Cdc42 and N-WASP regulate actin filament organization during exocytosis in PC12 cells.

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A series of recent studies has strengthened the link between the actin cytoskeleton and intracellular membrane trafficking[1]. In particular, attention has been paid to the mechanisms regulating actin filament organization and progress has been made in identifying specific actin binding proteins and actin signalling molecules. Interestingly, among them, several appear to stimulate the actin nucleation activity of the Arp2/3 complex. One of the best characterized example is the Wiskott-Aldrich syndrome protein (WASP) family of proteins that can be regulated by the Rho GTPase Cdc42[2].

In chromaffin cells, like in many secretory cells, the cortical network of actin filaments forms a physical barrier to exocytosis for the majority of secretory granules since they are excluded from the subplasmalemmal zone[3]. On the other hand, evidence is emerging that actin filaments may as well control and/or modulate an active step in exocytosis. We previously proposed that Cdc42 regulates an actin-dependent function in chromaffin cell secretion[4]. The aim of our recent work was to dissect the role of Cdc42 on the actin network re-organization process during exocytosis in PC12 cells. We will briefly review here our findings that led us to propose N-WASP and Arp2/3 as molecules that bridge Cdc42 signaling to the actin cytoskeleton and the exocytotic machinery[5].

RESULTS AND DISCUSSION

Using a growth hormones (GH) release assay[6], we established that a constitutively active mutant of Cdc42 ($Cdc42^{L61}$; defective in GTP hydrolysis) stimulates the secretory activity of PC12 cells (Figure 1A) whereas its corresponding dominant inactive mutant preferentially binding GDP ($Cdc42^{N17}$) slightly decreases it, thus demonstrating the active participation of Cdc42 in large dense-core granule exocytosis. Interestingly, exogenous $Cdc42^{L61}$ localized to the plasma membrane and triggered the formation of actin filament in stimulated PC12 cells (Figure 1B). This observation led us to the idea that stimulation of exocytosis induced by Cdc42 might be related to its ability to promote actin polymerization in the cell periphery, hence suggesting N-WASP as a potential effector for Cdc42.

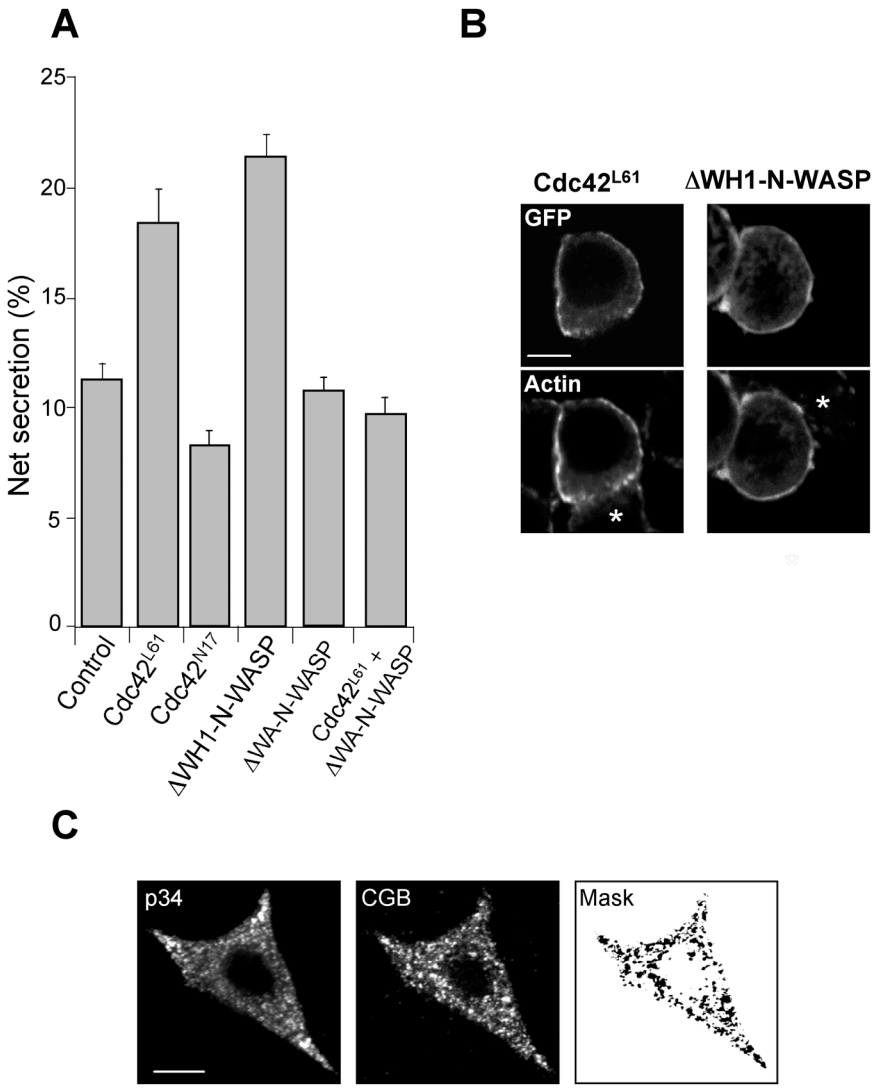

Figure 1. A) Cdc42 and N-WASP enhance the exocytotic release of GH from PC12 cells*:* PC12 cells co-expressing GH and the indicated proteins were incubated for 10 min in calcium-free Locke's solution or stimulated for 10 min with 59 mM K^+. Control cells were transfected with the empty pBR6 vector. Basal release (~ 8%) was unchanged and was subtracted from the release evoked by 59 mM K^+ to obtain the net secretory response. Data are given as the mean values ± SEM (n=3). **B) Active Cdc42 and N-WASP localized in the cell periphery and stimulated actin formation.** PC12 cells expressing GFP-$Cdc42^{L61}$ or GFP-ΔWH1-

N-WASP (GFP) were stimulated for 10 min with 59 mM K^+ and subsequently fixed and stained with rhodamine-conjugated phalloidin to visualize actin filaments (Actin). The asterisks indicate non-transfected cells displaying a classical disruption of the cortical actin network in response to K^+-stimulation. Scale bar represents 5 μm. **C) Intracellular distribution of Arp2/3 in PC12 cells:** PC12 cells were fixed and stained with anti-p34-Arc (Arp2/3 subunit) and anti-chromogranin B (CGB; a marker for large dense core secretory granules) antibodies. Masks representing the region of co-localization are obtained by selecting the pixels double-labeled. Scale bar represents 5 μm.

Accordingly, expression of a dominant active mutant of N-WASP (ΔWH1-N-WASP) stimulated GH release in PC12 cells and enhanced the formation of actin filaments in the subplasmalemmal region to a similar extent as the dominant active GTP-bound $Cdc42^{L61}$ (Figure 1A and B). Moreover, a dominant negative N-WASP mutant unable to induce actin polymerisation (ΔWA-N-WASP) had no effect on GH release (Figure 1A) and did not promote actin filament formation (not shown). Finally, co-expression of ΔWA-N-WASP completely abolished the stimulatory effect of $Cdc42^{L61}$ on GH release (Figure 1A). Taken together, these results elect N-WASP as one of the downstream effector by which Cdc42 organizes the actin architecture required for exocytosis.

The carboxy terminus of WASP family members initiates the growth of new actin filaments by bringing together actin monomers and the Arp2/3 complex[2]. In PC12 cells, Arp2/3 complexes are associated to large dense-core granules as revealed by double labeling experiments with chromogranin B and p34 antibodies (Figure 1C). According to this differential localization, it is tempting to postulate that the interaction between Cdc42, N-WASP, Arp2/3 and the actin monomers takes place only at the interface between granules and the plasma membrane in stimulated cells, providing a way to specifically target local actin filament polymerization to the granule docking and fusion sites.

To conclude, we propose that activation of secretion in neuroendocrine cells does not simply trigger the disassembly of the cortical actin barrier but rather induces a fine remodeling of the peripheral actin network into structures required for exocytosis. Further studies are now required to investigate whether the local

production of actin filaments during exocytosis would serve for docking, scaffolding, fusion and/or membrane retrieval.

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