Summary

The actin cytoskeleton plays an essential role in a variety of cellular processes, from cell division and cell motility to the intracellular trafficking of organelles. Mammalian actin-binding protein 1, Abp1, and syndapins are Src homology 3 (SH3) domain-containing proteins suggested to be the link between membrane trafficking and the actin cytoskeleton.
Mammalian Abp1 is a signal-responsive F-actin-binding protein involved in receptor internalisation by an association with the GTPase dynamin. The ability of Abp1 not only to bind to actin fibres but also to regulate actin polymerisation via an interaction with the neuronal Wiskott-Aldrich Syndrome Protein (N-WASP), a potent stimulator of the Arp2/3 complex was revealed in this study. Affinity purifications and overlay assays demonstrate that the SH3 domain of Abp1 binds specifically and directly to the proline-rich domain of N-WASP. Coimmunoprecipitation studies and reconstitution of the N-WASP/Abp1 complex at intracellular membranes demonstrate the physiological relevance of this interaction. Immunofluorescence studies in primary hippocampal neurons showed a colocalisation of Abp1 and N-WASP at actin-rich sites such as growth cones and synapses.
Syndapins associate with members of the endocytic and actin polymerisation machineries such as dynamin or N-WASP and have functionally been involved in receptor internalisation and cortical actin remodelling. Here, the molecular mechanism of regulation of the actin cytoskeleton by syndapins was further dissected.
In vitro reconstitution assays show that both Abp1 and syndapins induce actin polymerisation by regulating the activity of N-WASP in an SH3 domain-dependent manner. Moreover, the SH3 domains of Abp1 and syndapins coated to the surface of beads were sufficient to promote actin nucleation in homogenates while mutants that fail to bind N-WASP did not. The SH3 domain of Abp1 and syndapin I act in concert with the Rho-like GTPases Cdc42 and Rac1 in stimulating N-WASP-dependent Arp2/3 complex-mediated actin filament nucleation, as demonstrated in fluorimetric analyses of actin polymerisation using purified components.
It was therefore proposed that the binding of Abp1 or syndapins promotes the release of the autoinhibited N-WASP conformation and thereby stimulates Arp2/3 complex-dependent actin polymerisation. These findings, together with the described function of N-WASP in endocytosis, suggest that F-actin nucleation during endocytosis is an Arp2/3 complex-based mechanism controlled by proteins such as Abp1 or syndapins.
Whereas Arp2/3 complex-induced actin filament nucleation in membrane trafficking processes appears to be mediated by N-WASP, additional members of the WASP family, the Scar proteins, are likely to trigger the formation of cortical cytoskeletal structures such as lamellipodia. A potential interaction of Abp1 and syndapins with further members of the WASP family of Arp2/3 complex activators was therefore investigated. Coprecipitations and blot overlay studies revealed that the SH3 domains of Abp1 and syndapins associate directly with the proline-rich region of Scar 1, a binding that also occurred in vivo, as demonstrated by coimmunoprecipitation assays and reconstitution of the complexes in living cells. Whereas both Abp1 and syndapins colocalised with Scar at actin-rich sites, such as growth cones in young hippocampal neurons or synaptic sites in mature neurons, Abp1 and Scar, but not syndapins, were enriched at F- actin-rich structures observed in the cell body of developing and mature neurons, suggesting that Abp1/Scar and syndapin/Scar complexes may have different physiological functions. This was further supported by differences in the complex composition detected in immunoprecipitations assays.
These findings strongly suggest that Abp1 and syndapins are involved in the regulation of actin dynamics in membrane trafficking, via the interaction with N-WASP, and of cortical actin cytoskeletal dynamics, via the association with Scar.