

Multiplexed Live-Cell Signaling Dynamics of the Cytoskeletal and Phospholipid Scaffold, IQGAP1

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Short Abstract — Regulation by the scaffold protein IQGAP1 underlies the coordination of the actin cytoskeleton and phospholipid membranes. Live-cell dynamics studies of endosomal compartments revealed a temporal sequence of scaffold dissociation and actin bursting events. Domain-level mutations and statistical modeling have lead to an understanding of the multifaceted tethering modes of IQGAP1, including mutually opposing forces mediated by two distal protein domains.

Keywords — Scaffold proteins, live-cell protein dynamics, endosomes, IQGAP1, actin, phosphoinositides, fluctuation analysis, statistical modeling.

I. PURPOSE

UNDERSTANDING the adaptive material properties of the eukaryotic cell and its spatiotemporal regulation by complex networks of signaling macromolecules remains a major unsolved scientific problem. Full characterization of adaptive subcellular systems requires new quantitative descriptions and new experimental frameworks.

The actin cytoskeleton is an adaptive, mechanical network known for its ability to change cell morphology. The deformation of the phospholipid membrane via actin polymerization is crucial in cellular processes like phagocytosis, cell migration, and immune synapse formation. In each of these contexts, actin is regulated by multiple protein species of the Rho GTPase family, whose local, collective spatial assembly is dependent on organelle-specific phospholipid signatures such as the abundant phosphatidylinositol (4,5)-bisphosphate (PIP₂).

Also critical to the regulation of actin-membrane structures are scaffold proteins, which function as biomolecular ‘circuit boards’ and template the flow of intracellular information [2]. The best studied cytoskeletal-regulating scaffold protein, IQGAP1, binds Rho GTPases, PIP₂, and filamentous actin through multiple protein domains, while also facilitating Raf-MEK-ERK and PI3K-Akt-mTOR signaling pathways which are major signaling axes for growth and proliferation [3].

II. RESULTS

We use a combined live-cell imaging and statistical modeling approach to characterize the micron-scale, endosomal recruitment dynamics of the cytoskeletal and

phospholipid scaffold protein, IQGAP1.

A. Actin and IQGAP1 correlations at endosomes

Through super-resolution and time lapse epifluorescent microscopy, we have characterized the scaffold-regulated maturation process of endosomes at the basal actin cortex of human mammary epithelial cells. Here, the dissociation of IQGAP1 is always followed by excitable bursts of actin polymerization. By analyzing multi-protein trajectories from time lapse movies, we found that at the 60-minute time scale, actin and IQGAP1 are positively correlated, whereas on 60 second timescales this pair is anti-correlated. This suggests the scaffold plays activating and inhibitory roles in actin polymerization.

B. Domain-level contributions to dynamics

Via a series of protein domain- and residue-level mutations, we identify the regions of IQGAP1 responsible for the anti-correlations and endosomal tethering [5].

C. Statistical modeling of protein fluctuations

We construct multiple linear regression models of the pairwise-correlative structure of actin, membrane, and scaffold fluctuations. By model selection we conclude that using wild type and mutant scaffolds in conjunction yields the best prediction of actin fluctuations, whereas a scaffold mutant alone sufficiently predicts membrane fluctuations [5].

III. CONCLUSION

Our combination of live-cell microscopy and statistical modeling yielded vital insights into the adversarial dynamics of a scaffold-regulated, membrane and cytoskeletal system.

REFERENCES

- [1] Croisé, P, Estay-Ahumada, C, Gasman, S, & Ory, S (2014). “Rho GTPases, phosphoinositides, and actin: a tripartite framework for efficient vesicular trafficking.” *Small GTPases*, 5(2), e29469.
- [2] Good, MC, Zalatan, JG, & Lim, WA (2011). ‘Scaffold proteins: hubs for controlling the flow of cellular information.’ *Science*, 332(6030), 680-686.
- [3] Abel, AM, et al. (2015). IQGAP1: insights into the function of a molecular puppeteer. *Molecular immunology*, 65(2), 336-349.
- [4] Samson, EB et al. (2017). ‘The coordinating role of IQGAP1 in the regulation of local, endosome-specific actin networks.’ *Biology open*, 6(6), 785-799.
- [5] Tsao DS, McLaughlin RT, et al. (2018). “Live-cell delineation of multiple tethering modes by the scaffold IQGAP1,” submitted.

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