JAMES RENNIE BEQUEST

REPORT ON EXPEDITION/PROJECT/CONFERENCE

Expedition/Project/Conference Title: Research on ribosome biogenesis in plants.

Travel Dates: from June, 1st to August, 15th 2009

Location: Frankfurt, Germany

Group Member(s): Malwina Niemczyk

Aims: The aim of the research was to purify TAP-tagged proteins involved in ribosome biogenesis in plants and use the to complement *A.thaliana* T-DNA insertion lines.

OUTCOME (not less than 300 words):-

The twelve-week project took place at the Institute for Molecular Biosciences, University of Frankfurt, Germany. Here, I focused on ribosoms in *Arabidopsis thaliana* plants. The aim of the project was to generate constructs and use these for the transformation of plants carrying T-DNA insertions in the corresponding genes in order to check whether the tagged proteins can complement the phenotypes of the mutant plant lines.

The first step was to clone the genes of interest using the Gateway recombination system as well as conventional cloning. The constructs were then tested for transient expression in *Arabidopsis thaliana* protoplasts. Protoplasts were isolated either from plant leaves or from cell cultures and transformed with the expression constructs. Transient expression was confirmed by Western Blot. In case expression in plant protoplasts was observed, I transformed *Agrobacterium tumefaciens* cultures with the constructs for the expression of tagged genes and prepared glycerol stocks for further use in transformation of *A. thaliana* plants. PCR analysis of *Agrobacterium* colonies allowed the identification of transformants.

When *A. thaliana* plants were about to start blossoming, they were ready for transformation with the *Agrobacterium* clones harboring the expression constructs. The Floral-Dip method was used for plant transformation. Liquid cultures were inoculated with *Agrobacterium* stocks shortly before transformation and the early flowers of *Arabidopsis* plants were dipped into the transformation solution. Seeds were collected about 4 weeks later and sewed on BASTA plates to select for transformed seeds, containing the gene of interest and the BASTA resistance gene. Resistant plants were then tested by PCR on genomic DNA for successful insertion and by Western Blot for expression of the constructs.

Thanks to the James Rennie Bequest, I was able to travel to Frankfurt and carry out this interesting project as well as meet new people, and visit this beautiful city.