Profilin membrane dynamics in live cells

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Profilin is a ubiquitously expressed protein involved in the regulation of cell proliferation, apoptosis and motility. *In vitro*, it is essential in the coordination of actin filament assembly and disassembly. However, the dynamics of profilin in live cells is still largely unknown. The epithelial breast cancer cell line MDA-MB-231 was transfected with wild type GFP-profilin, or GFP-profilin mutants R88A or H119E. The R88A mutation affects binding to phosphoinositides and actin while the H119E mutation only affects binding to actin. Image stacks of the cell membrane were acquired by TIRF microscopy and analysed using image mean square displacement (iMSD) analysis to determine diffusion modes (isotropic, confined or transiently confined) and diffusion rates within the membrane. The effect of actin filament disruption by Cytochalasin D on profilin dynamics was also investigated.

Isotropic diffusion rates decreased significantly only in the wtGFP-profilin cells treated with Cytochalasin D. Confined diffusion rates were faster for both profilin mutants compared to the control. Cytochalasin D treatment reduced confined diffusion rates in H119E cells but increased the confined diffusion rates in R88A cells. Transiently confined diffusion rates were similar to the controls values for untreated R88A and H119E cells but these values increased for both mutants with Cytochalasin D treatment.

The iMSD value at time 0 (Sigma), is a combination of the particle size under observation and the trapping component of dynamic partitioning. In areas of isotropic diffusion, Sigma values increased in H119E cells. In areas of confined diffusion, Sigma increased in both R88A and H119E and in R88A cells treated with Cytochalasin D. There were no significant differences in Sigma values in areas of transiently confined diffusion.

The iMSD data allow us to add details from *in vivo* observations to existing models of the role of profilin in cell function.