HHS Public Access

Author manuscript

Cell Signal. Author manuscript; available in PMC 2024 October 01.

Published in final edited form as:

Cell Signal. 2023 October ; 110: 110846. doi:10.1016/j.cellsig.2023.110846.

Ca^{2+} Signaling and the Hippo Pathway: Intersections in Cellular Regulation

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Abstract

The Hippo signaling pathway is a master regulator of organ size and tissue homeostasis. Hippo integrates a broad range of cellular signals to regulate numerous processes, such as cell proliferation, differentiation, migration and mechanosensation. Ca^{2+} is a fundamental second messenger that modulates signaling cascades involved in diverse cellular functions, some of which are also regulated by the Hippo pathway. Studies published over the last five years indicate that Ca^{2+} can influence core Hippo pathway components. Nevertheless, comprehensive understanding of the crosstalk between Ca^{2+} signaling and the Hippo pathway, and possible mechanisms through which Ca^{2+} regulates Hippo, remain to be elucidated. In this review, we summarize the multiple intersections between Ca^{2+} and the Hippo pathway and address the biological consequences.

Keywords

Calcium; Calmodulin; S100; Hippo; Signaling; YAP

1. Introduction

The Hippo pathway and the Ca^{2+} signaling network have crucial roles in regulating diverse physiological processes, including cell proliferation, differentiation, and apoptosis. The highly conserved Hippo pathway [1] controls organ size and tissue homeostasis by regulating cell growth and proliferation [2]. Ca^{2+} influences a wide range of signaling

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Author contributions

S.S., L.T., D.B.S. conceptualization; S.S. and L.T. formal analysis, visualization, writing – original draft; D.B.S. writing – review and editing, supervision, funding acquisition.

Declaration of Competing Interest

Authors declare no competing interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

cascades via a complex network to control diverse cellular processes, ranging from muscle contraction and neurotransmitter release to gene expression [3]. The recent observations that Ca^{2+} regulates Hippo signaling has stimulated investigation into the interactions between Ca^{2+} and the Hippo pathway. In this review, we summarize the evidence and discuss potential mechanisms underlying the regulation of Hippo by Ca^{2+}.

2. Hippo signaling

Hippo regulates expression of genes that modulate numerous processes, including cell polarity, adhesion, nutrient sensing, proliferation, migration, anti-apoptosis, response to stress and self-renewal [4]. Unlike many other signaling pathways, Hippo is not controlled by a dedicated receptor; rather it functions as a cellular hub, constantly receiving signals from diverse receptors and stimuli [5].

The intracellular core components of the mammalian Hippo pathway comprise i) adaptor protein MOB1 and Salvador (SAV1); ii) a kinase module consisting of sterile 20-like protein kinase 1 and 2 (MST1, MST2) and large tumor suppressor kinase 1 and 2 (LATS1, LATS2); and iii) transcriptional co-activators Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) [5] (Figure 1).

Posttranslational modifications, predominantly phosphorylation, control the abundance and subcellular localization of YAP/TAZ, which ultimately determine their activity [6]. When the Hippo pathway is ON, SAV1 enables MST to catalyze phosphorylation of LATS, and MOB1 binds to LATS to enhance its catalytic activity [7] (Figure 1B). Active LATS directly phosphorylates multiple sites on YAP/TAZ [2]. When phosphorylated, YAP/TAZ are either sequestered in the cytoplasm or are ubiquitinated and undergo degradation in the proteasome [2]. Thus, phosphorylated YAP/TAZ do not enter the nucleus and are unable to stimulate gene transcription. When the Hippo pathway is OFF, hypophosphorylated YAP/TAZ translocate into the nucleus, where they induce gene expression by binding to the TEA domain (TEAD) family of transcription factors (Figure 1A) [8]. It is important to note that both *in vitro* and *in vivo* YAP/TAZ undergo rapid and dynamic phosphorylation-dephosphorylation states, which determine the spatial and temporal regulation of the Hippo pathway [9, 10]. The mechanisms mentioned above describe the canonical core Hippo pathway; however recent evidence has revealed additional regulatory mechanisms for YAP/TAZ that depend on both the cell type and microenvironment [11].

3. Ca^{2+} signaling

Ca^{2+} is a highly versatile messenger that modulates diverse signaling cascades and impacts nearly every aspect of cellular life [12]. The Ca^{2+} signaling network controls processes, such as embryonic development, cell differentiation, migration, apoptosis, and tumorigenesis [3].

The specific components of Ca^{2+} signaling vary among cell types, depending on the function of the cell [13]. In excitable cells, such as striated muscle and neurons, short-term Ca^{2+} spikes trigger rapid responses, while slow Ca^{2+} signals in non-excitable cells control functions, such as transcription and cell division [3]. The dynamics of Ca^{2+} signaling can be attributed to different types of receptors, sources of Ca^{2+}, Ca^{2+} transporters, Ca^{2+}

buffers, as well as Ca^{2+}-binding proteins (CaBPs) [13]. Thus, cells have evolved molecular machinery to decode the changes in intracellular free Ca^{2+} concentrations ($[Ca^{2+}]_i$) into distinct signaling cascades using Ca^{2+}-sensitive signaling molecules.

The large gradient of Ca^{2+} concentrations across the plasma membrane (1 mM in the extracellular space and 100 nM inside the cell) necessitates that $[Ca^{2+}]_i$ be tightly regulated by a delicate balance between "ON" mechanisms that promote Ca^{2+} entry into the cytosol and "OFF" mechanisms that remove Ca^{2+} from the cytosol (Figure 2). The Ca^{2+} signaling toolkit includes CaBPs (e.g., calmodulin), Ca^{2+}-buffering proteins (e.g., parvalbumin), pumps (e.g, Ca^{2+}-ATPase), channels (e.g., transient receptor potential (TRP) channels and Piezo1) and exchangers (e.g., Na^+/Ca^{2+} exchanger) [14] (Figure 2). The regulation of Ca^{2+} is highly intricate and has been comprehensively addressed in several excellent reviews [12–15]. Here, we provide a brief overview of Ca^{2+} signaling that focuses on the main components which may influence Hippo signaling.

Intracellular Ca^{2+} acts as a messenger to modulate numerous Ca^{2+}-sensitive processes. An increase in $[Ca^{2+}]_i$ is induced by a plethora of mechano-electrochemical and biological stimuli, including, but not limited to, membrane depolarization, mechanical stretch, extracellular agonists, and intracellular messengers. These stimuli generate Ca^{2+}-mobilizing signals (ON mechanisms), which increase $[Ca^{2+}]_i$ by promoting the entry of Ca^{2+} either from the extracellular environment or by the release of Ca^{2+} from intracellular stores, primarily the endoplasmic reticulum (ER), but also the Golgi, mitochondria, and lysosomes (Figure 2). Moreover, agonists such as growth factors, bind to transmembrane G protein-coupled receptors (GPCRs) or receptor tyrosine kinases (not shown), to generate intracellular second messengers, including inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). The latter can activate receptor-operated channels, enabling the influx of Ca^{2+} across the plasma membrane, while the binding of IP_3 to IP_3 receptors (IP_3R) located on the ER stimulates the release of Ca^{2+} from ER stores (Figure 2). As a consequence of depletion of Ca^{2+} from the ER, Ca^{2+} influx is triggered to replenish Ca^{2+} stores or to maintain $[Ca^{2+}]_i$. This feedback mechanism, known as store-operated Ca^{2+} entry (SOCE), is mediated by the coupling of Orai at the plasma membrane and STIM on the ER [16] (Figure 2). SOCE is a major mechanism for maintaining Ca^{2+} homeostasis in non-excitable cells [17]. The increased $[Ca^{2+}]_i$ influences a wide array of cellular processes by binding to and altering localization, interaction, and function of CaBPs [14].

Ca^{2+} signaling is terminated by removal of $[Ca^{2+}]_i$ from the cytosol (Figure 2, OFF). This occurs through channels, pumps and exchangers, which either extrude Ca^{2+} to the extracellular space or sequester Ca^{2+} in internal stores, as well as by Ca^{2+}-buffering proteins, which bind to and sequester free Ca^{2+}. As a result, only a small fraction of the Ca^{2+} that enters the cytosol remains as $[Ca^{2+}]_i$.

4. Ca^{2+}-binding proteins modulate Hippo signaling

Ca^{2+} ions exert their secondary messenger functions in part by binding to CaBPs. CaBPs are characterized by Ca^{2+}-binding motifs that can reversibly bind Ca^{2+}, allowing transitions between Ca^{2+}-free (apo) and Ca^{2+}-bound states [18]. CaBPs are conveniently divided into

(i) buffering proteins that regulate $[Ca^{2+}]_i$ by sequestering Ca^{2+}; *(ii)* transport proteins that shuttle Ca^{2+} across the membranes of the organelles; and *(iii)* signaling proteins that interact in a Ca^{2+}-regulated manner with selected targets to influence their activity, thereby converting changes in $[Ca^{2+}]_i$ into specific signaling outcomes [18]. The S100 proteins and calmodulin (CaM) are signaling CaBPs that contain two and four helix-loop-helix EF-hand Ca^{2+}-binding motifs, respectively. Binding of Ca^{2+} to these motifs induces a conformational change, leading to exposure of hydrophobic surfaces that mediate regulatory interactions with selected targets [18]. Although most published literature reports signaling functions for the Ca^{2+}-bound forms of S100 and CaM, their apo counterparts also regulate the activity of selected proteins [19, 20]. Via these Ca^{2+}-modulated interactions, S100 and CaM coordinate various essential cellular processes, ranging from cell growth and differentiation to cell cycle regulation and DNA transcription [18]. The CaBPs S100A1, S100B, and CaM have been documented to modulate Hippo signaling through direct binding to Hippo core proteins [21–23]. Moreover, studies identified that the S100 proteins S100A7, S100A8, S100A9, and S100A14 modulate Hippo signaling by influencing the activity of Hippo regulators [24–26]. Both inhibitory (S100A1, S100A8, S100A9, CaM) [22, 26, 27] and activating (S100B, S100A7, S100A14, CaM) [21, 24, 25, 28] effects on Hippo signaling have been reported, which illustrates the complex, tissue-specific crosstalk between Ca^{2+} and Hippo. It is important to note that, except for Ca^{2+}/CaM and Ca^{2+}/S100B, these studies did not distinguish whether the influence on Hippo activation was produced by the apo- and/or Ca^{2+}-bound proteins.

4.1 S100

4.1.1 Hippo inhibitory S100 proteins—S100A1 binds to LATS1 kinase in hepatocellular carcinoma cells [22]. Silencing S100A1 increases YAP phosphorylation, which accelerates YAP degradation and reduces its nuclear co-transcriptional activity. Interestingly, LATS1 knockdown reduces S100A1-mediated proliferation of liver carcinoma cells [22]. Consistent with this study, S100A1 knockdown in thyroid carcinoma cells increases YAP phosphorylation, which correlates with reduced cell proliferation and increased apoptosis [29]. In breast carcinoma cells, the long non-coding RNA FOXD2-AS1 upregulates S100A1 expression, likely by sponging S100A1 micro-RNAs, leading to S100A1-mediated reduction of LATS1 kinase activity and increased cell proliferation, migration, and invasion [30]. Together, these findings indicate that S100A1 binding to LATS1 reduces its kinase activity towards YAP, which increases oncogenic YAP nuclear activity (Figure 3A).

The S100A8/S100A9 heterocomplex also suppresses Hippo activation [26]. Secreted extracellular S100A8/S100A9 can bind to the receptor for advanced glycation end-products (RAGE) [31]. Exposure of triple-negative breast carcinoma cells to S100A8/S100A9 activates RAGE, which stimulates focal adhesion kinase (FAK). By initiating formation of focal adhesions, FAK inhibits MST1, which ultimately increases nuclear YAP (Figure 3A). S100A8/S100A9 stimulation promotes triple-negative breast carcinoma cell growth and migration, suggesting that it may have carcinogenic properties by inactivating Hippo through RAGE-FAK [26].

4.1.2 Hippo activating S100 proteins—S100A7 is an activator of Hippo [24]. Overexpression of S100A7 in epidermoid squamous carcinoma cells increases phosphorylation of LATS1 and YAP, and decreases YAP cellular abundance. S100A7 knockdown produces the opposite effect, which confirms that S100A7 inhibits YAP expression and activity. The proposed mechanism is that S100A7 activates the NF-κB pathway, thereby repressing expression of the transcription factor ΔNp63. ΔNp63 decreases MST1 and stimulates YAP expression [24]. Hence, by downregulating ΔNp63, S100A7 increases Hippo activation by MST1 and decreases YAP abundance [24] (Figure 3B). YAP phosphorylation is enhanced in squamous cell carcinoma tissues with increased expression of S100A7. Moreover, overexpression of S100A7 prevents YAP-induced apoptosis during chemotherapy [24]. Although the Hippo pathway is most commonly perceived as a tumor suppressor, these findings intimate that Hippo activation by S100A7 through NF-κB/ΔNp63 may promote carcinogenesis.

S100A14 also stimulates Hippo activation. Overexpression of S100A14 in prostate cancer cells increases phosphorylation of MST1, LATS1, and YAP, which correlates with upregulated expression of fat atypical cadherin-1 (FAT1) [25]. The intracellular region of FAT1 assembles a Hippo signalosome comprising MST1 and LATS1, and coordinates MST1 activation by the Tao kinases [32]. Together, these findings suggest that S100A14 activates Hippo by upregulating FAT1 (Figure 3B). In mice, S100A14 overexpression increases both FAT1 expression and YAP phosphorylation, which correlates with reduced prostate carcinoma growth. These observations led the authors to speculate that S100A14 is a tumor suppressor in prostate carcinoma through FAT1-mediated Hippo activation [25].

The nuclear Dbf2-related kinases 1 and 2 (Ndr1 and Ndr2), members of the same kinase family as LATS1, are additional Hippo proteins that can inhibit YAP activity by catalyzing its phosphorylation [33]. The Ca^{2+}-bound form of S100B binds to both Ndr1 and Ndr2, which stimulates their activating autophosphorylation [23, 28]. Although not experimentally demonstrated, the authors speculate that Ca^{2+}/S100B could inactivate YAP via the Ndr kinases (Figure 3B). S100B and S100P interact with the scaffold protein IQGAP1 [34, 35], which downregulates YAP co-transcriptional activity [36]. It is therefore feasible that YAP inhibition by S100 proteins could be coordinated by IQGAP1 scaffolding.

4.1.3 Hippo-mediated expression of S100 proteins—Reciprocally, the expression of several S100 proteins is regulated by Hippo in carcinoma cell lines, implying that Hippo signaling influences Ca^{2+}-regulated, S100-mediated signaling. Hippo activation stimulates expression of both S100A8 and S100A9 in squamous carcinoma cells via the TEAD1 transcription factor [37]. Similarly, Hippo activation induces TEAD1-mediated expression of S100A7 in lung carcinoma, epidermoid carcinoma, and squamous carcinoma cells [38–40]. These observations suggest that nuclear YAP and TEAD1, most commonly perceived as transcriptional co-activators, also repress transcription of selected target genes, as previously reported [41]. In contrast, YAP stimulates S100A4 expression [42]. Because S100A4, S100A7, S100A8, and S100A9 promote cell proliferation, invasion, and/or carcinoma phenotypic transition, these studies imply that Hippo-regulated expression of S100 proteins drives carcinogenesis [37–40].

4.2 Calmodulin

Unlike the S100 proteins that are expressed in a cell- and tissue-specific manner, CaM is ubiquitous [18]. Ca^{2+}/CaM was recently documented to bind LATS1 and YAP [21]. Ca^{2+} initiates the formation of a ternary complex of pure CaM:LATS1:YAP *in vitro*. The interaction of CaM with LATS1 and YAP was confirmed in HeLa cells. Functionally, CaM antagonism decreases YAP phosphorylation, increases YAP:TEAD1 interactions in the nucleus, and promotes YAP co-transcriptional activity. Moreover, Ca^{2+}/CaM directly stimulates LATS1 kinase activity [21]. Together, these observations demonstrate that Ca^{2+}/CaM stimulates LATS1-catalyzed phosphorylation of YAP, which increases YAP retention in the cytoplasm to inhibit its nuclear activity (Figure 3C). Ca^{2+}/CaM also binds Ndr1 and Ndr2 kinases [28] (Figure 3C), implying that it may regulate YAP phosphorylation by acting on several upstream kinases.

In contrast to the results summarized above, Ca^{2+}/CaM signaling was recently observed to increase the nuclear co-transcriptional activity of YAP in mouse intestinal organoids [27]. In this system, activation of non-canonical Wnt signaling triggers the release of Ca^{2+} from intracellular stores into the cytosol, leading to increased binding of Ca^{2+} to CaM and subsequent activation of the Ca^{2+}/CaM-dependent kinases CaMKK and CaMKII. Chemical inhibition of Ca^{2+}/CaM, CaMKK, or CAMKII suppresses YAP nuclear translocation induced by non-canonical Wnt. Moreover, inhibition of Ca^{2+}/CaM signaling reduces the cystic morphology of mouse intestinal organoids that is observed upon activation of non-canonical Wnt (Figure 3D). These results led the authors to postulate that Ca^{2+}/CaM signaling in intestinal cells with active non-canonical Wnt upregulates YAP nuclear activity, with consequences on tissue morphology [27].

There is evidence for bidirectional crosstalk between CaM and Hippo. CaM is encoded by three genes, *CALM1, CALM2*, and *CALM3* [43], one of which (*CALM2*) was identified as a YAP target gene in colon carcinoma cells [44]. YAP and PRDI-BF- & RIZ-homology-domain-containing transcriptional regulator, PRDM14, are enriched at the *CALM2* promoter. YAP suppression decreases CaM mRNA and protein levels [44]. Analysis in a mouse xenograft model indicates that PRDM14 rescues YAP suppression to promote colon carcinoma progression and maintain YAP-mediated growth in a process which is dependent on *CALM2* expression. Taken together, these findings emphasize the intricate interplay and feedback between Ca^{2+}/CaM and the Hippo pathway.

5. Increased intracellular Ca^{2+} enhances YAP nuclear translocation

Activation of mechanosensitive Ca^{2+} channels, including stretch activated channels (e.g., Piezo1), transient receptor potential (TRP) channels (e.g., TRPV), and gap junction channels (e.g., connexins), rapidly increases $[Ca^{2+}]_i$ and triggers Ca^{2+}-dependent signaling [45] in response to intrinsic and extrinsic mechanical cues, such as stretching, shear forces, cell-cell contact, substrate stiffness, increased extracellular viscosity, and contractile forces. Activation of the channels by mechanical cues enhances YAP nuclear localization and /or activity through increased $[Ca^{2+}]_i$ [46–49] (Figure 4A).

5.1 Piezo1

Piezo1 is activated by traction forces and mechanical tension [50]. Ca^{2+} influx through Piezo1 occurs early after mechanical stimulation of cells [51]. This Ca^{2+} influx results in RhoA-induced stress fiber formation [52, 53]. In a separate study of stress fiber formation, constitutively active RhoA enhanced YAP nuclear localization and increased the expression of YAP target genes [54]. Activation of Piezo1 in response to mechanical forces promotes YAP nuclear localization (Figure 4A), while knocking down Piezo1 prevents this translocation [49]. Moreover, pharmacological stimulation of Piezo1 by Yoda1 increases $[Ca^{2+}]_i$ and induces YAP-mediated transcription of genes that are related to mechanical stress [50]. Collectively, these data suggest that Piezo1 enhances YAP nuclear localization via Ca^{2+}-stimulated activation of RhoA.

Reciprocally, the expression of an active YAP mutant construct in oral squamous carcinoma cells significantly increases Piezo1 mRNA levels, implying that Piezo1 is a transcriptional target of YAP [55]. These observations raise the possibility of a positive feedback mechanism between Ca^{2+} signaling by Piezo1 and the Hippo pathway, whereby active Piezo1 induces YAP nuclear translocation, which potentially enhances both expression of and signaling by Piezo1.

5.2 Transient Receptor Potential (TRP) channels

TRPV4 is a Ca^{2+}-permeable channel and a member of a widely expressed subfamily of TRPs that crosstalks with integrins and adhesion molecules to transmit mechanical cues [56]. In response to mechanical forces, TRPV4 causes adhesion, spreading, and morphological changes of fibroblasts [57]. Knockdown or chemical inhibition of TRPV4 decreases $[Ca^{2+}]_i$, which suppresses actin re-organization resulting from RhoA/ROCK [48, 58]. Consistent with the role of the RhoA/ROCK/actin network in inhibiting the Hippo kinase LATS1/2 [59, 60], TRPV4-mediated Ca^{2+} influx initiates YAP/TAZ nuclear translocation [61, 62] (Figure 4A). Functionally, knockout or chemical antagonism of TRPV4 suppresses cytoskeletal remodeling and prevents mechanically-induced YAP/TAZ nuclear translocation [61, 62].

Actin polymerization is a priming signal for YAP/TAZ nuclear localization [63]. It is tempting to speculate that TRPV4 could also modulate YAP/TAZ through direct regulation of actin dynamics in response to Ca^{2+} signaling. The C-terminal domain of TRPV4 contains multiple actin and tubulin binding sites [64, 65]. TRPV4 activation disassembles microtubules, while promoting actin polymerization [64]. Moreover, the C-terminal region of TRPV4 binds to CaM [66, 67]. Ca^{2+}/CaM regulates actin remodeling by activating CaMKII and dissociating it from actin bundles to allow actin re-organization [68]. Overall, the Ca^{2+} influx induced by TRPV4 stimulates YAP/TAZ subcellular localization via RhoA activation and likely via direct action on actin.

5.3 Connexins

Connexons, the structural components of gap junctions, are composed of two sets of hexameric connexin hemichannels, one set contributed by each adjacent cell. Connexin hemichannels align on the plasma membrane, creating a pore between the cytoplasm

of adjacent cells, which enables the direct transfer of cations (Na^+, K^+, and Ca^{2+}), small molecules and metabolites [69]. At the plasma membrane, connexins associate with integrins and focal adhesions to transmit mechanical information to cells and tissues [70]. Connexin43 (Cx43), a widely expressed and well-studied member of the connexin family, participates in Ca^{2+} signaling [71]. siRNA depletion of Cx43 significantly reduces $[Ca^{2+}]_i$, while overexpression of Cx43 enhances Ca^{2+} influx and increases $[Ca^{2+}]_i$ [71, 72].

Cx43 modulates the Hippo pathway [46]. In mammary epithelial tissue Cx43 hemichannels are opened in cells located in regions of high mechanical stress. Channel opening promotes membrane depolarization, leading to nuclear translocation of YAP/TAZ and increased cell proliferation (Figure 4A) [46]. Selective inhibition of Cx43 reduces both nuclear translocation of YAP/TAZ and cell proliferation. Taken together, these findings suggest that nuclear localization of YAP/TAZ in response to high mechanical stress is contingent on Cx43 hemichannel function.

$[Ca^{2+}]_i$ modulates Cx43 gating. Increased $[Ca^{2+}]_i$ enhances Ca^{2+}/CaM binding to the C-terminal region of Cx43. This closes the channel and attenuates its permeability [73–75], and also inhibits phosphorylation of Cx43 by Src and Erk1/2 [74]. Cx43 phosphorylation influences its interaction with binding partners, such as the actin binding protein, drebrin [76]. Interestingly, both Cx43 [77] and Ca^{2+}/CaM [21] bind directly to YAP. Moreover, Ca^{2+}/CaM binds to LATS1 and stimulates LATS1 kinase activity [21]. Thus, Ca^{2+}/CaM could participate in the response to mechanical cues by integrating signals from Cx43 to the Hippo pathway, providing a potential mechanism for coordinating mechanical and molecular signaling.

6. Increased intracellular Ca^{2+} suppresses YAP nuclear localization

In contrast to the studies mentioned in the previous section, increased $[Ca^{2+}]_i$ has also been documented to suppress YAP and TAZ nuclear co-transcriptional activity. For example, elevation of $[Ca^{2+}]_i$ through chemical stimulation of SOCE in glioblastoma cells increases YAP/TAZ phosphorylation and cytoplasmic retention [78]. The increased $[Ca^{2+}]_i$ promotes the association of PKC-βII with F-actin, and activates PKC-βII. In turn, PKC-βII activates MST1/2 and LATS1/2 Hippo kinases, resulting in YAP/TAZ inhibition [78] (Figure 4B). Another study reports that increasing $[Ca^{2+}]_i$ by incubating cells with the Ca^{2+} ionophore A23187 leads to phosphorylation and cytoplasmic retention of YAP through activation of PKC-α [79]. Whether the PKC kinases directly phosphorylate the Hippo proteins or require intermediate effectors remains to be determined.

Chemically-induced SOCE also activates Hippo via Merlin, a membrane-associated protein that can activate MST1/2 and recruit LATS1/2 [80, 81]. Induction of SOCE by thapsigargin triggers both dephosphorylation and NEDD4L-mediated ubiquitination of Merlin. Merlin ubiquitination promotes its interaction with LATS1, which increases LATS1 activity [81] (Figure 4B). Additionally, there is a potential role for endolysosomal Ca^{2+} release in regulating YAP/TAZ activity brought about by SOCE. Knockdown of two-pore channel2 (TPC2), which causes Ca^{2+} release from endolysosomal compartments, significantly reduces the mRNA and protein levels of Orai1, a plasma membrane SOCE channel, which correlates

with increased YAP/TAZ co-transcriptional activity [82]. Together, these observations further suggest that SOCE-mediated increase of $[Ca^{2+}]_i$ inhibits YAP/TAZ.

7. Complexity of Ca^{2+}-Hippo crosstalk

A recent study investigated YAP cytoplasmic-nuclear shuttling in response to Ca^{2+} in breast carcinoma cells [83]. Wounding a cell monolayer transiently increased $[Ca^{2+}]_i$ and induced YAP nuclear accumulation within 1 min. Nuclear depletion of YAP then occurred over 20 min, followed by slow nuclear re-accumulation ~3 h after the initial stimulation. In contrast, increasing $[Ca^{2+}]_i$ by pharmacological induction of SOCE causes YAP accumulation in the cytoplasm for 25 min, followed by slow nuclear enrichment over 50 min [83]. These data indicate that YAP spatial dynamics are influenced by the nature of the Ca^{2+} stimulus and emphasize the complexity of the effect of Ca^{2+} on the Hippo pathway.

In human adipose-derived stem cells, Ca^{2+} influx induced by mechanical stretching at 37°C significantly enhances YAP nuclear localization, which promotes cell proliferation [84]. In contrast, stretching while simultaneously reducing temperature from 37°C to 10°C suppresses YAP nuclear translocation and cell proliferation, despite a significant increase in $[Ca^{2+}]_i$. Chemical inhibition of SOCE restores YAP nuclear activity at the low temperature [84]. Together, these studies emphasize that Ca^{2+}-Hippo crosstalk is influenced by several parameters, including temperature and the stimulus eliciting Ca^{2+} influx.

Actin remodeling impacts Hippo activation, thereby affecting YAP subcellular localization. Ca^{2+} influences actin both by binding directly to actin and via CaBP. A potential mechanism for regulation of YAP by Ca^{2+} is through Ca^{2+}-mediated actin reset (CaAR) [85, 86]. In this process, Ca^{2+} triggers rapid disassembly of cortical actin, which is followed by the translocation of actin filaments to the endoplasmic reticulum and perinuclear rim [85, 86]. Depletion of formin-2 impairs CaAR and decreases phosphorylation of LATS and YAP [87]. Therefore, CaAR activates Hippo. However, actin remodeling via a Ca^{2+}-mediated increase in active RhoA induces nuclear translocation of YAP [88]. This illustrates that the effects of cytoskeleton components on YAP/TAZ are complex and likely are regulated by both the dynamics and subcellular site of actin remodeling.

8. Ca^{2+} and Hippo crosstalk in health and disease

All the studies described above were carried out in cultured cell lines. The potential implications of the crosstalk between Ca^{2+} and Hippo in physiology and pathology are illustrated by *in vivo* studies using tissues from mouse models and human patients.

8.1 Skeletal remodeling

The skeleton is remodeled by removal of damaged bone by osteoclasts and synthesis of collagen by osteoblasts to form new bone tissue. Disruption of skeletal remodeling can lead to pathological conditions, such as osteoporosis. Patients with osteoporosis have decreased amounts of Piezo1, which is expressed in both osteoclasts and osteoblasts, in their bones [89]. Similarly, as described above, deletion of Piezo1 reduces bone mass in mice, while the Piezo1 agonist, Yoda1, increases mouse bone mass by promoting YAP

transcriptional activity [50]. In a conditional knockout mouse, osteoblast-specific loss of Piezo1 results in marked bone resorption and osteoporosis [90]. Loss of Piezo1 from the mouse osteoblasts significantly decreases YAP nuclear localization, thus suppressing YAP-mediated transcription of collagen. Moreover, knockdown of YAP by shRNA decreases collagen expression, while pharmacological inhibition of MST1/2 increases collagen abundance in stem cells [90]. Based on these findings, the authors propose that collagen expression induced by YAP provides protection against mechanical stress in bone tissue.

8.2 Atherosclerosis

High shear stress at the apical surface of arterial endothelial cells increases $[Ca^{2+}]_i$ [91], which can initiate inflammation and formation of atherosclerotic lesions [92]. These lesions can be effectively suppressed by treating patients with Ca^{2+}-channel blocking agents [93].

Cx43 was recently identified as a mediator of inflammation and atherogenesis [94, 95]. Pharmacological inhibition of Cx43 inhibits the release of inflammatory mediators and prevents the formation of atherosclerotic lesions in mice [94]. Under mechanical stress, phosphorylation of Cx43 by MST1 suppresses its signaling by inhibiting the opening of Cx43 channels *in vitro* and preventing translocation of Cx43 to lipid rafts *in vivo,* thus protecting against atherosclerosis (Figure 5A). Plaque formation in mice with knockout of both MST1 and YAP was comparable to that seen in mice with knockout of MST1 alone [94]. While the authors concluded that the role of MST1 in atherosclerosis is not mediated through YAP and is independent of the Hippo pathway, several other studies showed that activation of the Hippo cascade and consequent inhibition of YAP/TAZ attenuates endothelial cell inflammation and suppresses formation of atherosclerotic lesions [96–98]. It is likely that MST1 knockout alone is sufficient to promote plaque formation, and TAZ could potentially compensate for the lack of YAP in the MST1/YAP double knockout mouse model used in the study by Quan *et al* [94]. Activation of Cx43 substantially increases $[Ca^{2+}]_i$ in endothelial cells [99]. Therefore, it seems reasonable to postulate that MST1-mediated inhibition of Cx43 attenuates Ca^{2+} permeability, further contributing to the protective effect of MST1 against atherosclerosis.

8.3 Bullous skin diseases

Pemphigus vulgaris is an autoimmune blistering disorder of the skin or mucous membranes caused by autoantibodies to the Ca^{2+}-dependent adhesion proteins desmoglein1 and/or 3 (Dsg1, Dsg3). The desmogleins are present in desmosomes and maintain the structural integrity of the epidermis [100]. In cultured keratinocytes, pemphigus autoantibodies increase $[Ca^{2+}]_i$ via phospholipase C (PLC)-mediated activation of IP_3R [101, 102]. PLC-induced release of Ca^{2+} from the ER is recognized as an early event in the pathogenesis of pemphigus [100]. Increased $[Ca^{2+}]_I$ leads to Dsg3 depletion, which results in loss of intercellular adhesion [103]. Findings reported in a preprint suggest that Dsg3 forms a complex with YAP, sequestering YAP at cell-cell junctions and preventing its nuclear translocation, while depletion of Dsg3 by autoantibodies dissociates YAP from these junctions [104]. In agreement with the inhibitory effect of Dsg3 on YAP, incubating cultured keratinocytes with serum antibodies from patients with pemphigus increases both the total abundance of YAP and its translocation to the nucleus in a dose- and time-dependent manner

[105]. Consistent with the *in vitro* observations, immunohistological analysis revealed overexpression of YAP in pemphigus lesions [105]. Collectively, these findings suggest that crosstalk between Ca^{2+} signaling and YAP has a role in the pathogenesis of pemphigus.

8.4 Nonalcoholic steatohepatitis (NASH)

The incidence of nonalcoholic fatty liver disease (NAFLD) and the stage known as nonalcoholic steatohepatitis (NASH) are increasing worldwide and pose a substantial healthcare concern [106]. NASH is characterized by inflammation and hepatocellular damage, increased cholesterol in the blood and accumulation of fat in the liver, which can eventually lead to fibrosis, cirrhosis, and liver failure. The connection between cholesterol and NASH progression is not fully understood. However, a link between cholesterol, $Ca^{2+}$and TAZ was recently identified (Figure 5B). High levels of cholesterol increase $[Ca^{2+}]_i$ through IP_3R-mediated release of Ca^{2+} from the ER, which increases the amount of active RhoA in hepatocytes [107]. Active RhoA stabilizes TAZ by preventing the E3 ubiquitin ligase β-TrCP from inducing its proteasomal degradation, thereby enhancing TAZ activity. The transcriptional activation resulting from increased nuclear TAZ induces fibrosis and NASH [107]. The activation of TAZ by Ca^{2+} in hepatocytes may provide an opportunity for therapeutic intervention to prevent the progression of liver fibrosis in NASH.

8.5. Autosomal dominant polycystic kidney disease

The proteins PC1 and PC2 form a heterodimer that allows Ca^{2+} influx at the plasma membrane and cilia. Mutations within their corresponding genes (*PKD1* and *PKD2*, respectively) causes autosomal dominant polycystic kidney disease (ADPKD), which is the most common genetic disorder of the kidney [108]. The Ca^{2+} imbalance caused by mutations in PC1/PC2 contributes to the development of cysts in the kidney, liver and pancreas, ultimately leading to failure of all these organs [108]. YAP activity is increased in the cysts with low Ca^{2+} concentration [109, 110], which suggests that influx of Ca^{2+} through PC1/PC2 regulates Hippo. In a mouse model of ADPKD, knockout of YAP and TAZ reduces cystogenesis, implying that their activity contributes to the pathogenesis of ADPKD [110]. Knockout of TAZ from mouse kidneys also decreases ubiquitin-dependent degradation of PC2 [111], illustrating bidirectional crosstalk between the two proteins.

8.6 Immune responses

In macrophages, Piezo1 has an important role in coordinating toll-like receptor (TLR)-mediated innate immunity [112]. Binding of the cell wall of bacteria to macrophages activates Piezo1 and induces the assembly of Piezo1 and TLR4 complexes (Figure 5C). Active Piezo1 enables influx of Ca^{2+}, which activates CaMKII. In turn, CaMKII stimulates an increase in active Rac1 through MST1/2, leading to actin re-organization, which is required for efficient phagocytosis by the macrophages [112]. Elimination of extracellular or intracellular Ca^{2+} effectively inhibits MST1/2 activation and suppresses the bactericidal function of macrophages. Therefore, activation of the core Hippo kinase MST1/2 during macrophage phagocytosis is dependent on Ca^{2+}.

Several pathological conditions, including lower respiratory tract disease, coronary artery disease, hematologic malignancies and thrombocytosis, result from altered and/or attenuated

tension-induced signaling of dendritic cells [113]. General inhibition of Ca^{2+} signaling by ruthenium red or pharmacological antagonism of YAP/TAZ transcriptional activity suppresses the proinflammatory response of dendritic cells to mechanical stimulation [114]. Thus, the crosstalk between Ca^{2+} signaling and Hippo core components integrates mechanosensation with both nonspecific cellular innate immunity and humoral adaptive immunity.

8.7 Carcinoma

The role of dysregulated Ca^{2+} homeostasis in cancer initiation, progression, metastasis and multidrug resistance is an area of active investigation [115]. Dysregulation of the Hippo pathway is associated with the hallmarks of malignancy, including hyperproliferation, invasion, metastasis and chemotherapeutic resistance [116]. In this section we present examples of Ca^{2+} crosstalk with the Hippo pathway during tumorigenesis.

The Ca^{2+}-sensing receptor (CaSR), a member of the GPCR family, maintains systemic Ca^{2+} homeostasis by binding to and sensing free ionized Ca^{2+} in the blood. Upon activation, the CaSR induces intracellular Ca^{2+} release through signaling molecules, such as PLC and PKC [117]. The CaSR regulates cell-cell adhesion, differentiation, gene transcription, tumorigenesis and acts as a tumor suppressor or oncogene, depending on the tissue origin of the tumor [118, 119]. In normal human parathyroid cells, activation of the CaSR reduces expression of LATS1/2, which results in YAP nuclear accumulation and increased expression of YAP target genes [120]. This effect is abrogated by RhoA inhibitors, suggesting that it is mediated by RhoA/ROCK signaling. By contrast, in parathyroid carcinoma CaSR expression is downregulated [121]. YAP nuclear localization is reduced in parathyroid cells with CaSR downregulation [120], suggesting that the CaSR modulates the Hippo pathway and influences cellular processes involved in tumorigenesis.

The Ca^{2+} transporter, SPCA2 (secretory pathway $Ca^{2+/}Mn^{2+}$-ATPase 2), which conveys Ca^{2+} from the cytosol into the Golgi apparatus [84], is primarily expressed in secretory tissues, such as mammary glands. There is a negative correlation between the expression of SPCA2 and that of YAP/TAZ in patient samples from all subtypes of breast carcinoma, suggesting that Ca^{2+} transport by SPCA2 reduces YAP/TAZ expression [122]. Studies in cultured breast epithelial cells revealed that Ca^{2+} signaling brought about by SPCA2 suppresses the oncogenic effects of nuclear YAP/TAZ, likely by regulating the expression of E-cadherin [122]. E-cadherin mediates contact inhibition and suppresses epithelial mesenchymal transition (EMT), in part by activating Hippo and attenuating YAP/TAZ nuclear accumulation [122, 123].

Mechanosensitive Ca^{2+} channels, such as Piezo1 and TRPV4, participate in tumorigenesis by regulating Hippo signaling. Piezo1 activation promotes EMT and metastasis of ovarian carcinoma [124] and cholangiocarcinoma [125] cells by inhibiting Hippo and activating YAP nuclear function. Furthermore, TRPV4 and YAP promote cancer cell migration regulated by viscosity. TRPV4 activation increases RhoA-mediated contractility of cancer cells *in vitro* [47]. *In vivo,* enhanced contractility facilitates the escape of cancer cells with metastatic advantage from the primary tumor site in a mouse model of metastasis.

Tumor cells establish a mechanical memory, characterized by decreased expression of MST1 and LATS2, and increased expression of YAP and TEAD2, which enables the malignant cells to retain migratory capacity while exposed to different degrees of tissue stiffness during metastasis [47]. The YAP inhibitor, verteporfin, abrogates the mechanical memory, and reduces the migration and metastasis of the neoplastic cells. Taken together, these findings suggest a role for Ca^{2+}-mediated YAP regulation in hypermotility and metastasis of the malignant cells (Figure 5D).

In order to investigate a potential link between CaBPs and Hippo in cancer, we used the Cancer Genome Atlas (TCGA). The data available on the cBio Cancer Genomics Portal (http://cbioportal.org) allowed us to examine mRNA expression, gene alterations (e.g., mutations, copy number variations) to query a panel of 10, 967 tumor biopsies from 32 studies of all cancer types for the core Hippo components (MST1/2, LATS1/3, SAV1, MOB1/2, YAP, and TAZ) and CaM (*CALM1*, *CALM2*, *CALM3*) or S100 proteins known to modulate Hippo (*S100A1*, *S100A7*, *S100A8*, *S100A9*, *S100A14*, *S100B*). In examining post-disease survival, the only significant link between genetic alteration and decreased patient survival was observed between each CaM gene and TAZ across all cancer types (Table 1). Additionally, we examined the correlation between the expression of mRNA from multiple genes and identified a very weak, but significant, correlation between CaM expression and TAZ, but not YAP, despite previous reports that *CALM2* is a YAP target gene [44]. These findings imply a potential functional relationship between CaM and TAZ in the development and/or progression of cancer. However, the molecular mechanism by which genetic changes in CaM or TAZ may regulate one another to affect patient survival remains to be determined. Further investigations are required to elucidate the nature and extent of the possible interactions between CaM and TAZ in tumorigenesis.

Concluding remarks

Signaling pathways have historically been perceived as linear, independent networks. Instead, numerous studies over the last decade have demonstrated that different signaling cascades can intersect, with one pathway influencing the activation of another. As illustrated in this review, this concept applies to Ca^{2+} and Hippo signaling. Importantly, both activating and inhibitory effects of Ca^{2+} on Hippo have been reported, illustrating the complex nature of the crosstalk. The influence of Ca^{2+} on Hippo is most likely determined by the cellular context, since Ca^{2+} and Hippo signaling proteins, as well as their regulators, exhibit differential expression, localization, and/or activity in different cell types or tissues. In addition, Ca^{2+}-Hippo crosstalk appears to be influenced by the nature of the stimulus applied to the cells. Most studies that investigated mechanically-induced influx of Ca^{2+} through plasma membrane channels report that increased $[Ca^{2+}]_i$ activates YAP via RhoA and actin. By contrast, SOCE-induced elevation of $[Ca^{2+}]_i$ generally inhibits YAP, for example via PKC or Merlin. The apparent discrepancies in the role of Ca^{2+} as a Hippo regulator emphasize the importance of accurately reporting the experimental conditions under which signaling was studied in order to enable readers to interpret and integrate different studies.

Most results mentioned in this review were obtained using carcinoma cells with artificially-induced increase of $[Ca^{2+}]_i$, for example through application of mechanical stress or chemical treatment. The use of such cellular systems clearly demonstrates crosstalk and provides molecular insights into how the pathways intersect, both via CaBPs and Ca^{2+}-regulated proteins. Future research should focus on validating the identified crosstalk in systems with physiologically-induced activation of Ca^{2+} and Hippo. Due to the localized and transient nature of signaling, this requires tools with high spatial and temporal resolution, such as imaging protein interactions and phosphorylation in live cells, neither of which is a trivial undertaking. Importantly, the influence of Ca^{2+} on Hippo has also been observed in more complex *in vivo* systems, where it influences both physiology and pathology. Further elucidation of the molecular mechanisms underlying the intersection of Ca^{2+} and Hippo may lead to the development of new therapeutic agents for a wide array of diseases.

Acknowledgements

The authors thank the many wonderful colleagues in the Sacks laboratory who have contributed to studies investigating Ca^{2+} and Hippo crosstalk. We thank Andrew Hedman for his valuable insight and helpful discussion. We apologize to authors whose primary work was omitted because of space restrictions.

Funding

Funding was provided by the Intramural Research Program of the National Institutes of Health.

Data availability

The results reported in Table1 are in whole based upon data generated by the TCGA Pan Cancer Atlas Studies: https://www.cbioportal.org

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Highlights

- The Hippo signaling pathway is a master regulator of organ size and tissue homeostasis.
- Ca^{2+} is a fundamental second messenger that modulates diverse signaling cascades.
- Ca^{2+} can influence Hippo pathway components.
- This review summarises the biological consequences of the intersections between Ca^{2+} and Hippo.

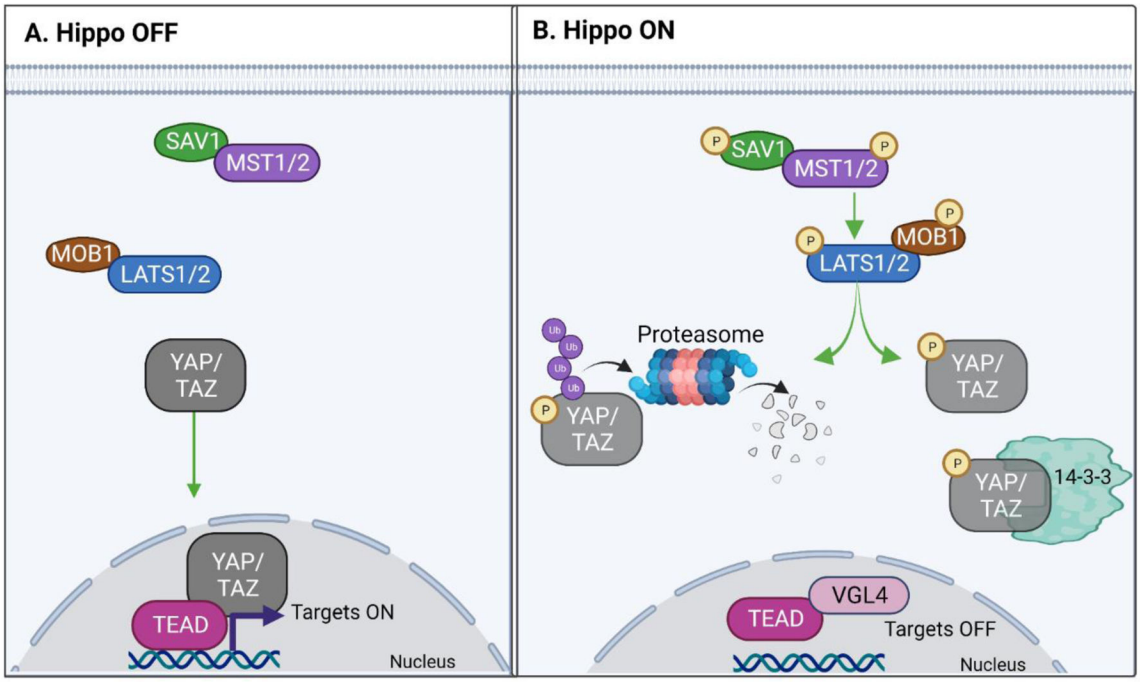

Figure 1. Core components of the Hippo pathway.

A. Schematic diagram illustrating the interactions among the core components of the Hippo pathway. When Hippo is OFF, YAP and TAZ accumulate in the nucleus, where they bind to and activate TEAD to promote the expression of target genes. **B.** When Hippo is ON, SAV1 allows MST to phosphorylate LATS, and MOB1 binds to LATS to enhance its catalytic activity. LATS then phosphorylates YAP/TAZ, causing their sequestration in the cytoplasm, either through binding to adaptor proteins, such as 14–3-3, or by inducing YAP/TAZ degradation in the proteasome. In the absence of nuclear YAP/TAZ, TEAD, which is bound to the transcription cofactor VGL4, suppresses the expression of YAP/TAZ target genes. Green arrows represent activation. Figure generated in BioRender.

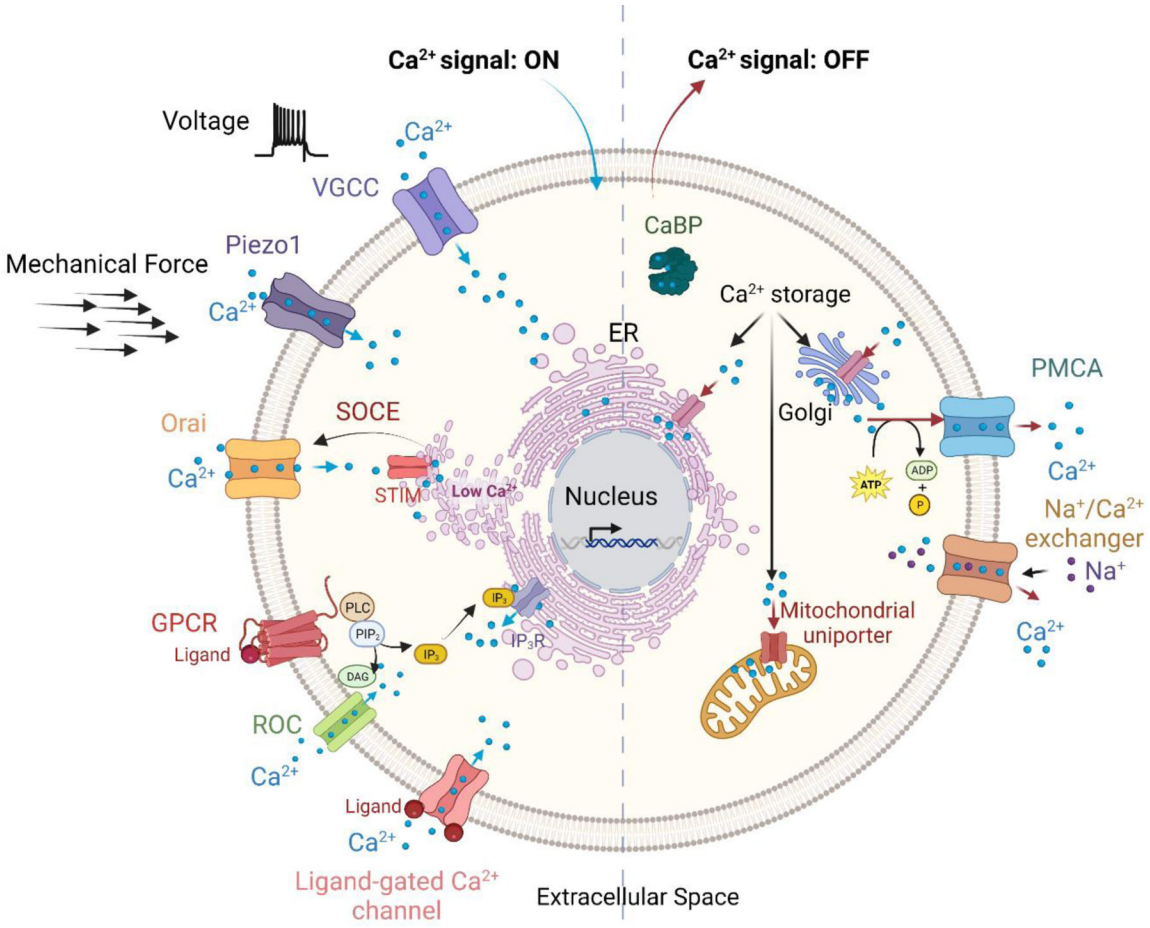

Figure 2. Ca^{2+} homeostasis in cells.

Intracellular free Ca^{2+} concentrations ($[Ca^{2+}]_i$) are tightly regulated by a network of proteins and channels, such as Ca^{2+}-binding proteins, Ca^{2+}pumps (e.g, Ca^{2+}-ATPase), Ca^{2+} channels (e.g., Piezo1) and exchangers (e.g., Na^+/Ca^{2+} exchanger). Diverse stimuli, including membrane depolarization, mechanical forces, receptor/ligand interactions, and intracellular messengers, induce "ON" mechanisms. These reactions increase $[Ca^{2+}]_i$ by promoting the entry of Ca^{2+} from the extracellular environment or by the release of Ca^{2+} from intracellular stores, e.g., the ER, Golgi and mitochondria. The mobilized Ca^{2+} acts as a messenger to modulate numerous Ca^{2+}-sensitive processes. When the ER Ca^{2+} reservoir is depleted (Low Ca^{2+}), Ca^{2+} influx is triggered by store-operated Ca^{2+} entry (SOCE), which results from coupling between Orai channels and STIM. Once Ca^{2+} has carried out its signaling function, it is rapidly removed from the cytosol through "OFF" mechanisms, which either extrude Ca^{2+} to the extracellular space or sequester Ca^{2+} into internal stores. Blue arrows indicate "ON" mechanisms, red arrows depict "OFF" mechanisms and black arrows indicate induction. Ca^{2+} is depicted by blue circles. ER, endoplasmic reticulum; CaBP, Ca^{2+}-binding proteins; TRP, transient receptor potential; PMCA, plasma membrane Ca^{2+} pumps; GPCR, G-protein coupled receptor; ROC, receptor-operated channel; VGCC, voltage-gated Ca^{2+} channel. The schematic depicts only the main transporters that regulate Ca^{2+}signaling. The figure was generated in BioRender.

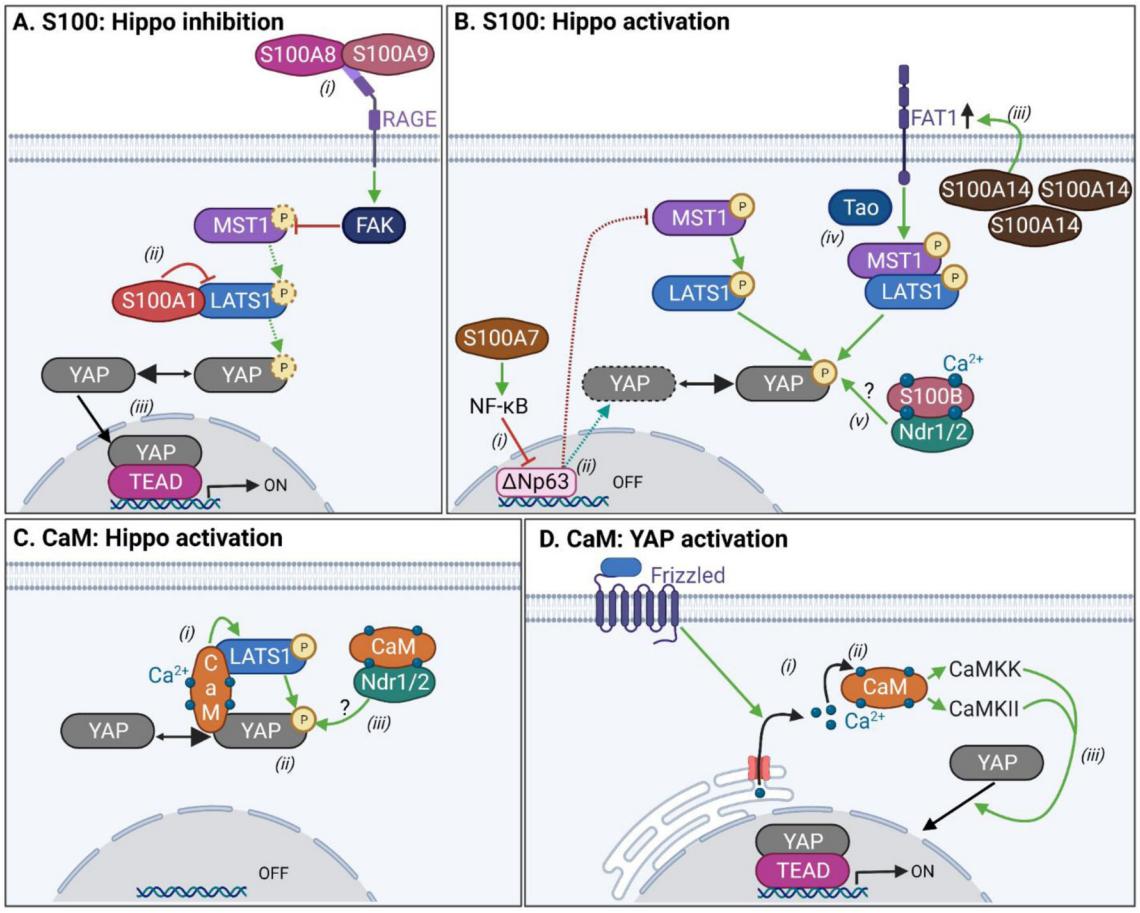

Figure 3. Ca^{2+}-binding proteins modulate Hippo activation.

A. Hippo inhibition by S100 proteins: *(i)* Activation of RAGE by extracellular S100A8/S100A9 activates FAK, which inhibits MST1. *(ii)* Binding of S100A1 to LATS1 inhibits its kinase activity towards YAP. *(iii)* In both cases, this increases active, non-phosphorylated YAP, which stimulates the expression of YAP/TEAD target genes. **B.** Hippo activation by S100 proteins. *(i)* S100A7 activates NF-κB signaling, which inhibits ΔNp63. *(ii)* This mechanism prevents ΔNp63-mediated reduction and stimulation of MST1 and YAP expression, respectively. *(iii)* Separately, S100A14 overexpression increases FAT1 cellular abundance. *(iv)* In turn, FAT1 stimulates Tao-mediated Hippo kinase activation. *(v)* Binding of Ca^{2+}/S100B to Ndr1/2 stimulates their kinase activities, which may also increase YAP phosphorylation. **C.** Hippo activation by calmodulin (CaM). *(i)* Ca^{2+}/CaM binds to both LATS1 and YAP in a ternary complex in which Ca^{2+}/CaM directly stimulates LATS1 kinase activity. (*ii*) This increases phosphorylated YAP, which impairs its nuclear translocation. *(iii)* Ca^{2+}/CaM also interacts with Ndr1/2 kinases, which could possibly stimulate Ndr1/2-catalyzed phosphorylation of YAP. **D.** YAP activation by CaM. *(i)* Activation of the Frizzled receptor initiates non-canonical Wnt signaling, which increases cytosolic Ca^{2+} concentration via Ca^{2+} release from intracellular stores. *(ii)* This increases Ca^{2+}/CaM which, via activation of CaMKK and CaMKII, *(iii)* stimulates YAP nuclear activity. Green, red, and dotted arrows represent activation, inhibition, and decrease, respectively. ? indicates speculative mechanisms not experimentally demonstrated. Figure generated in BioRender.

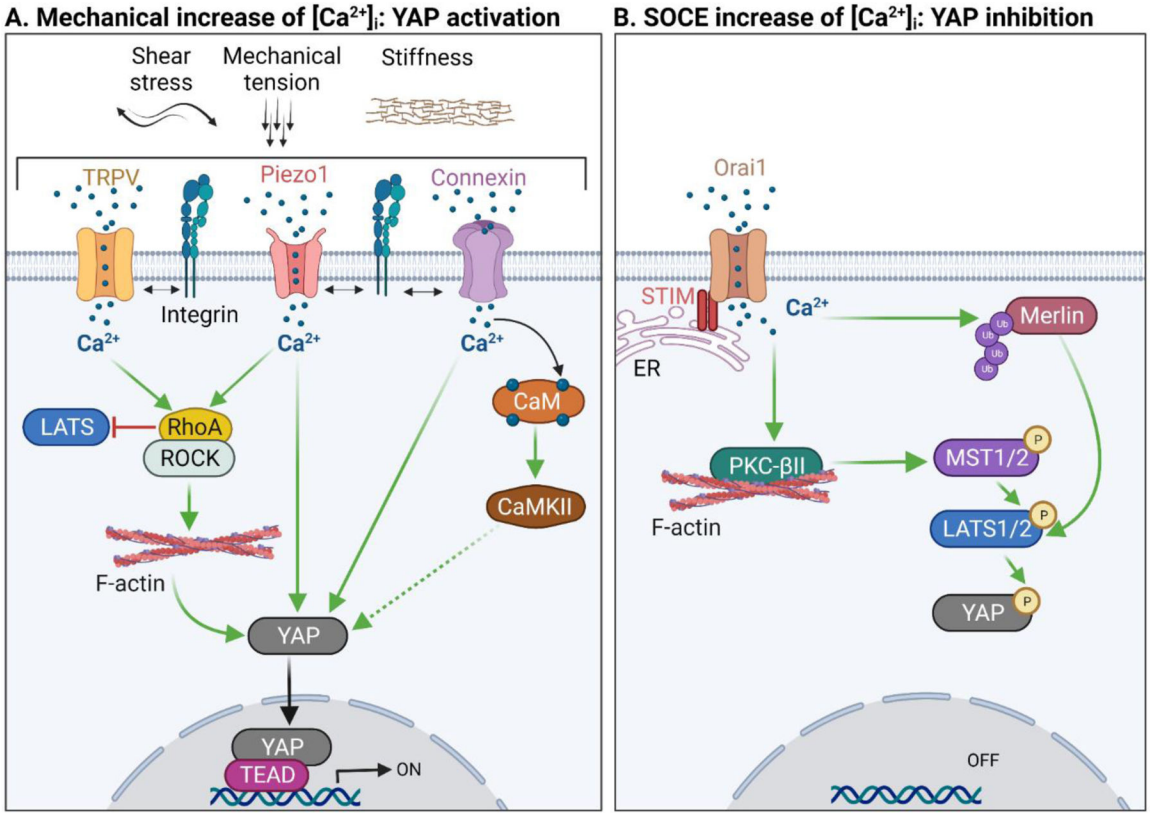

Figure 4. Ca^{2+} and Hippo signaling crosstalk.

A. Mechanical stimuli, such as shear stress, mechanical tension and substratum stiffness, elicit a cascade of signaling events by inducing an influx of Ca^{2+} through the activation of Ca^{2+}-permeable mechanosensitive channels, including the TRPV family of proteins, Piezo1, and connexins. The increased $[Ca^{2+}]_i$ activates downstream effectors, RhoA/ROCK and subsequent actin remodeling. Active RhoA inhibits LATS1/2 phosphorylation, leading to nuclear translocation and transcriptional activation of YAP. Increased $[Ca^{2+}]_i$ also activates CaMKII via CaM. CaMKII may promote nuclear translocation of YAP. **B.** Store-operated calcium entry (SOCE) triggers an increase in $[Ca^{2+}]_i$, leading to the activation of PKC-βII and ubiquitination of Merlin. Active PKC-βII stimulates the Hippo kinase cascade, while ubiquitinated Merlin promotes LATS1 activity, all resulting in increased YAP phosphorylation and inhibition of its nuclear activity. Green arrows represent activation. Dashed line depicts speculative mechanism. CaM, calmodulin; CaMKII, Ca^{2+}/calmodulin-dependent kinase II; ER, endoplasmic reticulum; ROCK, Rho kinase; and TRPV, Transient Receptor Potential Vanilloid. Figure generated in BioRender.

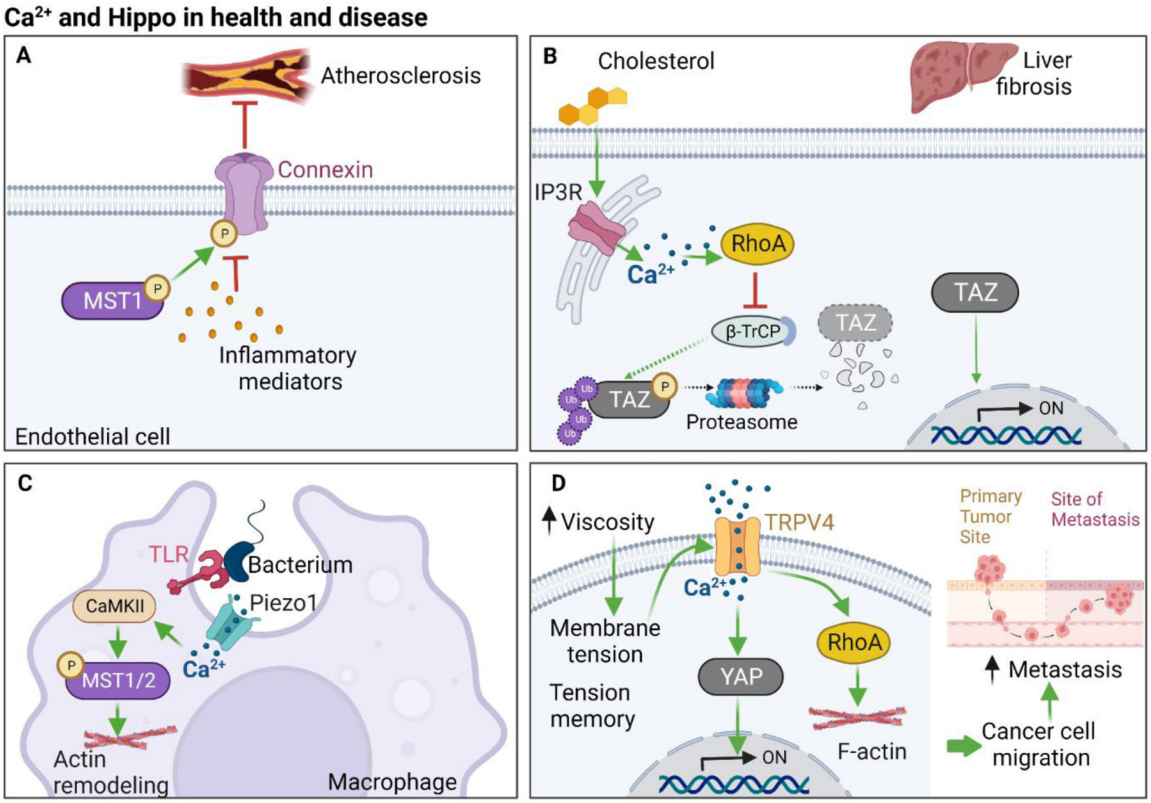

Figure 5. Ca^{2+} and Hippo crosstalk in health and disease.

A. MST1 phosphorylates Cx43 and attenuates its ability to release inflammatory molecules, thus protecting endothelial cells from atherosclerosis. **B.** Cholesterol loading in hepatocytes suppresses degradation of TAZ in the proteasome by β-TrCP, an E3 ubiquitin ligase, via an IP3R-Ca^{2+}-RhoA pathway, thereby promoting TAZ-mediated liver fibrosis. **C.** The interaction of bacterial cell wall components with toll-like receptors (TLRs) on macrophages initiates a cascade of events. Piezo1 couples with TLRs, mediating the influx of Ca^{2+}, which activates MST1. This leads to actin remodeling and culminates in phagocytosis of the bacteria by the macrophages. **D.** Carcinoma cells secrete macromolecules, which increase the viscosity of the extracellular environment. This induces membrane tension and increases $[Ca^{2+}]_i$ via TRPV channels. Ca^{2+} activates RhoA and promotes actin remodeling, leading to cancer cell migration and metastasis. Green, red and dashed arrows represent activation, inhibition, and decrease, respectively. CaMKII, Ca^{2+}/calmodulin-dependent kinase II; IP3R, inositol 1,4,5-triphosphate (IP_3) receptor; TLR, Toll-like receptor; and TRPV4, Transient Receptor Potential Vanilloid 4. Figure generated in BioRender.

Table 1.

mRNA correlation and gene alteration of CaM and TAZ across all types of cancer.

Gene (Protein)	mRNA correlation coefficient †Spearman (*p* value)	Cases with genetic alteration (%)	Decreased survival of patients with altered genes Logrank Test *p* value
CALM1 (Calmodulin) *WWTR1* (TAZ)	0.04 (p = 2.354e-5)	97/10950 (0.9%) 383/10950 (3%)	0.0302 *
CALM2 (Calmodulin) *WWTR1* (TAZ)	−0.11 (p = 4.27e-26)	108/10950 (1%) 383/10950 (3%)	0.0334 *
CALM3 (Calmodulin) *WWTR1* (TAZ)	−0.06 (p = 1.96e-9)	87/10950 (0.8%) 383/10950 (3%)	2.240e-3 *

*$P < 0.05$

†Spearman: 0–0.19 is regarded as very weak, 0.2–0.39 as weak, 0.40–0.59 as moderate, 0.6–0.79 as strong and 0.8–1 as very strong correlation.