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Role of YAP/TAZ Nuclear Shuttling Dynamics in Stiffness-Mediated Cell Fate Decisions

[Aiko Tanaka[1], Liam O'Connell[2], Priya Mehta[3]]

[1] Kyoto Institute of Technology, Kyoto, Japan
[2] University College Dublin, Dublin, Ireland
[3] Indian Institute of Science, Bangalore, India

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Abstract

Extracellular matrix (ECM) stiffness is a critical physical cue that directs stem cell lineage specification and tissue homeostasis, yet the molecular mechanisms decoding mechanical signals into transcriptional programs remain incompletely understood. Here, we investigate the dynamics of YAP/TAZ nucleocytoplasmic shuttling as a central mechanotransduction module in stiffness-mediated cell fate decisions. Using a combination of live-cell imaging, fluorescence recovery after photobleaching (FRAP), and pharmacological perturbations in mesenchymal stem cells (MSCs) cultured on tunable polyacrylamide hydrogels (1–50 kPa), we demonstrate that substrate stiffness modulates the frequency, amplitude, and duration of YAP/TAZ nuclear translocation events. On stiff matrices (25–50 kPa), YAP/TAZ exhibited sustained nuclear localization with rapid shuttling kinetics (mean nuclear residence time 12.3 ± 2.1 min), whereas on soft matrices (1–5 kPa), shuttling was sporadic with prolonged cytoplasmic retention. Mathematical modeling revealed that nuclear shuttling frequency correlates with osteogenic differentiation, while sustained nuclear localization promotes proliferation. Pharmacological inhibition of actin polymerization (latrunculin A) and Rho-associated kinase (Y-27632) abolished stiffness-dependent shuttling dynamics and disrupted lineage commitment. Furthermore, we identified that YAP/TAZ shuttling cooperates with Notch signaling to fine-tune cell fate decisions. Our findings establish YAP/TAZ nuclear shuttling dynamics as a key determinant of mechanosensitive cell fate, providing a framework for understanding how physical microenvironmental cues are encoded into discrete transcriptional outputs.

Keywords: YAP/TAZ, nuclear shuttling, mechanotransduction, matrix stiffness, stem cell fate, osteogenesis, Notch signaling, live-cell imaging

Introduction

Cells continuously sense and respond to mechanical cues from their microenvironment, including extracellular matrix (ECM) stiffness, topography, and applied forces. Among these, matrix stiffness has emerged as a potent regulator of cell behavior, influencing migration, proliferation, and differentiation (Dupont, 2016; Heng et al., 2020). In stem cells, substrate stiffness can direct lineage specification: for example, mesenchymal stem cells (MSCs) commit to osteogenic lineages on stiff matrices and adipogenic lineages on soft matrices (Pathak et al., 2014; Rosowski et al., 2015). The transcriptional coactivators YAP and TAZ are central mediators of mechanotransduction, transducing mechanical signals from the ECM to the nucleus to regulate gene expression (Driskill & Pan, 2023; Dupont, 2016). YAP/TAZ activity is controlled by nucleocytoplasmic shuttling: when active, they accumulate in the nucleus and bind TEAD transcription factors to promote proliferation and inhibit differentiation; when inactive, they are sequestered in the cytoplasm and degraded (Shreberk-Shaked & Oren, 2019).

Although the steady-state nuclear localization of YAP/TAZ in response to stiffness is well documented, the dynamic aspects of their shuttling—such as frequency, amplitude, and duration—remain poorly characterized. Recent studies have highlighted that transcription factors exhibit dynamic nuclear shuttling that encodes information beyond simple localization (Lasick et al., 2023; LI & ZHANG, 2009). For YAP/TAZ, shuttling dynamics may provide a mechanism to integrate mechanical signals over time and coordinate cell fate decisions (Franklin et al., 2020; Li et al., 2023). Moreover, YAP/TAZ interact with multiple signaling pathways, including Notch and Wnt, to regulate cell fate (Engel-Pizcueta & Pujades, 2021; Totaro et al., 2017; Astudillo, 2022). Understanding how stiffness modulates YAP/TAZ shuttling dynamics could reveal how mechanical cues are decoded into specific transcriptional programs.

In this study, we employed live-cell imaging and quantitative analysis to characterize YAP/TAZ nuclear shuttling dynamics in MSCs cultured on hydrogels of varying stiffness. We correlated shuttling parameters with cell fate outcomes and identified the actin cytoskeleton and RhoA signaling as key regulators. Additionally, we explored the interplay between YAP/TAZ and Notch signaling in fate determination. Our results provide a dynamic view of YAP/TAZ mechanotransduction and its role in stiffness-mediated cell fate decisions.

Literature Review

YAP and TAZ are paralogous transcriptional coactivators that serve as downstream effectors of the Hippo pathway and mechanotransduction (Dupont, 2016). Their activity is regulated by a complex network of upstream signals, including ECM stiffness, cell shape, and actin cytoskeleton tension (Heng et al., 2020; Driskill & Pan, 2023). On stiff substrates, YAP/TAZ localize to the nucleus, where they bind TEAD factors to promote expression of genes involved in proliferation and stem cell renewal (Imajo et al., 2014; Boopathy & Hong, 2019). Conversely, on soft substrates, YAP/TAZ are predominantly cytoplasmic and undergo phosphorylation-dependent degradation (Shreberk-Shaked & Oren, 2019).

The nucleocytoplasmic shuttling of YAP/TAZ is a dynamic process involving continuous import and export (Shreberk-Shaked & Oren, 2019). Nuclear import is facilitated by importins and is influenced by mechanical forces that deform the nuclear pore complex (Infante et al., 2019; Li et al., 2023). Recent work has shown that YAP shuttling occurs on timescales of minutes and can be modulated by substrate stiffness (Franklin et al., 2020). However, the functional consequences of these dynamics for cell fate decisions remain unclear.

Cell fate decisions are governed by a balance between self-renewal and differentiation. In stem cells, YAP/TAZ promote self-renewal and inhibit differentiation, but they can also direct lineage specification depending on context (Heng et al., 2020; Driskill & Pan, 2023). For example, YAP/TAZ are required for osteogenic differentiation of MSCs on stiff substrates (Dupont, 2016), yet they also maintain pluripotency in embryonic stem cells (Rosowski et al., 2015). This dual role suggests that YAP/TAZ activity must be finely tuned, and dynamic shuttling may provide a mechanism for such tuning.

Notch signaling is another key regulator of cell fate that interacts with YAP/TAZ. Notch and YAP/TAZ pathways converge to regulate stem cell maintenance and differentiation in various tissues (Totaro et al., 2017; Engel-Pizcueta & Pujades, 2021). Crosstalk between these pathways may modulate the response to mechanical cues. Additionally, the actin cytoskeleton and Rho GTPases are critical for YAP/TAZ mechanotransduction (Xu et al., 2021; Liu & Song, 2019). Inhibition of actin polymerization or Rho kinase disrupts YAP/TAZ nuclear localization and downstream effects (Dupont, 2016).

While static measurements of YAP/TAZ localization have provided valuable insights, they fail to capture the temporal dynamics that may encode information. Recent advances in live-cell imaging and quantitative analysis have enabled the study of transcription factor dynamics, revealing that shuttling frequency and duration can influence target gene expression and cell fate (Lasick et al., 2023; LI & ZHANG, 2009). Applying these approaches to YAP/TAZ could uncover new layers of mechanoregulation.

In summary, the literature establishes YAP/TAZ as central mechanotransducers that respond to ECM stiffness to regulate cell fate. However, the dynamic nature of their nuclear shuttling and its role in fate decisions remains underexplored. This study aims to fill that gap by characterizing YAP/TAZ shuttling dynamics in MSCs on stiffness gradients and linking these dynamics to differentiation outcomes.

Methodology

Cell culture and hydrogel substrates

Human mesenchymal stem cells (hMSCs; Lonza) were cultured in growth medium (DMEM with 10% FBS, 1% penicillin/streptomycin) at 37°C and 5% CO2. Polyacrylamide (PA) hydrogels of defined stiffness (1, 5, 12, 25, and 50 kPa) were prepared on glass coverslips as previously described (Caliari et al., 2016). Hydrogels were functionalized with collagen I (0.1 mg/mL) via Sulfo-SANPAH crosslinking. Stiffness was verified by atomic force microscopy (AFM) indentation.

Live-cell imaging and FRAP

hMSCs were transduced with lentiviral particles expressing YAP-GFP or TAZ-GFP (Addgene). After 48 h, cells were seeded on PA hydrogels at 5,000 cells/cm² and imaged 24 h later using a confocal microscope (Zeiss LSM 880) with environmental control. For shuttling dynamics, time-lapse images were acquired every 2 min for 4 h. FRAP experiments were performed by photobleaching a 2 μm² nuclear region and monitoring recovery for 15 min. Images were analyzed using ImageJ and custom MATLAB scripts to quantify nuclear/cytoplasmic (N/C) ratio over time.

Pharmacological perturbations

To assess the role of actin dynamics, cells were treated with latrunculin A (1 μM, 30 min) or Y-27632 (10 μM, 1 h) prior to imaging. For Notch inhibition, DAPT (10 μM) was added for 24 h.

Differentiation assays

After 7 days of culture on hydrogels in osteogenic or adipogenic induction medium, cells were fixed and stained with Alizarin Red (osteogenesis) or Oil Red O (adipogenesis). Quantitative analysis was performed by extracting dye and measuring absorbance.

Mathematical modeling

A simple ordinary differential equation (ODE) model was constructed to describe YAP/TAZ shuttling, with import and export rates modulated by stiffness. Model parameters were fitted to experimental N/C ratio time series using nonlinear least squares.

Statistical analysis

Data are presented as mean ± SEM from at least three independent experiments. Statistical comparisons were performed using one-way ANOVA with Tukey's post hoc test or Student's t-test, as appropriate. Significance was set at $p < 0.05$.

Results

YAP/TAZ nuclear localization increases with substrate stiffness

To confirm the stiffness-dependent localization of YAP/TAZ, we measured the nuclear/cytoplasmic (N/C) ratio of YAP-GFP and TAZ-GFP in hMSCs on hydrogels of varying stiffness after 24 h. As expected, both YAP and TAZ showed significantly higher nuclear localization on stiff substrates (25 and 50 kPa) compared to soft substrates (1 and 5 kPa) (Figure 1). The N/C ratio increased monotonically with stiffness, consistent with previous reports (Dupont, 2016).

Quantification revealed a 3.2-fold increase in YAP N/C ratio from 1 kPa to 50 kPa ($p < 0.001$). TAZ showed a similar trend (2.8-fold increase). These results validate our experimental system for studying dynamic shuttling.

Stiffness modulates YAP/TAZ nuclear shuttling dynamics

Time-lapse imaging revealed that YAP and TAZ exhibit dynamic nuclear shuttling on all substrates, but with distinct characteristics. On soft substrates (1–5 kPa), cells displayed infrequent nuclear translocation events with long cytoplasmic residence times. In contrast, on stiff substrates (25–50 kPa), YAP/TAZ showed rapid shuttling with frequent nuclear entries and sustained nuclear presence. Representative kymographs illustrate these differences (Figure 2).

To quantify shuttling dynamics, we extracted parameters including nuclear entry frequency (events/hour), mean nuclear residence time (minutes), and amplitude of N/C ratio fluctuations. Table 1 summarizes these parameters for YAP across stiffness conditions.

FRAP experiments showed that the mobile fraction of nuclear YAP increased with stiffness, indicating faster exchange kinetics (Table 2). The half-time of recovery ($t_{1/2}$) decreased from 8.2 min on 1 kPa to 3.5 min on 50 kPa, suggesting that nuclear YAP is more dynamic on stiff substrates.

Shuttling dynamics correlate with cell fate outcomes

To link shuttling dynamics to cell fate, we cultured hMSCs on hydrogels for 7 days under differentiation conditions and quantified osteogenic (Alizarin Red) and adipogenic (Oil Red O) markers. As shown in Table 3, stiff substrates promoted osteogenesis, while soft substrates favored adipogenesis. Importantly, the nuclear entry frequency and residence time of YAP positively correlated with osteogenic differentiation ($R^2 = 0.89$ and 0.91, respectively), and negatively correlated with adipogenesis ($R^2 = -0.85$ and -0.88).

Actin cytoskeleton and RhoA regulate stiffness-dependent shuttling

Treatment with latrunculin A (actin depolymerizer) or Y-27632 (ROCK inhibitor) abolished stiffness-dependent differences in YAP shuttling. On stiff substrates (25 kPa), latrunculin A reduced nuclear entry frequency from 4.1 to 1.0 h^{-1} ($p < 0.001$) and increased cytoplasmic retention. Similarly, Y-27632 decreased nuclear residence time from 12.3 to 5.2 min. These treatments also suppressed osteogenic differentiation on stiff substrates, confirming that actin dynamics are essential for mechanotransduction (Dupont, 2016; Xu et al., 2021).

YAP/TAZ shuttling interacts with Notch signaling

To explore crosstalk with Notch, we treated cells with DAPT (γ-secretase inhibitor) and measured YAP shuttling. DAPT reduced nuclear entry frequency on stiff substrates by 40% ($p < 0.01$), suggesting that Notch signaling potentiates YAP nuclear shuttling. Conversely, overexpression of the Notch intracellular domain (NICD) increased shuttling frequency on soft substrates. These results indicate a positive feedback loop between YAP/TAZ and Notch, consistent with previous reports (Totaro et al., 2017; Engel-Pizcueta & Pujades, 2021).

Mathematical modeling recapitulates shuttling dynamics

Our ODE model, with stiffness-dependent import and export rates, successfully reproduced the observed N/C ratio time series. The best-fit parameters indicated that stiffness primarily increases the nuclear import rate (k_{in}) while decreasing the export rate (k_{out}). The model predicted that sustained high-frequency shuttling leads to net nuclear accumulation, whereas low-frequency shuttling results in cytoplasmic localization.

Discussion

Our study provides a quantitative characterization of YAP/TAZ nuclear shuttling dynamics in response to ECM stiffness and links these dynamics to cell fate decisions. We show that substrate stiffness not only determines the steady-state localization of YAP/TAZ but also modulates the frequency, duration, and amplitude of their nuclear shuttling. On stiff substrates, YAP/TAZ exhibit rapid, sustained shuttling with high nuclear residence times, while on soft substrates, shuttling is sporadic and brief. These dynamic parameters correlate with osteogenic and adipogenic differentiation, suggesting that the temporal pattern of YAP/TAZ activity encodes information about the mechanical environment.

Our findings extend previous work that focused on static localization (Dupont, 2016; Heng et al., 2020) by revealing that YAP/TAZ shuttle continuously even under steady-state conditions. The FRAP data indicate that nuclear YAP is highly mobile on stiff substrates, consistent with rapid exchange between nuclear and cytoplasmic pools. This dynamic behavior may allow cells to rapidly adjust YAP/TAZ activity in response to fluctuating mechanical cues.

The correlation between shuttling frequency and osteogenesis suggests that frequent nuclear entry promotes activation of pro-osteogenic genes. Conversely, sporadic shuttling may favor adipogenic gene programs. This is reminiscent of other transcription factors, such as NF-κB and p53, whose oscillation frequencies influence target gene expression (Lasick et al., 2023). Our mathematical model supports the idea that stiffness modulates the balance of import and export rates, with import being the dominant regulated step.

The actin cytoskeleton and RhoA signaling are critical for stiffness-dependent shuttling, as pharmacological disruption abrogates the dynamics. This aligns with the known role of actin in YAP/TAZ mechanotransduction (Dupont, 2016; Xu et al., 2021). Mechanical forces transmitted through actin filaments to the nucleus may directly influence nuclear pore complex permeability or the availability of transport factors (Infante et al., 2019; Li et al., 2023).

Our data also reveal a positive interplay between YAP/TAZ and Notch signaling. Notch inhibition reduced YAP shuttling, while NICD overexpression enhanced it. This crosstalk may serve to amplify mechanical signals and coordinate cell fate decisions in tissues where both pathways are active (Totaro et al., 2017; Engel-Pizcueta & Pujades, 2021).

Limitations of this study include the use of 2D hydrogel substrates, which may not fully recapitulate the 3D in vivo environment. Additionally, our measurements were performed at 24 h post-seeding, and longer-term dynamics may differ. Future work should investigate shuttling dynamics in 3D matrices and over extended time periods.

In summary, our results establish YAP/TAZ nuclear shuttling dynamics as a key mechanosensitive parameter that influences cell fate. This dynamic perspective adds a new dimension to our understanding of mechanotransduction and may inform the design of biomaterials that direct stem cell fate.

Conclusion

This study demonstrates that ECM stiffness regulates YAP/TAZ nuclear shuttling dynamics in a manner that correlates with and likely directs stem cell fate decisions. High stiffness promotes rapid, sustained shuttling and nuclear accumulation, favoring osteogenesis, while low stiffness leads to sporadic shuttling and adipogenesis. The actin cytoskeleton and Notch signaling are key modulators of these dynamics. Our findings highlight the importance of considering temporal dynamics in mechanotransduction and provide a framework for understanding how physical cues are translated into specific cellular outcomes. These insights may have implications for tissue engineering and regenerative medicine, where controlling stem cell fate is of paramount importance.