

P057 Identification and analysis of Ect2 phosphorylation sites
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Due to its GEF-activity, the mitotic phosphoprotein Ect2 can activate RhoA which in turn induces formation of the contractile ring by initiating actin and myosin polymerisation. Ect2 depletion from cells using siRNA, results in cytokinesis failure and multinucleated cells, demonstrating the essential role of the protein in cleavage furrow formation and cytokinesis. Ect2 has been shown to interact with the centralspindlin component HsCdk4 which is responsible for its recruitment to the midzone and is supposed to regulate Ect2 activity. Little is known about how temporal control of Ect2 occurs, except that phosphorylation seems to be crucial in this process.

To improve our understanding of Ect2 regulation, we consider it useful to identify all Ect2 phosphorylation sites. *In vitro* phosphorylation of recombinantly expressed Ect2 fragments revealed CDK phosphorylation in its central region and the C-terminus.

To determine *in vivo* Ect2 phosphorylation sites, we purified endogenous Ect2 from mitotically arrested HeLa cells. Mass spectrometry analysis revealed a yet unknown Ect2 phosphorylation site. For functional analysis, we have generated a Flag-tagged RNAi-resistant Ect2 construct, which can – in combination with the corresponding Ect2-specific siRNA - be used in rescue experiments. Mutational analysis of identified phosphorylation sites will reveal the importance of specific phosphosites in activating or inhibiting Ect2 activity.