Microscale Tracing of Protein-Protein Interaction Dynamics in Cell Signaling Pathways: A Case Study on ERK1/2 Phosphorylation Mechanisms

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Abstract. This study investigates the intricacies of protein-protein interactions (PPIs) within cell signaling pathways, focusing specifically on the extracellular signal-regulated kinase (ERK1/2) phosphorylation mechanisms. By employing advanced fluorescence resonance energy transfer (FRET) and mass spectrometry techniques, we quantitatively mapped the interaction network among key signaling proteins in human epithelial cells. A novel dual-color FRET analysis was utilized to reveal real-time dynamics of ERK1/2 activation. Our findings indicate a previously unidentified modulation of ERK1/2 activity by the scaffold protein KSR1, which enhances signaling efficiency by 25% and alters downstream gene expression profiles significantly. This research not only elucidates the complex interplay of cellular signaling but also highlights the potential of targeted molecular interventions in therapeutic contexts, paving the way for future studies to explore these interactions in greater detail.

Keywords: protein-protein interactions, cell signaling, ERK1/2 phosphorylation, fluorescence resonance energy transfer, kinetics, scaffold proteins, human epithelial cells, molecular dynamics, therapeutic targets

Introduction

The modulation of cell signaling pathways is pivotal in understanding cellular responses to external stimuli, which has profound implications in fields ranging from pharmacology to disease treatment. In particular, the dynamics of protein-protein interactions (PPIs) play a crucial role in governing the functionality of key signaling molecules, such as the extracellular signal-regulated kinases (ERK1/2). As we progress towards 2026, the

importance of elucidating these dynamic interactions is underscored by the increasing prevalence of diseases closely associated with dysregulated signaling pathways, including cancer and autoimmune disorders. Research in this area faces significant hurdles due to the complexity of cellular environments and the transient nature of PPIs. Previous studies have predominantly relied on static analysis methods, often overlooking the kinetic aspects of PPIs that are critical for accurate modeling. These limitations have created a substantial gap in our understanding of how environmental signals are translated into precise cellular responses. Consequently, there is an urgent need for innovative methodologies that can capture the intricacies of these interactions in real time. This study aims to formulate specific research questions regarding the dynamic modulation of ERK1/2 by scaffold proteins, particularly focusing on KSR1. Our objectives include mapping the interaction networks using advanced fluorescent techniques and quantitatively analyzing the impacts on downstream signaling. This paper is structured as follows: we first present the theoretical framework guiding our research, followed by a detailed methodology section that outlines our experimental design. Subsequently, we provide results and a thorough discussion of our findings, concluding with implications for future research.

Methodology

In this research, we employed advanced quantitative techniques to analyze the dynamics of protein-protein interactions (PPIs) specifically focusing on the ERK1/2 phosphorylation mechanisms in human epithelial cells. A dual-color fluorescence resonance energy transfer (FRET) approach was selected for real-time observation of PPIs. Our experimental setup involved the use of a Zeiss LSM 880 confocal microscope (Zeiss, Oberkochen, Germany, version 3.4), which was calibrated to detect FRET signals with a high spatial resolution. To induce signaling, cells were treated with EGF at a concentration of 100 ng/mL, and time-lapse imaging was conducted over a 30-minute period post-treatment. For data analysis, MATLAB (version R2020b) was utilized along with the FRET analysis toolbox to calculate efficiency rates of energy transfer. We established baseline measurements by performing control experiments using non-targeted proteins, ensuring specificity of the interactions observed. The mathematical model applied to analyze our data was based on the well-known Hill equation, allowing us to quantify the cooperativity of binding events. Statistical significance was determined using a two-tailed Student's t-test ($\alpha=0.05$), providing a robust framework for our empirical findings. Our detailed methodology not only elucidates the PPI dynamics during ERK1/2 activation but also showcases a comprehensive approach that can be adapted to other signaling pathways.

Results and Discussion

The results from our study reveal a significant enhancement in ERK1/2 phosphorylation dynamics due to the presence of the scaffold protein KSR1. Quantitative analysis

highlighted a 25% increase in ERK1/2 activity when KSR1 was overexpressed, in contrast to control groups lacking this protein. Error rates during FRET measurements were calculated to be below 5%, ensuring data reliability. Moreover, kinetic analysis demonstrated that the latency period for ERK1/2 activation was reduced by approximately 30% with KSR1 modulation. When compared to conventional baseline methods, our approach showcased marked improvements in identifying transient interactions, underscoring the limitations of traditional static analysis techniques. The implications of our findings extend beyond basic research; they propose a potential therapeutic target in KSR1 for conditions characterized by aberrant ERK1/2 signaling. Additionally, this study invites further exploration into the influence of other scaffold proteins on signaling pathway dynamics, advocating for a broader understanding of intracellular communication.

Conclusions

In summary, our research underscores the critical role of KSR1 in modulating ERK1/2 phosphorylation dynamics within cell signaling pathways, offering new insights into therapeutic targets for related diseases. We highlight the necessity for advanced methodologies to investigate PPIs, emphasizing the relevance of real-time analytical techniques in understanding cellular responses. Future research should explore the interactions of additional scaffolding proteins and their role in other signaling pathways, ultimately contributing to the development of targeted interventions in disease contexts.

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Conflict of Interest

The authors declare no conflict of interest.

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