Profilin Associates with Microtubules in Cultured cells

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Abstract

The microtubule and actin filament systems participate in many cellular processes such as intracellular organelle trafficking, cell adhesion, migration and mitosis. The two filament systems are closely connected, operate in parallel and are essential for proper cell function. Profilin is a small protein involved in the control of actin polymerization. It enhances nucleotide exchange on actin, binds proline-rich sequences present in actin nucleation and elongation promoting factors and thereby contributes to bring polymerization-competent actin to sites where filament formation takes place.
Determination of the dynamics of profilin distribution in living cells has been hampered by the lack of well working fluorescently tagged profilin since typical fusions of fluorophore-proteins to either its N-terminus or C-terminus have been shown to compromise its poly-(L-proline) binding capacity to various degree. This problem has been overcome in our laboratory by making an internal fusion where citrine, a mutant form of yellow fluorescent protein (YFP), has been placed in a loop extending away from the actin and proline-binding surfaces in the profilin molecule. In vitro characterizations demonstrate that citrine-profilin binds polyproline, phosphatidylinositol lipids and actin and it also appears to distribute properly in cells after expression. This construct is now used to study a previously observed profilin-microtubule association in our laboratory (*Grenklo et al. EJCB 83:413-23, 2004*). We have found that profilin and/or profilin:actin localize in close proximity to microtubules and kinesin, and we have corroborated this by co-immunoprecipitation as well as biochemical analysis where polymerized and unpolymerized microtubules are partitioned after drug treatment of the cells. Further, profilin was pulled down by GST-WHAMM, a potential candidate connecting profilin to the microtubule system. Currently we are searching to understand the functional implications of this interaction.