Final report of grant (62p1) within the Aktion framework - „Structural analysis of the binding of MAP2c to Plectin's SH3 domain using NMR".

Month	Person	Function	Duration	Purpose	Place
February 2012	J. Nováček	PhD student	1 month	Data aqquisition	Vienna
	L. Janda	Applicant	1 week	Data aqquisition	Vienna
August/September 2012	L. Janda	Applicant	1 week	Data aqquisition	Vienna
March 2012	G. Wiche	Professor, co-applicant	2 days	Lecture	Brno
November 2012	G. Walko	Post-Doc	1 week	Lecture, data aqquisition	Brno

The Implementation Plan for grant 62p1 was as follows:

February 2012/Vienna: The task was to transfer the know-how of the preparation of proteins (SH3 domain, MAP2c, tubulin) to the Vienna-based laboratory (Lubomir Janda, Jiri Novacek). Jiri Novacek (PhD student) should then continue in Vienna with testing the biological activities of the above mentioned proteins (cosedimentation assays, turbidity measurements).

March 2012/Brno: The aim was to familiarize the partner laboratory about the details of the project in form of a lecture and to formulate specific objectives in relation to the measured data from Vienna (Prof. Wiche).

August / September 2012/Vienna: The goal was to process the results from both labs, and/or complete the measurements in order to publish the results (Lubomir Janda).

November 2012/Brno: This mission should complete with the processing of results and their publication in the form of writing of a scientific article (Gernot Walko).

Month	Person	Function	Duration	Purpose	Place
February 2012	J. Nováček	PhD student	1 month	Data aqquisition	Vienna
	L. Janda	Applicant	1 week	Data aqquisition	Vienna
August 2012	L. Janda	Applicant	1 week	Data aqquisition	Vienna
October 2012	G. Wiche	Professor, co-applicant	2 days	Lecture	Brno
June 2012	G. Walko	Post-Doc	1 week	Lecture, data aqquisition	Brno

The actual implementation of the plan for grant 62p1:

February 2012/Vienna : January 30, 2012 - February 3, 2012 (Lubomír Janda), January 30, 2012 - March 2, 2012 (Jiri Novacek); Lubomir Janda and Jiri Novacek visited the group of Prof. Gerhard Wiche, Max F. Perutz Laboratories, University of Vienna. The aim of this visit was to complete experiments for the writing of a manuscript with the provisional title "Intermediate filament-associated plectin 1c cytolinker destabilizes microtubules in keratinocytes". One of the most important results that would significantly promote the article was to provide evidence that the SH3 domain of plectin interacts with microtubule-associated proteins (MAPs) of the MAP2/tau family. For this purpose it was necessary to prepare different MAPs and tubulin for biochemical interaction assays. PC (phosphocelulose purified) tubulin needed to be freshly purified form from hog brain, as it was a key protein of the whole experiment. Following the expression and purification of MAP proteins and the isolation the PC tubulin, the interaction between plectin`s SH3 domain and MAPs in the presence of tubulin should was quatnified by co-sedimentation experiments, which are able to quantitatively show the interaction between different proteins. During this period, we were able to carry out the planned experiments . The journey was made by car, because in addition to passenger transport it was necessary to transport frozen samples (DNA and proteins - MAP2c, SH3domain of Plectin (exons 16-21). The stay was finished by an oral presentation of Jiri Novacek entitled “Nuclear magnetic resonance as a tool for characterization of intrinsically disordered proteins”. The presentation summed up the results achieved during the stay in Vienna, and gave an overview over nuclear magnetic resonance methodology and its application for the studies of intrinsically disordered proteins, which is the main topic of the future collaborations of both partners.

June 2012/Brno:

Gernot Walko`s visit to Brno, which was originally scheduled in November, took place in June 18-22.6.2012.

This stay included data analysis as well as several important discussions for the conception of a manuscript entitled "Intermediate filament-associated plectin 1c cytolinker destabilizes microtubules in keratinocytes," which was submitted to Molecular Biology of the Cell in November 2012. In addition, Gernot Walko was giving an oral presentation entitled “Role of plectin 1c in microtubule dynamics”.

August 2012/Vienna: August 19, 2011 - August 24, 2012. Lubomir Janda visited group of prof. Gerhard Wiche, Max F. Perutz Laboratories, University of Vienna. The aim of this visit was to determine the sequence of MAP2c protein that is directly involved in binding to plectin`s SH3 domain. Limited proteolysis (trypsin, chymotrypsin and Glu-C) was used for identification of a protein fragment which was protected from protease cleavage by binding to the SH3 domain of plectin. Using bioinformatic sequence analysis the region of MAP2c binding to the SH3 domain should be identified. During his stay, we were able to carry out all the experiments that were planned. The journey was made by car, because in addition to passenger transport it was necessary to transport samples on ice (MAP2c, SH3domain of Plectin (exons 16-21)) and on the return journey as well as frozen (polyclonal antibodies against MAP2 and cartridge samples of the proteases Glu-C and Chymotrypsin).

October 2012/Brno:

Prof. Gerhard Wiche`s visit to Brno, which was originally scheduled in March, took place in October 18-22.6.2012.

Prof. Gerhard Wiche was giving a lecture to the faculty "The many faces of plectin and plectinopathies" and in lab seminars discussed future plans.

Submitted article after minor revision.

Intermediate filament-associated cytolinker plectin 1c destabilizes microtubules in keratinocytes

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