac development and the major form of heart growth after birth. Cardiomyocyte hypertrophy also happens under pathological conditions such as I/R-induced infarction, hypertension, and valvular heart disease, in which elevated wall stress normally induces an adaptive heart hypertrophy to compensate for insufficient contractile mass. An increase in wall thickness by cardiac hypertrophy can reduce wall stress (by Laplace law), which in turn reduces both oxygen consumption and cell death.

A role of the Hippo pathway in inhibiting pathological hypertrophy was first observed in *Mst1* heart-specific transgenic mice. Consistent with the kinase activity–dependent role of MST1/2 in promoting apoptosis, transgenic expression of *Mst1* but not a kinase inactive mutant under α-MHC promoter clearly increases cardiomyocyte apoptosis and extensive fibrosis in adult hearts, leading to wall thinning and DCM. However, detailed examination indicates that cardiac dilation is because of lateral myocyte slippage under elevated wall stress rather than compensatory hypertrophy. Thus, although myocardium damage and stress to the heart were evident, a default hypertrophy program was not initiated, suggesting a role of the Hippo pathway in inhibiting this process. In other pathological conditions such as pressure overload, MST1 is activated in the myocardium, in correlation with apoptosis. Interestingly, α-MHC promoter–driven *Rassf1A* cKO blocks MST1/2 activation and attenuates the hypertrophic response likely because of inhibition of apoptosis and fibrosis and thus reduced heart damage. Thus, inhibition of the Hippo pathway may also inhibit cardiomyocyte hypertrophy because of an indirect effect in repressing apoptosis and heart injury. However, it should be noted that α-MHC promoter–driven expression of *DN-Mst1* or *DN-Rassf1A*, which also show inhibitory effect on MST1 phosphorylation, apoptosis, and fibrosis to a similar level as *Rassf1A* cKO, do not block cardiomyocyte hypertrophy. The reason for this discrepancy is unclear.

Different from *Mst1*, α-MHC promoter–driven *Lats2* transgenic hearts show reduced size and no apoptosis at baseline. LATS2 leads to increased cardiomyocyte size and biventricular hypertrophy at baseline. Thus, both MST1 and LATS2 seem to inhibit hypertrophy. However, it is unclear whether they work in a linear pathway fashion. Furthermore, the possibility of MST and LATS affecting hypertrophy by a secondary effect because of a more pleiotropic role of these proteins in myocardium proliferation and apoptosis has not been unequivocally excluded.

Interestingly, cKO of *Yap* leads to a phenotype similar to *Mst1* overexpression. Early deletion of *Yap* using knockin Nkx2.5 Cre leads to demise of the embryo, which prevents analysis of the effect of long-term loss of *Yap* in cardiac function. Ablation of *Yap* using α-MHC-Cre, which expresses as early as E10.5 and mainly postnatally, circumvented embryonic lethality. However, these mutants die by 20 weeks of age because of DCM and heart failure. Consistent with a low expression of TAZ in myocardium, deletion of *Taz* using the same Cre does not cause obvious abnormality of the heart. However, combination of *Yap* and *Taz* knockout dose dependently worsens the phenotype suggesting functional redundancy of the 2 genes. Examination of myocardium indicates reduced proliferation and increased cardiac apoptosis in neonatal α-MHC-Cre *Yap* cKO; *Taz* conditional heterozygous knockout mice and 8-week-old α-MHC-Cre *Yap* cKO mice. Noteworthy, *Yap* cKO by Nkx2.5 Cre does not induce apoptosis in embryonic hearts. Postnatal heart endures much more mechanical stress than fetal heart. Thus, the observed apoptosis in α-MHC-Cre–driven *Yap* cKO mice is possibly secondary to compromised cardiac function and elevated wall stress because of insufficient cardiomyocyte proliferation. In *Yap* cKO myocardium, cardiomyocyte hypertrophy is obvious as indicated by cross-sectional area of cells. However, the observed hypertrophy is likely secondary to heart injury. The role of *Yap* in cardiomyocyte hypertrophy has also been studied in myocardium with mosaic deletion of *Yap* by delivering of Tntt2-Cre–encoding adenovirus to *Yap* floxed neonatal mice. Results indicate that YAP does not affect cardiomyocyte hypertrophy in neonatal hearts or after ascending aortic constriction in adult hearts. In this experimental setting, *Yap* deletion happens only postnatally, which minimizes the secondary effect of *Yap* deletion on cardiomyocyte hypertrophy owing to insufficient proliferation and induced apoptosis. Furthermore, examination of *Yap* transgenic myocardium did not find obvious cardiomyocyte hypertrophy in vivo. In addition, during development, YAP is downregulated in hypertrophic phase of heart growth. These studies suggest that YAP plays a role in heart hypertrophy secondary to its role in regulation of cardiomyocyte proliferation and apoptosis but may not directly regulate cardiomyocyte hypertrophy. In adult hearts, α-MHC-Cre–driven condition deletion of only 1 allele of *Yap* moderately decreases cardiomyocyte hypertrophy after myocardial infarction. In cardiomyocytes cultured in vitro, expression of YAP increased cell size and knockdown of YAP attenuated phenylephrine-induced cardiomyocyte hypertrophy. Interestingly, it was recently reported that YAP expression is enhanced while YAP phosphorylation is dampened with reduced Mst1 expression in myocardium of patients with hypertrophic cardiomyopathy, suggesting a role of YAP in pathogenesis of human hypertrophic heart disease. Taken
together, functions of YAP and the Hippo pathway in cardiac hypertrophy might be more complex and context-dependent.

The PI3K–AKT–mTOR pathway is a critical regulator of cell size. The Hippo pathway may modulate mTOR and protein synthesis through YAP-dependent induction of miR-29 and inhibition of phosphatase and tensin homolog (PTEN), thus activation of AKT. Interestingly, AKT is also activated by YAP in myocardium, which may involve induced expression of Pik3cb. Knockdown of Pik3cb reduces ectopic cardiomyocyte proliferation in vivo and expression of Pik3cb ameliorates cardiomyopathy on YAP cKO. Therefore, the Hippo-mTOR cross-talk likely plays a role in regulation of cardiomyocyte hypertrophy in vivo. Damage-induced mechanical overload is a common cause of cardiac hypertrophy. Interestingly, the Hippo pathway is known to respond to mechanical stress. However, the precise nature and signaling mechanism of mechanical stress to impinge on the Hippo pathway in the context of cardiac hypertrophy and dilation would be an important question for future study.

Hippo Pathway in Heart Regeneration

Although some organs in the human body have substantial regeneration capacity, the renewal potential of the heart is limited. Nevertheless, recent evidence indicates that adult human and mouse heart is renewing slowly and such potential can be overwhelmed by sudden loss of cardiomyocytes in pathological conditions. Several different approaches have been attempted such as direct supplement of cardiac progenitor cells and reprogramming by cardiac genes or small molecules. Some of these manipulations improve regeneration but are generally not robust. Although both cardiac progenitor cells and cardiomyocytes renewal have been documented, lineage tracing suggests that cells contribute to ventricular regeneration are primarily cardiomyocytes. In fact in species such as zebrafish the potential of cardiomyocyte proliferation in pathological conditions. Several different approaches have been attempted such as direct supplement of cardiac progenitor cells and reprogramming by cardiac genes or small molecules. Some of these manipulations improve regeneration but are generally not robust. Although both cardiac progenitor cells and cardiomyocytes renewal have been documented, lineage tracing suggests that cells contribute to ventricular regeneration are primarily cardiomyocytes. In fact in species such as zebrafish the potential of cardiomyocyte proliferation in pathological conditions.

In newborn mice before postnatal day 7 (P7), cardiomyocytes could also proliferate to reach substantial cardiac regeneration. However, such ability is quickly lost after P7, leaving behind fibrosis and scar tissue after damage. The molecular mechanism that switches off the regeneration potential of cardiomyocytes is unclear but is likely associated with the switch of heart growth from cardiomyocyte proliferation to cellular hypertrophy. Therefore, attempts have been made to force cardiomyocyte proliferation by overexpression of various cell cycle regulators such as cyclin A2, CDK2, and cyclin D1. However, although DNA synthesis and karyokinesis could readily be observed, complete cytokinesis and proliferation remain inefficient in most cases. A better understanding of mechanisms of cardiac regeneration is thus in need.

The Hippo pathway is known to play important roles in regeneration of intestines after damage. Although cKO of Yap does not seem to affect general development and function of mouse intestine, the damage-induced regeneration program is largely impaired without Yap. Considering functions of the Hippo pathway in control of heart size and cardiomyocyte proliferation during development, it is possible that the Hippo pathway also exerts vital functions during repair and regeneration of the heart. Such possibility has been directly tested in conditions of heart injury. Resection of mouse cardiac apex after P7 normally results in scarring in contrast to regeneration if resection is done before P7. However, in 2 different Sav1 cKO models, 1 specifically in cardiomyocytes by Myh6 Cre/Ert2 induced from P7 and the other during development by knock-in Nkx2.5 Cre, myocardium resected at P8 regenerated with reduced scar size compared with control animals. Study of the function of the Hippo pathway in acute resection-induced heart regeneration avoids complications by the role of the Hippo pathway in damage-induced apoptosis, although this kind of damage is nonphysiological Hippo pathway.

In human hearts, cardiomyocyte loss is more commonly caused by myocardium infarction because of coronary artery disease, which could be mimicked by left anterior descending coronary artery occlusion. Similar to that in apex resection, heart injury induced by left anterior descending occlusion at P8 or P7 is also much better tolerated with reduced scar size and improved heart functional recovery in cardiomyocyte-specific Sav1 cKO (α-MHC-Cre) mice, respectively. To further examine the role of the Hippo pathway in regeneration of adult hearts, left anterior descending occlusion was done at 1 or 2 months of age in the same Yap transgenic or Sav1 cKO mice. In both cases, improved heart regeneration was indicated by reduced fibrotic scarring and improved recovery in heart functional parameters such as fractional shorting, ejection fraction, and stroke volume. Noteworthy, Yap expression or Sav1 cKO does not completely block heart injury (scarring), although in Sav1 cKO model, fractional shorting and ejection fraction recovered to a level similar to sham-operated animals. In contrast, cardiomyocyte-specific Yap cKO by α-MHC-Cre impairs neonatal heart regeneration induced by left anterior descending occlusion at P2 leaving behind extensive fibrotic infarct zone and gross deficiency of healthy myocardium.

Proliferating cardiomyocytes are observed in Hippo pathway–deficient hearts, which is likely the reason for improved cardiac regeneration. Lineage tracing of regenerated myocardium in resected Sav1 cKO mice indicates that the regenerated cTnt staining–positive cardiomyocytes are also positive for green fluorescent protein (GFP) resulted from recombination of the mTmG allele, indicating pre-existing cardiomyocyte lineage. Thus, regenerated myocardium is largely from proliferating cardiomyocytes, although some contribution from resident stem cells could not be completely ruled out. In fact, cardiomyocyte-specific inactivation of Sav1 could even induce complete mitosis in myocardium of mice 4 months of age. Conversely, conditional heterozygous knockout of Yap decreases proliferating cells in infarcted myocardium. These studies suggest that the Hippo pathway is active in suppressing mitosis in adult heart. In support of this notion, YAP protein is clearly detected in neonatal hearts and declines with age, while YAP phosphorylation increases with age. However, in infarcted adult heart, YAP expression reappeared at the border of the infarction zone, which could be because of increased stiffness of the infarcted area. The functional role of YAP re-expression in these areas has not been demonstrated. Nevertheless, it has been known for a while that injury of 1 area of the heart induces cell cycle re-entry of cardiomyocytes throughout the whole organ in zebrafish. Similar phenomenon has also been observed in Hippo-deficient
mouse hearts. Therefore, in zebrafish hearts or neonatal mouse hearts, cues upstream to the Hippo pathway may exist to propagate damaged signals to instruct cardiomyocyte proliferation distant from the site of injury. Whether the Hippo pathway is directly responsive to myocardium injury or simply limits cardiomyocyte proliferation needs to be further examined.

Transcriptional Regulation of Heart Size and Regeneration Downstream of YAP/TAZ

As transcription coactivators, the function of YAP/TAZ depends on their interacting transcription factors (Figure 2). Evidence to date supports that the TEAD family is the major transcription factor target of YAP/TAZ in vitro and in vivo. Functions of TEADs in YAP-regulated cardiomyocyte proliferation and heart development have also been demonstrated in vivo. Cardiomyocyte-specific knockin mutation of mouse Yap-S79A (equivalent to human YAP-S94A mutant), which abolishes its interaction with TEADs, leads to cardiomyocyte hypoplasia comparable with that caused by Yap cKO in fetal hearts. In addition, introduction of a peptide disrupting YAP–TEAD interaction significantly inhibits YAP-induced expression of cell cycle–related genes such as Aurkb, cdc20, Ccna2, and proliferation of cultured cardiomyocytes. Furthermore, whole body Tead1 knockout mice die around embryonic day 11.5 with abnormally thin ventricular wall and a dramatic reduction of myocardium trabeculation. These phenotypes closely resemble those observed in Yap cKO mice and strongly support that TEAD1 is critical for YAP to regulate cardiomyocyte proliferation and cardiac development. Noteworthy, in human, all Sveinsson chorioretinal atrophy patients are heterozygous for TEAD1 mutation. Heart defects of these patients, however, have not been described, which also suggests that different from the optic disc, 1 allele of Tead1 is sufficient to sustain myocardium development and function.

Wnt signaling is one of the most recognized pathways in regulation of development. β-CAT is a transcription coactivator and major effector of the Wnt pathway. Wnt stimulation leads to disassembly of the destruction complex and stabilization and nuclear enrichment of β-CAT. In Sav1 cKO myocardium, nuclear localization of β-CAT and expression of β-CAT target genes were found to be elevated. Furthermore, dephosphorylated and active, but not phosphorylated and inactive, YAP interacts with β-CAT. It has also been reported that in epithelial cells, cytoplasmic inactive YAP directly binds to and sequesters β-CAT in the cytoplasm. Thus, activity of the Hippo pathway may dictate a stimulatory or inhibitory role of YAP on β-CAT activity, although the applicability of such mechanism to myocardium is unknown. In cardiomyocytes, sequential chromatin immunoprecipitation (ChIP) showed that YAP and β-CAT co-occupy the promoters of target genes such as Sox2 and Snai2. More importantly, heterozygous knockout of β-catenin in Sav1 cKO mice normalizes ventricular cardiomyocyte proliferation rate, and myocardial thickness, supporting a functional role of β-CAT in cardiac overgrowth induced by Hippo pathway inactivation. Several mechanisms of β-CAT activation by the Hippo pathway have been reported including those affecting β-CAT stability, subcellular localization, and transcriptional activity. In cardiomyocytes, 1 possible mechanism for YAP-induced activation of β-CAT is the elevation of insulin-like growth factor 1R expression and subsequent activation of AKT and inhibition of glycosynthase kinase-3 beta (GSK3β), which could then cause β-CAT accumulation and nuclear enrichment. The mechanism for insulin-like growth factor 1R induction by Hippo pathway inhibition remains unknown. It should be noted that the Wnt/β-CAT and Hippo signaling show substantial functional differences in heart development in regard to progenitors of the SHF. However, activity of β-CAT as Wnt effector may be limited by the Hippo pathway in cardiomyocytes, which may be reactivated under certain conditions such as heart injury.

YAP and TAZ are also reported to associate with TBX5, a T-box transcription factor mutated in Holt–Oram syndrome, which is characterized by a variety of cardiac and other abnormalities. YAP/TAZ-TBX5 stimulate expression of cardiac-specific genes such as Nppa. TBX5 directly binds to Nppa promoter and co-expression of TAZ or YAP with TBX5 potently stimulates luciferase expression driven by Nppa promoter, suggesting that Nppa is a direct target gene of YAP/TAZ-TBX5. Interestingly, some of the Holt–Oram syndrome patient–associated TBX5 mutants lost interaction with YAP, suggesting the involvement of this interaction in pathogenesis of subtypes of Holt–Oram syndrome. The functional significance of this interaction is yet to be validated by genetic models. YAP–TBX5 interaction has also been implicated in cancer. A TBX5–YAP–β-CAT–YES complex is shown to bind to promoters of antiapoptotic genes such as Birc5 and Bcl2L1, thus regulates survival and transformation of Wnt-dependent cancer cells. It is currently unknown whether the function of YAP/TAZ-TBX5 in cardiomyocytes is also Wnt dependent. However, this connection could provide another possibility for cross-talk between Hippo and Wnt pathways in regulation of cardiac physiology.

Figure 2. Transcription effectors of the Hippo pathway in regulation of cardiac physiology. Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) transcription factor partners in cardiomyocytes and their downstream target genes are shown. The Hippo pathway likely regulates cardiac physiology through a coordinated transcriptional program. DVL indicates Dishevelled; p300, E1A binding protein p300; MEIS1, Meis homeobox 1; pCAF, p300/CBP-associated factor, KAT2B; and TCF/LEF, transcription factor/lymphoid enhancer-binding factor.
FoxO1 is a Forkhead transcription factor known to regulate expression of antioxidant genes such as catalase and Sod2, thus protects cardiomyocytes from oxidative stress.\(^{212-214}\) YAP is reported to directly bind to FoxO1 and stimulate antioxidant gene expression.\(^{215}\) In condition of I/R in the heart, activation of MST1/2 leads to inhibition of YAP and thus attenuates antioxidant gene expression.\(^{216}\) Indeed, inhibition of the Hippo pathway by dominant-negative or knockdown of LATS2 rescues catalase and Sod2 expression, restores antioxidant capacity, and reduces cardiomyocyte apoptosis and myocardium infarction under I/R setting in a FoxO1-dependent manner.\(^{215}\)

However, FoxO1 is also well known to induce apoptosis.\(^{216}\) How would the conflicting roles of YAP-FoxO1 in generating antioxidant potential and promoting apoptosis be reconciled in the context of cardiac injury by I/R would need further study. In addition, YAP is known to activate AKT in cardiomyocytes,\(^{17}\) which is a major kinase phosphorylating and inactivating FoxOs. Whether and how a balance between YAP-induced FoxO1 activation and YAP-AKT-induced FoxO1 inhibition is reached to regulate cardiomyocyte survival under stressed condition is another issue requiring further investigation.

Other YAP/TAZ target transcription factors may also mediate the effect of the Hippo pathway in heart development and regeneration. For example, YAP/TAZ are known to interact with SMADs to regulate stemness downstream of transforming growth factor-β/BMP pathways.\(^{60,217}\) The interaction between YAP and SMAD1 after BMP stimulation is particularly interesting because BMP signaling is known to be involved in cardiac development and antiapoptotic in neonatal hearts.\(^{218}\) However, the potential role of Hippo-BMP signaling cross-talk in cardiac development is merely hypothetical at this point. In addition, Meis1, a TALE family homeodomain protein, was recently found to be critical in regulation of the cardiac growth switch from proliferation to hypertrophy.\(^{219}\) Leukemia deletion in mouse cardiomyocytes extends the postnatal proliferative window of cardiomyocytes, and overexpression of Meis1 in cardiomyocytes decreases neonatal cardiomyocyte proliferation and regeneration.\(^{219}\) Interestingly, Homothorax, the *Drosophila* homolog of Meis1, interacts with Yki to induce expression of microRNA *bantam* and to regulate proliferation and apoptosis in specific compartment of *Drosophila* eye imaginal disc.\(^{220}\)

Whether YAP-Meis1 could interact in cardiomyocytes to coordinately regulate cell proliferation and hypertrophy has not been examined. One model is that Meis1 functions as a transcriptional repressor with other cofactors to inhibit cardiomyocyte proliferation, which is blocked by competitive binding of YAP to Meis1.

Evidence to date supports that multiple transcriptional complexes downstream of the Hippo pathway are involved in regulation of cardiac development and regeneration (Figure 2). More YAP/TAZ transcription factor partners and functional downstream target genes are likely to emerge in the near future.

**Perspectives and Concluding Remarks**

Proper heart development is vital to life and heart repair/regeneration postinjury is a topic of paramount importance in biomedical research. Current research has provided abundant evidence for the important functions of the Hippo pathway in heart development, injury, and regeneration. However, our understanding of basic mechanisms of the Hippo pathway is still incomplete, such as the signal transduction mechanisms of GPCRs and mechanical stress to regulate activity of LATS1/2 and YAP/TAZ; additional signals in physiological and pathological conditions in regulation of Hippo pathway activity; and contribution and coordination of downstream effectors in mediating biological outcome of the Hippo pathway. Although the Hippo pathway has been demonstrated to regulate cardiomyocyte proliferation during development, the cardiac-specific upstream signal remains an enigma. The proliferation to hypertrophy switch of cardiomyocytes soon after birth is accompanied by an acute increase of oxygen pressure and mechanical load, which can modulate the Hippo pathway activity. Whether regulation of the Hippo pathway by these signals influences the switch of cardiomyocyte fate would be an important question for future study. During heart regeneration, cardiomyocyte proliferation could happen distant from the damage site, suggesting the involvement of diffusible signal(s). It would be interesting to further investigate whether such a signal would be a Hippo inhibitor such as a GPCR ligand or secreted growth factors encoded by YAP target genes. The Hippo pathway and YAP are known to regulate epithelial–mesenchymal transition in the context of development and cancer metastasis.\(^{34,163}\) In the heart, epithelial–mesenchymal transition has a critical function in the transdifferentiation and formation of heart valve from endothelial cells.\(^{212,222}\)

Whether the Hippo pathway and YAP are involved in valve development and defects are topics worth further investigation. MicroRNAs (miRNAs) play important roles in heart development and homeostasis.\(^{223-225}\) This is indicated by heart-specific cKO of *Dicer*, the miRNA-processing enzyme, which leads to lethality because of heart failure.\(^{226}\) Disruption of miRNA production postnatally also leads to cardiac remodeling and dysfunction.\(^{227,228}\) YAP is known to induce expression of specific miRNAs and broadly repress miRNA production by sequestering p72, a regulatory component of the miRNA-processing machinery.\(^{140,222}\) The possibility of altered miRNA expression, either globally or individually, in mediating YAP regulation of cardiac physiology and disease is of interest and potential therapeutic value.

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