© 2014. Published by The Company of Biologists Ltd | Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

REVIEW

The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease

Xaralabos Varelas*

ABSTRACT

Studies over the past 20 years have defined the Hippo signaling pathway as a major regulator of tissue growth and organ size. Diverse roles for the Hippo pathway have emerged, the majority of which in vertebrates are determined by the transcriptional regulators TAZ and YAP (TAZ/YAP). Key processes regulated by TAZ/YAP include the control of cell proliferation, apoptosis, movement and fate. Accurate control of the levels and localization of these factors is thus essential for early developmental events, as well as for tissue homeostasis, repair and regeneration. Recent studies have revealed that TAZ/YAP activity is regulated by mechanical and cytoskeletal cues as well as by various extracellular factors. Here, I provide an overview of these and other regulatory mechanisms and outline important developmental processes controlled by TAZ and YAP.

KEY WORDS: Hippo pathway, Stem cells, Pre-implantation development, Organ patterning, Mechanosensing

Introduction

A coordinated balance between proliferation, apoptosis and differentiation is essential for the accurate formation and maintenance of tissues and organs. Recent studies have indicated that the fidelity of these processes relies on cues transduced by the Hippo pathway, a conserved signaling pathway crucial for integrating cytoskeletal changes with the extracellular environment. The Hippo pathway was identified in genetic studies of *Drosophila melanogaster* as a suppressor of tissue overgrowth (Huang et al., 2005; Jia et al., 2003; Justice et al., 1995; Pantalacci et al., 2003; Tapon et al., 2002; Udan et al., 2003; Wu et al., 2003). This initial work outlined a core group of factors that control transcriptional events important for cell proliferation and apoptosis. A wealth of studies since then, including those in vertebrates, has greatly expanded the complexity of the Hippo pathway network.

This Review provides an overview of the Hippo pathway in development, with a particular focus on the mammalian transcriptional regulators TAZ (transcriptional co-activator with a PDZ-binding domain; also known as WW domain containing transcription regulator 1, or WWTR1) and YAP (Yes-associated protein; also known as YAP1). TAZ and YAP function as key downstream effectors of the Hippo pathway, and throughout this Review I refer to these factors collectively as TAZ/YAP, as many aspects of their regulation and function are shared. New mechanisms directing the nuclear/cytoplasmic localization of TAZ/YAP have been revealed and will be discussed. These regulatory mechanisms are closely integrated with extracellular stimuli that influence cytoskeletal dynamics, such as mechanical forces exerted by matrix stiffness (Dupont et al., 2011), as well as modulators of G protein-coupled receptors (GPCRs) (Yu et al., 2012). Notably, regulation of TAZ/YAP localization is implicated in the control of various developmental processes, ranging from pre-implantation embryogenesis to the patterning of many organs. Clear redundancy in the developmental roles and molecular activity of TAZ/YAP exist, but evidence also indicates that TAZ and YAP have divergent functions, which are discussed below.

The core Hippo pathway: a conserved network of signals

A search for mutations that led to tissue overgrowth in *D. melanogaster* identified a conserved kinase cascade that comprises the Hippo kinase, the Warts kinase and the adaptor proteins Salvador and Mob (Fig. 1A) (Jia et al., 2003; Justice et al., 1995; Pantalacci et al., 2003; Tapon et al., 2002; Udan et al., 2003; Wu et al., 2003). Activation of these signals promotes phosphorylation of the transcriptional regulator Yorkie by the Warts kinase, resulting in its exclusion from the nucleus (Huang et al., 2005). Nuclear Yorkie promotes proliferation and inhibits apoptosis by associating with the transcription factor Scalloped (Goulev et al., 2008; Wu et al., 2008; Zhang et al., 2008). Nuclear Yorkie relieves the action of transcriptional repressors associated with Scalloped, thereby activating the expression of a variety of target genes (Koontz et al., 2013). Uncontrolled nuclear Yorkie activity induces cellular overgrowth phenotypes, acting as the primary driver of phenotypes identified in the early genetic screens. A wealth of additional studies in *Drosophila*, including recent mass spectrometry-based proteomic approaches (Kwon et al., 2013), has broadened the *Drosophila* Hippo pathway network, which now extends to factors that respond to alterations in cell adhesion and polarity, as well as to core regulators of the actin cytoskeleton (Fernandez et al., 2011; Sansores-Garcia et al., 2011).

The conservation of the core Hippo pathway factors is striking, as the kinase cascade is conserved throughout the eukaryotic kingdom. These factors have taken on diverse essential roles (see Box 1), which include the regulation of mitotic exit in *Saccharomyces cerevisiae* (Fig. 1B), thermal stress resistance in *Caenorhabditis elegans* (Fig. 1C) and control of cell fate in mammals (Fig. 1D). Much of the insight into the molecular functions of Hippo pathway effectors has come from studies in mammalian model systems. YAP, which is a functional homolog of the transcriptional regulator Yorkie, was the first protein identified with a WW domain (Sudol, 1994; Sudol et al., 1995), a protein-interaction domain comprising a distinct arrangement of two tryptophan (W) residues. TAZ, a paralog of YAP, was subsequently characterized (Kanai et al., 2000). The finding that TAZ phosphorylation on a conserved serine residue (Ser89 in human TAZ; equivalent to Ser127 in human YAP) promotes binding to 14-3-3 proteins and subsequent cytoplasmic retention revealed a key mechanism of Hippo pathway function (Kanai et al., 2000). Phosphorylation of this residue by the LATS1 and LATS2 kinases (homologs of Warts) has emerged as the signature mode of action for the Hippo pathway, and dissection of this phosphorylation event has

Department of Biochemistry, Boston University School of Medicine, 72 East Concord Street, Room K-620, Boston, MA 02118, USA.

*Author for correspondence (xvarelas@bu.edu)

Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

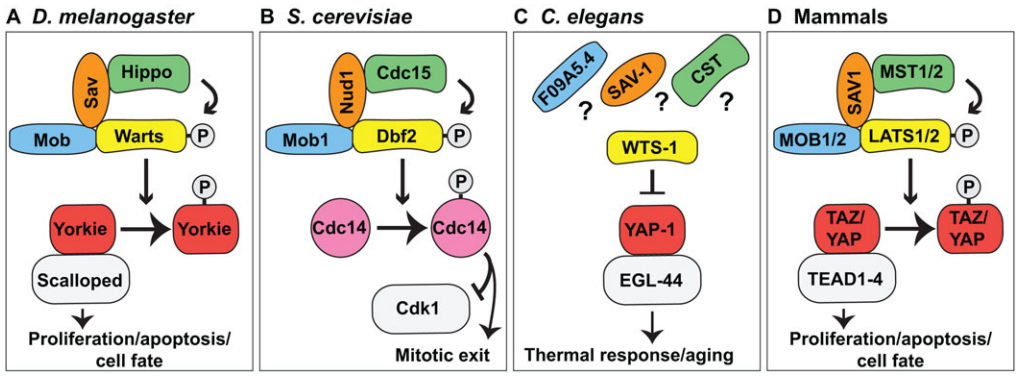

Fig. 1 . Conservation of the core Hippo pathway. The core components of the Hippo signaling pathway in (A) *Drosophila melanogaster*, (B) *Saccharomyces cerevisiae*, (C) *Caenorhabditis elegans* and (D) mammals. The functionally conserved factors are matched by color. In *S. cerevisiae* these signals are known as the mitotic exit network, which controls mitotic exit and cytokinesis. In *C. elegans* these signals control transcriptional events important for thermal response and aging, whereas in *D. melanogaster* and mammals this network controls transcriptional events that direct proliferation, apoptosis and cell fate.

charted many regulators of TAZ/YAP activity. Most upstream components in the Hippo pathway are conserved in mammals, including the MST1 and MST2 kinases (homologs of Hippo, also known as STK4 and STK3), SAV1 (a homolog of Salvador, also known as WW45), and MOB1A and MOB1B (homologs of Mob). However, as is the case with flies, numerous studies, including recent mass spectrometry-based proteomic studies (Couzens et al., 2013; Wang et al., 2013), have extended the effectors of the pathway, many of which have not yet been dissected in detail.

Box 1. Evolutionary conservation of the Hippo pathway

The Hippo pathway is highly similar to the mitotic exit network (MEN) in *Saccharomyces cerevisiae*, a system of signaling effectors that serve as a checkpoint for proper exit from mitosis (Bosl and Li, 2005). The MEN includes Cdc15, a member of the Ste20-like kinase family that is homologous to *Drosophila* Hippo, which phosphorylates the scaffold protein Nud1 to assemble a complex consisting of Dbf2 and the adaptor Mob1 (Rock et al., 2013). Dbf2 is part of the NDR family of kinases, which include *Drosophila* Warts and mammalian LATS1/2 (Hergovich et al., 2006). Mob1 facilitates Dbf2 activity, which is homologous to the role that Mob proteins play in the activation of Warts and LATS1/LATS2 (Lee et al., 2001; Mah et al., 2001). Activated Dbf2 promotes the phosphorylation and activation of the Cdc14 phosphatase, which dephosphorylates and inhibits Cdk1, ensuring exit from mitosis and accurate cytokinesis. Despite their importance in higher eukaryotes, homologs of TAZ/YAP or TEAD transcription factors remain elusive in yeast (Hilman and Gat, 2011).

The emergence of genes encoding the transcriptional effectors of the Hippo pathway appears to have occurred prior to the origin of metazoans, as unicellular *Capsaspora owczarzaki*, a unicellular amoeboid, possess functional homologs of YAP and TEAD-like factors (Sebe-Pedros et al., 2012). Recent work in *C. elegans* has also identified a YAP homolog, which was named YAP-1 (Iwasa et al., 2013). The gene duplication leading to TAZ and YAP occurred in vertebrates, and their dynamic expression patterns play essential roles in the development of various organs in *Xenopus tropicalis* and *Danio rerio* (Hong et al., 2005; Hu et al., 2013; Jiang et al., 2009; Nejigane et al., 2011). Interestingly, a divergence in several upstream regulators of TAZ/YAP appears to have specifically evolved in vertebrates. For example, the adaptor protein angiomotin (AMOT), which has no obvious homolog in flies, has vital roles in regulating TAZ/YAP activity in vertebrates (Chan et al., 2011; Yi et al., 2013; Zhao et al., 2011). Nonetheless, despite differences in some aspects of regulation, the conservation of core components underscores the evolutionary importance of the Hippo pathway.

The regulation of TAZ/YAP in vertebrates

TAZ/YAP are the primary downstream effectors of Hippo pathway signaling in vertebrates, and these factors share many regulatory features and structural domains (Fig. 2). Diverse factors, including kinases, adaptor proteins and miRNAs control TAZ/YAP activity. Many of these factors are highlighted in Table 1. Moreover, as outlined in Table 2, many post-transcriptional modifications have been reported to control TAZ/YAP localization and activity. Below, I emphasize the prominent regulatory features found within TAZ/YAP and provide an overview of some of the key factors and pathways that control TAZ/YAP activity.

Structural features of TAZ and YAP

The most discernible domain within TAZ or YAP that confers signaling specificity is the WW domain, which consists of two tryptophan residues separated by 20-23 amino acids (Salah et al., 2012). The WW domains within TAZ/YAP recognize a PPxY motif (proline/proline/any amino acid/tyrosine) that is found in a variety of proteins, many of which control TAZ/YAP localization and activity. The human TAZ isoform that is commonly studied possesses one WW domain, whereas the human YAP isoform contains two tandem WW domains. However, isoforms of TAZ and YAP with either one or two WW domains have been identified (Sudol, 2013; Webb et al., 2011).

TAZ and YAP also share a C-terminal PDZ-binding motif, which mediates interactions with PDZ domains. PDZ domains are 80-90 amino acid protein-interaction domains that are found in several proteins, many of which are transmembrane or cytoskeleton associated (Ye and Zhang, 2013). Functionally, the PDZ-binding domains are suggested to direct TAZ/YAP localization (Oka and Sudol, 2009; Remue et al., 2010). Mono-methylation of lysine 494 in YAP, which lies very close to the PDZ-binding domain, promotes the cytoplasmic retention of YAP (Oudhoff et al., 2013). Given this proximity it is reasonable to speculate that methylation might regulate the binding of YAP to PDZ-domain proteins, consequently impacting YAP localization.

The extended C-terminus of TAZ/YAP constitutes an unstructured transcriptional activation domain. Within this domain lies a conserved tyrosine residue (Y321 in human TAZ; Y407 in human YAP), which is phosphorylated by c-ABL, SRC and YES (Jang et al., 2012; Levy et al., 2008; Zaidi et al., 2004). Although the processes controlled by this modification are not fully understood, evidence indicates that it regulates the transcriptional roles of TAZ/YAP (Jang et al., 2012;

Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

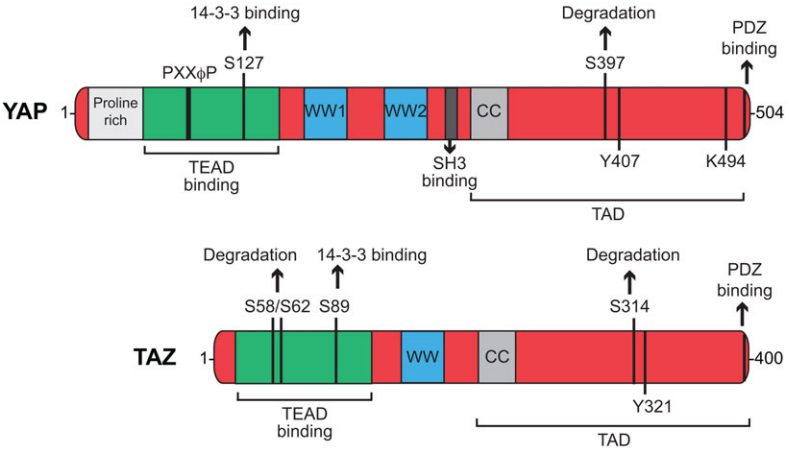

Fig. 2. Regulatory domains of the Hippo pathway effectors TAZ/YAP. Important domains and regulatory modifications within YAP and TAZ. Prominent regions include the WW domain(s), the coiled-coil (CC) domain, the SH3-binding domain, the TEAD transcription factor-binding domain, the transcriptional activation domain (TAD) and the PDZ-binding motif. Some key post-translational modifications are also shown (see Table 2 for a more complete list).

Zaidi et al., 2004). Recent observations suggest that the SRC kinase promotes YAP binding to TEAD transcription factors in cancer-associated fibroblasts (Calvo et al., 2013). Similarly, YES1-induced tyrosine phosphorylation of YAP in colon cancer cells induces its binding to the transcriptional regulator β-catenin and the transcription factor TBX5, which together inhibit apoptosis (Rosenbluh et al., 2012). Conversely, c-ABL-mediated phosphorylation of YAP promotes apoptosis in response to DNA damage (Levy et al., 2008). Recent data also indicate that tyrosine phosphorylation of YAP is associated with increased DNA damage and loss of epithelial homeostasis, which contributes to the defective repair of RASSF1A-depleted tissues following inflammation-induced injury (Gordon et al., 2013). Thus, given the varying effects mediated by tyrosine phosphorylation, our understanding of the molecular mechanisms by which this modification affects TAZ/YAP activity is incomplete.

A shared domain in the N-terminal region of TAZ/YAP mediates binding to TEAD transcription factors. In YAP this domain consists of two short alpha helices with an extended loop that contains a PxxΦP motif (where x is any amino acid and Φ is a hydrophobic residue) (Chen et al., 2010; Li et al., 2010). Although much of the TEAD-binding domain is conserved between TAZ and YAP, TAZ lacks the PxxΦP motif. Thus, differences between TAZ and YAP binding to TEAD transcription factors exist, which is supported by recent molecular modeling studies and mutational analysis (Hau et al., 2013). However, whether these differences translate to disparate TAZ/YAP activities *in vivo* is yet to be determined. What is clear is that point mutations that disrupt the binding of TAZ or YAP to TEADs abolish their ability to promote proliferation and tumorigenic overgrowth phenotypes (Lamar et al., 2012; Zhang et al., 2009; Zhao et al., 2008), suggesting that control of TEAD activity is crucial for many of the TAZ/YAP functions described.

The TEAD-binding region in TAZ/YAP is in very close proximity (possibly overlapping) with that required for 14-3-3 binding. 14-3-3 binding fosters cytoplasmic sequestration (Basu et al., 2003; Kanai et al., 2000), and is one of the major mechanisms by which the Hippo pathway controls TAZ/YAP localization and activity. 14-3-3 binding is induced by LATS1/2 kinase-mediated phosphorylation of the well-studied Ser89 in human TAZ and Ser127 in human YAP. Substitution of the phosphoserine with an alanine promotes nuclear TAZ/YAP localization and activity, driving cellular overgrowth phenotypes both *in vitro* and *in vivo*.

Although several features are shared between TAZ and YAP, distinctions are evident. For example, the extreme N-terminus of YAP contains a proline-rich region not found in TAZ. This region is reported to interact with heterogeneous nuclear ribonuclear protein U (hnRNPU) (Howell et al., 2004), a nuclear matrix RNA-binding protein that plays a role in mRNA processing. YAP also possesses an SH3-binding motif (amino acids PVKQPPPLAP) that is lacking in TAZ. This region mediates interactions with the SH3 domains of several proteins, including the YES and SRC kinases, as well as the adaptor proteins NCK and CRK (Sudol, 1994). However, these differing features of TAZ and YAP have not been well explored.

TAZ/YAP stability and turnover

The C-terminal region of TAZ/YAP contains a serine-rich phospho-degron motif that, when phosphorylated, targets TAZ/YAP for ubiquitylation and proteasome-mediated degradation. LATS1/2-mediated phosphorylation of a conserved serine in this region (Ser311 in human TAZ; Ser397 in human YAP) primes for further phosphorylation by CK1ε/δ kinases (Ser314 on human TAZ; Ser400/403 in human YAP) (Liu et al., 2010; Zhao et al., 2010). A consequence of these modifications is the recruitment of the β-TrCP/SCF ubiquitin ligase, which facilitates TAZ/YAP ubiquitylation and degradation (Liu et al., 2010; Zhao et al., 2010). Phosphorylation of TAZ on Ser314 by the kinase NEK1 also recruits β-TrCP, but, interestingly, in this scenario TAZ is suggested to function as an adaptor for β-TrCP to promote ubiquitylation of the calcium-permeable cation channel protein polycystin 2 (PC2, or PKD2) (Tian et al., 2007), thereby controlling cilia-directed signaling (Yim et al., 2011).

Other regions are also important for regulating TAZ stability. Phosphorylation of TAZ by glycogen synthase kinase 3β (GSK3β) on Ser58 and Ser62 in human TAZ also recruits β-TrCP, targeting TAZ for degradation (Huang et al., 2012). GSK3β associates with Axin and APC to make up a complex that targets the transcriptional regulator β-catenin for degradation (Stamos and Weis, 2013). Recent work shows that this complex destabilizes TAZ/β-catenin complexes (Azzolin et al., 2012). Stimulation of cells with Wnt, which inhibits GSK3β activity, increases TAZ and β-catenin levels and nuclear activity. In this context GSK3β is reported not to target the phospho-degron in the N-terminal region of TAZ, suggesting that additional modifications control TAZ stability. GSK3β-mediated regulation of YAP has not been reported. A specific regulator of YAP stability that has recently been uncovered is the homeodomain-interacting protein kinase (HIPK2), which stabilizes YAP to promote its nuclear activity (Poon et al., 2012). Thus, disparate regulation of TAZ and YAP levels exists.

Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

Table 1. Mediators of TAZ/YAP activity

Transcriptional regulators	Phosphatases	G protein signaling
ARC105	PP1	Gα(12/13)-coupled receptors
β-catenin	PP2A (STIPAK)	Gα(i/o)-coupled receptors
MYC	PTPN14	Gα(q/11)-coupled receptors
EGR1	**Adaptor proteins**	Gα(s)-coupled receptors
GABP	14-3-3	**Rho-GTPases**
GLIS3	α-catenin	CDC42
KLF5	AJUBA	RAC1
MYOD	AMOT/AMOTL1/AMOTL2	RHOA
NKX2.1 (TTF1)	ASPP1/2	**Actin capping/severing**
p53	BMI1	CAPZB
p63	CD44	CFL1/2
p73	CRB3	GSN
PAX3/8	CTLA4 (CD152)	**Lysine methyltransferase**
PML	DCHS1	SET-7
PPARγ	E-cadherin	**Chromatin modulators**
RUNX1/2/3	CFL1/2	CBP
SMAD1/2/3/4	CFL1/2	CHD4
TBX5	FAT4	GATAD2/3
TEAD1/2/3/4	FRMD4A/6	MBD3
YBX1	GPC3	MTA1/2/3
Kinases	HSP90	p300
ABL1	IQGAP	SIRT1
AKT1	LIFR	**Ubiquitin ligases**
ATM	LIMD1	β-TrCP
CDK1	LIN7C	NEDD4
CK1ε/δ	MASK1/2	NEDD4L
ERBB4	MOB1A/B	**miRNAs (target)**
GSK3β	MPDZ	miR-31 (*Lats2*)
HIPK2	NCK1/2	miR-93 (*Lats2*)
JNK1/2	NF2 (Merlin)	miR-133b (*Mst2*)
LATS1/2	NHERF1/2	miR-135b (*Lats2*)
LKB1	NPHP4	miR-138 (*Mst1*)
MEK1 (MAP2K1)	PALS1	miR-141 (*Yap*)
MARK1/2/3/4	PAR6B	miR-195 (*Lats2*)
MST1/2	PATJ	miR-200a (*Yap*)
NDR1/2	PKD2	miR-302a (*Lats2*)
NEK1	RASSF1-6	miR-372 (*Lats2*)
Pi3K	SAV1 (WW45)	miR-373 (*Lats2*)
PKA	SCRIB	miR-375 (*Yap*)
PKCι/ζ	SHP2	miR-483-3p (*Yap*)
ROCK1/2	SMAD7	
SRC	TRIB2	
TAOK1	WBP1/2	
YES1	WTIP	
	WWC1 (Kibra)	
	ZO-1/2	

Mechanical cues and GPCRs: novel upstream regulators of TAZ/YAP

An important class of TAZ/YAP regulators that has been uncovered recently is the GPCR family, a large group of receptors that make up ∼4% of the protein-coding genome (Bjarnadottir et al., 2006). GPCRs sense extracellular molecules and relay signals through associated G proteins. In response to phospholipids, such as serum-borne lysophosphatidic acid (LPA) or sphingosine 1-phosphophate (S1P), receptors coupled to Gα12/13 GTP-binding proteins have been shown to inhibit the LATS1/2 kinases, inducing nuclear TAZ/YAP activity (Yu et al., 2012). Protease-activated receptor [PAR, also known as thrombin receptor (F2R)], which also transduces signals via Gα12/13 proteins, similarly stimulates nuclear TAZ/YAP following its activation by the serine protease thrombin (Mo et al., 2012). Conversely, stimulation of Gαs-coupled GPCRs with hormones, such as epinephrine or glucagon, increases LATS1/2-mediated phosphorylation of YAP, thereby decreasing nuclear YAP activity (Yu et al., 2012). Additionally, cyclic adenosine monophosphate (cAMP), a secondary messenger downstream of Gαs-coupled receptors, acts via protein kinase A (PKA) to stimulate LATS1/2-mediated YAP phosphorylation to maintain high levels of cytoplasmic YAP (Kim et al., 2013; Yu et al., 2013). Thus, a diverse array of GPCR-mediated signals appears to direct TAZ/YAP localization in opposing manners, providing a large number of potential mechanisms for TAZ/YAP control.

Observations suggest that GPCR-regulated cues are transduced to TAZ/YAP via Rho-GTPases, a family of GTPases that influence actin cytoskeleton dynamics (Yu et al., 2012). Activation of Rho-kinase (ROCK), a downstream Rho-GTPase effector, has been shown to promote nuclear TAZ/YAP activity (Dupont et al., 2011). Mechanical cues are regulators of Rho-GTPase and ROCK activity, and stiffness changes in the cellular environment have emerged as mediators of TAZ/YAP activity (Aragona et al., 2013; Dupont et al., 2011). For example, stiff extracellular conditions or surfaces that allow cell spreading induce nuclear TAZ/YAP localization. Alterations in filamentous actin (F-actin) assembly are related to these mechanical cues, as disruption of F-actin stress fibers represses nuclear TAZ/YAP (Dupont et al., 2011). Conversely, soft microenvironments or surfaces that confine the physical cellular environment restrict nuclear TAZ/YAP. F-actin capping or severing proteins, such as cofilin, CapZ and gelsolin, facilitate the restriction of TAZ/YAP in the cytoplasm, as depletion of these factors induces nuclear TAZ/YAP activity in a compacted microenvironment (Aragona et al., 2013).

The mechanical signals directed by TAZ/YAP are important biologically, as they influence cell fate decisions, the best-described example of which is the specification of mesenchymal stem cell (MSC) fate (Fig. 3) (Dupont et al., 2011; Hong et al., 2005). The ability of cytoskeletal dynamics to influence cell fate was initially described in MSCs (Engler et al., 2006; McBeath et al., 2004). Roles for TAZ/YAP in directing MSC fate were later described, as depletion of TAZ was found to promote adipogenesis, and increased nuclear TAZ activity promoted osteogenesis (Hong et al., 2005). Thus, the recent observations indicating that TAZ/YAP localization is influenced by mechanical cues provide a molecular explanation for how cytoskeletal dynamics are integrated with cell fate. Although the regulation of TAZ/YAP localization by mechanical stimuli is still poorly understood, recent work indicates that an MT1-MMP/β1-integrin/Rho-GTPase signaling cascade is involved, as activation of these signals promotes skeletal stem cell commitment in a TAZ/YAP-dependent manner (Tang et al., 2013). Intriguingly, recent work suggests that the regulation of TAZ and YAP localization by mechanical cues is independent of the LATS1/2 kinases. For example, knockdown of LATS1/2 in human MSCs, dermal microvascular endothelial cells or breast cancer cells is reported to have no effect on TAZ/YAP nuclear accumulation or activity in a soft microenvironment (Aragona et al., 2013; Dupont et al., 2011). Thus, it appears that control of TAZ/YAP localization extends beyond the core upstream Hippo pathway regulators.

Input from cell adhesion and polarity signals

Mechanical signals lie at the epicenter of cell-cell contacts, the formation of which is an event long known to restrict cell proliferation and migration (Abercrombie, 1979). Cell contact-mediated adhesion has been shown to potently restrict TAZ/YAP in the cytoplasm, contributing to the contact inhibition phenotypes observed in non-transformed cells (Zhao et al., 2007). Cells with aberrant nuclear TAZ/YAP escape contact inhibition, primarily through transcriptional regulation. Recent work indicates that contact-mediated changes in TAZ/YAP localization also influence global miRNA biogenesis (Chaulk et al., 2013; Mori et al., 2014). In part,

Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

Table 2. Post-translational modifications controlling TAZ/YAP activity

Modification	Regulatory enzyme	Functional consequence
YAP		
S61-p	LATS1/2	Cytoplasmic retention?
S109-p	LATS1/2	Cytoplasmic retention?
T119-p	CDK1	Cell cycle regulation
S127-p	LATS1/2	14-3-3 binding/cytoplasmic retention
S164-p	LATS1/2	Cytoplasmic retention?
S289-p	CDK1	Cell cycle regulation
S367-p	CDK1	Cell cycle regulation
S397-p	LATS1/2	Primer for S400/403-p
S400-p	CK1ε/δ	Degradation/β-TrCP recruitment
S403-p	CK1ε/δ	Degradation/β-TrCP recruitment
Y407-p	ABL/SRC/YES	Altered nuclear activity
K494-meth	SET-7	Cytoplasmic retention
TAZ		
S58-p	GSK3β	Degradation
S62-p	GSK3β	Degradation
S66-p	LATS1/2	Cytoplasmic retention?
S89-p	LATS1/2	14-3-3 binding/cytoplasmic retention
S117-p	LATS1/2	Cytoplasmic retention?
S311-p	LATS1/2	Primer for S314-p
S314-p	NEK1/CK1ε/δ	Degradation/β-TrCP recruitment
Y321-p	ABL	Altered nuclear activity

this is achieved via the regulation of the Let-7 family of miRNAs, which control the levels of the miRNA-processing enzyme Dicer (Chaulk et al., 2013). Thus, cell contact-mediated TAZ/YAP signaling influences a variety of events, many of which are likely yet to be discovered.

Cell contacts are also a prerequisite for the acquisition and maturation of epithelial cell polarity. A very close relationship between Hippo pathway components and proteins that control epithelial polarity has thus emerged over the last few years (Fig. 4). Major TAZ/YAP-interacting proteins include components of Crumbs, a protein complex that is important for establishing the epithelial apical domain (Varelas et al., 2010b). Depletion of Crumbs complex components, including Pals1 and the Crumbs family member Crb3, in polarized epithelial cells was found to increase nuclear TAZ/YAP localization (Varelas et al., 2010b). Angiomotin (AMOT), a factor that is recruited to the Crumbs complex in epithelial cells (Wells et al., 2006), also tightly associates with TAZ/YAP (Varelas et al., 2010b; Wang et al., 2011; Zhao et al., 2011). Interactions between TAZ/YAP and AMOT family members (AMOTL1, AMOTL2 and the p130 isoform of AMOT) are involved in directing TAZ/YAP localization. Knockdown of AMOTL2 in confluent epithelial cells increases YAP nuclear accumulation and promotes cellular transformation (Wang et al., 2011; Zhao et al., 2011). This might relate to the ability of AMOTL2 to bridge complexes of MST, LATS and YAP (Paramasivam et al., 2011). Interestingly, recent work shows that p130-AMOT also binds YAP-TEAD complexes in the nucleus to specify transcriptional responses (Yi et al., 2013). In this context, p130-AMOT was found to disrupt YAP interactions with LATS1/2, thus inhibiting YAP-Ser127 phosphorylation and increasing nuclear YAP levels. Supporting this role *in vivo*, the deletion of *Amot* rescues the hepatomegaly and tumorigenesis (processes driven by increased levels of nuclear YAP) that are observed following loss of the tumor suppressor *Nf2* (which encodes the protein Merlin). However, how AMOT family members direct these seemingly opposing roles is unclear.

Nf2/Merlin has emerged as an important mediator of TAZ/YAP activity, and is one of the few upstream Hippo pathway regulators frequently found to be mutated in cancers (Harvey et al., 2013). Merlin associates with Par3 and α-catenin (Gladden et al., 2010), which are scaffold proteins that assemble with epithelial tight and adherens junctions, respectively. Recent evidence from studies of *D. melanogaster* indicates that Merlin directly interacts with Warts, recruiting it to the plasma membrane and in turn facilitating its activation. Merlin also binds F-actin (James et al., 2001), and the loss of F-actin stress fibers promotes interactions between Merlin and Warts (Yin et al., 2013), indicating that Merlin relays signals in response to cytoskeletal changes.

Like Merlin, YAP also binds α-catenin (Schlegelmilch et al., 2011; Silvis et al., 2011). Recruitment of α-catenin to adherens junctions restricts YAP in the cytoplasm, and knockdown of α-catenin induces nuclear TAZ/YAP accumulation (Schlegelmilch et al., 2011; Silvis et al., 2011; Varelas et al., 2010b). This phenotype is also observed *in vivo*, as depletion of α-catenin in the epidermis increases nuclear YAP activity and consequently leads to hyperproliferation and tumor formation (Schlegelmilch et al., 2011; Silvis et al., 2011). Loss of Scribble, an epithelial basal-lateral domain determinant, from mammary epithelial cells also increases the nuclear abundance and activity of TAZ (Cordenonsi et al., 2011). At the molecular level, Scribble is suggested to form a complex that bridges interactions between TAZ, LATS and MST to promote TAZ phosphorylation and subsequent degradation. Opposing the assembly of TAZ/YAP-LATS-MST complexes are the LIM domain Ajuba proteins (Ajuba, LIMD1 and WTIP), which when expressed ectopically decrease S127-YAP phosphorylation and induce nuclear YAP activity (Das Thakur et al., 2010). Ajuba proteins associate with cadherin complexes and the actin cytoskeleton and thus may participate in mechanotransduction via TAZ/YAP, but such roles have not yet been explored. Of note, TAZ/YAP cytoplasmic recruitment relies on binding to 14-3-3 proteins, which themselves have extended connections with a wide range of polarity regulators (Morrison, 2009). Therefore, a complex but as yet poorly understood network of TAZ/YAP regulation exists that encompasses mediators of cell adhesion, apical-basal polarity and the actin cytoskeletal.

Roles for TAZ/YAP in stem cell regulation and early development

The importance of Hippo signaling is illustrated in early animal development, as precise changes in TAZ/YAP localization are essential for determining some of the first cell fate events. These roles include the renewal of embryonic stem cell populations and the

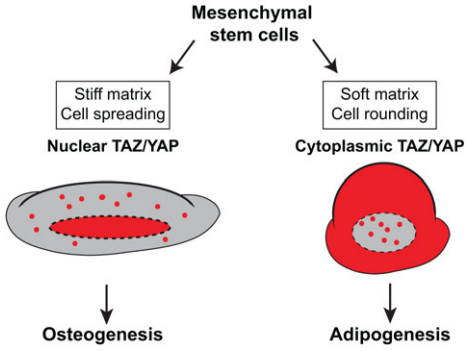

Fig. 3. The mechanical environment controls cell fate by directing the localization and activity of TAZ/YAP. The mechanical cues translated by TAZ/YAP have recently been shown to influence mesenchymal stem cell differentiation. High mechanical stress induced by cell spreading or by culture on a hard surface promotes the nuclear localization of TAZ/YAP (red) and drives osteogenesis. By contrast, the growth of cells on a soft surface, or an environment that induces cell rounding, restricts TAZ/YAP to the cytoplasm and facilitates adipogenesis.

Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

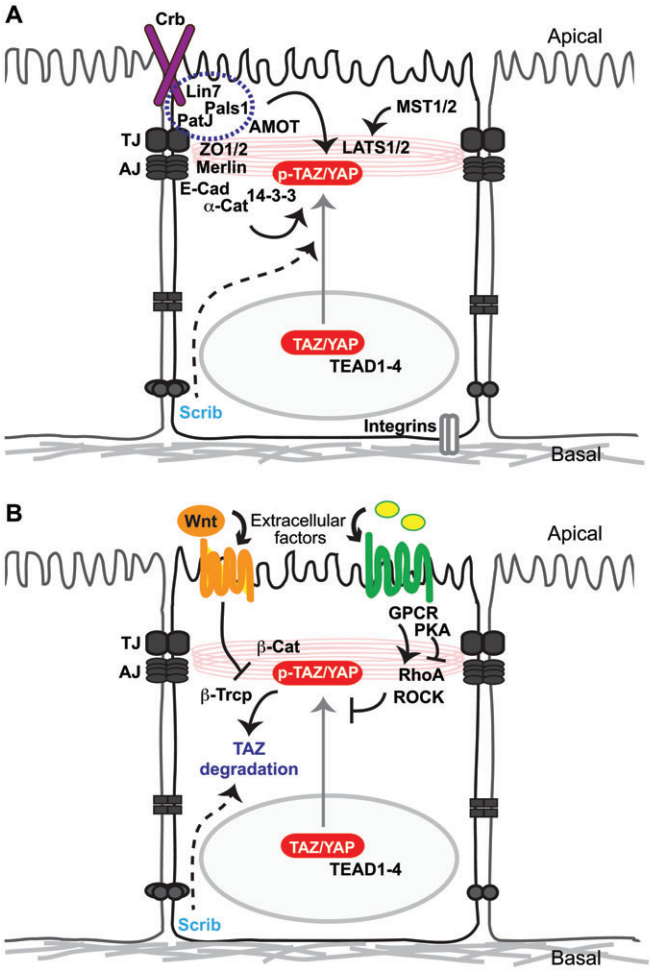

Fig. 4. Network integrating extracellular and epithelial polarity cues with TAZ/YAP. (A) Factors important for establishing epithelial cell polarity exclude TAZ and YAP from the nucleus. Key polarity regulators that relay signals to induce cytoplasmic TAZ and YAP localization are illustrated, and include the apical determinant Crumbs (Crb, purple) and the basal-lateral regulator Scribble (Scrib). The factors acting downstream of these polarity regulators are indicated. Major points of adhesion, such tight junctions (TJs) and adherens junctions (AJs), are also highlighted, as well as the apical and basal domains of cells. The cortical belt of actin filaments found in epithelial cells is represented in pink. (B) Major extracellular factors controlling TAZ/YAP localization and stability are shown in the context of an epithelial cell. The stimulation of G protein-coupled receptors (GPCRs, green) by extracellular factors (yellow) regulates the phosphorylation and localization TAZ/YAP. Activation of Gα12/13, Gαq/11 and Gαi/o GTP-binding proteins (not shown) inhibits TAZ/YAP phosphorylation, inducing nuclear TAZ/YAP localization. Conversely, activation of Gαs-coupled receptors relays PKA-dependent signals that promote LATS1/2-medidated TAZ/YAP phosphorylation, restricting TAZ/YAP to within the cytoplasm. These GPCR-mediated events are transduced by poorly understood mechanisms that involve Rho-ROCK and the actin cytoskeleton. Another extracellular factor impacting TAZ dynamics is the Wnt growth factor (orange; the Wnt receptor is also shown in orange), which stabilizes TAZ protein levels by inhibiting the β-TrCP-mediated destruction of TAZ/β-catenin protein complexes. Interestingly, the epithelial polarity regulator Scrib facilitates the β-TrCP-mediated destruction of TAZ.

control of cell differentiation signals. Our current knowledge of these processes is discussed below.

Hippo pathway signaling in pre-implantation embryonic development

The nuclear/cytoplasmic distribution of TAZ/YAP defines the first cell fate choice in the mouse embryo – the decision of embryonic cells to become either trophectoderm (TE) or inner cell mass (ICM). One of the first processes to occur in the embryo is ‘compaction’, during which cells at the ∼8-cell stage form adherens and tight junctions and acquire apical-basal polarity (Cockburn and Rossant, 2010). As these cells divide, the innermost and more compacted cells lose their polarity, and their differences to the outer cells result in a disparate distribution of TAZ/YAP (Fig. 5). By the blastocyst stage, TAZ/YAP accumulate in the nuclei of outer TE cells, but are distributed throughout the cytoplasm in cells of the ICM (Nishioka et al., 2009). Nuclear TAZ/YAP control the activity of TEAD transcription factors to direct a TE-specific transcriptional program that includes the induction of *Cdx2* (Home et al., 2012). In line with this, deletion of *Tead4* results in the loss of *Cdx2* expression, giving rise to embryos that fail to establish the TE (Nishioka et al., 2008; Yagi et al., 2007). Deletion of both *Taz* and *Yap* also results in cell fate specification defects, but with embryos dying at the morula stage prior to the specification of TE or ICM (Nishioka et al., 2009). Of note, deletion of either *Taz* or *Yap* alone does not result in pre-implantation defects (Hossain et al., 2007; Morin-Kensicki et al., 2006), indicating redundant *Taz/Yap* activity at this stage of development. Recent work indicates that TEAD4-deficient embryos can develop into properly specified blastocysts when cultured under conditions that alleviate oxidative stress (Kaneko and DePamphilis, 2013). The presence of TEAD4 in the embryo prevents reactive oxygen species (ROS) accumulation, suggesting that this unexplored role might be crucial for early cell fate events.

Increased nuclear localization of TAZ/YAP, resulting from the deletion of *Lats1/Lats2*, leads to amplified *Cdx2* expression, which prevents proper specification of the ICM (Nishioka et al., 2009). Temporal reduction of *Lats1/Lats2* with siRNA leads to similar defects, even when 8-cell stage LATS1/LATS2 knockdown embryos are aggregated with wild-type morulas (Lorthongpanich et al., 2013). In these aggregation experiments, LATS1/LATS2 knockdown cells were found to maintain high levels of *Cdx2* despite their positioning, whereas the wild-type inner cells did not. The knockout of both *Mob1a* and *Mob1b*, which are regulators of LATS1/2 activity, also results in developmental defects, with embryos arresting at around embryonic day (E) 6.5, prior to gastrulation (Nishio et al., 2012). Analysis of MOB1A/B-deficient blastocysts revealed aberrant nuclear YAP localization and modest growth failure in the ICM region, with few defects associated with the TE. Like *Lats1/2*-deleted embryos, depletion of both *Amot* and *Amotl2* also results in increased nuclear YAP localization and *Cdx2* expression throughout both inner and outer cell populations, resulting in severe pre-implantation defects. These defects were not apparent when either *Amot* or *Amotl2* was depleted alone, indicating redundancy between AMOT family members (Hirate et al., 2013). Mechanistic studies have revealed that LATS1/2 induce the phosphorylation of AMOT in the inner cells of the pre-implantation embryo, promoting its association with NF2 at cell membranes, and consequently amplifying TAZ/YAP phosphorylation (Hirate et al., 2013).

The role of TAZ/YAP in early embryo development has been examined using several other mouse models of the Hippo pathway, including knockouts of *Mst1/2*, *Sav1* and *Nf2*, all of which display severe developmental defects (Lee et al., 2008; Lu et al., 2010; McClatchey et al., 1997; Oh et al., 2009; Song et al., 2010). Maternal contributions in many of these mice, however, lead to the emergence of these defects much later than the pre-implantation stage. For example, *Nf2* knockout embryos do not exhibit severe phenotypes until ∼E7.0 (McClatchey et al., 1997). Yet, examination of embryos with maternal-zygotic *Nf2* mutation or embryos injected

Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

with dominant-negative NF2 reveals increased *Cdx2* expression, high nuclear YAP localization and aberrant TE specification (Cockburn et al., 2013).

Although the mechanisms are still unclear, cell polarity changes are linked to the regulation of TAZ/YAP localization in the pre-implantation embryo. Knockdown of PAR6B, knockout of both atypical PKCs (PKCλ and PKCζ), or double knockdown of both PAR1A and PAR1B, all result in decreased nuclear YAP in the outer cells of the blastocyst (Hirate et al., 2013). However, unlike epithelial cells in adult tissues or culture, where apical-basal polarity restricts nuclear TAZ/YAP, the relationship with cell polarity in the blastocyst appears to be reversed, as TAZ/YAP are exclusively nuclear in apical-basal polarized cells. Thus, there is much that remains to be uncovered with respect to how TAZ/YAP are regulated in the early embryo.

Hippo pathway signaling in embryonic stem cells

Numerous studies have shown that TAZ/YAP play important roles in embryonic stem cells (ESCs), which are derived from the ICM of the blastocyst and have the capacity to self-renew and give rise to all functional cell types in an adult animal. A precise balance of growth factor-induced and cytoskeletal-associated cues is required for the maintenance of ESC pluripotency. These signals ultimately control the levels and action of a core transcriptional circuitry consisting of OCT4 (also known as POU5F1), NANOG and SOX2 (Young, 2011). Several studies have indicated that nuclear TAZ/YAP activity is required to integrate growth factor signals with these core transcriptional regulators to maintain the ESC pluripotent state.

Human ESCs require signals induced by fibroblast growth factors (FGFs) and members of the transforming growth factor-β (TGFβ) family (Beyer et al., 2013a). TGFβ stimulates the action of serine/threonine kinase receptors that phosphorylate and activate the SMAD2/3 class of transcription factors (Weiss and Attisano, 2013). Studies have shown that TAZ/YAP form complexes with phosphorylated SMAD2/3 (Varelas et al., 2008, 2010b). In the nucleus, the TAZ/YAP-SMAD2/3 complexes bind to TEAD transcription factors, as well as to the core stem cell regulator OCT4, and together mediate the pluripotent state (Beyer et al., 2013b). Mechanistically, this complex assembles with factors that make up the nucleosome remodeling and deacetylation (NuRD) complex to buffer the expression of pluripotency genes and repress genes that define mesendoderm (Fig. 6). Upon mesendoderm specification, the TAZ/YAP-TEAD-OCT4 complex dissociates from the SMADs, allowing the SMADs to activate the forkhead transcription factor FOXH1 and drive differentiation (Beyer et al., 2013b).

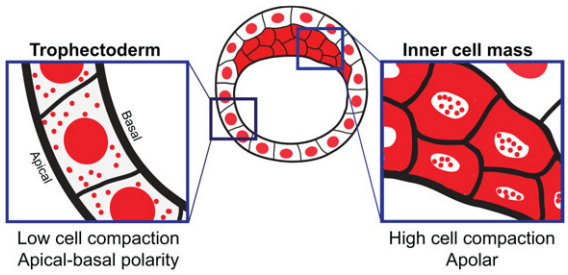

Fig. 5. Dynamic changes in TAZ/YAP localization direct pre-implantation development. As the mouse embryo develops from the morula to the blastocyst stage (illustrated), the inner and outer cells acquire differences in apical-basal polarity that alter the localization of TAZ/YAP (red). TAZ/YAP is nuclear localized in the less compacted, but polarized, outer cells that give rise to the trophectoderm. By contrast, compaction of the apolar cells within the inner cell mass promotes cytoplasmic TAZ/YAP localization.

Mouse ESCs also require precise YAP levels to maintain their pluripotent state. Knockdown of YAP in mouse ESCs leads to a loss of OCT4 and SOX2, and consequent differentiation (Lian et al., 2010). YAP is also essential for reprogramming of fibroblasts into the induced pluripotent stem cell (iPSC) state, which closely resembles that of ESCs in phenotype and differentiation potential (Takahashi and Yamanaka, 2006). As in human ESCs, YAP binding to TEADs directs transcriptional events important for maintaining pluripotency. Ectopic expression of a nuclear-localized mutant YAP promotes mouse ESC self-renewal and increases the efficiency of iPSC reprogramming (Lian et al., 2010). Similar observations have been made with human iPSCs, where LATS2 knockdown, which elevates nuclear TAZ/YAP levels, increases reprogramming efficiency (Qin et al., 2012). One key difference between human and mouse ESCs is that the latter rely on signals induced by bone morphogenetic protein (BMP) and leukemia inhibitory factor (LIF) (Beyer et al., 2013a). BMPs are a subclass of the TGFβ superfamily and, like TGFβ, promote the phosphorylation and activation of SMAD1/5/8 transcription factors. YAP interacts with BMP-activated SMAD transcription factors (Alarcon et al., 2009) and, as in the case of TGFβ signaling, YAP-TEAD complexes may direct SMAD activity in mouse ESCs. Supporting a co-dependency of TAZ/YAP and TEADs, the simultaneous knockdown of TEAD1/3/4 results in a loss of mouse ESC pluripotency (Lian et al., 2010). Additionally, LIF increases TEAD transcriptional activity by reducing YAP phosphorylation and promoting nuclear YAP-TEAD complexes that enhance mouse ESC self-renewal (Lian et al., 2010; Tamm et al., 2011). Intriguingly, expression of LIF receptor (LIFR) in breast cancer cells conversely promotes YAP phosphorylation and inhibits breast cancer metastasis in mice (Chen et al., 2012). Thus, further studies are required to understand the mechanisms connecting LIF and YAP.

While nuclear TAZ/YAP have crucial roles in ESCs cultured *in vitro*, *in vivo* studies in mouse blastocysts clearly indicate that TAZ/YAP are in the cytoplasm of the ICM, the region from which ESCs are derived. Such observations suggest that pluripotent ESCs exist very transiently *in vivo*, and that changes in TAZ/YAP localization might provide a mechanism to integrate microenvironmental cues with cell differentiation. Indeed, a wealth of work has shown that the mechanical microenvironment can have dramatic consequences for stem cell fate (Lutolf et al., 2009). Therefore, the mechanisms controlling TAZ/YAP localization might be a fundamental determinant of cell fate specification, and understanding these has obvious implications for regenerative medicine.

Control of organ development, homeostasis and disease by the Hippo pathway

The Hippo pathway was described as a regulator of organ size in *D. melanogaster*, and recent evidence is confirming this premise in mammals. Context-dependent roles for TAZ and YAP are also emerging, highlighting functional redundancy as well as divergent roles for these factors. Recent studies in mice suggest that nuclear TAZ/YAP promote progenitor renewal and proliferation in a range of organs, and that cytoplasmic restriction of TAZ/YAP is a requisite for tissue homeostasis. TAZ/YAP localization is dramatically altered upon tissue damage, and in some tissues nuclear TAZ/YAP abundance facilitates regeneration. However, how TAZ/YAP are regulated is still an open question, particularly with respect to the potential differences between these factors. Moreover, the precise roles for TAZ/YAP in these settings are likely to extend beyond transcriptional regulation, and these roles are starting to be uncovered. Our current knowledge of these *in vivo* roles for TAZ and YAP is

Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

briefly discussed below, with a focus on what is known about Hippo signaling in different organs.

Liver

Unlike most other organs, the liver has the ability to tolerate substantial changes in size and high levels of stress, and possesses a distinct ability to regenerate following partial damage. For these reasons, the liver was an early choice for studying Hippo signaling *in vivo*. Expression of *Yap*, or of a constitutively nuclear mutant, from a kidney-specific inducible promoter was observed to increase liver mass following only 3 days (Camargo et al., 2007; Dong et al., 2007). Prolonged expression of *Yap* was found to promote hepatocellular carcinoma phenotypes, providing *in vivo* evidence that elevated YAP is oncogenic. Consistent with these observations, YAP is amplified in human liver cancers (Zender et al., 2006). A similar onset of hepatocellular carcinoma is associated with liver-specific deletions of *Sav1*, *Nf2* or both *Mst1* and *Mst2*, all of which result in elevated nuclear YAP levels (Lee et al., 2010; Lu et al., 2010; Song et al., 2010; Zhang et al., 2010; Zhou et al., 2009). In all of these models nuclear YAP was found to promote the proliferation of oval cells, a potential progenitor population of the liver that might be capable of generating both hepatocytes and biliary cells. Recent evidence has revealed that AMOT associates with YAP in the nucleus to direct transcriptional events required for oval cell proliferation (Yi et al., 2013). Similar increases in oval cell expansion have also been observed following bile acid-induced injury of the liver, which elevates nuclear YAP levels by enhancing the expression of IQGAP, an inhibitor of cell adhesion (Anakk et al., 2013). Human patients with advanced stage cholestatic liver disease, which results in bile acid-induced ductal injury, display aberrantly high nuclear YAP levels (Bai et al., 2012). Thus, changes in YAP levels appear to be crucial for liver repair and regeneration. Indeed, YAP levels are dramatically induced following hepatectomy (Apte et al., 2009), and the deletion of *Yap* in the mouse liver compromises the regenerative response of hepatocytes (Bai et al., 2012).

Pancreas

Recent work has shown that changes in YAP localization are essential for proper pancreas development (Gao et al., 2013; George et al., 2012). Analyses of pancreas-specific *Mst1/2* knockout mice have revealed severe developmental defects that correlate with increased nuclear hypophosphorylated YAP (Gao et al., 2013; George et al., 2012). The most obvious defect observed was the failure for acini to form their classic rosette-like structure, resulting in severely reduced acinar-ductal ratios. Ectopic expression of a nuclear-localized YAP mutant (S112A-Yap) mirrored many aspects of the *Mst1/2* knockouts (Gao et al., 2013). Additionally, heterozygous deletion of *Yap* reversed many of the phenotypes observed in the *Mst1/2* knockout (Gao et al., 2013). Thus, YAP functions downstream of MST1/2 in the pancreas. Immune cell infiltration was associated with both the *Mst1/2* knockouts and the S112A-Yap-expressing pancreases, resembling phenotypes observed in human acute pancreatitis. These studies therefore suggest that dysregulation of Hippo signaling might be an unappreciated mechanism contributing to human pancreatic disease phenotypes.

Salivary glands

The morphogenesis of the murine salivary submandibular gland (SMG) relies on dynamic changes in cell adhesion and polarity that occur during epithelial tubule branching. These changes have recently been shown to rely on accurate Hippo pathway signaling

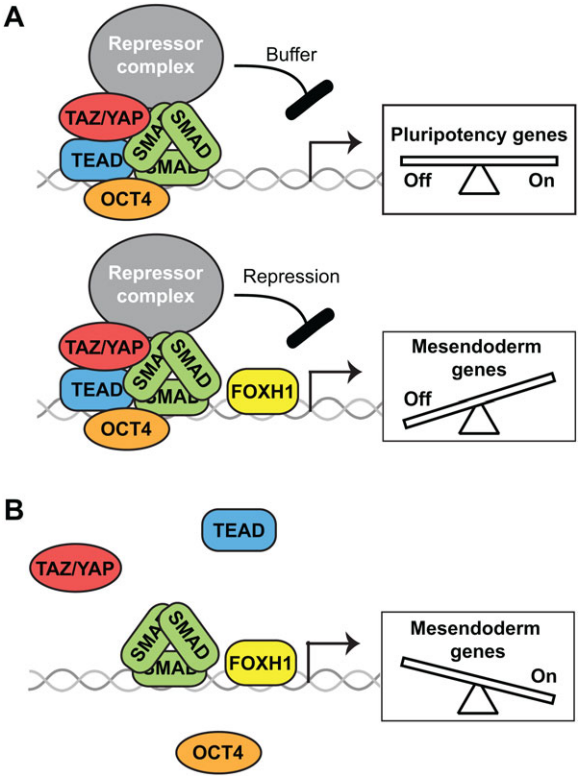

Fig. 6. Roles for TAZ/YAP in human embryonic stem cell (ESC) specification. TAZ/YAP, TEADs, TGFβ-induced SMAD2/3-SMAD4 complexes, and OCT4 assemble on the promoters of genes important for controlling embryonic pluripotency and mesendoderm specification in human ESCs. (A) TAZ and YAP recruit the NuRD repressor complex (gray) to buffer and maintain an optimal expression level of pluripotency genes (top), while suppressing the expression of mesendoderm genes (bottom). (B) Upon mesendoderm specification, the TAZ/YAP-TEAD-OCT4 complex dissociates, allowing the TGFβ-induced SMAD2/3-SMAD4 complexes to activate the FOXH1 transcription factor, consequently driving mesendoderm gene expression.

(Enger et al., 2013). In particular, increases in TAZ/YAP levels, along with enhanced phospho-S89-TAZ levels, were found to be associated with the assembly of junctional E-cadherin/α-catenin complexes during SMG branching (Enger et al., 2013). Depletion of LATS2 in *ex vivo* SMG cultures delocalizes TAZ, resulting in aberrant branching and inhibition of ductal extensions. The defects associated with LATS2 knockdown showed many similarities to phenotypes observed in human Sjogren's syndrome (Enger et al., 2013), an autoimmune disease of the salivary and lacrimal glands. Similar dysregulation of TAZ localization was confirmed in human Sjogren's syndrome tissues, indicating that dysregulated Hippo signaling might be a contributing factor in this disease.

Kidney

The first evidence for the importance of Hippo signaling in kidney development emerged from analyses of *Taz* knockout mice, which develop polycystic kidneys (Hossain et al., 2007; Makita et al., 2008; Mitani et al., 2009; Tian et al., 2007). The large cysts found in *Taz* knockout kidneys exhibit elevated levels of PC2, a calcium-permeable cation channel that has roles in ciliogenesis and is commonly dysregulated in polycystic kidney disease (Tian et al., 2007). The cystic regions in *Taz* knockout kidneys also display aberrantly localized β-catenin (Varelas et al., 2010a), suggesting that hyperactive Wnt/β-catenin signaling might contribute to these defects.

Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

Conditional deletion of *Taz* in the progenitor cap mesenchyme population of the developing kidney leads to the formation of cystic kidneys that are very similar to those observed in whole-body *Taz* knockouts (Reginensi et al., 2013). Strikingly, however, the conditional deletion of *Yap* in the same populations results in hypoplastic kidneys with few detectable glomeruli or proximal tubules, and defects in the formation of the Henle's loop and distal tubules (Reginensi et al., 2013). Dynamic changes in the nuclear/cytoplasmic localization of YAP in the kidney are also observed, which, interestingly, were found to be regulated by the GTPase CDC42. Deletion of *Cdc42* in the developing kidney reduces the levels of nuclear YAP, resulting in phenotypes similar to those observed upon *Yap* deletion. Intriguingly, analyses of kidneys with deletion of both *Taz* and *Yap* indicate that these factors have divergent roles in the kidney, as double knockouts exhibit phenotypes that are mostly similar to the *Yap* deletion, but with the few developed proximal tubules displaying cystic phenotypes like those found in the *Taz* deletion (Reginensi et al., 2013). Whether upstream Hippo pathway regulators of TAZ or YAP direct their distinct roles has not been extensively explored. A superficial analysis of *Mst1/2*- and *Sav1*-deleted kidneys indicates that they develop normally, suggesting that signals relayed via CDC42 might be the primary mode of regulation.

Lung

In addition to the kidney defects discussed above, *Taz* knockout mice were observed to display alveolarization defects that resemble those seen in human emphysema (Hossain et al., 2007; Makita et al., 2008; Mitani et al., 2009; Tian et al., 2007). TAZ associates with and promotes the activity of TTF1 (NKX2.1), a transcription factor essential for specifying the lung (Park et al., 2004). However, whether this relationship is important for the roles of TAZ in lung development is unclear, as *Taz* knockout lungs do not exhibit any changes in TTF1 target gene expression. Intriguingly, heterozygous deletion of *Taz* was found to confer resistance to bleomycin-induced lung fibrosis (Mitani et al., 2009), suggesting that TAZ might be a key factor promoting fibrotic responses. Conditional knockout of *Mst1/2* in the respiratory epithelium also leads to lethal respiratory failure (Chung et al., 2013), but these defects were not attributed to changes in TAZ or YAP localization. Rather, *Mst1/2*-deficient lungs were found to have dysregulated FOXA2 transcription factor activity. The function of other Hippo pathway components in the lung is still unclear, but given the dramatic morphogenetic changes that occur during lung development, and the close connections between lung cancer and TAZ/YAP (Su et al., 2012; Zhou et al., 2011b), it is likely that the Hippo pathway will prove to be important.

Heart

The mammalian heart undergoes dramatic changes in size during development, driven in large part through the proliferation of cardiomyocytes. These cells withdraw from the cell cycle shortly after birth as a result of decreased nuclear levels of YAP. Deletion of upstream Hippo pathway effectors, such as conditional deletion of *Sav1* or *Lats2* (Heallen et al., 2011), or induced expression of nuclear-localized S112A-Yap (von Gise et al., 2012; Xin et al., 2013), drives aberrant cardiomyocyte proliferation. Conversely, conditional deletion of *Yap* in mouse cardiomyocytes causes cardiac hypoplasia (von Gise et al., 2012; Xin et al., 2011). Cardiac-specific deletion of *Taz*, however, does not result in obvious defects until combined with heterozygous deletion of *Yap* (Xin et al., 2013). Loss of both *Taz* and *Yap* results in severe defects in cardiomyocyte proliferation, with increased apoptosis of these cells. Therefore, TAZ and YAP have redundant roles in the heart. Nuclear TAZ/YAP have been reported to direct cardiomyocyte proliferation by mediating Wnt/β-catenin and insulin-like growth factor (IGF) signaling (Heallen et al., 2011; Xin et al., 2011). Supporting the relationship between TAZ/YAP and Wnt, heterozygous deletion of β-catenin partially rescues the phenotypes observed in cardiac-deleted *Sav1* animals (Heallen et al., 2011). Of note, despite the normal dormancy of cardiomyocytes in adult hearts, these cells can be potently driven to proliferate by forcing the expression of nuclear S112A-Yap (Xin et al., 2013). As a result, S112A-Yap expression improves contractility following myocardial infarction. Additionally, conditional deletion of *Sav1* or *Lats1/2* enhances cardiomyocyte regeneration after adult myocardial infarction (Heallen et al., 2013). Directing YAP activity might therefore provide a means for therapeutic regeneration following heart damage.

Intestine

Several recent studies have examined the roles of the Hippo pathway in intestinal development and repair, and what has emerged is a complex story surrounding the activity of YAP. Nuclear YAP levels are elevated in stem cell compartments of the intestinal epithelium (Camargo et al., 2007). Deletion of *Mst1/2* or *Sav1* drives crypt hyperplasia, leading to an expansion of undifferentiated intestinal progenitors, suggesting that nuclear YAP promotes progenitor proliferation (Cai et al., 2010; Camargo et al., 2007; Zhou et al., 2011a). Supporting this idea, deletion of *Yap* impairs the regenerative response following dextran sulfate sodium salt (DSS)-induced damage (Cai et al., 2010). Deletion of *Yap* caused no obvious defects in intestinal development or homeostasis, suggesting that these roles for *Yap* are specific to the regenerative response. Complicating matters, however, another study has shown that deletion of *Yap* causes hyperplasia and overgrowth following irradiation-induced damage (Barry et al., 2013). Moreover, ubiquitous expression of S127A-YAP can promote dysplasia along the entire intestinal epithelium (Camargo et al., 2007), whereas expression of S127A-YAP specifically in the intestinal epithelium results in a degenerative phenotype associated with the rapid loss of proliferating crypts (Barry et al., 2013). Related to these intestinal roles for YAP is its ability to inhibit Wnt/β-catenin signaling by binding to and inhibiting the Wnt effector Dishevelled (Barry et al., 2013). Such observations are reminiscent of similar roles for TAZ, which also restrict Wnt/β-catenin signaling (Varelas et al., 2010a). Supporting this Wnt-repressive role for YAP, stimulation of *Yap*-deleted intestines with the Wnt agonist R-spondin leads to massive hyperplasia (Barry et al., 2013). Thus, Hippo pathway and YAP regulation of the intestinal epithelium appears to be complex and might rely on the presence or absence of local niches, such as those that express Wnt, to define distinct contextual signaling events.

Skin

The development and homeostasis of the epidermis rely on a balance between proliferation and differentiation of progenitor populations. These populations are dramatically influenced by changes in YAP levels and localization. Nuclear YAP is evident in undifferentiated progenitors found in early single-layered developing epithelia (Schlegelmilch et al., 2011; Zhang et al., 2011). YAP then shifts to the cytoplasm concomitant with differentiation, suggesting that this change in localization is crucial for maturation of the epidermal epithelium. Indeed, deletion of *Sav1* results in hyperproliferation of basal progenitors (Lee et al., 2001). Similarly, the conditional expression of a nuclear-localized mutant YAP in the skin is reported to drive extensive proliferation of basal progenitors, resulting in

Development (2014) 141, 1614-1626 doi:10.1242/dev.102376

the onset of squamous cell carcinoma-like tumors (Schlegelmilch et al., 2011). These events are primarily mediated by interactions between nuclear YAP and TEADs, as disruption of TEAD binding rescues the effect of YAP expression. Conversely, conditional deletion of *Yap* in epidermal progenitors has been shown to result in failed skin expansion and a complete loss of the epidermal barrier (Schlegelmilch et al., 2011). Surprisingly, skin-specific depletion of *Mst1* and *Mst2*, or the depletion of *Lats1/2* kinases, does not result in epidermal cell fate abnormalities. Rather, the adherens junction-associated protein α-catenin appears to have a crucial role in the regulation of YAP in this context, as it binds YAP to promote cytoplasmic restriction (Schlegelmilch et al., 2011; Silvis et al., 2011). Loss of α-catenin leads to dramatic nuclear YAP accumulation and elevated YAP transcriptional activity, stimulating epidermal stem cell expansion at the expense of differentiation.

Nervous system

Some of the first studies to examine the roles of Hippo signaling in progenitor cells focused on neuroepithelial cells, which are a self-renewing population of multipotent progenitors that generate the central nervous system. This work indicated that reduced YAP levels result in a decreased number of neuroepithelial cells in the developing chick neural tube and *Xenopus laevis* embryo, whereas increased nuclear YAP-TEAD activity drives the expansion of these cells (Cao et al., 2008; Gee et al., 2011). The expression of dominant-negative MST2 or knockdown of LATS1/2 activity was similarly found to drive neuroepithelial proliferation, suggesting that the Mst-Lats cascade mediates YAP function in this context. The protocadherin FAT4 has also been implicated in the regulation of YAP activity in neural progenitors. Knockdown of FAT4 in the developing chick or mouse neural tube increases neural progenitor numbers in a YAP-dependent fashion (Cappello et al., 2013; Van Hateren et al., 2011). Additionally, NF2/Merlin has recently been shown to suppress mouse neural progenitor expansion by inhibiting TAZ/YAP activity (Lavado et al., 2013). How nuclear TAZ/YAP activity controls progenitor proliferation is poorly understood, but is likely to involve the activation of genes encoding cell cycle regulators, such as cyclin D1, and inhibition of pro-differentiation factors, such as NeuroM (Cao et al., 2008). The ability of YAP to activate the Sonic hedgehog signaling pathway also appears to be functionally relevant in neuronal progenitors (Lin et al., 2012), but how YAP intersects with this pathway is unclear.

Conclusions

The broad importance of the Hippo pathway in animal development has prompted a profusion of research devoted to this field over the past decade. Detailed examination of genetic models together with biochemical characterization of pathway components has provided a glimpse into the complex network of signals controlling the activity of TAZ and YAP. These studies have also highlighted that the nuclear/cytoplasmic distribution of TAZ/YAP has key roles in directing cell fate, proliferation and apoptosis. However, these functions are not always observed synchronously and often are evident in a context-specific manner. A thorough characterization of the cytoplasmic functions of TAZ/YAP that goes beyond their nuclear transcriptional roles might provide better insight into these differences. Moreover, events that distinctly regulate TAZ or YAP are poorly described, and thus a better understanding of these signals might offer important insight.

It is noteworthy that TAZ and/or YAP localization and levels are dysregulated in a broad range of cancers, which I have not covered in depth here (for a recent review see Harvey et al., 2013). The degree of TAZ/YAP dysregulation is frequently correlated with cancer progression, and recent evidence indicates that, much like their roles in development, uncontrolled nuclear TAZ/YAP activities may drive an undifferentiated state of cancer cells (Cordenonsi et al., 2011). Thus, further knowledge of the mechanism of TAZ/YAP regulation might offer previously unappreciated insight into disease progression that will hopefully lead to new therapeutic approaches. Provided that we learn how to control the nuclear functions of TAZ/YAP, these factors also offer a potential approach for modulating tissue regeneration.

Acknowledgements

I would like to acknowledge, with apology, the wealth of high quality recent work focused on the Hippo pathway that was not cited owing to space constraints.

Competing interests

The author declares no competing financial interests

Funding

X.V. is supported by the March of Dimes Foundation and Concern Cancer Foundation.

References

Abercrombie, M. (1979). Contact inhibition and malignancy. *Nature* **281**, 259-262.

Alarcön, C., Zaromytidou, A.-I., Xi, Q., Gao, S., Yu, J., Fujisawa, S., Barlas, A., Miller, A. N., Manova-Todorova, K., Macias, M. J. et al. (2009). Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways. *Cell* **139**, 757-769.

Anakk, S., Bhosale, M., Schmidt, V. A., Johnson, R. L., Finegold, M. J. and Moore, D. D. (2013). Bile acids activate YAP to promote liver carcinogenesis. *Cell Rep.* **5**, 1060-1069.

Apte, U., Gkretsi, V., Bowen, W. C., Mars, W. M., Luo, J.-H., Donthamsetty, S., Orr, A., Monga, S. P. S., Wu, C. and Michalopoulos, G. K. (2009). Enhanced liver regeneration following changes induced by hepatocyte-specific genetic ablation of integrin-linked kinase. *Hepatology* **50**, 844-851.

Aragona, M., Panciera, T., Manfrin, A., Giulitti, S., Michielin, F., Elvassore, N., Dupont, S. and Piccolo, S. (2013). A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell* **154**, 1047-1059.

Azzolin, L., Zanconato, F., Bresolin, S., Forcato, M., Basso, G., Bicciato, S., Cordenonsi, M. and Piccolo, S. (2012). Role of TAZ as mediator of Wnt signaling. *Cell* **151**, 1443-1456.

Bai, H., Zhang, N., Xu, Y., Chen, Q., Khan, M., Potter, J. J., Nayar, S. K., Cornish, T., Alpini, G., Bronk, S. et al. (2012). Yes-associated protein regulates the hepatic response after bile duct ligation. *Hepatology* **56**, 1097-1107.

Barry, E. R., Morikawa, T., Butler, B. L., Shrestha, K., de la Rosa, R., Yan, K. S., Fuchs, C. S., Magness, S. T., Smits, R., Ogino, S. et al. (2013). Restriction of intestinal stem cell expansion and the regenerative response by YAP. *Nature* **493**, 106-110.

Basu, S., Totty, N. F., Irwin, M. S., Sudol, M. and Downward, J. (2003). Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis. *Mol. Cell* **11**, 11-23.

Beyer, T. A., Narimatsu, M., Weiss, A., David, L. and Wrana, J. L. (2013a). The TGFbeta superfamily in stem cell biology and early mammalian embryonic development. *Biochim. Biophys. Acta* **1830**, 2268-2279.

Beyer, T. A., Weiss, A., Khomchuk, Y., Huang, K., Ogunjimi, A. A., Varelas, X. and Wrana, J. L. (2013b). Switch enhancers interpret TGF-β and hippo signaling to control cell fate in human embryonic stem cells. *Cell Rep.* **5**, 1611-1624.

Bjarnadöttir, T. K., Gloriam, D. E., Hellstrand, S. H., Kristiansson, H., Fredriksson, R. and Schioth, H. B. (2006). Comprehensive repertoire and phylogenetic analysis of the G protein-coupled receptors in human and mouse. *Genomics* **88**, 263-273.

Bosl, W. J. and Li, R. (2005). Mitotic-exit control as an evolved complex system. *Cell* **121**, 325-333.

Cai, J., Zhang, N., Zheng, Y., de Wilde, R. F., Maitra, A. and Pan, D. (2010). The Hippo signaling pathway restricts the oncogenic potential of an intestinal regeneration program. *Genes Dev.* **24**, 2383-2388.

Calvo, F., Ege, N., Grande-Garcia, A., Hooper, S., Jenkins, R. P., Chaudhry, S. I., Harrington, K., Williamson, P., Moeendarbary, E., Charras, G. et al. (2013). Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. *Nat. Cell Biol.* **15**, 637-646.

Camargo, F. D., Gokhale, S., Johnnidis, J. B., Fu, D., Bell, G. W., Jaenisch, R. and Brummelkamp, T. R. (2007). YAP1 increases organ size and expands undifferentiated progenitor cells. *Curr. Biol.* **17**, 2054-2060.
Cao, X., Pfaff, S. L. and Gage, F. H. (2008). YAP regulates neural progenitor cell number via the TEA domain transcription factor. *Genes Dev.* **22**, 3320-3334.
Cappello, S., Gray, M. J., Badouel, C., Lange, S., Einsiedler, M., Srour, M., Chitayat, D., Hamdan, F. F., Jenkins, Z. A., Morgan, T. et al. (2013). Mutations in genes encoding the cadherin receptor-ligand pair DCHS1 and FAT4 disrupt cerebral cortical development. *Nat. Genet.* **45**, 1300-1308.
Chan, S. W., Lim, C. J., Chong, Y. F., Pobbati, A. V., Huang, C. and Hong, W. (2011). Hippo pathway-independent restriction of TAZ and YAP by angiomotin. *J. Biol. Chem.* **286**, 7018-7026.
Chaulk, S. G., Lattanzi, V. J., Hiemer, S. E., Fahlman, R. P. and Varelas, X. (2013). The hippo pathway effectors TAZ/YAP regulate dicer expression and miRNA biogenesis through let-7. *J. Biol. Chem.* **289**, 1886-1891.
Chen, L., Chan, S. W., Zhang, X., Walsh, M., Lim, C. J., Hong, W. and Song, H. (2010). Structural basis of YAP recognition by TEAD4 in the hippo pathway. *Genes Dev.* **24**, 290-300.
Chen, D., Sun, Y., Wei, Y., Zhang, P., Rezaeian, A. H., Teruya-Feldstein, J., Gupta, S., Liang, H., Lin, H.-K., Hung, M. C. et al. (2012). LIFR is a breast cancer metastasis suppressor upstream of the Hippo-YAP pathway and a prognostic marker. *Nat. Med.* **18**, 1511-1517.
Chung, C., Kim, T., Kim, M., Kim, M., Song, H., Kim, T.-S., Seo, E., Lee, S.-H., Kim, H., Kim, S. K. et al. (2013). Hippo-Foxa2 signaling pathway plays a role in peripheral lung maturation and surfactant homeostasis. *Proc. Natl. Acad. Sci. USA* **110**, 7732-7737.
Cockburn, K. and Rossant, J. (2010). Making the blastocyst: lessons from the mouse. *J. Clin. Invest.* **120**, 995-1003.
Cockburn, K., Biechele, S., Garner, J. and Rossant, J. (2013). The Hippo pathway member Nf2 is required for inner cell mass specification. *Curr. Biol.* **23**, 1195-1201.
Cordenonsi, M., Zanconato, F., Azzolin, L., Forcato, M., Rosato, A., Frasson, C., Inui, M., Montagner, M., Parenti, A. R., Poletti, A. et al. (2011). The hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* **147**, 759-772.
Couzens, A. L., Knight, J. D. R., Kean, M. J., Teo, G., Weiss, A., Dunham, W. H., Lin, Z.-Y., Bagshaw, R. D., Sicheri, F., Pawson, T. et al. (2013). Protein interaction network of the Mammalian hippo pathway reveals mechanisms of kinase-phosphatase interactions. *Sci. Signal.* **6**, rs15.
Das Thakur, M., Feng, Y., Jagannathan, R., Seppa, M. J., Skeath, J. B. and Longmore, G. D. (2010). Ajuba LIM proteins are negative regulators of the Hippo signaling pathway. *Curr. Biol.* **20**, 657-662.
Dong, J., Feldmann, G., Huang, J., Wu, S., Zhang, N., Comerford, S. A., Gayyed, M. F., Anders, R. A., Maitra, A. and Pan, D. (2007). Elucidation of a universal size-control mechanism in Drosophila and mammals. *Cell* **130**, 1120-1133.
Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S. et al. (2011). Role of YAP/TAZ in mechanotransduction. *Nature* **474**, 179-183.
Enger, T. B., Samad-Zadeh, A., Bouchie, M. P., Skarstein, K., Galtung, H. K., Mera, T., Walker, J., Menko, A. S., Varelas, X., Faustman, D. L. et al. (2013). The Hippo signaling pathway is required for salivary gland development and its dysregulation is associated with Sjogren's syndrome. *Lab. Invest.* **93**, 1203-1218.
Engler, A. J., Sen, S., Sweeney, H. L. and Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677-689.
Fernandez, B. G., Gaspar, P., Bras-Pereira, C., Jezowska, B., Rebelo, S. R. and Janody, F. (2011). Actin-capping protein and the hippo pathway regulate F-actin and tissue growth in Drosophila. *Development* **138**, 2337-2346.
Gao, T., Zhou, D., Yang, C., Singh, T., Penzo-Mendez, A., Maddipati, R., Tzatsos, A., Bardeesy, N., Avruch, J. and Stanger, B. Z. (2013). Hippo signaling regulates differentiation and maintenance in the exocrine pancreas. *Gastroenterology* **144**, 1543-1553, 1553 e1541.
Gee, S. T., Milgram, S. L., Kramer, K. L., Conlon, F. L. and Moody, S. A. (2011). Yes-associated protein 65 (YAP) expands neural progenitors and regulates Pax3 expression in the neural plate border zone. *PLoS ONE* **6**, e20309.
George, N. M., Day, C. E., Boerner, B. P., Johnson, R. L. and Sarvetnick, N. E. (2012). Hippo signaling regulates pancreas development through inactivation of Yap. *Mol. Cell. Biol.* **32**, 5116-5128.
von Gise, A., Lin, Z., Schlegelmilch, K., Honor, L. B., Pan, G. M., Buck, J. N., Ma, Q., Ishiwata, T., Zhou, B., Camargo, F. D. et al. (2012). YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. *Proc. Natl. Acad. Sci. USA* **109**, 2394-2399.
Gladden, A. B., Hebert, A. M., Schneeberger, E. E. and McClatchey, A. I. (2010). The NF2 tumor suppressor, Merlin, regulates epidermal development through the establishment of a junctional polarity complex. *Dev. Cell* **19**, 727-739.
Gordon, M., El-Kalla, M., Zhao, Y., Fiteih, Y., Law, J., Volodko, N., Mohamed, A., El-Kadi, A. O. S, Liu, L., Odenbach, J. et al. (2013). The tumor suppressor gene, RASSF1A, is essential for protection against inflammation -induced injury. *PLoS ONE* **8**, e75483.
Goulev, Y., Fauny, J. D., Gonzalez-Marti, B., Flagiello, D., Silber, J. and Zider, A. (2008). SCALLOPED interacts with YORKIE, the nuclear effector of the hippo tumor-suppressor pathway in Drosophila. *Curr. Biol.* **18**, 435-441.
Harvey, K. F., Zhang, X. and Thomas, D. M. (2013). The Hippo pathway and human cancer. *Nat. Rev. Cancer* **13**, 246-257.
Hau, J. C., Erdmann, D., Mesrouze, Y., Furet, P., Fontana, P., Zimmermann, C., Schmelzle, T., Hofmann, F. and Chene, P. (2013). The TEAD4-YAP/TAZ protein-protein interaction: expected similarities and unexpected differences. *Chembiochem* **14**, 1218-1225.
Heallen, T., Zhang, M., Wang, J., Bonilla-Claudio, M., Klysik, E., Johnson, R. L. and Martin, J. F. (2011). Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. *Science* **332**, 458-461.
Heallen, T., Morikawa, Y., Leach, J., Tao, G., Willerson, J. T., Johnson, R. L. and Martin, J. F. (2013). Hippo signaling impedes adult heart regeneration. *Development* **140**, 4683-4690.
Hergovich, A., Stegert, M. R., Schmitz, D. and Hemmings, B. A. (2006). NDR kinases regulate essential cell processes from yeast to humans. *Nat. Rev. Mol. Cell Biol.* **7**, 253-264.
Hilman, D. and Gat, U. (2011). The evolutionary history of YAP and the hippo/YAP pathway. *Mol. Biol. Evol.* **28**, 2403-2417.
Hirate, Y., Hirahara, S., Inoue, K.-i., Suzuki, A., Alarcon, V. B., Akimoto, K., Hirai, T., Hara, T., Adachi, M., Chida, K. et al. (2013). Polarity-dependent distribution of angiomotin localizes Hippo signaling in preimplantation embryos. *Curr. Biol.* **23**, 1181-1194.
Home, P., Saha, B., Ray, S., Dutta, D., Gunewardena, S., Yoo, B., Pal, A., Vivian, J. L., Larson, M., Petroff, M. et al. (2012). Altered subcellular localization of transcription factor TEAD4 regulates first mammalian cell lineage commitment. *Proc. Natl. Acad. Sci. USA* **109**, 7362-7367.
Hong, J.-H., Hwang, E. S., McManus, M. T., Amsterdam, A., Tian, Y., Kalmukova, R., Mueller, E., Benjamin, T., Spiegelman, B. M., Sharp, P. A. et al. (2005). TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. *Science* **309**, 1074-1078.
Hossain, Z., Ali, S. M., Ko, H. L., Xu, J., Ng, C. P., Guo, K., Qi, Z., Ponniah, S., Hong, W. and Hunziker, W. (2007). Glomerulocystic kidney disease in mice with a targeted inactivation of Wwtr1. *Proc. Natl. Acad. Sci. USA* **104**, 1631-1636.
Howell, M., Borchers, C. and Milgram, S. L. (2004). Heterogeneous nuclear ribonuclear protein U associates with YAP and regulates its co-activation of Bax transcription. *J. Biol. Chem.* **279**, 26300-26306.
Hu, J., Sun, S., Jiang, Q., Sun, S., Wang, W., Gui, Y. and Song, H. (2013). Yes-associated protein (yap) is required for early embryonic development in zebrafish (Danio rerio). *Int. J. Biol. Sci.* **9**, 267-278.
Huang, J., Wu, S., Barrera, J., Matthews, K. and Pan, D. (2005). The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila homolog of YAP. *Cell* **122**, 421-434.
Huang, W., Lv, X., Liu, C., Zha, Z., Zhang, H., Jiang, Y., Xiong, Y., Lei, Q.-Y. and Guan, K. L. (2012). The N-terminal phosphodegron targets TAZ/WWTR1 protein for SCFbeta-TrCP-dependent degradation in response to phosphatidylinositol 3-kinase inhibition. *J. Biol. Chem.* **287**, 26245-26253.
Iwasa, H., Maimaiti, S., Kuroyanagi, H., Kawano, S., Inami, K., Timalsina, S., Ikeda, M., Nakagawa, K. and Hata, Y. (2013). Yes-associated protein homolog, YAP-1, is involved in the thermotolerance and aging in the nematode Caenorhabditis elegans. *Exp. Cell Res.* **319**, 931-945.
James, M. F., Manchanda, N., Gonzalez-Agosti, C., Hartwig, J. H. and Ramesh, V. (2001). The neurofibromatosis 2 protein product merlin selectively binds F-actin but not G-actin, and stabilizes the filaments through a lateral association. *Biochem. J.* **356**, 377-386.
Jang, E. J., Jeong, H., Han, K. H., Kwon, H. M., Hong, J.-H. and Hwang, E. S. (2012). TAZ suppresses NFAT5 activity through tyrosine phosphorylation. *Mol. Cell. Biol.* **32**, 4925-4932.
Jia, J., Zhang, W., Wang, B., Trinko, R. and Jiang, J. (2003). The Drosophila Ste20 family kinase dMST functions as a tumor suppressor by restricting cell proliferation and promoting apoptosis. *Genes Dev.* **17**, 2514-2519.
Jiang, Q., Liu, D., Gong, Y., Wang, Y., Sun, S., Gui, Y. and Song, H. (2009). yap is required for the development of brain, eyes, and neural crest in zebrafish. *Biochem. Biophys. Res. Commun.* **384**, 114-119.
Justice, R. W., Zilian, O., Woods, D. F., Noll, M. and Bryant, P. J. (1995). The Drosophila tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. *Genes Dev.* **9**, 534-546.
Kanai, F., Marignani, P. A., Sarbassova, D., Yagi, R., Hall, R. A., Donowitz, M., Hisaminato, A., Fujiwara, T., Ito, Y., Cantley, L. C. et al. (2000). TAZ: a novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins. *EMBO J.* **19**, 6778-6791.
Kaneko, K. J. and DePamphilis, M. L. (2013). TEAD4 establishes the energy homeostasis essential for blastocoel formation. *Development* **140**, 3680-3690.

Kim, M., Kim, M., Lee, S., Kuninaka, S., Saya, H., Lee, H., Lee, S. and Lim, D.-S. (2013). cAMP/PKA signalling reinforces the LATS-YAP pathway to fully suppress YAP in response to actin cytoskeletal changes. *EMBO J.* **32**, 1543-1555.

Koontz, L. M., Liu-Chittenden, Y., Yin, F., Zheng, Y., Yu, J., Huang, B., Chen, Q., Wu, S. and Pan, D. (2013). The Hippo effector Yorkie controls normal tissue growth by antagonizing scalloped-mediated default repression. *Dev. Cell* **25**, 388-401.

Kwon, Y., Vinayagam, A., Sun, X., Dephoure, N., Gygi, S. P., Hong, P. and Perrimon, N. (2013). The Hippo signaling pathway interactome. *Science* **342**, 737-740.

Lamar, J. M., Stern, P., Liu, H., Schindler, J. W., Jiang, Z.-G. and Hynes, R. O. (2012). The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. *Proc. Natl. Acad. Sci. USA* **109**, E2441-E2450.

Lavado, A., He, Y., Pare, J., Neale, G., Olson, E. N., Giovannini, M. and Cao, X. (2013). Tumor suppressor Nf2 limits expansion of the neural progenitor pool by inhibiting Yap/Taz transcriptional coactivators. *Development* **140**, 3323-3334.

Lee, S. E., Frenz, L. M., Wells, N. J., Johnson, A. L. and Johnston, L. H. (2001). Order of function of the budding-yeast mitotic exit-network proteins Tem1, Cdc15, Mob1, Dbf2, and Cdc5. *Curr. Biol.* **11**, 784-788.

Lee, J.-H., Kim, T.-S., Yang, T.-H., Koo, B.-K., Oh, S.-P., Lee, K.-P., Oh, H.-J., Lee, S.-H., Kong, Y.-Y., Kim, J.-M. et al. (2008). A crucial role of WW45 in developing epithelial tissues in the mouse. *EMBO J.* **27**, 1231-1242.

Lee, K.-P., Lee, J.-H., Kim, T.-S., Kim, T.-H., Park, H.-D., Byun, J.-S., Kim, M.-C., Jeong, W.-I., Calvisi, D. F., Kim, J.-M. et al. (2010). The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. *Proc. Natl. Acad. Sci. USA* **107**, 8248-8253.

Levy, D., Adamovich, Y., Reuven, N. and Shaul, Y. (2008). Yap1 phosphorylation by c-Abl is a critical step in selective activation of proapoptotic genes in response to DNA damage. *Mol. Cell* **29**, 350-361.

Li, Z., Zhao, B., Wang, P., Chen, F., Dong, Z., Yang, H., Guan, K.-L. and Xu, Y. (2010). Structural insights into the YAP and TEAD complex. *Genes Dev.* **24**, 235-240.

Lian, I., Kim, J., Okazawa, H., Zhao, J., Zhao, B., Yu, J., Chinnaiyan, A., Israel, M. A., Goldstein, L. S. B., Abujarour, R. et al. (2010). The role of YAP transcription coactivator in regulating stem cell self-renewal and differentiation. *Genes Dev.* **24**, 1106-1118.

Lin, Y.-T., Ding, J.-Y., Li, M.-Y., Yeh, T.-S., Wang, T.-W. and Yu, J.-Y. (2012). YAP regulates neuronal differentiation through Sonic hedgehog signaling pathway. *Exp. Cell Res.* **318**, 1877-1888.

Liu, C.-Y., Zha, Z.-Y., Zhou, X., Zhang, H., Huang, W., Zhao, D., Li, T., Chan, S. W., Lim, C. J., Hong, W. et al. (2010). The hippo tumor pathway promotes TAZ degradation by phosphorylating a phosphodegron and recruiting the SCF{beta}-TrCP E3 ligase. *J. Biol. Chem.* **285**, 37159-37169.

Lorthongpanich, C., Messerschmidt, D. M., Chan, S. W., Hong, W., Knowles, B. B. and Solter, D. (2013). Temporal reduction of LATS kinases in the early preimplantation embryo prevents ICM lineage differentiation. *Genes Dev.* **27**, 1441-1446.

Lu, L., Li, Y., Kim, S. M., Bossuyt, W., Liu, P., Qiu, Q., Wang, Y., Halder, G., Finegold, M. J., Lee, J.-S. et al. (2010). Hippo signaling is a potent in vivo growth and tumor suppressor pathway in the mammalian liver. *Proc. Natl. Acad. Sci. USA* **107**, 1437-1442.

Lutolf, M. P., Gilbert, P. M. and Blau, H. M. (2009). Designing materials to direct stem-cell fate. *Nature* **462**, 433-441.

Mah, A. S., Jang, J. and Deshaies, R. J. (2001). Protein kinase Cdc15 activates the Dbf2-Mob1 kinase complex. *Proc. Natl. Acad. Sci. USA* **98**, 7325-7330.

Makita, R., Uchijima, Y., Nishiyama, K., Amano, T., Chen, Q., Takeuchi, T., Mitani, A., Nagase, T., Yatomi, Y., Aburatani, H. et al. (2008). Multiple renal cysts, urinary concentration defects, and pulmonary emphysematous changes in mice lacking TAZ. *Am. J. Physiol. Renal Physiol.* **294**, F542-F553.

McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriraju, K. and Chen, C. S. (2004). Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* **6**, 483-495.

McClatchey, A. I., Saotome, I., Ramesh, V., Gusella, J. F. and Jacks, T. (1997). The Nf2 tumor suppressor gene product is essential for extraembryonic development immediately prior to gastrulation. *Genes Dev.* **11**, 1253-1265.

Mitani, A., Nagase, T., Fukuchi, K., Aburatani, H., Makita, R. and Kurihara, H. (2009). Transcriptional coactivator with PDZ-binding motif is essential for normal alveolarization in mice. *Am. J. Respir. Crit. Care Med.* **180**, 326-338.

Mo, J.-S., Yu, F.-X., Gong, R., Brown, J. H. and Guan, K.-L. (2012). Regulation of the Hippo-YAP pathway by protease-activated receptors (PARs). *Genes Dev.* **26**, 2138-2143.

Mori, M., Triboulet, R., Mohseni, M., Schlegelmilch, K., Shrestha, K., Camargo, F. D. and Gregory, R. I. (2014). Hippo signaling regulates microprocessor and links cell-density-dependent miRNA biogenesis to cancer. *Cell* **156**, 893-906.

Morin-Kensicki, E. M., Boone, B. N., Howell, M., Stonebraker, J. R., Teed, J., Alb, J. G., Magnuson, T. R., O'Neal, W. and Milgram, S. L. (2006). Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65. *Mol. Cell. Biol.* **26**, 77-87.

Morrison, D. K. (2009). The 14-3-3 proteins: integrators of diverse signaling cues that impact cell fate and cancer development. *Trends Cell Biol.* **19**, 16-23.

Nejigane, S., Haramoto, Y., Okuno, M., Takahashi, S. and Asashima, M. (2011). The transcriptional coactivators Yap and TAZ are expressed during early Xenopus development. *Int. J. Dev. Biol.* **55**, 121-126.

Nishio, M., Hamada, K., Kawahara, K., Sasaki, M., Noguchi, F., Chiba, S., Mizuno, K., Suzuki, S. O., Dong, Y., Tokuda, M. et al. (2012). Cancer susceptibility and embryonic lethality in Mob1a/1b double-mutant mice. *J. Clin. Invest.* **122**, 4505-4518.

Nishioka, N., Yamamoto, S., Kiyonari, H., Sato, H., Sawada, A., Ota, M., Nakao, K. and Sasaki, H. (2008). Tead4 is required for specification of trophectoderm in pre-implantation mouse embryos. *Mech. Dev.* **125**, 270-283.

Nishioka, N., Inoue, K.-i., Adachi, K., Kiyonari, H., Ota, M., Ralston, A., Yabuta, N., Hirahara, S., Stephenson, R. O., Ogonuki, N. et al. (2009). The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. *Dev. Cell* **16**, 398-410.

Oh, S., Lee, D., Kim, T., Kim, T.-S., Oh, H. J., Hwang, C. Y., Kong, Y.-Y., Kwon, K.-S. and Lim, D.-S. (2009). Crucial role for Mst1 and Mst2 kinases in early embryonic development of the mouse. *Mol. Cell. Biol.* **29**, 6309-6320.

Oka, T. and Sudol, M. (2009). Nuclear localization and pro-apoptotic signaling of YAP2 require intact PDZ-binding motif. *Genes Cells* **14**, 607-615.

Oudhoff, M. J., Freeman, S. A., Couzens, A. L., Antignano, F., Kuznetsova, E., Min, P. H., Northrop, J. P., Lehnertz, B., Barsyte-Lovejoy, D., Vedadi, M. et al. (2013). Control of the hippo pathway by Set7-dependent methylation of Yap. *Dev. Cell* **26**, 188-194.

Pantalacci, S., Tapon, N. and Leopold, P. (2003). The Salvador partner Hippo promotes apoptosis and cell-cycle exit in Drosophila. *Nat. Cell Biol.* **5**, 921-927.

Paramasivam, M., Sarkeshik, A., Yates, J. R., III, Fernandes, M. J. G. and McCollum, D. (2011). Angiomotin family proteins are novel activators of the LATS2 kinase tumor suppressor. *Mol. Biol. Cell* **22**, 3725-3733.

Park, K.-S., Whitsett, J. A., Di Palma, T., Hong, J.-H., Yaffe, M. B. and Zannini, M. (2004). TAZ interacts with TTF-1 and regulates expression of surfactant protein-C. *J. Biol. Chem.* **279**, 17384-17390.

Poon, C. L. C., Zhang, X., Lin, J. I., Manning, S. A. and Harvey, K. F. (2012). Homeodomain-interacting protein kinase regulates Hippo pathway-dependent tissue growth. *Curr. Biol.* **22**, 1587-1594.

Qin, H., Blaschke, K., Wei, G., Ohi, Y., Blouin, L., Qi, Z., Yu, J., Yeh, R.-F., Hebrok, M. and Ramalho-Santos, M. (2012). Transcriptional analysis of pluripotency reveals the Hippo pathway as a barrier to reprogramming. *Hum. Mol. Genet.* **21**, 2054-2067.

Reginensi, A., Scott, R. P., Gregorieff, A., Bagherie-Lachidan, M., Chung, C., Lim, D.-S., Pawson, T., Wrana, J. and McNeill, H. (2013). Yap- and Cdc42-dependent nephrogenesis and morphogenesis during mouse kidney development. *PLoS Genet.* **9**, e1003380.

Remue, E., Meerschaert, K., Oka, T., Boucherie, C., Vandekerckhove, J., Sudol, M. and Gettemans, J. (2010). TAZ interacts with zonula occludens-1 and -2 proteins in a PDZ-1 dependent manner. *FEBS Lett.* **584**, 4175-4180.

Rock, J. M., Lim, D., Stach, L., Ogrodowicz, R. W., Keck, J. M., Jones, M. H., Wong, C. C. L., Yates, J. R., III, Winey, M., Smerdon, S. J. et al. (2013). Activation of the yeast Hippo pathway by phosphorylation-dependent assembly of signaling complexes. *Science* **340**, 871-875.

Rosenbluh, J., Nijhawan, D., Cox, A. G., Li, X., Neal, J. T., Schafer, E. J., Zack, T. I., Wang, X., Tsherniak, A., Schinzel, A. C. et al. (2012). beta-Catenin-driven cancers require a YAP1 transcriptional complex for survival and tumorigenesis. *Cell* **151**, 1457-1473.

Salah, Z., Alian, A. and Aqeilan, R. I. (2012). WW domain-containing proteins: retrospectives and the future. *Front. Biosci.* **17**, 331-348.

Sansores-Garcia, L., Bossuyt, W., Wada, K.-I., Yonemura, S., Tao, C., Sasaki, H. and Halder, G. (2011). Modulating F-actin organization induces organ growth by affecting the Hippo pathway. *EMBO J.* **30**, 2325-2335.

Schlegelmilch, K., Mohseni, M., Kirak, O., Pruszak, J., Rodriguez, J. R., Zhou, D., Kreger, B. T., Vasioukhin, V., Avruch, J., Brummelkamp, T. R. et al. (2011). Yap1 acts downstream of alpha-catenin to control epidermal proliferation. *Cell* **144**, 782-795.

Sebe-Pedros, A., Zheng, Y., Ruiz-Trillo, I. and Pan, D. (2012). Premetazoan origin of the hippo signaling pathway. *Cell Rep.* **1**, 13-20.

Silvis, M. R., Kreger, B. T., Lien, W.-H., Klezovitch, O., Rudakova, G. M., Camargo, F. D., Lantz, D. M., Seykora, J. T. and Vasioukhin, V. (2011). alpha-catenin is a tumor suppressor that controls cell accumulation by regulating the localization and activity of the transcriptional coactivator Yap1. *Sci. Signal.* **4**, ra33.

Song, H., Mak, K. K., Topol, L., Yun, K., Hu, J., Garrett, L., Chen, Y., Park, O., Chang, J., Simpson, R. M. et al. (2010). Mammalian Mst1 and Mst2 kinases play

essential roles in organ size control and tumor suppression. *Proc. Natl. Acad. Sci. USA* **107**, 1431-1436.
Stamos, J. L. and Weis, W. I. (2013). The beta-catenin destruction complex. *Cold Spring Harb. Perspect. Biol.* **5**, a007898.
Su, L. L., Ma, W. X., Yuan, J. F., Shao, Y., Xiao, W. and Jiang, S. J. (2012). Expression of Yes-associated protein in non-small cell lung cancer and its relationship with clinical pathological factors. *Chin. Med. J. (Engl.)* **125**, 4003-4008.
Sudol, M. (1994). Yes-associated protein (YAP65) is a proline-rich phosphoprotein that binds to the SH3 domain of the Yes proto-oncogene product. *Oncogene* **9**, 2145-2152.
Sudol, M. (2013). YAP1 oncogene and its eight isoforms. *Oncogene* **32**, 3922.
Sudol, M., Bork, P., Einbond, A., Kastury, K., Druck, T., Negrini, M., Huebner, K. and Lehman, D. (1995). Characterization of the mammalian YAP (Yes-associated protein) gene and its role in defining a novel protein module, the WW domain. *J. Biol. Chem.* **270**, 14733-14741.
Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676.
Tamm, C., Bower, N. and Anneren, C. (2011). Regulation of mouse embryonic stem cell self-renewal by a Yes-YAP-TEAD2 signaling pathway downstream of LIF. *J. Cell Sci.* **124**, 1136-1144.
Tang, Y., Rowe, R. G., Botvinick, E. L., Kurup, A., Putnam, A. J., Seiki, M., Weaver, V. M., Keller, E. T., Goldstein, S., Dai, J. et al. (2013). MT1-MMP-dependent control of skeletal stem cell commitment via a beta1-integrin/YAP/TAZ signaling axis. *Dev. Cell* **25**, 402-416.
Tapon, N., Harvey, K. F., Bell, D. W., Wahrer, D. C. R., Schiripo, T. A., Haber, D. A. and Hariharan, I. K. (2002). salvador Promotes both cell cycle exit and apoptosis in Drosophila and is mutated in human cancer cell lines. *Cell* **110**, 467-478.
Tian, Y., Kolb, R., Hong, J.-H., Carroll, J., Li, D., You, J., Bronson, R., Yaffe, M. B., Zhou, J. and Benjamin, T. (2007). TAZ promotes PC2 degradation through a SCFbeta-Trcp E3 ligase complex. *Mol. Cell. Biol.* **27**, 6383-6395.
Udan, R. S., Kango-Singh, M., Nolo, R., Tao, C. and Halder, G. (2003). Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. *Nat. Cell Biol.* **5**, 914-920.
Van Hateren, N. J., Das, R. M., Hautbergue, G. M., Borycki, A.-G., Placzek, M. and Wilson, S. A. (2011). FatJ acts via the Hippo mediator Yap1 to restrict the size of neural progenitor cell pools. *Development* **138**, 1893-1902.
Varelas, X., Sakuma, R., Samavarchi-Tehrani, P., Peerani, R., Rao, B. M., Dembowy, J., Yaffe, M. B., Zandstra, P. W. and Wrana, J. L. (2008). TAZ controls Smad nucleocytoplasmic shuttling and regulates human embryonic stem-cell self-renewal. *Nat. Cell Biol.* **10**, 837-848.
Varelas, X., Miller, B. W., Sopko, R., Song, S., Gregorieff, A., Fellouse, F. A., Sakuma, R., Pawson, T., Hunziker, W., McNeill, H. et al. (2010a). The Hippo pathway regulates Wnt/beta-catenin signaling. *Dev. Cell* **18**, 579-591.
Varelas, X., Samavarchi-Tehrani, P., Narimatsu, M., Weiss, A., Cockburn, K., Larsen, B. G., Rossant, J. and Wrana, J. L. (2010b). The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-beta-SMAD pathway. *Dev. Cell* **19**, 831-844.
Wang, W., Huang, J. and Chen, J. (2011). Angiomotin-like proteins associate with and negatively regulate YAP1. *J. Biol. Chem.* **286**, 4364-4370.
Wang, W., Li, X., Huang, J., Feng, L., Dolinta, K. G. and Chen, J. (2013). Defining the protein-protein interaction network of the human Hippo pathway. *Mol. Cell. Proteomics* **13**, 119-131.
Webb, C., Upadhyay, A., Giuntini, F., Eggleston, I., Furutani-Seiki, M., Ishima, R. and Bagby, S. (2011). Structural features and ligand binding properties of tandem WW domains from YAP and TAZ, nuclear effectors of the Hippo pathway. *Biochemistry* **50**, 3300-3309.
Weiss, A. and Attisano, L. (2013). The TGFbeta superfamily signaling pathway. Wiley interdisciplinary reviews. *Dev. Biol.* **2**, 47-63.
Wells, C. D., Fawcett, J. P., Traweger, A., Yamanaka, Y., Goudreault, M., Elder, K., Kulkarni, S., Gish, G., Virag, C., Lim, C. et al. (2006). A Rich1/Amot complex regulates the Cdc42 GTPase and apical-polarity proteins in epithelial cells. *Cell* **125**, 535-548.
Wu, S., Huang, J., Dong, J. and Pan, D. (2003). hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts. *Cell* **114**, 445-456.
Wu, S., Liu, Y., Zheng, Y., Dong, J. and Pan, D. (2008). The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway. *Dev. Cell* **14**, 388-398.
Xin, M., Kim, Y., Sutherland, L. B., Qi, X., McAnally, J., Schwartz, R. J., Richardson, J. A., Bassel-Duby, R. and Olson, E. N. (2011). Regulation of insulin-like growth factor signaling by Yap governs cardiomyocyte proliferation and embryonic heart size. *Sci. Signal.* **4**, ra70.
Xin, M., Kim, Y., Sutherland, L. B., Murakami, M., Qi, X., McAnally, J., Porrello, E. R., Mahmoud, A. I., Tan, W., Shelton, J. M. et al. (2013). Hippo pathway effector Yap promotes cardiac regeneration. *Proc. Natl. Acad. Sci. USA* **110**, 13839-13844.
Yagi, R., Kohn, M. J., Karavanova, I., Kaneko, K. J., Vullhorst, D., DePamphilis, M. L. and Buonanno, A. (2007). Transcription factor TEAD4 specifies the trophectoderm lineage at the beginning of mammalian development. *Development* **134**, 3827-3836.
Ye, F. and Zhang, M. (2013). Structures and target recognition modes of PDZ domains: recurring themes and emerging pictures. *Biochem. J.* **455**, 1-14.
Yi, C., Shen, Z., Stemmer-Rachamimov, A., Dawany, N., Troutman, S., Showe, L. C., Liu, Q., Shimono, A., Sudol, M., Holmgren, L. et al. (2013). The p130 isoform of angiomotin is required for Yap-mediated hepatic epithelial cell proliferation and tumorigenesis. *Sci. Signal.* **6**, ra77.
Yim, H., Sung, C. K., You, J., Tian, Y. and Benjamin, T. (2011). Nek1 and TAZ interact to maintain normal levels of polycystin 2. *J. Am. Soc. Nephrol.* **22**, 832-837.
Yin, F., Yu, J., Zheng, Y., Chen, Q., Zhang, N. and Pan, D. (2013). Spatial organization of Hippo signaling at the plasma membrane mediated by the tumor suppressor Merlin/NF2. *Cell* **154**, 1342-1355.
Young, R. A. (2011). Control of the embryonic stem cell state. *Cell* **144**, 940-954.
Yu, F.-X., Zhao, B., Panupinthu, N., Jewell, J. L., Lian, I., Wang, L. H., Zhao, J., Yuan, H., Tumaneng, K., Li, H. et al. (2012). Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling. *Cell* **150**, 780-791.
Yu, F.-X., Zhang, Y., Park, H. W., Jewell, J. L., Chen, Q., Deng, Y., Pan, D., Taylor, S. S., Lai, Z.-C. and Guan, K.-L. (2013). Protein kinase A activates the Hippo pathway to modulate cell proliferation and differentiation. *Genes Dev.* **27**, 1223-1232.
Zaidi, S. K., Sullivan, A. J., Medina, R., Ito, Y., van Wijnen, A. J., Stein, J. L., Lian, J. B. and Stein, G. S. (2004). Tyrosine phosphorylation controls Runx2-mediated subnuclear targeting of YAP to repress transcription. *EMBO J.* **23**, 790-799.
Zender, L., Spector, M. S., Xue, W., Flemming, P., Cordon-Cardo, C., Silke, J., Fan, S.-T., Luk, J. M., Wigler, M., Hannon, G. J. et al. (2006). Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. *Cell* **125**, 1253-1267.
Zhang, L., Ren, F., Zhang, Q., Chen, Y., Wang, B. and Jiang, J. (2008). The TEAD/TEF family of transcription factor Scalloped mediates Hippo signaling in organ size control. *Dev. Cell* **14**, 377-387.
Zhang, H., Liu, C.-Y., Zha, Z.-Y., Zhao, B., Yao, J., Zhao, S., Xiong, Y., Lei, Q.-Y. and Guan, K.-L. (2009). TEAD transcription factors mediate the function of TAZ in cell growth and epithelial-mesenchymal transition. *J. Biol. Chem.* **284**, 13355-13362.
Zhang, N., Bai, H., David, K. K., Dong, J., Zheng, Y., Cai, J., Giovannini, M., Liu, P., Anders, R. A. and Pan, D. (2010). The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. *Dev. Cell* **19**, 27-38.
Zhang, H., Pasolli, H. A. and Fuchs, E. (2011). Yes-associated protein (YAP) transcriptional coactivator functions in balancing growth and differentiation in skin. *Proc. Natl. Acad. Sci. USA* **108**, 2270-2275.
Zhao, B., Wei, X., Li, W., Udan, R. S., Yang, Q., Kim, J., Xie, J., Ikenoue, T., Yu, J., Li, L. et al. (2007). Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev.* **21**, 2747-2761.
Zhao, B., Ye, X., Yu, J., Li, L., Li, W., Li, S., Yu, J., Lin, J. D., Wang, C.-Y., Chinnaiyan, A. M. et al. (2008). TEAD mediates YAP-dependent gene induction and growth control. *Genes Dev.* **22**, 1962-1971.
Zhao, B., Li, L., Tumaneng, K., Wang, C.-Y. and Guan, K.-L. (2010). A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF(beta-TRCP). *Genes Dev.* **24**, 72-85.
Zhao, B., Li, L., Lu, Q., Wang, L. H., Liu, C.-Y., Lei, Q. and Guan, K.-L. (2011). Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein. *Genes Dev.* **25**, 51-63.
Zhou, D., Conrad, C., Xia, F., Park, J.-S., Payer, B., Yin, Y., Lauwers, G. Y., Thasler, W., Lee, J. T., Avruch, J. et al. (2009). Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. *Cancer Cell* **16**, 425-438.
Zhou, D., Zhang, Y., Wu, H., Barry, E., Yin, Y., Lawrence, E., Dawson, D., Willis, J. E., Markowitz, S. D., Camargo, F. D. et al. (2011a). Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance. *Proc. Natl. Acad. Sci. USA* **108**, E1312-E1320.
Zhou, Z., Hao, Y., Liu, N., Raptis, L., Tsao, M.-S. and Yang, X. (2011b). TAZ is a novel oncogene in non-small cell lung cancer. *Oncogene* **30**, 2181-2186.