

Spatiotemporal Distribution of YAP in Nucleus and Cytosol after Mechanical Wounding of Adjacent Cells

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(隣接細胞損傷時における YAP の細胞内時空間分布)

区 分 : 甲

論 文 内 容 の 要 旨

Cells sense mechanical stimuli from their surroundings and respond to them, thereby regulating life activities such as development, differentiation, and tissue repair. Among the multiple signaling pathways involved in the transmission of mechanical stimuli, the Hippo pathway and its downstream effector, Yes-associated protein (YAP), play an important role. YAP plays a central role in regulating organ size, cell regeneration, and tumorigenesis. When the Hippo signaling pathway is activated, YAP is phosphorylated and retained in the cytoplasm. Mechanical stimulation or loss of cell-cell contact causes YAP to be dephosphorylated and translocated to the nucleus, where it activates downstream gene expression. Therefore, YAP is considered to be a mechanosensitive transcription regulator that plays a central role in cells sensing and responding to the external physical environment. Abnormalities in YAP function have been suggested to be associated with various pathological conditions, including fibrosis and cancer. Understanding how YAP responds to mechanical stimuli is important for elucidating its physiological and pathological roles. This study focuses on the spatiotemporal distribution of YAP after mechanical wounding of adjacent cells, with the aim of elucidating the main factors that regulate the intracellular translocation of YAP and clarifying the mechanism of directional cell migration.

Chapters 1 and 2 introduce the research background and previous studies, providing an overview of the context and purpose of this study. In scratch experiments on monolayer cells, it has been shown that YAP rapidly accumulates in the cell nucleus. However, the specific process of YAP movement in the cytoplasm remains unclear. On the other hand, when cells are damaged, the surrounding cells normally migrate to the damaged site. In this process, proteins related to cell migration (e.g., protein kinase C α (PKC α) and cell division control protein 42 homolog (Cdc42)) accumulate at the leading edge of the cells. Considering the activation relationships between YAP, PKC, and Cdc42, it is necessary to examine whether YAP is also involved in the dynamic distribution associated with directional migration. The cytoskeleton not only maintains cell shape and tension but also plays a crucial role in regulating the intracellular localization and transport of proteins. Due to its relatively large molecular weight, YAP requires active transport via the nuclear pore complex (NPC) to enter the nucleus. The Importin- β complex functions as a key mediator, recognizing nuclear localization signals and promoting nuclear transport. However, YAP lacks a typical nuclear localization sequence, and its nuclear transport mechanism remains unclear. Against this background, this study aims to clarify the roles of the cytoskeleton and Importin- β in YAP movement and, by combining numerical simulations, to explore the spatial and temporal distribution of YAP within cells following mechanical wounding of adjacent cells.

In chapter 3, the experimental methods employed in this study are introduced. A micropipette was

used to puncture a confluent monolayer of cells and create a single-cell wound, in order to observe the response of neighbouring cells. Fluorescent reporters, including EGFP-YAP, mRFP and EGFP-Importin- β , were expressed in neighbouring cells. Time-lapse fluorescence microscopy was used to capture the redistribution of YAP in real time. Image analysis involved measuring fluorescence in three regions of the cell: Nucleus, Near (facing the wound) and Far (opposite the wound). To assess YAP translocation dynamics, the fluorescence intensity in the three regions was measured over time and normalised to baseline levels prior to wounding. Various pharmacological inhibitors were used to test specific mechanisms: Cytochalasin D (to depolymerise F-actin), nocodazole (to disrupt microtubules) and importazole (to block importin- β).

In chapter 4, live cell imaging results show that YAP can respond quickly, within 1–2 minutes, to neighbouring cells after mechanical wounding. Specifically, EGFP-YAP exhibited a significant decrease in 'Near' and a significant increase in 'Nucleus' and 'Far'. Compared to the control protein mRFP, the asymmetric redistribution of YAP indicates that its transport does not occur via diffusion, but rather represents an active response to mechanical stimulation.

In chapter 5, experiments using inhibitors revealed that F-actin polymerization plays a critical role in YAP transport. Inhibition of actin polymerization can significantly weaken the polarized transport of YAP and reduce its nuclear accumulation after damage. In contrast, microtubule disruption had no effect on YAP redistribution. When Importin- β was inhibited, YAP redistribution in the cytoplasm still occurred, but its rapid nuclear accumulation was significantly suppressed.

In chapter 6, a numerical model was constructed to simulate the intracellular dynamics of YAP following mechanical stimulation. The simulation successfully reproduced the rapid nuclear accumulation of YAP within tens of seconds, consistent with experimental observations. However, the simulated cytoplasmic distribution remained nearly uniform and did not capture the spatial asymmetry observed in experiments. These findings suggest that, in addition to diffusion and nuclear import, other mechanisms may be involved in shaping the cytoplasmic distribution of YAP.

In chapter 7, the main findings of this study were summarized. Following wounding of adjacent cells, the rapid redistribution of YAP is controlled by two mechanisms. The first mechanism is nuclear transport mediated by Importin- β , which actively transports YAP through the nuclear pore complex (NPC). The second mechanism is cytoskeletal regulation, which may influence the spatial distribution of YAP within the cytoplasm. These two mechanisms demonstrate YAP's role in modulating cellular responses to mechanical stimuli, providing important insights into the broader context of tissue repair and cellular adaptation.