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Review

Response to Mechanical Cues by Interplay of YAP/TAZ Transcription Factors and Key Mechanical Checkpoints of the Cell: A Comprehensive Review

Seyed Ali Mosaddad[a] Yalda Salari[b] Samira Amookhteh[a] Reza Sayyad Soufdoost[c] Alexander Seifalian[d] Shahin Bonakdar[e] Fahimeh Safaeinejad[f] Mehrdad Moosazadeh Moghaddam[g] Hamid Tebyanian[h]

[a]School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran, [b]Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran, [c]Imam Khomeini Clinic of Dentistry, Tehran, Iran, [d]Nanotechnology and Regenerative Medicine Commercialization Centre (Ltd), London Bioscience Innovation Centre, London, UK, [e]National Cell Bank Department, Pasteur Institute of Iran, Tehran, Iran, [f]Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [g]Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, [h]Research Center for Prevention of Oral and Dental Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran

Key Words
Mechanotransduction • YAP/TAZ activity • Focal adhesion • Cytoskeletal tension • Rho GTPase • Nuclear proteins

Abstract
Many factors including growth factors (GF), scaffold materials, and chemical and physical cues determine the cell behaviors. For many years, growth factors have been considered as the pivotal cell behavior regulators, whereas recent studies emphasize also the key role of physical factors such as mechanical forces, cell shape, surface topographies, and extracellular matrix (ECM) in regulating the cell proliferation, apoptosis, differentiation, etc. through mechanotransduction pathways. In this process, the cell morphology and mechanical properties of the cell's micro/nano-environments and ECM can be conveyed to the nucleus by regulating transcriptional factors such as Yes-associated protein and transcriptional coactivator with PDZ-binding motif (TAZ). Generally, YAP/TAZ activity is considered as the key factor for the growth of whole organs, however, recent studies have also repeatedly addressed the role of YAP/TAZ in mechanotransduction. In this review, the biological functions of the YAP/TAZ pathway and its contribution to the mechanotransduction and cell behavior regulation in response to the mechanical cues have been summarized. Also, the role of key mechanical checkpoints in the

Mehrdad Moosazadeh Moghaddam and Hamid Tebyanian

Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran (Iran), E-Mail mm.genetics@gmail.com; rsr.moosazadeh@bmsu.ac.ir

Research Center for Prevention of Oral and Dental Disease, Baqiyatallah University of Medical Sciences, Tehran (Iran), Tel. +989198045743, E-Mail tebyan.hamid@yahoo.com

cell including focal adhesions, cytoskeletal tension, Rho small GTPases, and nuclear membrane protein elements involved in the transfer of environmental mechanical cues from the cell surface to the nucleus and their effect in regulating the YAP/TAZ activity are discussed.

Introduction

Many elements participate in cell fate, including soluble factors and adhesive mediators, that can physiologically activate relevant differentiation responses [1, 2]. While biologists contemplate the main role of soluble cues (e.g. GFs and cytokines) in controlling cell proliferation, apoptosis, and differentiation, many researches demonstrate that the physical forces and mechanical cues as micro/nano-environmental factors also influence strongly the cellular functions. Then, these factors may effectually be involved in controlling cell differentiation and proliferation. Accordingly, many varied extrinsic and intrinsic factors in the cell environment can impact the interactions between the cell and substrate that can lead to change in cell behavior and function [3, 4]. In this regard, during tissue development, cells are in contact with different micro/nanoscale topographies in their environment, especially the components of extracellular matrix (ECM), that have important roles in tissue development and organization [5, 6]. ECM contains pores, ridges, and fibers with micro/nanometer scales whose mechanical properties as stiffness, rigidity, elasticity, etc. can influence the behavior of cells such as proliferation and differentiation via mechanotransduction pathways [7-9]. Mechanotransduction is the process of converting a mechanical signal into a cellular response [10]. In other words, mechanotransduction describes the molecular mechanisms by which cells respond to changes in their physical environment via translating mechanical stimuli into biochemical signals [11-13]. In this biological event, a broad variety of mediators cooperates in a coordinated manner, so that a physical cue within the cell microenvironment eventually alters gene expressions. Until now, various effectors ranging from proteins participating in focal adhesion complex to different transcription factors have been discovered in this regard. In the meantime, the role of some transcription factors in the mechanosensing of the cell has been well-highlighted too.

YAP/TAZ transcription factors are the main linker between proteins involved in the mechanotransduction cascade following physical cues and genomic regulation. Understanding the interaction between each component of mechanotransduction signaling and YAP/TAZ transcription factors in response to mechanical features of ECM would help to better clarify the mechanotransduction pathways.

Topographical Features and Cell Mechanosensing

Some of the mechanical stimuli are surface topography and geometry, fluid shear stress, or cell shape induction that activate the downstream signaling cascades which result in the mechanical-dependent altering of gene expression, then, cell proliferation and differentiation in turn [13-16]. In addition to impacting the lipid bilayer structure, these forces affect cell signaling pathways by activating specific receptors attached to the cytoskeleton [17-19]. Recently, more studies have been carried out based on the influence of mechanical cues especially the effect of surface topographies on the differentiation of stem cells, which have highlighted the remarkable effects of these cues. In these studies, direct differentiation of stem cells is reported using substrates with imprinted cell-like topographies and geometry. These imprinted substrates could mimic the cell-specific shape, plasma membrane micro-/nano-topographies, and specific mechanical forces associated with cell shape [20-24]. Given the importance of this issue, many efforts have been made over the recent years to understand the aspects of cell-substrate interaction through recreating the topography [25-27].

Topological features can profoundly influence the stem cell self-renewal. For instance, in a cytoskeleton-dependent manner, as the stiffness of tropoelastin substrates increased, the hematopoietic stem cells and progenitor cells exhibited higher proliferation rate compared with control [28]. The culture of IPSCs on different electrospun materials with various physiochemical features also showed that there was an inverse relationship between the substrate stiffness and sphericity of IPSC colony, while the substrate stiffness increased IPSCs self-renewal and spontaneous differentiation [29]. In addition to the self-renewal, mechanical cues have a remarkable impact on stem cell differentiation. The MSCs grown on materials with various stiffness showed different cell fates, so that those cultured on soft, intermediate, and hard materials differentiated toward neurogenic, chondrogenic, and osteogenic lineages, respectively [30].

Based on the discussed topics, embryological studies have demonstrated that micromechanical models that exert mechanical stress and morphology alterations are one of the crucial elements of phenotype generation and determination of the cell fate. These parameters act through shape- or tension-dependent changes in cytoskeletal structure by mechanosensing of mechanical forces. The main links between the mechanosensing task of the cytoskeleton and cell fate are the interactions between integrins, cytoskeletal proteins, protein kinases, and mechanical forces that affect cells in order to change shape, proliferation, differentiation, and apoptosis. Cosgrove et al. reported that the interaction between N-cadherin (HAVDI motifs) and RDG motifs of fibronectin changes the fate of mesenchymal stem cells. According to their findings, MSCs cultured on HAVDI/RGD hydrogels showed lower nuclear accumulation of RUNX2 and consequently less differentiation into osteogenic lineage compared with those grown on Scram/RGD hydrogels [31, 32]. In translating mechanical stimuli into biochemical signals, there are molecular switches considered to be adjusting several signal transduction pathways in cells. Many studies highlight the key role of Rho-dependent signaling in the regulation of the actin cytoskeleton in mechanotransduction [33, 34].

One of the important signaling mechanisms which describe the differentiation of stem cells based on geometric control of cell morphology is the RhoA-Rho-associated kinase pathway [33]. This kinase is categorized within the Rho family of small GTPase [35]. The role of RhoA is to control the stress fiber assembly and tension stress in the cell, as with altering the outside mechanical forces, RhoA is activated and stimulates tension through its effector, RhoA-Rho associated kinase, which indirectly increases the phosphorylation level of the myosin light chain [36]. Hence, the assembly of the actomyosin stress fiber is promoted by the activation of Rho, changing the mechanical features of the cell and consequently regulation of gene expression. Therefore, the RhoA activation modulates cell lineage commitment via cell morphology. On the other hand, it is also proved that cell morphology and mechanosensing of the ECM features can be conveyed to the nucleus by regulating the transcriptional factors such as Yorkie-homologs Yes-associated protein [37] and transcriptional coactivator with PDZ-binding motif (TAZ) [38]. Accordingly, studies about the role of surface topographies, micro-/nano-environmental features, mechanotransduction in cell behavior, and their connection with activation of cellular transcription factors have shown that YAP and TAZ as nuclear factors link these mechanical signals to the genomic activity of the cell and thereby lead to induction of the cells' features [39, 40]. These factors generally regulate the size of the organs by regulating the transcription of many genes especially growth factors like TGFβ [41]. However, their pre-activation also depends on the Rho GTPase and actomyosin activities from the cellular skeleton. Patterned substrates based on the fibronectin-coated micro-sizes (Micropattered fibronectin islands) and capillary epithelial cells of the lung have been used to study the differentiating role of YAP/TAZ. The different sizes of patterns allow the cell to take a spherical to oval shape [42]. Besides, the survival of cells depends on the YAP/TAZ activity which is regulated by cell geometry and morphology. Based on these studies, cells are able to detect the elasticity of the substrate, shape, topographical property, and micro-/nano-environmental forces based on the activity stage of YAP/TAZ factors (Fig. 1). Along with morphogenes, this process plays an important role during embryonic formation and configuration of tissue organs [43, 44].

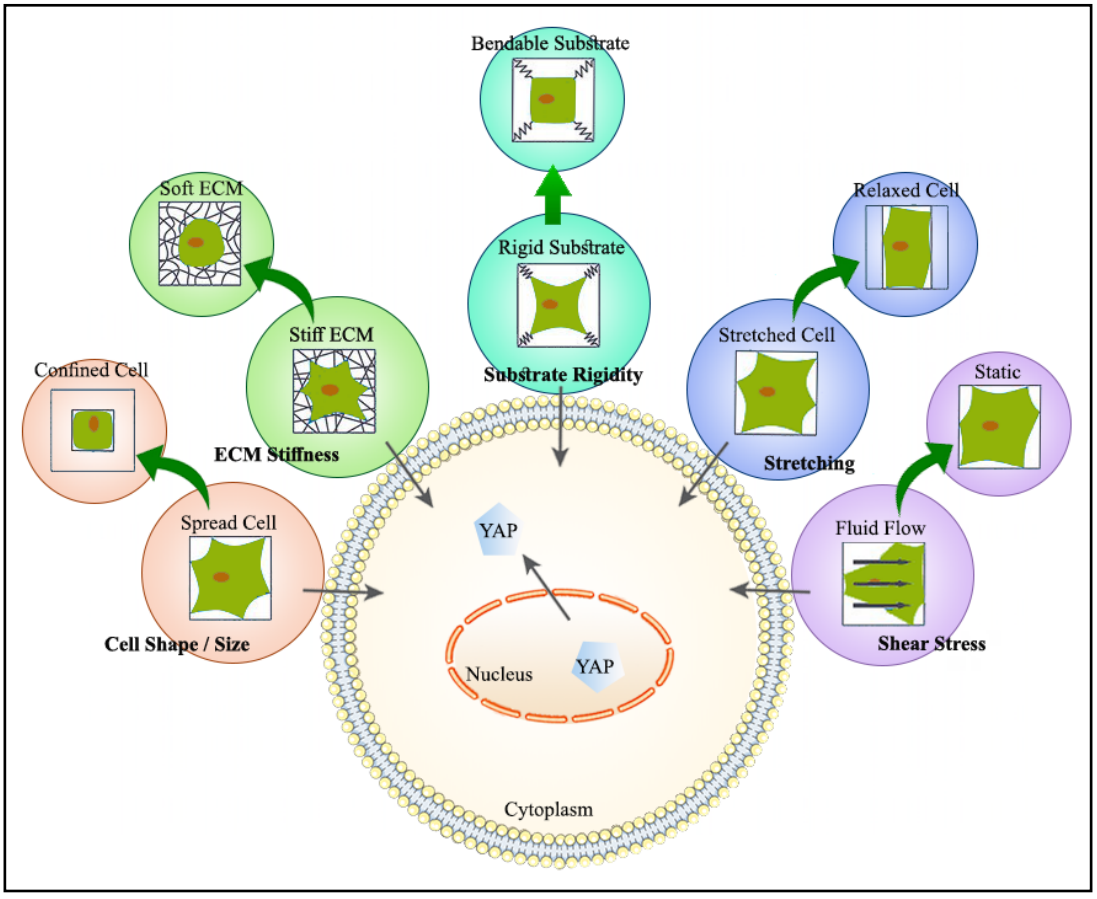

Fig. 1. Schematic representation of regulating YAP activity by different mechanical cues. YAP is localized to the nucleus and active under mechanical conditions that lead to high intracellular tension such as a large adhesive area, stiff extracellular matrix (ECM), non-bendable substrates, cell stretching, or fluid shear stress. Conditions favoring low contractile forces in the cell, such as small adhesive areas, soft ECM, bendable substrates, relaxation of stretching forces, or culture in static media lead to YAP inactivation by nuclear exclusion.

Generally, the morphologic changes during the differentiation of stem cells and their alteration into various cellular lineages are considered as a notable issue. Based on the studies, these morphological changes are caused by various actions and tensions of the cellular skeleton, resulting in changes in the expression rate of cellular proteins and their interactions. Studies also demonstrated that the mechanical forces which lead to changing the cell density and morphology can influence the mechanical properties of the cell skeleton and regulate the activities of the YAP/TAZ mechanical sensors which change the behavior and function of the cell [45, 46].

YAP/TAZ Biological Functions

YAP and TAZ, as mechano-responsive transcription factors, are paralog proteins with molecular weights of 65-kDa and 43-kDa, respectively [47]. YAP was first discovered by Marius Sudol (1999) over 20 years ago [48]. YAP contains a PDZ-binding motif (PDZ-BM) in its C-terminus, and a proline-rich region (P-rich) in its N-terminus which is absent in TAZ. Moreover, YAP protein contains one or two WW domains, a Tea Domain Transcription Factor (TEAD) binding domain, an Src homology domain-3 binding motif (SH3-BM), and a

coiled-coil domain (CC) [49]. The WW domain is one of the smallest protein modules which mediates specific protein-protein interactions with short proline-rich or proline-containing motifs. Despite the similar domain organization of TAZ, it has only one WW domain and no SH3-BM. TAZ or WW domain-containing transcription regulator 1 (WWTR1) is one of the 8 isoforms of YAP which has lost one WW domain and its TAD has undergone some changes. In a general explanation, the Hippo pathway begins by MST1/2-STE20 family protein kinases that activate LATS1/2 by phosphorylating its hydrophobic motifs which in turn phosphorylates the serine residues in YAP/TAZ. YAP/TAZ phosphorylation results in binding to the 14-3-3 regulatory proteins, a family of conserved regulatory molecules that are expressed in all eukaryotic cells, which keeps them in the cytoplasm [46, 50]. Due to lacking any DNA-binding site, YAP/TAZ mediates transcribing growth-promoting, antiapoptotic, and cell fate genes via binding to the TEAD DNA-binding transcription factors (Fig. 2). The transcriptional coactivator role of YAP was first observed in interaction with Runt Related Transcription Factor 2 (RUNX2)/PEBP2αA acting as a transcriptional coactivator of RUNX2 similar to TAZ. RUNX2 is a protein that, in humans, acts as a key transcription factor associated with osteoblast differentiation. The function of YAP causes a significant increase in RUNX2-mediated transactivation activity [50].

Despite all thriving researches and discoveries on the YAP/TAZ biology and regulatory function, there are still many unknown fundamental questions about their role in developmental signaling pathways [41, 48]. A suggested mechanism for YAP/TAZ function is to act as nuclear transducers of the Hippo signaling pathway. Hippo pathway has been indexed within the family of signaling pathways that control organ size and development via regulating the cell proliferation, apoptosis, and stem cell self-renewal (Fig. 2). Realizing the physical measures of a growing organ is the most remarkable capability of YAP/TAZ that helps them to involve in controlling the growth of the organs up to reaching their correct size. Hippo pathway prevents cell proliferation and induces apoptosis by kinase factors such as Hpo and Wts. The hippo pathway performs this regulation function through contact inhibition of proliferation (CIP) procedure [51]. CIP is the state of cell division inhibition by reaching a defined cell stationary density [41, 52]. Another recently well-characterized mechanism of YAP regulating function via Hippo signaling has been cell-cell contact. This classic paradigm is evidenced by the nucleus aggregation of YAP and its activity in cells growing at low density, while, in confluent cultures, it aggregates in the cytoplasm. In addition, YAP is phosphorylated on the Ser112 position during contact inhibition, while

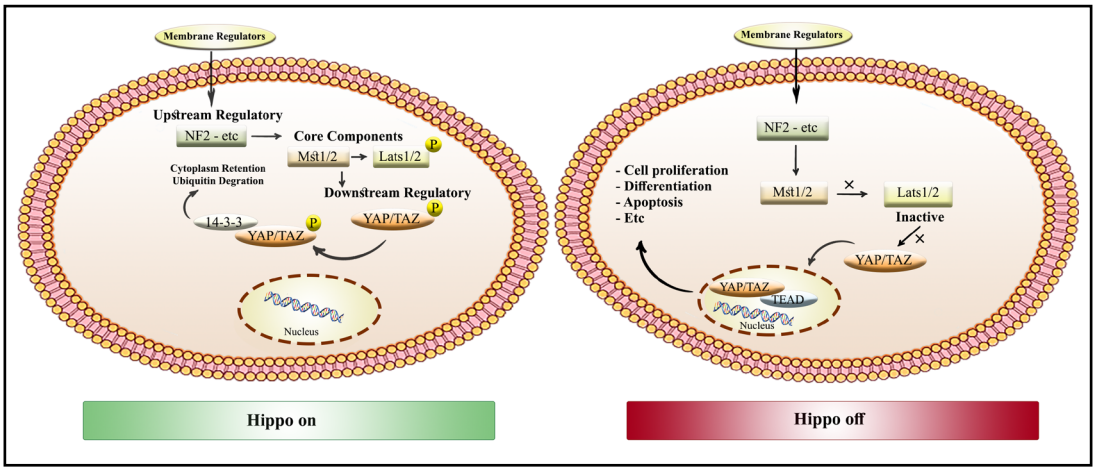

Fig. 2. Schematic representation of the Hippo pathway and its effects on the activation of TAP/TAZ. 'Hippo On' leads to the phosphorylation and inactivation of TAP/TAZ via LATS1/2, ultimately leading to the cytoplasmic retention of TAP/TAZ (left). 'Hippo Off' abrogates the TAP/TAZ inactivity, thus they are translocated into the nucleus to induce cell proliferation and tumor growth (right). P, phosphorylation; R, receptors; ↓, activation; x, block.

when its non-phosphorylatable form is overexpressed, the proliferation arrest is postponed and facilitates cell proliferation up to higher densities (Fig. 3A, B & C) [53].

YAP/TAZ plays an influential role in transducing the cell structural features such as polarity, shape, and cytoskeletal organization which are in close relation with the cell-cell and cell-ECM attachment ability of cells and their micro/nano-environment. Therefore, while other conventional signaling pathways contain dedicated ligand-receptor pairs, the Hippo-YAP signal transduction pathway involves a variety of biochemical signals as well as architectural and mechanical cues such as ECM stiffness, cell-cell, and cell-matrix adhesion, as well as density, shape, and polarity of the cells [44, 53]. Mechanical cues such as stress, strain, and distortion that physiologically affect the cell density, extracellular environment stiffness, and cell geometry can influence the YAP/TAZ localization and activity by regulating their nuclear accumulation rate via their nuclear exclusion (transducing to the cytoplasm) and inactivation (Fig. 1) [54]. For example, a stiff ECM causes YAP/TAZ accumulation in the nucleus in transcriptionally active form, while a soft ECM leads to their nuclear exclusion and inhibits their function [38]. However, it is not fully-understood how mechanical signals influence gene expression in cells. Some studies refer to YAP/TAZ transcriptional coactivators as key mediators of the biological responses to the ECM physiological properties and cell shape [38].

YAP/TAZ Responses to Mechanical Features

YAP and TAZ act as shuttle gene transcription regulators in both the cytoplasm and nucleus which their nuclear accumulation plays a key role in regulating cell function. The presence of active YAP and TAZ factors in the nucleus results in organ growth, cell size augmentation, cellular proliferation, suppressing apoptotic signals, tumor growth induction, and loss of contact inhibition. As mentioned earlier, the mechanical cues affect the activities of these transcriptional factors. Based on in vitro studies, cell cultures on micropatterned ECMs with similar stiffness and different degrees of cell spreading show a resembling regulatory effect on the activity and nuclear-cytoplasmic transduction of YAP and TAZ (Fig. 3D & G) [53, 55]. Accordingly, the YAP and TAZ nuclear localization (active form) is more abundant in cells spread on the large fibronectin islands, while they are more inactive (cytoplasmic localization) in cells cultured on small adhesive islands which confine cells spread (Fig. 3D, E, F). In addition, cells grown on the stiffer surfaces have a higher concentration of YAP and TAZ in the nucleus (active form) (Fig. 3G, H, I). Calvo et al. showed that stiff matrixes through actomyosin contractility and Src function promote YAP nucleus localization and activation [56].

Active YAP/TAZ also remains in the nucleus when the cell is stretched, while in the case of high cell density, the inactivated form of YAP/TAZ localizes in the cytoplasm (Fig. 4) [53]. Convincing evidence has been provided for the mediator involvement of activated YAP/TAZ mechano-regulated proteins in the effecting procedure of environmental mechanical properties on cellular proliferation, migration, and differentiation fundamental procedures such as adipogenic/osteogenic switch of MSCs. Accordingly, using the regulation of the substrate mechanics and nano-topography or the cell spreading conditions in a way that induces a low cytoskeletal tension, MSCs could be induced towards adipogenesis or osteoblastogenesis. It was shown that cell confinement and compliant substrates induce adipogenic phenotype, whereas osteoblastic phenotype develops due to high cell spreading and stiff surfaces. Besides, *in vitro* studies have shown that during adipogenesis, Hippo kinases are activated and YAP nuclear activity decreases (Fig. 5) [57].

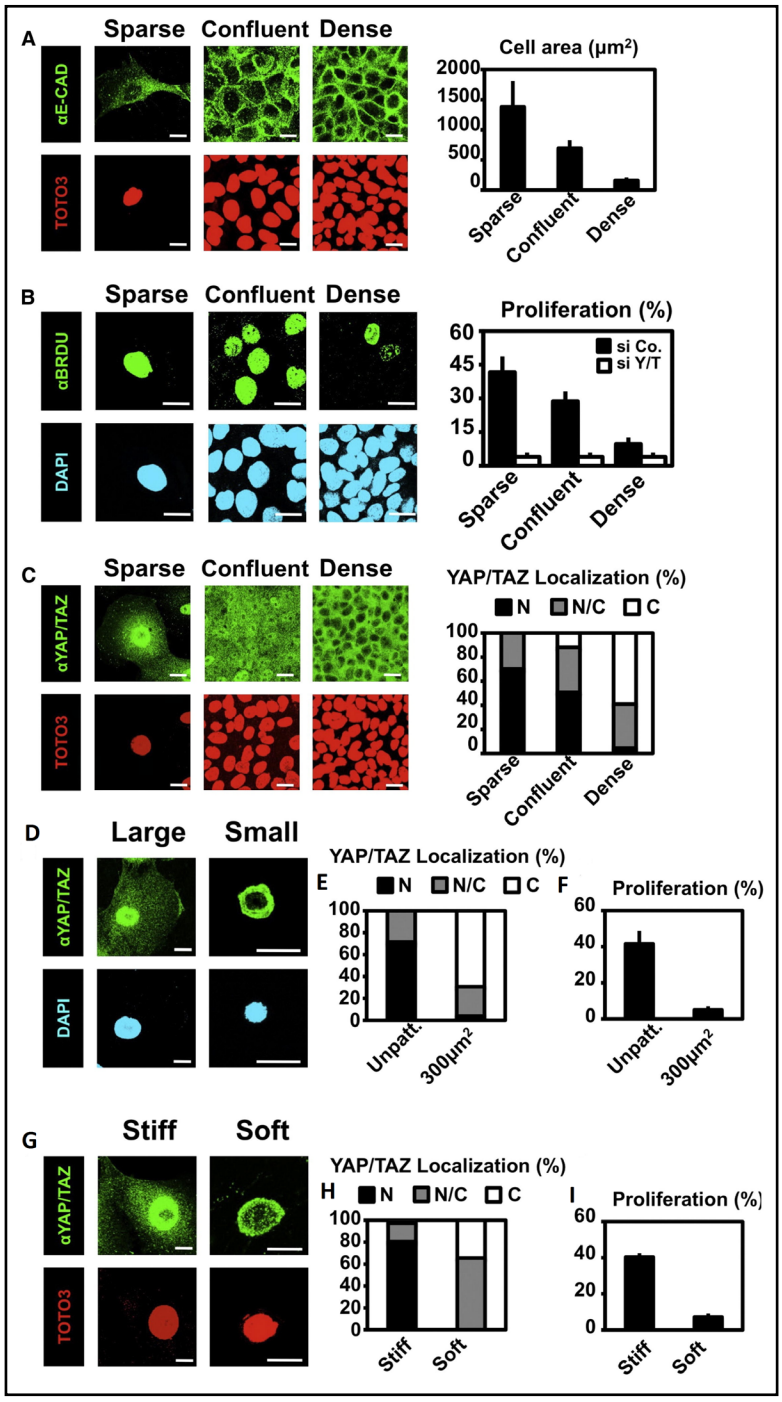

Fig. 3. The effect of cell density, cell geometry, and ECM stiffness on YAP/TAZ localization and cell proliferation. High cell Density, small cell geometry, and soft ECM lead to YAP/TAZ inactivation, and growth arrest. (A) Cell area in the three seeding conditions. Cells plated at different densities display increasingly smaller cell-substrate adhesion areas. Cells seeded to obtain sparse cells and confluent or dense monolayers. After 2 days, cells fixed for immunofluorescence with anti-E-cadherin antibody (αE-CAD) to visualize the formation of cell-cell contacts. TOTO3 is a nuclear counterstain. Scale bar, 20 μm. (Right) Average cell area in the three seeding conditions. (B) Cell proliferation in the three seeding conditions which measured as the percentage of BrdU-positive cells. Cell plated as in (A); after 2 days, cells were incubated with BrdU to label cells undergoing DNA duplication. Then cells were fixed and processed for anti-BrdU immunofluorescence (αBRDU). (Right) Quantitation of cell proliferation in the three seeding conditions. (C) Nuclear/cytoplasmic localization of YAP/TAZ in the three seeding conditions. Cells plated as in (A) and stained for immunofluorescence with anti-YAP/TAZ antibody (αYAP/TAZ). TOTO3 is a nuclear counterstain. Scale bar, 20 μm. (Right) The proportion of cells displaying preferential nuclear YAP/TAZ localization (N, black), even distribution of YAP/TAZ in nucleus and cytoplasm (N/C, gray), or cytoplasmic YAP/TAZ (C, white). (D) Effects of restricting cell-substrate adhesion area (Cell geometry) and (G) ECM substrate stiffness on YAP/TAZ localization and cell proliferation. In (D), immunofluorescence images of YAP/TAZ with anti-YAP/TAZ antibody (αYAP/TAZ). DAPI is a nuclear counterstain. Scale bar, 20 μm. (E) YAP/TAZ nuclear/cytoplasmic localization and (F) percentage of cell proliferation which processed for BrdU. In (G), Immunofluorescence images of YAP/TAZ in plated cells on fibronectin-coated stiff (plastic) and soft (acrylamide hydrogels of 0.7 kPa) substrates using anti-YAP/TAZ antibody (αYAP/TAZ). TOTO3 is a nuclear counterstain. Scale bar, 20 μm. (H) YAP/TAZ nuclear/cytoplasmic localization and (I) percentage of cell proliferation which processed for BrdU. Down: Cells seeded as individual cells plated on fibronectin-coated glass (large) or square microprinted fibronectin islands of 300 μm2 (small). (Reprinted with permission, Cell Press, for citation see [53]).

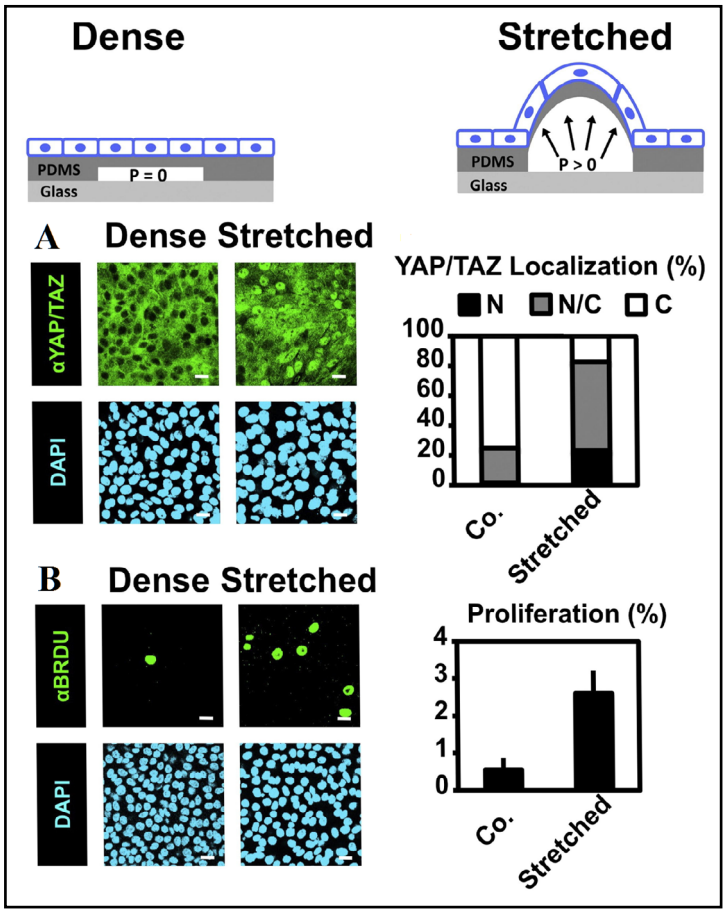

Fig. 4. Effects of stretching of cells on YAP/TAZ localization and cell proliferation. The stretching of a cell monolayer overcomes YAP/TAZ and growth inhibition in contact-inhibited cells. (A) Cells plated on the stretching device. After 2 days, cells were subjected to static stretching, fixed with the device still under pressure, and then stained with anti-YAP/TAZ antibody (aYAP/TAZ) for immunofluorescence imaging. DAPI is a nuclear counterstain. Scale bar, 20 mm. (Right) The proportion of cells displaying preferential nuclear YAP/TAZ localization (N, black), even distribution of YAP/TAZ between the nucleus and the cytoplasm (N/C, gray), or prevalently cytoplasmic YAP/TAZ (C, white). (B) Cells plated on the stretching device. After 2 days, cells were subjected to static stretching in the presence of BrdU to label cells undergoing DNA duplication. Scale bar, 20 mm. (Right) Quantitation of proliferation measured as the percentage of BrdU-positive cells (Reprinted with permission, Cell Press, for citation see [53]).

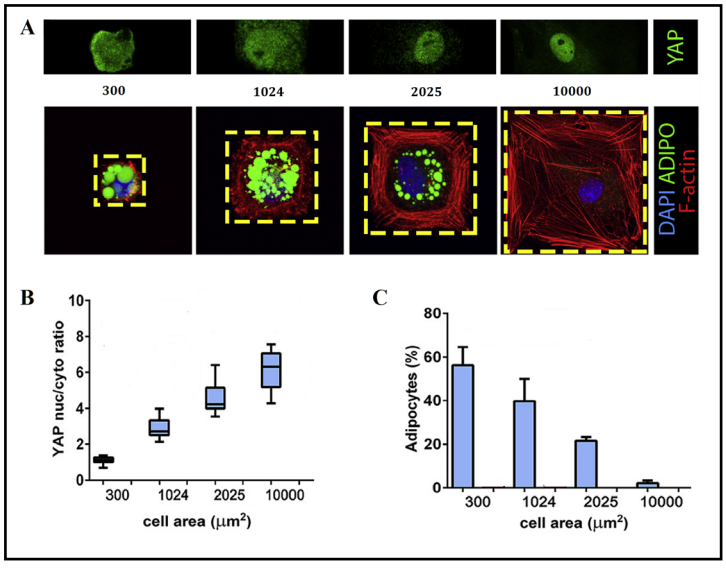

Fig. 5. The mechanical regulation of MSC differentiation to adipocyte via cell spreading and YAP activation in single MSCs seeded onto micropatterned surfaces with controlled areas (300 to 10000 μm^2). (A) Immunofluorescence imaging of YAP/TAZ (green) localization in MSC seeded onto micropatterned surfaces with controlled areas. (B) Immunofluorescence imaging of single MSC grown onto micropatterns with the increasing surface area for 3 days in adipogenic medium. Single-cell stained for lipids (green), F-actin (red), and nuclear (blue). (B) YAP/TAZ nuclear/cytoplasmic localization and (C) percentage of adipocytes for each area. Dashed lines indicate the adhesion area (Reprinted with permission, Elsevier, for citation see [57]).

Using the knockdown and overexpression of YAP/TAZ, studies have shown the leading role of YAP and TAZ activity is not only the biological response to mechanical cues but also mediating the mechanical signals. For example, cells with reduced expression of YAP and TAZ were cultured on large adhesive areas or stiff ECM but showed a phenotype that commonly happens in small adhesive areas or on soft ECM. Conversely, increased expression of YAP and TAZ in cells grown on a soft matrix caused cells to show the behavior of cells in the presence of a stiff matrix [58]. Furthermore, the inactivation of YAP and TAZ, in conditions of disrupted F actin or inhibited Rho, confirmed the close dependence of mechanotransduction procedures on the integral actomyosin cytoskeleton. In the same regard, YAP and TAZ activity have been shown to increase when overexpression of the Rho-regulated F actin nucleator diaphanous causes F actin polymerization [39]. Another evidence that proved YAP and TAZ acting as mediators of mechanical signals is that weakening the innate tensile forces by inhibiting the myosin or its regulators such as Rho-associated kinase (ROCK) and myosin light-chain kinase (MLCK) inactivate YAP and TAZ which make cell to show a phenotype belonging to the presence of a soft ECM or restricted cell size [58].

According to the aforementioned explanations, the rate of YAP/TAZ accumulation in the cytoplasm or nucleus plays a key role in regulating many cellular behaviors. Many factors and molecular elements are involved in sensing and transmitting environmental mechanical signals from the cell surface to the nuclear and regulating the YAP/TAZ localization. For example, at a low mechanical stress, binding the ARID1A–SWI/SNF complex to YAP/TAZ inhibits their activation. In contrast, binding nuclear F-actin to the ARID1A–SWI/SNF complex in response to high mechanical stresses, inhibits ARID1A–SWI/SNF-YAP/TAZ complex formation, facilitating association between TEAD and YAP/TAZ [59].

Four key mechanical checkpoints play the most significant role in transmitting mechanical signals and YAP/TAZ regulation, including focal adhesions, cytoskeletal tension, Rho small GTPases, and nuclear membrane protein elements (Fig. 6) [60]. These hubs act as key upstream mechanical checkpoints in the cell that affected YAP/TAZ activity depending on the environmental mechanical cues. Generally, all the mechano-responsive factors are directly or indirectly associated with these mechanical checkpoints. Considering the importance of these checkpoints in mechanical-depended YAP/TAZ regulation, in the following, we discuss how mechanical cues and upstream mechanical checkpoints in cells influence the activity of YAP/TAZ mechanotransducers.

Cellular Mechanical Checkpoints in Regulating YAP/TAZ Activity

Focal Adhesion Complex Mediators and YAP/TAZ Regulation. The main hub for cell mechanosensing is complex protein structures called focal adhesions [13] where congregated integrin receptors interact with both ECM and actin cytoskeleton [61]. Over 30 years ago, integrins were recognized as a widely expressed family of adhesion receptors with a principle mediating role in platelet and leukocyte aggregation and endothelial adhesion [62]. Transmembranal integrins are proteins that bridge the ECM substrates attached to their extracellular domains to various intracellular structures including the cytoskeleton by their intracellular domains. This bridging function of integrins is denominated as cell-ECM crosstalk [39, 63]. Integrin complexes promote cell survival, spreading, migration, proliferation, and differentiation by cell-ECM crosstalk that include sensing mechanical cues such as substrate stiffness and responding to them via its close interactions with cytoplasmic receptors and the cytoskeleton.

It has been observed that the extracellular fibronectin along with stress fibers colocalize with actin filaments at the cell surface terminating at the adhesion plaques. The matrix/actin cytoskeleton coupling function of ECM receptors has been supported with further findings too [64]. For instance, fibronectin has been shown to release from the cell surface in case of inducing disruption of the actin cytoskeleton using cytochalasin B. This observation suggested the fibronectin contribution to organizing the actin cytoskeleton-connected attachment plaques [62].

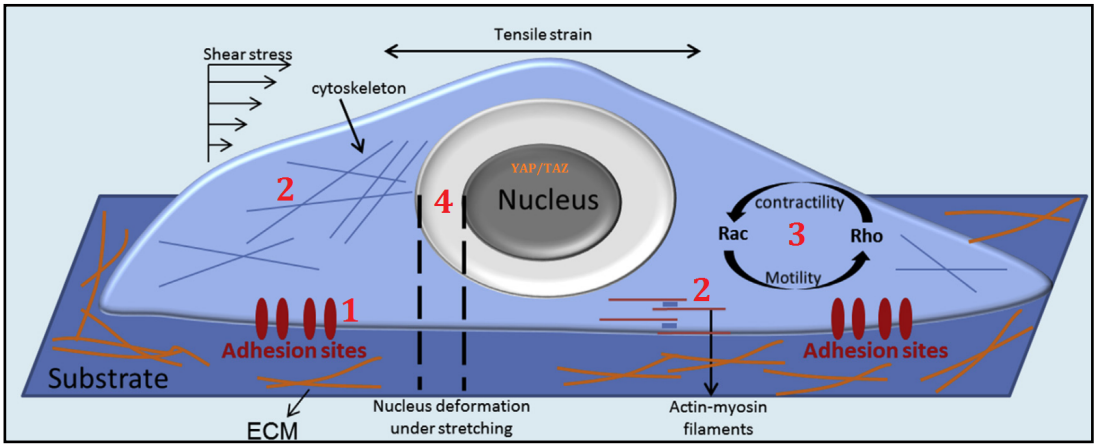

Fig. 6. The dominant elements, as upstream mechanical checkpoints, regulate YAP/TAZ localization in cell response to mechanical stimuli. In cells exposed to mechanical cues, YAP/TAZ localization (nuclear or cytoplasm) regulated through changes in the adhesion sites (1), cytoskeletal elements (2), the Rho signaling pathway (3), and nucleus deformation (4) (Reprinted with permission, Elsevier, for citation see [60]).

The FA mechanosensing complex is a large bidirectional network constituting almost 180 adaptors (intermediate), signaling, and structural modules that together provide a force-mediated system for different cell functions and control the integrin/actin adhesome (cell-matrix focal adhesion system) [65]. All these three functional proteins have a distinct regulatory function in mechanosensing and cellular mechano-response [10]. These modules with their adaptor, cytoskeletal, and signaling roles together regulate the dynamics of FAs and adjust the actin cytoskeleton-integrin link. FA structural proteins have represented the role of connecting the actin cytoskeleton components such as talin, vinculin, and tensin1 to the ECM-bound integrins [10]. FA signaling proteins include for example FAK and paxillin that contribute to adhesion-based signaling [66]. Other proteins, including kindlin2 (or FERMT2) and α-actinin, exert intermediating functions known as adaptors [67].

Despite the recognition of these pivotal components of the FAs' molecular architecture, the dynamic processes happening within FAs, and the functional relevance of these components to mechanotransduction is not much disclosed [66]. An interesting difference between these groups of core FA proteins is their various mobility and turnover that correlates with their function, as structural proteins are significantly less mobile and have the slowest turnover, while the signaling proteins are more mobile with a very fast turnover having a considerable higher transient residency time within the adhesion [67, 68]. The signaling proteins seem to be not involved in sensing the rigidity of ECM since, in contrast to structural proteins that change mobility regarding the substrate stiffness, the signaling protein turnover rates show no relation to the substrate stiffness. Talin, vinculin, and several isoforms of tensin are the main structural proteins of FA that physically link to the integrins and actin cytoskeleton. Talin activates integrins by binding to them and contributes to the cell adhesion and spreading, vinculin is essential for FA function and force transduction, and different isoforms of tensin are involved in its localization pattern [69]. Accordingly, while tensin1 localizes to both focal adhesions and fibrillar adhesions (FBs), tensin2 is mainly found in FAs, and tensin3 presents in FBs almost exclusively [70]. However, the activation and regulation mechanism of tensin family proteins is not much known [71]. Tension-dependent conformational changes activate talin and vinculin showing that these proteins can sense the force-generated signals from the environment. Active talin and vinculin contribute to a trilateral compound (including both of them plus the actomyosin machinery) that causes FA maturation and stabilization. Then, tension-dependent FA maturation is a process discussed to be occurring concerning actomyosin tension and actin crosslinker α-actinin. α-actinin is suggested that transmits the forces, and actin stress fibers serve as a template for FA maturation. There are other factors detected to be involved in adhesion regulation and mechanotransduction such as

Kindlin2, FAK, and paxillin. While an increased level of FAK and paxillin phosphorylation is observed on stiff substrates, Kindlin2 activates integrin and contributes to the paxillin recruitment [71].

FAK phosphorylation contribution in cell response to substrate rigidity is shown to be through forming a focal complex (FX) with Src kinase which matures to FAs later. This FAK-Src complex-driven phosphorylation also contributes to cell spreading, cellular response to cyclic stretching, mechanotransduction, and paxillin localization at cell-ECM adhesions [72]. Different forms of mutated paxillin have been shown that contribute to cell-ECM adhesions, however, it is not exactly determined that whether they are involved in initial sensing of substrate stiffness or the cellular response. As a piece of the puzzle, it has been shown that a tyrosine to glutamate mutation creates a phosphomimetic form of paxillin which is mostly localized to the FXs, while the tyrosine to phenylalanine mutation creates the phospho-null mutants that preferentially localize to FBs [73]. Several studies strongly support the idea of integrins function as signaling receptors and mediators of cell adhesion [74]. For instance, it has been detected that every interaction between the integrin and extracellular substrates activates the FAK and Src tyrosine kinases. FAK can also be activated with some GFs and other agonists that also activate the sodium-proton antiporter and protein kinase C (PKC) [75]. FAK needs to cluster to be activated by integrins and GFs. FAK clustering critically depends on the cytoskeletal processes such as actin polymerization and actomyosin contractility. Although FAK is not a necessity for FA formation, it can bind to cytoskeletal proteins and activate Rho GTPases via *Crk*-associated substrate (Cas) [74, 76].

FAs and stress fibers are induced to assemble in presence of stiffer substrates which in turn activates the FAK kinase, induces cell spreading, triggers the Hippo pathway, and increases YAP/TAZ activity in a talin-dependent manner. As was mentioned above, talin is one of the tension-sensing FA proteins [77]. Then, YAP/TAZ activity is another factor that is regulated by ECM stiffness sensing mechanisms of cells through cell spreading. It has been discussed that cells' nuclear localization has a correlation with YAP/TAZ activity within the cells spreading and growth on stiff ECM [78]. Nuclear localization can be controlled by the mechanical forces drove by the cell morphology, spreading, and connection with ECM. These motives apply the mechanical forces through LATS1/2-dependent YAP phosphorylation. Talin and the LINC complex are mostly in association with the cell-ECM mechanical connections rather than any other cytoskeletal structure. Mechanistically, the LINC complex connects the nucleus with the stress fibers, while talin forms FAs and stress fibers via unfolding [79]. Therefore, talin and LINC complex in combination transfer the extracellular forces to the nucleus and cause YAP translocation by connecting the ECM and focal adhesions to the cytoskeleton and nucleoskeleton [80].

The level of FA tension can also regulate the YAP/TAZ signaling hub. As in large actomyosin force and stiff ECM, the FA tension is high which causes FAK, Src, and Cas to be phosphorylated sequentially. Whereas, by a small actomyosin force and/or soft ECM, the FA tension is low which leaves the FAK, Src, and Cas non-phosphorylated. In the first state that Src and Cas are phosphorylated, they trigger PI3K–PDK2 and Rac1–PAK pathways, respectively, which facilitate YAP translocation to the nucleus by preventing its LATS-mediated phosphorylation [80]. Despite all molecular findings, the knowledge about the highly complex mechanism of the FA-regulation of the Hippo pathway and YAP/TAZ especially in different cell types and morphologies is incomplete. What is disclosed is that FA signaling leads to cell proliferation and survival on stiff substrates by suppressing the Hippo pathway and inducing the YAP/TAZ activity and that the integrin signaling is an involved molecular mechanism [38]. Stiff ECM can cause YAP translocation to the nucleus and activating the transcription of some genes. These fibronectin-rich substrates can suppress the LATS1/2 activity by triggering the β1-integrin–FAK–Src–PI3K–PDK1 pathway off. LATS1/2 is also involved in the YAP phosphorylation inhibition by the Src–Rac1–PAK pathway [80]. We still do not know if these signaling axes (FAK–Src–PI3K–PDK1 and Src–Rac1–PAK) act within a common cascade or function parallelly. Activated PAK also promotes the Merlin phosphorylation that attenuates YAP phosphorylation by pausing function as a scaffold for YAP and LATS1/2 [81]. Evaluating

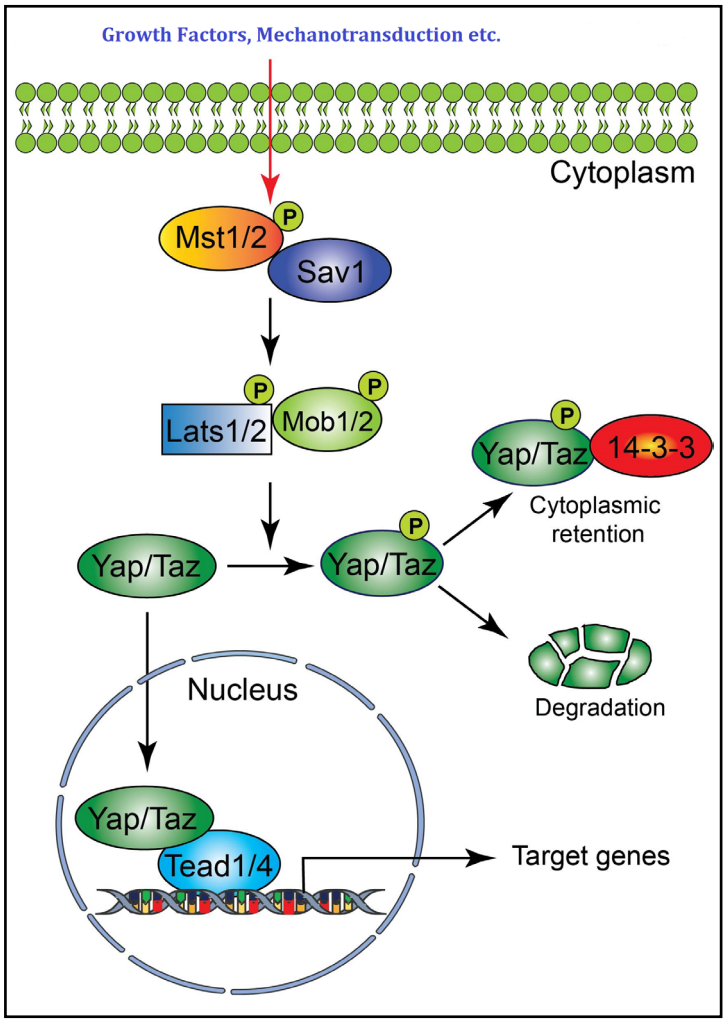

Fig. 7. The Hippo pathway effectors which involved in regulating the localization of YAP/TAZ. When the Hippo pathway is not active, YAP/TAZ can interact with TEAD1-4 transcription factors and promote the transcription of genes involved in cell proliferation. When the Hippo pathway is active, YAP/TAZ is inhibited due to their phosphorylation by core components of the Hippo pathway such as SAV1, MST1/2, and LATS1/2 (shown in the central rectangle). In a phosphorylated form, cytoplasmic YAP/TAZ may interact with (a) the 14-3-3 protein, (b) components of cell junctional complexes like AMOT or b-catenin, or (c) may be degraded in proteasomes [84].

the gene expression profiles of a cell line on a stiff substrate compared to a soft one has shown that YAP/TAZ and LATS1/2 involve in most gene expression alterations. Hippo signaling and YAP/TAZ have also been shown to be critically involved in FA-mediated substrate stiffness-regulated transcription in association with the FAK and Src tyrosine kinases [81].

FAK has been also shown that can increase the YAP activity by removing the inhibitory phosphorylation on S397 of mice YAP either indirectly via promoting the association of YAP with the protein phosphatase 1A (PP1A) or directly by tyrosine phosphorylating of Y357 in YAP and Y26 in the regulatory protein MOB1 (scaffold proteins MOB Kinase Activator 1) which causes inhibition of its binding to LATS1/2 [82]. YAP can be also phosphorylated on 3 sites (Y341/357/394) by Src family kinases that result in increased transcription stimulation activity of YAP. An underlying reason is suggested to be its increased interaction with TEAD. Src also regulates Hippo proteins directly as Src-mediated phosphorylation of LATS1 and LATS2 is triggered by cell adhesion and suppresses their activity. Studies on mammalian Hippo pathway-dependent YAP/TAZ activity have reported a pivotal kinase cascade in the Hippo pathway in which the activated MST1 or MST2 (Mammalian Sterile 20-like Kinase 1 or 2) binds to the scaffold protein SAV1 (Salvador Homolog 1) and causes its phosphorylation [83]. Then, this activated complex (MST/SAV1) activates the LATS1 kinase, LATS2 kinase, MOB1A, and/or MOB1B via phosphorylating them and creating another complex consisting of LATS and MOB. Afterward, the active LATS/MOB complex inhibits YAP/TAZ by phosphorylation via two different mechanisms. First is the phosphorylation of YAP on serine 127 and TAZ on serine 89 causing the 14-3-3 binding and their sequestration in the

cytoplasm. The second is the phosphorylation of YAP on serine 381 and TAZ on serine 311 that lead to more phosphorylation by Casein Kinase I δ/ε. Finally, proteasomal degradation occurs by the contribution of the E3 ubiquitin ligase SCF (β-TRCP) (Fig. 7) [84]. This is while the non-phosphorylated YAP/TAZ complex transfers to the nucleus and stimulates some gene expression in association with other transcription factors, including TEAD. FAK and Src impact on the Hippo signaling pathway can also occur indirectly via other signaling pathways at their downstream [85]. For instance, in a study, breast epithelial cells (MCF10A) suppressed the LATS1/2 activity after adhering to the fibronectin-coated substrates via a FAK–Src–PI3K–PDK1 pathway [86].

Cytoskeletal Tension and YAP/TAZ Regulation. According to the available literature, the cytoplasmic actomyosin cytoskeleton in cooperation with FAs-associated proteins pivotally regulates the mechanotransduction pathways such as Hippo-YAP signaling through various mechanical and biochemical cues and contractility micro-devices [87, 88]. The actin cytoskeleton is a dynamic structure constituted from actin globular monomers (G-actin), filamentous actin (F-actin), and a variety of actin-binding proteins such as myosin II. This motor molecule in cooperation with F-actin is responsible for the cell's mechanical properties by generating tension forces in actomyosin complexes. Myosin II is regulated by the Rho GTPases that phosphorylate the myosin light chains (MLC) by the mediation of the myosin light chain phosphatase (MLCP). Phosphorylated MLC can generate adhesion and tension forces by interacting with F-actin. The noncovalent interactions of the actomyosin cytoskeleton confer it dynamicity which facilitates its active responding and rearrangement to the physical and chemical stimulators (e.g., GFs, chemokines, and substrate stiffness) and balancing different forces exerted from tension, integrin clusters, and cytoskeletal stress fibers by equilibrating between filamentous and monomeric forms [88, 89].

Actin dynamicity means switching between G-actin and F-actin that can be activated by environmental stimuli and trigger a series of intracellular signaling cascades leading to rearrangement of the cytoskeleton and other structures in the cell. A very important regulatory function of the F-actin/G-actin ratio is reciprocal cell shape transforming between spherical and spread states [89]. For example, F-actin creates adhesion contacts, stress fibers, and membrane projection resulting in the spread shape (Fig. 8) [57]. In response to environmental stimuli, the actomyosin cytoskeleton may reorganize and form stress fiber, and F-actin structures undergo polymerization/depolymerization cycles which may lead to force distribution and cellular spreading. These dynamic changes in the cell, in turn, stimulate the adhesion molecules and activate signal transduction pathways including

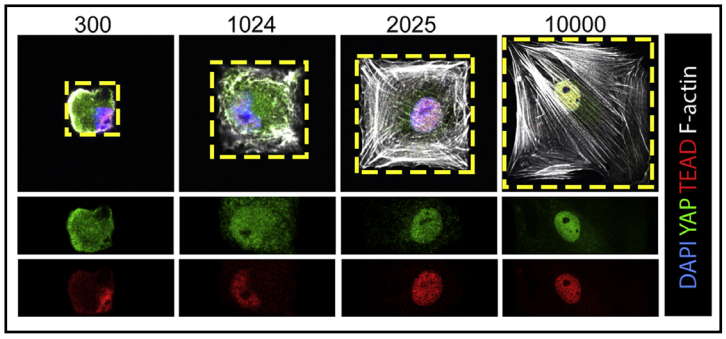

Fig. 8. Effect of focal adhesion areas on cytoskeleton tension (F-actin), YAP/TAZ localization, and cell shape. Focal adhesion areas can affect the cytoskeleton tension by changing the F-actin/G-actin ratio. cytoskeleton tension can also affect the YAP/TAZ localization. A very important regulatory function of the F-actin/G-actin ratio is the reciprocal cell shape transforming between spherical (low tension) and spread (high tension) states. Cytoskeleton tension can be regulated by restricting single cells on fibronectin-coated micropatterned surfaces with controlled areas (300 to 10000 μm^2). Cells in larger patterns have more focal adhesion areas, leading to increased cytoskeleton tension, the YAP nuclear/cytoplasmic ratio, and spread shape. Immunofluorescence image represents single MSCs grown onto fibronectin-coated micropatterns having the indicated adhesion area and stained for F-actin (white), YAP (green), and TEAD (red) (Reprinted with permission, Elsevier, for citation see [57]).

mitogen-activated protein kinases and Rho GTPases which regulate the expression of some genes largely by on/off switching [90].

All of the above-mentioned cellular contractility and intracellular actin dynamics eventually result in cell differentiation, migration, motility, and even cellular division and proliferation [78, 90]. Therefore, the role of the cytoskeleton as "a complex scaffold of filaments scattered all through the cytoplasm" is much determinative in supporting the cell, forming its structure and rigidity, its subcellular organization, and intracellular transport of molecules and mechanotransduction, as well as responding to environmental stimuli. Among all, YAP/TAZ in close relation to the F-actin cytoskeleton plays a critical role in cell adhesion to the substrate by adapting different conformation and tension [78]. In cells cultured on large or stiff substrates, YAP/TAZ expression increases due to high cytoskeletal tension driven by ROCK and non-muscle-myosin-II, while in cells cultured on softer or smaller substrates, the reduced adhesive area and the round shape of cells inhibit YAP/TAZ (Fig. 6) [91]. Therefore, the mechanical forces that regulate the YAP/TAZ cellular signals are derived from the architecture and properties of the actin cytoskeleton which are mainly controlled by tissue/cell shape and 3D ECM [58].

Apparently, high mechanical and low mechanical forces lead to concentrated activating and inhibiting YAP/TAZ proteins, respectively, representing YAP/TAZ as a connector of tissue/cell architecture and cellular functions. F-actin cytoskeleton reorganization due to YAP and TAZ activation/inhibition is mediated by F-actin capping (e.g., CapZ) and severing (e.g., Cofilin) proteins [53]. In this regard, in contact-inhibited central cells in multicellular sheets with low mechanical stresses, F-actin-capping and -severing proteins play a critical role in suppressing YAP/TAZ since a minimal level of CapZ or Cofilin significantly increases localizing YAP/TAZ in the nucleus, activates the transcription, and induces the proliferation [92]. Correspondingly, the peripheral cells with high cytoskeletal contractility proliferate in a YAP/TAZ-dependent manner and are minimally influenced by the loss of CapZ or Cofilin (Fig. 9) [58].

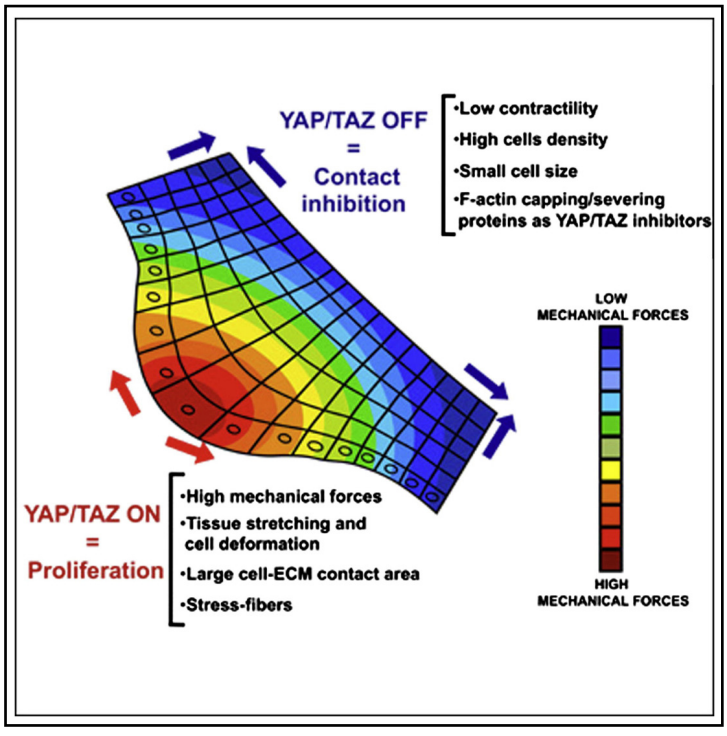

Fig. 9. Schematic representation of the role of environmental micro-mechanical forces and other extra- and intracellular conditions on the activation of TAP/TAZ which can lead to inhibition of cell growth or proliferation (Reprinted with permission, Cell Press, for citation see [53]).

Then, it can be inferred from the available data that YAP/TAZ promotion due to mechanical forces is considerably mediated by inhibiting capping and severing proteins. For instance, inactivated capping and severing proteins have been shown to reorganize the contractile F-actin bundles (actin stress fibers). Whereas, YAP/TAZ inactivation causes decreased stress fiber formation and cellular contractility- a phenocopy of suppressed formin and myosin [58]. According to this, studies have shown that manipulation of F-actin levels through mutation or knockdown of regulators of the actin cytoskeleton or treatment with F-actin–inhibitory drugs had dramatic effects on Hippo signaling and YAP/TAZ activity. For example, knockdown of actin-capping protein (CapZb) causes an increase in F-actin, nuclear YAP/TAZ, and YAP/TAZ target gene expression (Fig. 10) [53].

YAP/TAZ activity is also controlled by the filamentous actin not only through the Hippo pathway (LATS1/2)-dependent mechanism but also through the Hippo pathway (LATS1/2)-independent mechanism each of which might predominate in a not well-understood particular cell type or set of conditions. Inhibition of F-actin activates LATS1/2 leading to YAP/TAZ regulation through the LATS1/2-MOB1 complex, nevertheless, some recent findings discuss that LATS has a marginal role in actin-dependent mechanotransduction via YAP/TAZ. For example, suppressing TAZ and making it unstable by an actin end-blocking factor-like latrunculin A has not been stopped by LATS1/2 deletion mutation which means that the activity of actin cytoskeleton on the TAZ regulation is independent of LATS [93, 94]. Also, mechanical cues like a physically soft environment could inhibit YAP/TAZ in LATS1/2 deleted mutants using a LATS-independent pathway [94]. According to the same research, the depletion of CapZ could reorganize the F-actin networks stating a debatable inhibitory effect for YAP/TAZ on the actin cytoskeleton due to a soft environment [94]. Concluding from all of the aforesaid observations, LATS is just one factor among several regulators that affect YAP/TAZ retaining the cytoskeleton functional in response to the mechanical cues [95].

Several Ste20 family kinases such as MST1/2, MAP4K-family, and TAO on the upstream of LATS1/2 are responsible for its activation due to F-actin disruption [95]. LATS1/2 activation loop triggers with phosphorylation of the hydrophobic motif (HM) which induces kinase cascade to fully active LATS1/2 by autophosphorylating it at the activation loop (AL) domain. However, recently, a phosphorylating enzyme called STK25 has been recognized that directly phosphorylate the AL site of LATS1/2 and activate it in response to a disrupted

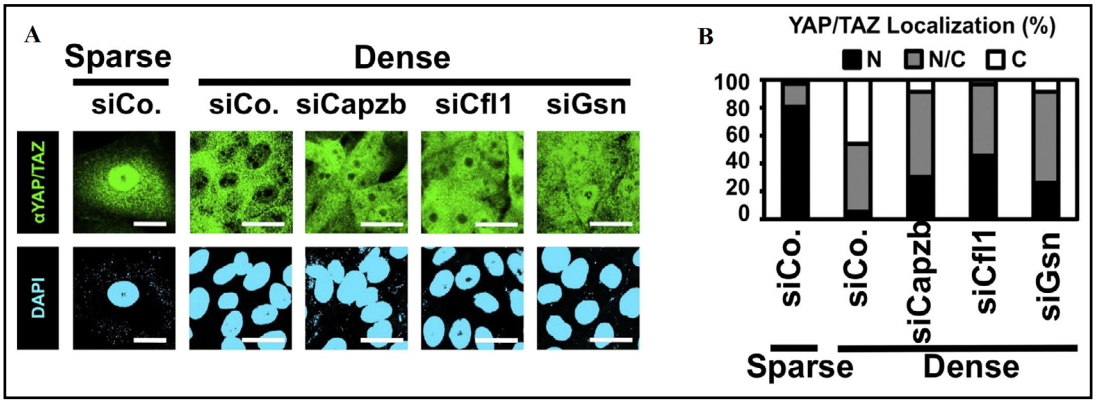

Fig. 10. Knockdown effects of F-actin-capping and -severing factors on contact inhibition of proliferation. The knockdown of these factors can rescue the contact inhibition of proliferation. (A) Immunofluorescence imaging of YAP/TAZ with anti-YAP/TAZ antibody (αYAP/TAZ). Cells transfected with inhibitory siRNA (si Capzb, siCfl1, siGsn, and siCO that is relative to GAPDH expression) and seeded to obtain sparse cells or a dense monolayer. Loss of Capzb, Cfl1, or Gsn, as F-actin-capping and -severing factors, lead to YAP/TAZ nuclear localization in dense monolayers. DAPI is a nuclear counterstain. Scale bar, 20 μm. (B) The proportion of cells displaying preferential nuclear YAP/TAZ localization (N, black); even distribution of YAP/TAZ between the nucleus and the cytoplasm (N/C, gray); or cytoplasmic YAP/TAZ (C, white) (Reprinted with permission, Cell Press, for citation see [53]).

F-actin network [96]. The regulation mechanism of LATS1/2 upstream kinases is not exactly understood to be exerted directly by F-actin or LATS1/2-mediated [97]. Additionally, protein kinase A (PKA) is another regulator that independently activates LATS1/2 in response to F-actin disruption through an unknown mechanism by phosphorylating sites different from the previous kinases [97, 98].

The fact that cell distortion and spreading are the main factors that affect YAP/TAZ activity via rearranging the F-actin cytoskeleton is confirmed by studies that discuss the involvement of FA components such as integrins, Src, and focal adhesion kinase (FAK) in YAP/TAZ activity. This ternary interaction between cell shape, F-actin, and YAP/TAZ activity is considered to be involved in various biological and pathological processes.

Furthermore, YAP/TAZ regulation is controlled by some physical and subcellular properties of F-actin rather than its total amount in contrast to the actin-mediated regulation of myocardin-related transcription factor (MRTF) family. YAP/TAZ mechanotransduction is controlled by the subcellular organization, fine structure, tension, microtubules, intermediate filaments, and the whole nucleus. On the contrary, MRTF is sensitive to F-actin/G-actin ratio since it binds directly to free G-actin in the nucleus and prevents its binding to the serum response factor (SRF) which is a DNA-binding partner [99]. YAP activation and localization in the nucleus can be stimulated by different factors such as extended ECM, stiff or stretched ECM, or fluid shear tension through pathways mediated by or independent from the Hippo signaling pathway. These cell types (e.g., sparse cells) with low mechanical stress situation, show no inhibition effect by LATS1/2 inactivation on YAP. Instead, the nuclear accumulation of YAP is prevented by inhibition of actin assembly or myosin II ATPase using pharmacological agents. In these situations, YAP localization is mechanically regulated by cell attachment through the maintenance of tension in an integral contractile actomyosin cytoskeleton without involving the integrin. Therefore, in sparse cells, YAP seems to be regulated by sustained YAP transcriptional activities and/or nuclear accumulation which is provided by mechanical tension rather than mechanosensation [100].

In a high cell contact situation as what can be observed in human epithelial cells and mouse embryonic fibroblasts, YAP nuclear exclusion due to contact inhibition appears dominant over the actomyosin cytoskeleton. However, in the absence of cell-cell contacts, YAP nuclear localization is mainly regulated by F-actin cytoskeleton network. In such a situation, ROCK-mediated myosin II contractility shows no regulatory effect on the YAP, and the suppressive effect on actomyosin contractility on YAP phosphorylation at Ser112 is dominated by actin cytoskeletal integrity in the absence of cell-cell contact and contractility. As aforesaid, Ser112 phosphorylation causes YAP sequestration in the cytoplasm. These two cytoskeleton-mediated regulation mechanisms for YAP has also been confirmed by YAP phosphomutant studies. The first mechanism involves YAP phosphorylation under the influence of actomyosin contractility, while in the second mechanism, cytoskeletal integrity dominates the phosphoregulation. However, some studies have shown that YAP accumulates in the nucleus due to sensing a stiff ECM through an F-actin-dependent mechanism and independent from the Ser112 phosphorylation. This data shows that the cytoskeletal integrity-mediated mechanism also operates during mechanotransduction [100].

Role of Rho GTPases in YAP/TAZ Regulation. As previously described, the YAP/TAZ pathway is an important transducing factor of cell structure and cytoskeletal organization that contributes to cell reaction to physiochemical changes in the whole tissue. Small GTPases, on the other hand, are the main regulators of the actin cytoskeleton organization. These Rho GTPases are a subset of the rat sarcoma virus (RAS) superfamily consisting of >30 small G proteins with chains of 188 to 189 amino acids and <21 kDa molecular weight [101]. The most notable member of this family is RAS homolog family member A (RhoA), RAS-related C3 botulinum toxin substrate 1 (RAC1), and cell division cycle 42 (CDC42). These Rho family members are well-characterized regulators of signaling pathways in reaction to developmental cues, mechanical stress, and inflammation and control various cellular functions including cytoskeletal dynamics, polarity, and morphogenesis [102]. It is reported

that there is a correlation between biochemical pathways and and YAP/TAZ activation. In a study, Sorrentino et al. reported that the geranylgeranyl pyrophosphate synthesized by the mevalonate cascade activate YAP/TAZ via a Rho GTPases-mediated manner [103].

RhoA is recognized to act as a transduction node for signals through GPCRs and is activated by G proteins (e.g., $G_{12/13}$) binding to Rho guanine nucleotide exchange factors (Rho-GEFs). The GPCRs are themselves stimulated by thrombin, lysophosphatidic acid (LPA), thromboxane, and sphingosine-1-phosphate (S1P) A2 as efficacious ligands for RhoA activation [104]. According to the recent genetic findings, in contrast to the Hippo pathway, small GTPases contribute to YAP/TAZ activation. Also, based on the biochemical investigations, small GTPases suppress the YAP phosphorylation in response to serum stimulation that promotes the YAP-TEAD interaction, whereas the Hippo pathway increases YAP phosphorylation leading to YAP-RUNX3 interaction [105]. It can be concluded that Rho-family small GTPases are molecular switches of YAP/TAZ DNA-binding transcription factors that can modulate several signaling pathways including mechanotransduction [101].

At the molecular level, the activation process of Rho GTPases includes converting the inactive GDP-bound form of Rho GTPases to their active GTP-bound form by the Rho-GEFs. On the other hand, its self-inactivation process is mediated by Rho-GTPase activating proteins (Rho-GAPs) that convert GTP-bound Rho GTPases form to GDP-bound forms by activating their intrinsic activity of hydrolyzing GTP to GDP [102]. Around 80 Rho-GEFs and 70 Rho-GAPs are coded on the human genome and the fundamental role of Rho-dependent signaling in actin-mediated mechanotransduction is extensively discussed in the literature [104]. RhoA-Rho-associated coiled-coil containing protein kinase (ROCK) pathway is among the principal signaling mechanisms involved in mechano-regulation of cell proliferation and/or differentiation. ROCK is responsible for the stress fiber assembling in response to environmental mechanical forces and controlling the tension stress within the cell. At the beginning of the ROCK pathway, RhoA is activated and promotes the phosphorylation level of the myosin light chain via RhoA-Rho-associated kinase which increases the tension by increasing the actin-myosin interaction [106].

The resulting cellular contraction, stress fiber assembling, cellular dynamic modulation, and mechanical feature changes can regulate cellular functions via affecting several gene expression profiles leading to cell differentiation and proliferation (Fig. 11) [106, 107]. Considering the regulatory effect of small GTPase signaling on the actin cytoskeleton, F-actin structures can be considered the common node of cross-talk between small GTPase signaling, Hippo pathway, and YAP nuclear localization. Also, RhoA activation by GTP binding leads to RhoA kinase (RAC) activation that promotes the activation of ROCK, PAK, and LIM6 kinase-1 (LIMK) in order. Consequently, cofilin (a potent actin-depolymerizing factor) is inactivated and F-actin stress fibers are formed [108, 109].

Cofilin inactivation through this pathway lead to YAP inactivation, decreased ARHGAP29 expression, and promoted RhoA activity. The latter, in turn, inhibits cofilin and stabilizes the actin filaments. ARHGAP29 inactivates RhoA by converting the GTP-bound RhoA to GDP-bound RhoA. ROCK can only be activated by GTP-RhoA and phosphorylates the Thr508 and Thr505 on LIM kinase 1 and 2 (LIMK1/2), respectively, to activate them [110]. The main substrate for LIMK is cofilin that is inactivated by phosphorylation at Ser3 leading to F-actin network stabilization. All these pathways cause the ECM feature, cell mechanics, and the cytoskeleton status function as a fundamental regulatory mechanism for YAP/TAZ activity [111]. Although there are several LATS-mediated regulatory mechanisms reported for YAP activity, YAP/TAZ regulation has been shown that can remain active in LATS1/2 depletion mutants cultured in soft gels. Therefore, despite the necessary contribution of Rho GTPase in this regulatory mechanism, it can be also performed independently from the Hippo/LATS pathway. Therefore, YAP regulation is performed through both the LATS-independent and -dependent pathways. One of the LATS-dependent pathways includes LPA and S1P stimulatory activity on G12/13-mediated Rho activation that causes Rho-mediated F-actin accumulation leading to YAP activity via inactivating LATS1/2 kinase. LATS1/2 kinases associate with GPCRs, LPA receptor (LPAR), and S1P receptor (S1PR) in order to activate and stabilize YAP

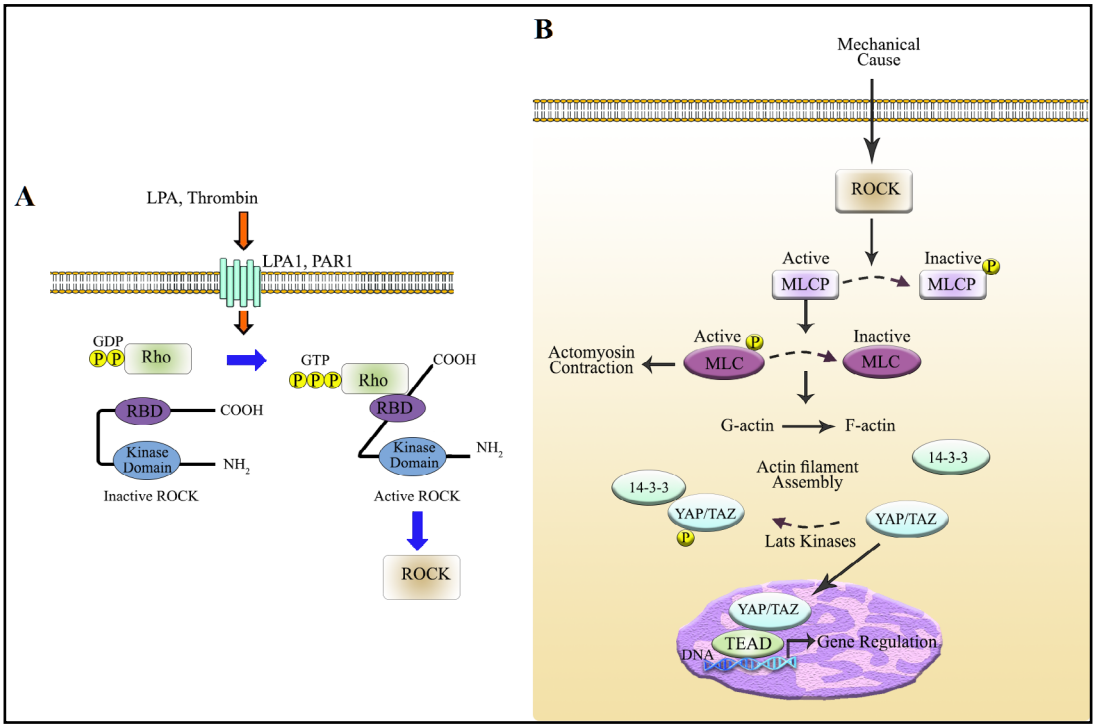

Fig. 11. ROCK controls the cytoskeletal dynamics and YAP/TAZ localization and activation. (A) Activation of ROCK isoforms by GTP-bound RhoA downstream of G protein-coupled receptors, such as LPA1 and PAR1. (B) Activated ROCKs phosphorylate MLC phosphatase, inhibiting its ability to dephosphorylate (and inactivate) MLC. Persistently phosphorylated active MLC is then able to induce stress fiber and focal adhesion formation, and cell contraction that leads to YAP/TAZ activation and nuclear localization [109].

and TAZ. Another LATS-dependent pathway of YAP regulation includes activating YAP by cyclic stretch through c-Jun N-terminal kinase (JNK) that promotes binding of LIM domains containing protein-1 (LIMD1, a LATS inhibitor) and LATS1 through direct phosphorylation of LIMD1 [112].

LIM domain is a unique structural domain containing two contiguous zinc finger motifs in a variety of proteins that contribute to several biological processes including cytoskeleton organization, cell differentiation, and tissue development. So, these studies show that Rho signaling regulates YAP activity in a LATS-dependent manner. Therefore, Rho signaling regulates YAP activity via both LATS-dependent and -independent mechanisms. As was mentioned above, actin dynamics is the intersection of YAP and Hippo-mediated mechanisms. However, these pathways exert different effects on actin organization as activated YAP inhibits RhoA activity preventing F-actin from linear elongation, but Hippo kinase induces F-actin capping proteins that promote actin branching process [113]. Moreover, YAP involves in the regulation of ARHGAPs transcription [111]. In addition to the RhoA-mediated regulatory effect of ECM and actomyosin tension mechanical cue on YAP/TAZ transcription, a reverse regulatory pathway has also been reported through which YAP upregulates the RhoA-actomyosin axis promoting an actomyosin-based tension in tissues [114].

Thus, YAP and RhoA signaling pathways are reciprocally regulated via a feedback loop because YAP promotes the expression of both the activator (ARHGEF17) and inhibitor (ARHGAP18/29) of RhoA [111]. In addition to RhoA activation via promoting the expression of ARHGEF17 (a Rho-GEFs), YAP induces either transcriptional or non-transcriptional expression of several other actomyosin cytoskeleton regulators and components, including myosin IIB, myosin regulatory light chain 2, and filamin A [37].

Nuclear Membrane Elements and YAP/TAZ Regulation. The YAP/TAZ nucleo-cytoplasmic distribution as a fundamental regulator of cell function is controlled by both mechanical and biochemical cues. The integrity of cell density, cytoskeletal integrity, and structures, matrix stiffness, adhesive complexes, cellular tension, metabolic state, kinases, soluble mediators, etc. all contribute to mechanotransduction from the plasma membrane to the nucleus and affect the YAP/TAZ nuclear accumulation [115, 116]. As previously described, YAP localization is an essential mediator of transferring the mechanical cues from the cellular environment, cytoskeleton, and nuclear membrane to the cell functions through both mechanical- and physical-dependent mechanisms. The inner nuclear membrane [3] and an outer nuclear membrane (ONM) of the nuclear envelope (NE) are separated by a perinuclear space (PNS) that develops into the ER lumen within ONM joining to the endoplasmic reticulum (ER) [117]. Lamina is a network of lamin A/C (LMNA) proteins locating on the inner surface of the INM that are mechanically connected to the NE and a variety of cytoskeletal proteins through the LINC complex and play a key role in the nucleus-cytoplasm connections. Therefore, LINC complexes facilitate the nuclear spatial and structural integrity and also involve transferring the external mechanical cues to the nucleus. The components of LINC complexes are connected to INM and ONM through their SUN (Sad1p, UNC-84) and KASH (Klarsicht/ANC-1/Syne Homology) domains. Proteins containing the SUN domains contain a single transmembrane segment followed by a short luminal sequence enabling them to correlate with nuclear lamins on one side and the chromatin locating across the INM which is connected to the nesprins (nuclear envelope spectrin repeat proteins) on the ONM on the other hand. The nesprin proteins contain the C-terminal KASH domain that extends into the PNS and forms various LINC complex isoforms in association with the SUN domain of one or more complementary SUN proteins. Nesprins contain variable domains on their N-terminal that facilitate their binding to various cytoskeletal components. Four isoforms of nesprins are detected in mammals that include nesprin-1, nesprin-2, nesprin-3, and nesprin-4. Nesprin-1 and -2 are called giant nesprins and contain the calponin homology (CH) domain at their N-terminal that mediate nesprins' binding to F-actin. The N-terminal domain of nesprin-3 can bind to plectin, the intermediate filament (IF) linker protein that acts as a cytoskeletal crosslinker and signaling scaffold. Finally, nesprin-4 makes indirect interactions with microtubules that are expanded across the F-actin to the membrane lamins and contribute to the LINC complex (Fig. 12) [109].

Additionally, nesprin proteins bind to the actin cytoskeleton, nucleoplasmic SUN protein trimer in the periplasmic space, and nuclear lamina via their calponin homology (CH) domains, KASH domains, and other transmembrane domains, respectively. The nuclear lamina is closely associated with chromatin. Emerin is another transmembrane protein component of the LINC complex that crosses over the inner envelope of the nucleus and binds to both SUN protein and lamina [117]. The LINC complex contributes to several structural and dynamical functions of the cell (e.g., linking the centrosome to the ONM, moving meiotic chromosomes, and nuclear motion and orientation). Also, it reacts to the extracellular mechanical cues that modulate cytoskeletal physical forces by connecting these stresses and strains to the nucleus. This nucleus/cytoskeleton mechanical coupling by the LINC complex is essential for Hippo-independent YAP nuclear translocation. Another cytoskeletal structure required for this pathway is talin whose unfolding helps FA and F-actin formation which pivotally contributes to transmitting forces to the nucleus and translocating YAP [117]. The intracellular mechanical connections made by talin result in coupling the ECM, FAs, cytoskeleton, and nucleoskeleton. Overall, the collection of the actin cytoskeleton, the mechanical forces within the cytoskeletal network, and its physical connections to the nucleus (mediated by nesprin 1 giant) control the influence of stress and strain to the nucleus (Fig. 13) [118].

Nesprin-1 is necessary for rapid activation of Src kinase to phosphorylate the emerin at cytoskeletal intersection points. This actin-binding LINC complex component exerts its activating function by stretching on the INM. Emerin is associated with the nuclear lamina and its phosphorylation causes changes in its binding to the lamin A (a nuclear structural protein).

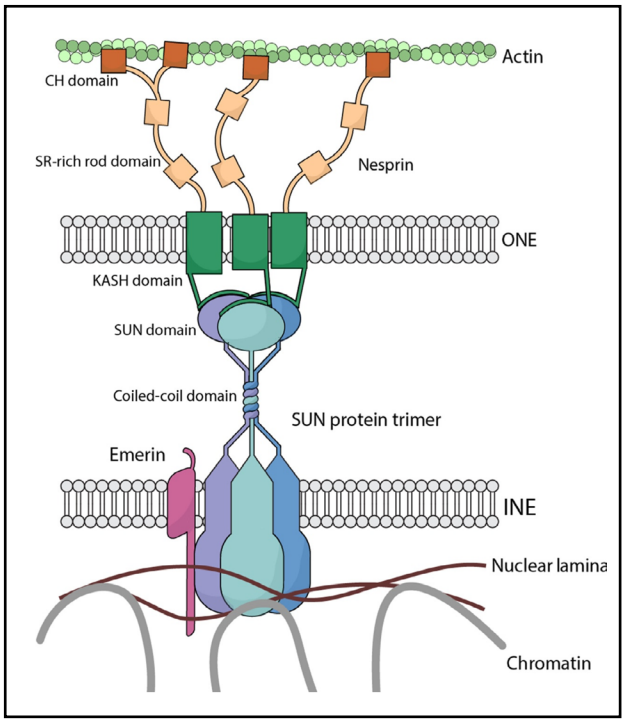

Fig. 12. Schematic representation of LINC complex and SUN-KASH proteins that span the nuclear envelope. LINC complexes facilitate transferring the external mechanical cues to the nucleus. The components of LINC complexes are connected to INM and ONM through their SUN domains and KASH domains. These domains as part of the LINC complex molecular chain connecting the cytoskeleton with the nucleoskeleton (Reprinted with permission, Elsevier, for citation see [109]).

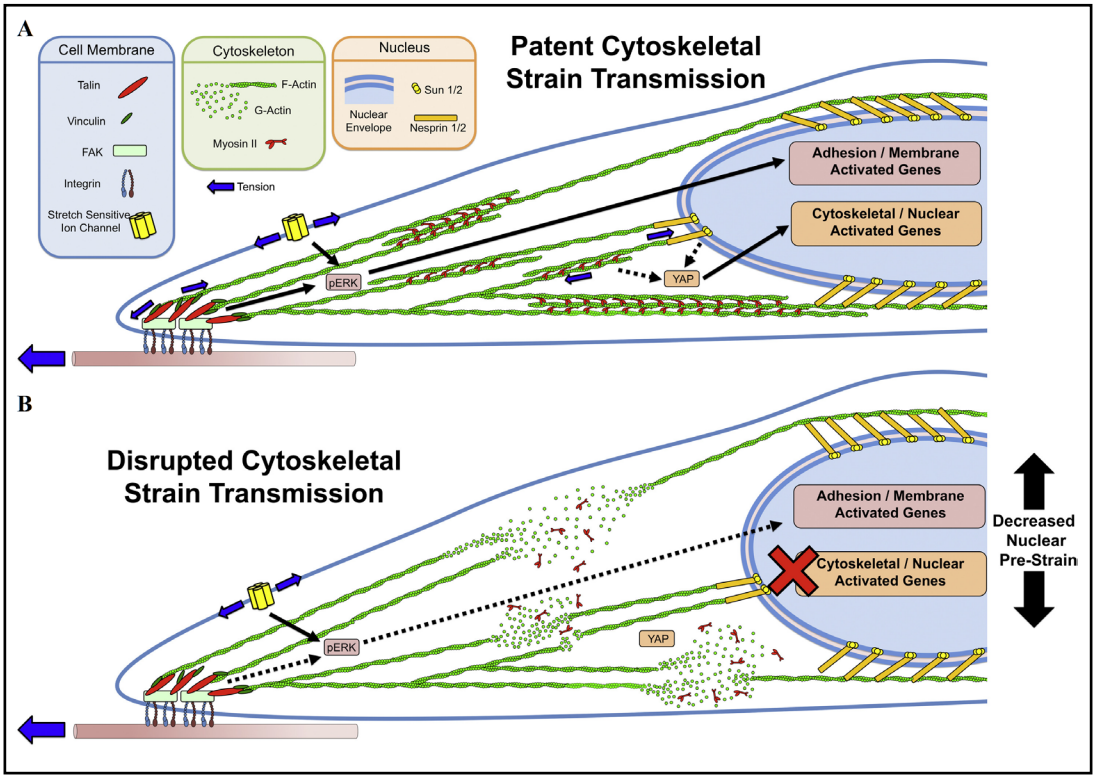

Fig. 13. Schematic illustration of the different routes of mechanotransduction from the cell surface to cell nuclear. Mechanoactivation may induce via cell membrane-mediated mechanotransduction elements or through cytoskeletal/nuclear strain transfer-mediated mechanotransduction elements. (A) Under basic conditions, both routes are probably operational. (B) By inhibiting ROCK, a decrease in nuclear prestrain and actin depolymerization has occurred; when the level of Nespirin 1/2 decreases, the transfer of cytoskeletal strain to the nucleus is also compromised. This leads to a loss in the cytoskeletal-to-nuclear strain transfer that is necessary for YAP nuclear localization and activation (Reprinted with permission, Cell Press, for citation see [118]).

These changes are associated with the nucleus stiffening, YAP accumulation in the nucleus, and inducing the expression of mechanically regulated genes. YAP nuclear accumulation also depends on the free nuclear YAP level and the availability of merlin in the nucleus. Free YAP includes proteins that are not trapped by TEAD or other transcription factors. Merlin involves in the YAP export signals in the nucleus. Some studies have also shown that the nucleus can directly act as a mechanotransducer for YAP nuclear translocation by increasing molecular transport through nuclear pores. This decrease in the mechanical restriction of nuclear pores originated from the forces created by the stiffness of the extracellular environment which are transmitted to the nucleus through the mechanical connection between the cytoskeleton and nucleus via focal adhesions and other afore-mentioned contributors. Then, these forces cause nuclear flattening which, in turn, generates some physical stretches in the nuclear pores. These stretches open up the nuclear pores and lessen their mechanical restriction to the molecular transport which leads to promoting YAP relocation to and accumulation in the nucleus (Fig. 14) [119].

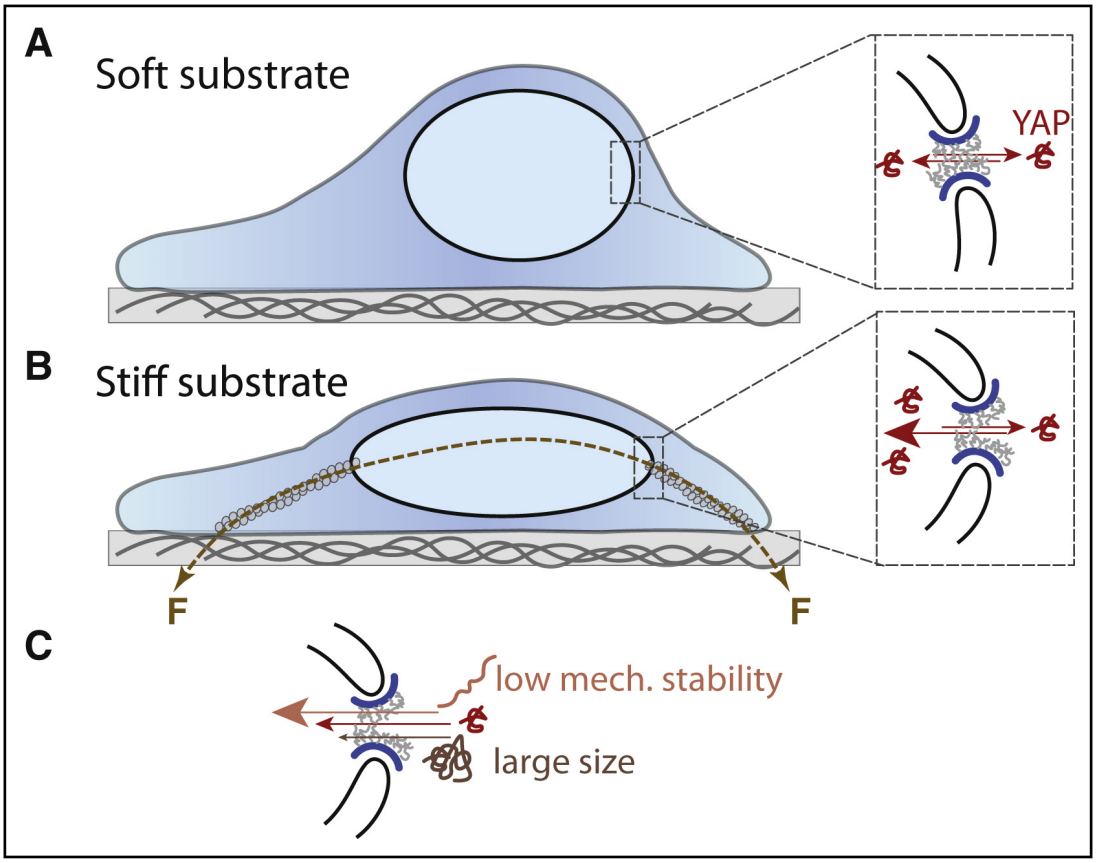

Fig. 14. Suggested model for mechanosensitive nucleocytoplasmic shuttling of YAP. (A) On soft substrates, the nucleus is mechanically uncoupled from the substrate and not submitted to forces. The import and export of YAP through nuclear pores are balanced. (B) On stiff substrates, focal adhesions and stress fibers (F-actin) are formed, the forces are applied to the nucleus and leads to flattening. This process led to stretching and curving nuclear pores that increased YAP import from the cytoplasm to nuclear. (C) Transmission through these pores depends on two factors: molecular weight and mechanical stability. Proteins with lower molecular weight or lower mechanical stability (easily unfolded protein) can pass through these pores much more easily. This is a general rule for transmission through such pores, which also includes the Yep molecule (Reprinted with permission, Cell Press, for citation see [119]).

The direct mechanosensing mechanism of nuclear pores continues when transported proteins show regulation function on the restriction of transport by their mechanical stability. The regulatory function of these proteins determinately applies to active transport of YAP and passive transport of small proteins, and even potentially on transcriptional activities. The active mechanism of protein transportation to and from the nucleus is an energy-dependent procedure exerted by importins and exportins. Nuclear passive diffusion through pores applies to small proteins (< 40-50 kDa). However, the existence of other associated mechanisms such as ion channels is undeniable. In the same regard, nuclear flattening can also impact the folds of the nuclear envelope which embody the mechanosensitive ion channels. Then, the forces change the protein concentration gradients by varying the nuclear/cell volume ratios. Studies have shown that actomyosin fibers are the main component of the actin cytoskeleton that mechanically connects the FAs to the LINC complexes at the apical surface of the nuclear envelope on stiff substrates. Whereas, such connection is not observed in cells spread on the soft substrates [119]. According to the literature, the forces generated by actomyosin are of contractile types that result in nucleus flattening and pore expansion leading to accelerating the YAP/TAZ import. This mechanism that does not alter the YAP/TAZ nuclear export rate is described as to be underlying the YAP/TAZ localization depending on the cell area and shape. Although the theoretical molecular weights of YAP and TAZ are ~65 and ~43 kDa, respectively, they both follow the active mechanism protein nuclear localization mechanism. Interestingly, after expanding the nuclear pore diameter due to actin cytoskeleton tension due to ECM stiffness, the nucleus-cytoplasm transport mechanism of YAP/TAZ does not switch to passive one and is still performed by importin and exportin. In summary, two mechanisms are considered responsible for nucleus-cytoplasm shuttling of YAP/TAZ both mediated by cell-ECM mechanical interaction. The first one includes inhibition of LATS1/2-mediated phosphorylation of YAP/TAZ. The second one includes stretching the nuclear pores and promoting the YAP/TAZ import. These two distinct mechanisms are suggested to be synergistically involved in regulating the YAP/TAZ transcriptional activity [114].

Conclusion

Physical forces, especially mechanical cues in the cellular environments, along with chemical factors such as growth factors, play a significant role in determining cellular behavior, which should be considered in related studies in the field of cell and tissue engineering. According to what was discussed, mechanical cues alone can also affect cell proliferation, differentiation, etc. In this regard, YAP and TAZ, are considered as the main mechano-responsive transcription factors whose function is affected by mechanical cues. YAP/TAZ are nucleocytoplasmic shuttling regulators in the cell where their localization or nucleus-to-cytoplasm ratio plays a key role in regulating cellular behavior. This ratio can be changed under the influence of chemical and physical factors in cellular micro-/nano-environments. Respectively, focal adhesions, cytoskeletal tension, Rho small GTPases, and nuclear membrane protein elements - as dominant cell mechanical checkpoints - are involved in sensing and transmitting mechanical signals from the cell environment to the cell nucleus and regulation of YAP/TAZ function. Understanding the cellular and molecular functions of these mechanical effectors whose impact on the activity of YAP/TAZ transcription factors will have important implications for comprehension of cell fate and function, discerning disease, and understanding how these factors might be targeted for cell/tissue engineering based-therapeutic applications.

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Author Contributions

The authors declare that the authors named in this article did this work. SAM, MMM, HT, AS, and SB were involved in study design and data collections. MMM, HT, SAM, YS, SA, RSS, and AS were involved in critically reviewing the data and help in writing the review article. MMM, HT, and FS were involved in revising the manuscript based on the reviewer's comments.

Disclosure Statement

The authors have no conflicts of interest to declare.

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