Project outline

1. Project outline describing the scientific rationale of the project (max 4,000 characters incl. spaces and returns)

The RAS family of small GTPases act as signalling hubs regulating cell proliferation and differentiation. Importantly, about 20% of all human cancers harbour mutations in RAS genes (COSMIC). It is generally assumed that, when oncogenically mutated, RAS molecules over-activate all the downstream effectors. We recently showed, however, that strikingly, a biased over-activation of one of the RAS effectors occurs upon a constitutive RAS signal input (Kelsall, Vertesy et al., bioRxiv, 2018). In this work, we exploited a relatively simple yet physiological setting of fission yeast mating process, where Ras1, the unique fission yeast RAS homologue, activates MAPK^Spk1 and Cdc42 pathways. We demonstrated that a constitutively active Ras1 mutant causes a prolonged activation of Cdc42, whilst MAPK^Spk1 activation stays transient as is the case for the wildtype Ras1.

Compared to the well-characterised process of RAS-mediated MAPK activation, molecular mechanism of RAS-mediated Cdc42/Rac activation is largely unknown. We hypothesised that Ras1 induces Cdc42 activation by directly interacting with Scd1, a GDP-GTP exchanging factor (GEF) for Cdc42. By acting as a GEF for Cdc42, Scd1 is expected to activate Cdc42. In order to obtain structural insights into the RAS-mediated Scd1 activation process, we produced bacterially expressed recombinant Ras1 and Scd1 proteins. Direct interaction between them was confirmed for the first time by NMR and pull-down assays.

Having established the RAS-mediated Cdc42 activation pathway using the powerful model system fission yeast, we now wish to address the next question; How a single signalling component, RAS, manages to activate multiple downstream targets in a coordinated manner. Does RAS simultaneously interacts with multiple targets? Or
does RAS somehow jump between different targets? The issue has been a challenging open question especially because of complex nature of RAS signalling involving a number of effectors in higher eukaryotes. We believe that the simple fission yeast system enables us to address the question and to build a core concept of the RAS signalling.

We have already obtained an intriguing observation as follows. It has been well-established that RAS directly interacts with MAP kinase kinase kinase (MAPKKK) to prime activation of MAPK pathway. We confirmed that fission yeast Ras1 also directly interacts with fission yeast MAPKKK, Byr2. Strikingly the Ras1-Byr2 complex formation was interfered by the presence of Scd1. Our result indicated that RAS may develop preferences towards particular downstream targets.

In the PhD project, we further address the question by fully exploiting the fission yeast model and using structural, cell biological and genetic approaches.

Techniques that will be undertaken during the project

In this project, we exploit highly tractable model organism, fission yeast, employing cell biology (extensive live cell imaging using a confocal microscope and deconvolution), biochemistry (Western blotting and immunoprecipitation) and genetic approaches (random mutagenesis and screenings). We will conduct extensive structural analyses using NMR, X-ray crystallography, SEC-MALLS (size exclusion chromatography-multiple angle laser light scattering), ITC (isothermal titration calorimetry), Fluorescence Polarization and cryo electron microscopy.