Formin and myosin II involvement in myosin X induced filopodia dynamics

N. O. Alieva[1*], M. Natarajan[1], X. Yuan[1], A. D. Bershadsky[1,2]

[1] Mechanobiology Institute, National University of Singapore, T-Lab, #10-01, 5A Engineering Drive 1, Singapore 117411, mbinoka@nus.edu.sg,

[2] Weizmann Institute of Science, 234 Herzl St. Rehovot 7610001 Israel

[*] Corresponding Author

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Abstract:

Filopodia are dynamic cellular protrusions composed of actin filament bundles. They are important in many cellular activities including cell migration, neuronal growth, cell-cell junction formation and others. Unconventional myosin X (MYO10) is a powerful inducer of filopodia formation and elongation once it undergoes over-expression. MYO10 is present at the filopodia tip as a bright puncta and remains there as the filopodia extend and retract. The exact mechanism by which MYO10 induces filopodia formation remains unclear. Members of the formin family have been found in filopodia, though the exact role they play in filopodia dynamics remains controversial. Here we show that MYO10 induced filopodia formation is highly dependent on formins. We found that at least a few formins, namely DIAPH1, DIAPH3, FMNL2 and DAAM1, were present in MYO10 induced filopodia of HeLa cells. The formin inhibitor SMIFH2 dramatically decreased the number and length of MYO10 induced filopodia. The phenotype observed after SMIFH2 exposure is consistent with knockdown experiments on individual formins. Low doses of SMIFH2 caused gradual changes in the dynamics of MYO10 enriched puncta at the filopodia tip. We observed numerous MYO10 positive patches being pinched out of the MYO10 puncta at filopodia tip. These moved rearward at rates corresponding to the retrograde flow of actin in filopodia. We also observed some MYO10 patches that were moving rearward at approximately ten times the speed of actin flow. Applying ROCK inhibitor Y-27632 to SMIFH2 pre-treated cells blocked the fast rearward movement of MYO10 patches, suggesting possible involvement of the molecular motor myosin II pathway in MYO10 induced filopodia dynamics. Subsequently, we demonstrated that myosin II was physically present at the base region of MYO10 induced filopodia. Our findings show, therefore, that formins are involved in MYO10 induced filopodia dynamics and that this involvement might be orchestrated by acto-myosin II mechanosensing machinery.