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Regulation of GRAF1 membrane sculpting function during cell movement

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Abstract
All eukaryotic cells rely on endocytic events to satisfy a constant need for nutrient and fluid uptake from their surroundings. Endocytosis-dependent turnover of cell surface constituents also serves to control signal transduction and establish morphological changes in response to extracellular stimuli. During endocytosis, distinct protein machineries re-sculpt the plasma membrane into vesicular carriers that enclose molecules that are to be taken up into the cell. Besides those produced from the canonical clathrin-mediated endocytic machinery, it is becoming increasingly clear that other membrane carriers exist. The indisputable connection between the function of these uptake systems and various disease states, highlights why it is so important to increase our knowledge about the underlying molecular machineries.

The aim of this thesis was therefore to characterise the function of GRAF1, a protein suggested to be a tumour suppressor due to that the gene has been found to be mutated in certain cancer patients. My work focused on understanding how this protein operates during formation of clathrin-independent carriers, with possible implications for disease development.

Previous *in vitro* studies showed that GRAF1 harbours a GTPase activating domain to inactivate Rho GTPase Cdc42, a major actin cytoskeleton regulator. Herein, microscopy based approaches used to analyse HeLa cells demonstrated the importance of a transient interaction between GRAF1 and Cdc42 for proper processing of GRAF1-decorated carriers. Although GRAF1-mediated inactivation of Cdc42 was not vital for the budding of carriers from the plasma membrane, it was important for carrier maturation. In addition, studies of purified GRAF1 and its association with lipid bilayers identified a membrane scaffolding-dependent oligomerisation mechanism, with the ability to sculpt membranes. This was consistent with the assumption that GRAF1 possesses an inherent banana shaped membrane binding domain. Remarkably, this function was autoinhibited and in direct competition with the Cdc42 interaction domain.

Finally, other novel GRAF1 interaction partners were identified in this study. Interestingly, many of these partners are known to be associated with protein complexes involved in cell adherence, spreading and migration. Although never actually seen localising to mature focal adhesions that anchor cells to their growth surface, dynamic GRAF1 carriers were captured travelling to and from such locations. Moreover, GRAF1 was recruited specifically to smaller podosome-like structures. Consistent with this, the tracking of GRAF1 in live cells uncovered a clear pattern of dynamic carrier formation at sites of active membrane turnover – notably protrusions at the cell periphery. Furthermore, the silencing of GRAF1 gave rise to cells defective in spreading and migration, indicating a targeting of GRAF1-mediated endocytosis to aid in rapid plasma membrane turnover needed for morphological changes that are a prerequisite for cell movement. Since these cells exhibited an increase in active Rab8, a GTPase responsible for polarised vesicle transport, the phenotype could also be explained by a defect in Rab8 trafficking that results in hyperpolarisation.

Taken together, the spatial and temporal regulation of GRAF1 membrane sculpting function is likely to be accomplished via its membrane binding propensity, in concert with various protein interactions. The importance of GRAF1 in aiding membrane turnover during cell movement spans different functional levels – from its local coordination of membrane and actin dynamics by interacting with Cdc42, to its global role in membrane lipid trafficking.

Keywords
Endocytosis, migration, polarisation, tension, CLIC/GEEC, GRAF1, Rho GTPase, Cdc42, Rab8

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