Summary

Cellular processes as diverse as cell morphology control, the formation of multicellular networks, cell migration, cytokinesis and membrane trafficking processes critically rely on actin filament polymerization. Likewise important are both a tight control of actin nucleation in time and space and the formation of different actin superstructures for the individual cellular functions. Despite the wealth of different actin structures formed, only two actin nucleation factors are well established in vertebrates, the Arp2/3 complex and formins. Syndapins (synaptic dynamin-associated proteins) and Abp1 (actin binding proteins) are Src homology 3 (SH3) domain-containing proteins linking membrane trafficking processes and the actin cytoskeleton via their association with proteins involved in both processes.

In this study, Cordon bleu (Cobl), a novel actin nucleator was identified in yeast-two hybrid screen as an interaction partner for syndapin I. Cobl is a brain-enriched protein that contains several proline-rich regions and three Wiskott-Aldrich syndrome protein homology 2 (WH2) domains. The interaction of syndapins with Cobl was verified using different in vitro and in vivo methods including coimmunoprecipitation and in vivo reconstitution studies. In parallel, the interaction between Cobl and Abp1 was revealed and characterized. Functional studies unraveled a role of Cobl/syndapin and Cobl/Abp1 complexes in receptor-mediated endocytosis.

In the second part of the study, the molecular mechanism of Cobl-mediated actin nucleation was investigated. Being a multiple WH2 domain-containing protein, Cobl is herein characterized as a novel actin nucleator, thus adding to the astonishingly limited group of so far identified actin filament nucleation machineries. Biochemical analyses and in vitro reconstitutions demonstrate that all three WH2 domains are used for actin binding albeit with different affinities. Kinetic studies show that Cobl-induced actin nucleation is as powerful as that triggered by fully activated Arp2/3 complex and gives rise to non-bundled and unbranched filaments. Cobl-mediated filament formation hereby relies on barbed end growth and does not lead to pointed end protection. In order to nucleate actin, Cobl requires all three Cobl WH2 domains and the extended linker L2 between the second and third WH2 domain. This study demonstrates that the nucleation power of Cobl is based on the assembly of three actin monomers in a cross-filament orientation. These findings thereby provide direct experimental evidence for the idea that minimally the assembly of three actin monomers is required for effective nucleation and that formation of cross-filaments seeds is the major source for actin filaments.

In line with Cobl being involved in actin dynamics also in vivo, Cobl localizes to sites of high actin dynamics and modulates the morphology of fibroblasts via the induction of intense, three-dimensional ruffling. In primary hippocampal cultures, Cobl has drastic effects on neuromorphogenesis, both the induction of neurites and neurite branching is dramatically increased. These effects critically depend on Cobl's actin nucleation ability. Nucleation-incompetent Cobl mutants did not lead to any effects. Consistently, Cobl knock-down results in decreased dendritic arborization thereby unraveling that Cobl is indeed a crucial factor for cellular morphogenesis.

These data therefore reveal Cobl to be a novel actin nucleator controlling neuronal morphology and development and provide detailed mechanistic insights into Cobl’s function in actin dynamics.