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Identification and characterization of proteins interacting with RanGAP

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Ran is a key factor in the transport of proteins harbouring nuclear localization signals (NLS) and nuclear export signals (NES) across the nuclear pore complex. Ran functions as a molecular switch by cycling between a GDP-bound inactive state and a GTP-bound active state. The change between the GDP- and GTP- bound state is tightly controlled by a guanine nucleotide exchange factor RCC1 and a GTPase activating protein RanGAP. RanGAP exists in an unmodified and in a SUMO-1 modified form. SUMO-RanGAP is docked to the nuclear pore complex via RanBP2. RanGAP can dramatically stimulate the low intrinsic GTPase activity of Ran. Guanine-nucleotide exchange is catalysed by the chromatin-bound nucleotide exchange factor RCC1 inside the nucleus. Disruption of the Ran GTPase system was shown to result in perturbation of numerous cellular processes including DNA replication, cell cycle progression and RNA export.

In this work, a yeast two-hybrid system was applied to identify RanGAP interacting partners. The N-terminal and the C-terminal domain of RanGAP (NRanGAP and CRanGAP) and full-length RanGAP were used as baits in the screening of a human testis cDNA library, respectively. The catalytic α1 subunit of 5´-AMP-activated protein kinase and Sds22, a regulatory subunit of protein phosphatase 1 were found to bind to the N-terminal region of RanGAP.

To confirm the interaction of RanGAP and these interacting proteins, *in vitro* binding assays were performed. GST-NRanGAP was shown to interact with AMPK and Sds22 as well. RanGAP and SUMO-RanGAP from HeLa were also found to interact with GST-AMPK α1 and GST-Sds22 in pulldown assays. AMPKinase from HeLa lysate was co-precipitated with RanGAP/SUMO-RanGAP in a co-immunoprecipitation assay.

To assay whether RanGAP could be phosphorylated by AMPK, AMPKinase from HeLa cells was affinity-purified and used to phosphorylate recombinant RanGAP and SUMO-RanGAP.

Both forms of RanGAP were not phosphorylated by AMPKinase. However, Crm1, the export mediator for nuclear export signal harbouring proteins, which was co-precipitated with RanGAP, was phosphorylated by a kinase bound to the anti-RanGAP immuno-complex.

The subcelluar localization of RanGAP and its interacting partners was determined in order to verify a possible co-localization. RanGAP was predominantly localized to the nuclear envelope, and a small fraction of AMPK $\alpha 1$ was also found to be associated with the nuclear envelope.

In order to understand whether AMPKinase, Sds22 and Sds22/PP1c regulate RanGAP, RanGAP activity was assayed upon addition of these components. None of these proteins modulate RanGAP activity, suggesting that in the RanGAP-bound form they may modify export substrates to stabilize their cytoplasmic location and to prevent re-import.