Universität Basel

Research Project

Elucidation of mechanisms of Arf1-dependent cellular processes

Third-party funded project

Project title Elucidation of mechanisms of Arf1-dependent cellular processes
Principal Investigator(s) Spang, Anne ;
Organisation / **Research unit**
Departement Biozentrum / Biochemistry (Spang)
Department
Project start 01.04.2019
Probable end 31.12.2023
Status Completed

The ability to communicate with and to sense the environment is essential for cells. Exo- and endocytosis are the processes promoting the discharge and uptake of proteins, lipids and other molecules at the plasma membrane, respectively. These processes are controlled by small GTPases mainly of the Arf and Rab family. At the same time signaling events occur at the plasma membrane that may drive the internalization of receptors and will subsequently lead to the termination of the initial signal transduction. Likewise, external cues read by receptors in the plasma membrane can influence membrane transport event. Thus, there is a crosstalk between membrane traffic and signaling. In recent years, however, it became clear that this crosstalk may be more complicated and intense and that small GTPases, such Arf1, may operate beyond their canonical functions

We have been working on the elucidation of different Arf1 functions. One feature that stood out was that Arf1 is important for the subcellular localization of a subset of mRNAs, and that in an arf1 mutant, some RNAs became better translated, even though translation was generally attenuated, and processing body (PB) formation was increased. PBs are the major decay compartment in yeast. To gain more insights, we performed next generation sequencing (NGS) on total RNA and ribosome associated mRNA (Riboseq) in an arf1 mutant and wild type. The NGS analysis extended the knowledge on differentially translated mRNAs. More importantly, two processes stood out that were extremely affected in the arf1 mutant.

Pathways that were the most strongly upregulated would yield an increase in methylation. We confirmed the hits and that the methyl donor (AdoMet) is increased in arf1 mutant cells. However, we do not know what molecular species are methylated. We can exclude so far lipids and histones. Nothing is known about the connection between Arf1 and methylation. Therefore, we will determine in the first aim the molecules that are methylated and the responsible methyltransferases. From there, we will determine the mechanism and signaling pathway that leads to the increase of methylation.

The most downregulated pathways were connected to ribosome biogenesis and amino acid metabolism. The regulation of these pathways are hallmarks of TORC1 signaling. Therefore, we will determine in the second aim how Arf1 controls TORC1 signaling. Even though, it was proposed that Arf1 controls TORC1 signaling in Drosophila, the underlying mechanism remains unknown.

In the third aim, we will aim to understand how mRNAs are recruited into PBs under various stress conditions and how the decision is made between storage and decay in PBs. In this aim, we will capitalize on our recently established method to purify PBs to determine the mRNA and protein content and combine this with Riboseq. This system-wide analysis will be followed up by in-depth mechanistic analyses. With this aim we will gain unprecedented molecular insights on how mRNA fate is determined.

Keywords ARF, mRNA metabolism, intracellular traffic, yeast, intracellular transport, methylation, signaling small GTPases, mRNA, TORC1
Financed by

Add publication

Add documents

Specify cooperation partners