INTEGRATION OF RNA BIOLOGY FOR NEXT-GENERATION SCIENTISTS

H2020-WIDESPREAD-2020-5
Project Number: 952541

D1.4 PhD internship reports

Work Package:	WP1
Deliverable due date:	31/12/2022
Responsible partner:	MU
Editors:	Michaela Musilová
Deliverable number:	D4
Deliverable type:	Report
Dissemination level:	Confidential
Created:	13/12/2022
Version:	1.0

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1 INTRODUCTION

Work Package 1, "Pathway to Excellence in RNA Cluster", focuses on establishing, enhancing and nurturing the collaboration between partners in the consortium and equipping CEITEC MU researchers with the necessary knowledge and expertise in techniques and methods to perform excellent research in RNA cluster and beyond. Among the main objectives is enhancing the excellence capacity of CEITEC MU ESRs and researchers in RNA biology.

The Deliverable D1.4 relates to Task 1.4 ESRs internships. PhD students (in total 16) from CEITEC MU went to the matching labs at collaborating institutions and in most of cases worked on collaborative projects from the seed funding. This deliverable includes reports from stays in laboratories of partner institutes in the period March 2022 – December 2023.

Unfortunately, the outgoing problems with COVID during the 1st reporting period affected the start of secondments and internships. No one was allowed to join another lab last year due to measures applied by national governments and by the director of CEITEC MU. We resumed the secondments and internships in March 2022. The list of the travels is presented in Table 1 below.

2 REPORTS BY ESR

In this section of the Deliverable, the reports of PhD students throughout the years 2022 to 2023 are included (as per Table 1).

Table 1. Secondments in WP1

Secondee	**Hosting institution**	**Term**
Alžběta Kusová	IMB	07.03.2022 -13.03.2022
Praveenkumar Rengaraj	EMBL	17.07.2022-07.08.2022
Juan Francisco Sanchez Lopez	JGU	05.09.2022-07.10.2022
Karolína Trachtová	EMBL	06.11.2022-12.11.2022
Terézia Kurucová	EMBL	06.11.2022-12.11.2022
Qiupei Du	UEDIN	29.01.2023-10.03.2023
Pedro Zeni	EMBL	10.10.2023-29.10.2023
Tomáš Reigl	EMBL	02.07.2023-29.07.2023
Janka Melicherová	EMBL	01.09.2023-30.09.2023
Jiří Rudolf	JGU	06.10.2023-06.11.2023
Anna Rudolfová	IMB	06.10.2023-06.11.2023
Karolína Trachtová	UEDIN	18.06.2023-25.06.2023
Dagmar Al Tukmachi	UEDIN	18.06.2023-25.06.2023
Terézia Kurucová	EMBL	5.11.2023-25.11.2023
Praveenkumar Rengaraj	EMBL	03.12.2023-06.12.2023
Karolína Černovská	EMBL	13.11.2023-24.11.2023

2.1 ALŽBĚTA KUSOVÁ, IMB - MAINZ, GERMANY

BUSINESS TRIP REPORT

INTEG – RNA: INTEGRATION OF RNA BIOLOGY FOR NEXT-GENERATION SCIENTISTS

Name, Surname, Title: Alžbeta Kusová Mgr

Date of business trip, No. of Travel Order: 7.3.2022-13.3.2022, CES/7101/0049/22

Name of Institution/Conference: Institute of Molecular Biology (IMB), Mainz

Address of the Institution/Conference venue:
Ackermannweg 4, 55128 Mainz, Germany

Aim of the business trip:
To identify proteins associated with RNA subunit of telomerase in *Arabidopsis thaliana*

Description of activities:
During my short stay at the IMB, I isolated nuclear extracts from *A. thaliana* 7-day-old seedlings. The extracts were subsequently used for the RNA pull-down with construct of AtTR subunit of telomerase. Once the pull-down was done, the samples were digested in gel and analyzed by MS.

Business trip /secondment /conference outcomes:
We were not able to obtain a lot of enriched proteins from the RNA pull-down, and considering this, thereafter we focused on *in vivo* pull-down rather than on *in vitro*.

Useful contacts:
Falk Butter – group leader
Patricia Schupp – PhD student who supervised me during the stay

Future Cooperation with the Institution (not valid for Conferences):
In future, we may perform the RNA pull-down also with other constructs of RNA with modified 5´- or 3´-end.

Date:
27.3.2023

Signature:

2.2 PRAVEENKUMAR RENGARAJ, EMBL - HEIDELBERG, GERMANY

BUSINESS TRIP REPORT

INTEG – RNA: INTEGRATION OF RNA BIOLOGY FOR NEXT-GENERATION SCIENTISTS

Name, Surname, Title: Praveenkumar Rengaraj

Date of business trip, No. of Travel Order: 16.7.22 to 5.8.2022, zakazka is 7101 and suborder is podzakazka is 10

Name of Institution/Conference: EMBL, Heidelberg

Address of the Institution/Conference venue: Meyerhofstr. 1, D-69117 Heidelberg, Germany

Aim of the business trip: To learn big data analysis

Description of activities:

The purpose of the visit was to learn big data analysis for our RNA sequencing data. Which is sequenced at Gencore Heidelberg So, I went to Dr. Vladimir Benes lab. Stayed for three week and I was able to process the entire data. I travelled on to 16.7.22 to 5.8.2022. EMBL Genomics Core Facility [GeneCore] , Meyerhofstr. 1, D-69117 Heidelberg, Germany

Business trip /secondment /conference outcomes: I learnt how to do the big data analysis.

Useful contacts: benes@embl.de

Future Cooperation with the Institution (not valid for Conferences):
We still have samples to sequence at EMBL. We are preparing the samples for the sequencing and once it's ready I would like to visit one more time.

Date: 06.03.2023

Signature:

2.3 JUAN FRANCISCO SANCHEZ LOPEZ, JGU - MAINZ, GERMANY

Name, Surname, Title: Juan Francisco Sánchez López

Date of business trip, No. of Travel Order: 05/09/2022-07/10/2022

Name of Institution/Conference: Johannes Gutenberg University Mainz

Address of the Institution/Conference venue: Hanns-Dieter-Hüsch-Weg 17 (BioZ II), 55128 Mainz

Aim of the business trip: Internship in the research group headed by Andreas Wachter

Description of activities: Perform analysis to evaluate alternative splicing in samples from plants grown at high temperature growth conditions

Business trip /secondment /conference outcomes: We found some genes that undergo alternative splicing in our stress samples

Useful contacts: sanchez.lopez@ceitec.muni.cz; helene.robert.boisivon@ceitec.muni.cz

Future Cooperation with the Institution: yes

2.4 KAROLÍNA TRACHTOVÁ, EMBL - HEIDELBERG, GERMANY

Name, Surname, Title: Karolína, Trachtová, Mgr

Date of business trip, No. of Travel Order: 6.-12.11.2022, CES/7101/0431/22

Name of Institution/Conference: EMBL, Heidelberg

Address of the Institution/Conference venue: Meyerhofstraße 1, 69117 Heidelberg, Germany

Aim of the business trip: Spatial transcriptomics data analysis

Description of activities:

This work trip was focused on knowledge transfer about spatial transcriptomics, novel method that enables the spatial mapping of gene expression within a tissue section. Mainly, we focused on the bioinformatic analysis of spatial transcriptomics data.

We discussed projects of several EMBL research groups that also use spatial transcriptomics and described our intended spatial transcriptomic analysis of prostate cancer tissues. We exchanged knowledge about respective steps of the analysis as well as the best practises.

Business trip /secondment /conference outcomes:

We managed to obtain state-of-the-art procedures related to bioinformatic analysis of spatial transcriptomics data from excellent EMBL scientists. This greatly enhanced our current understanding spatial transcriptomics and will improve and advance our own research.

Useful contacts:

Vladimir Benes, Head of Genomics Core Facility, EMBL
Jonathan Landry, Bioinformatician at Genomic Core Facility, EMBL
Future Cooperation with the Institution (not valid for Conferences):

We intend to continue our collaboration with Genomic Core Facility of EMBL and exchange knowledge in the field of bioinformatics.

2.5 TERÉZIA KURUCOVÁ, EMBL - HEIDELBERG, GERMANY

BUSINESS TRIP REPORT

INTEG – RNA: INTEGRATION OF RNA BIOLOGY FOR NEXT-GENERATION SCIENTISTS

Name, Surname, Title: Terézia, Kurucová, Mgr.

Date of business trip, No. of Travel Order: 06.11.22-12.11.22, CES/7101/0434/22

Name of Institution/Conference: EMBL Heidelberg

Address of the Institution/Conference venue: Meyerhofstraße 1 69117 Heidelberg, Germany

Aim of the business trip: to broaden the knowledge of spatial transcriptomics, to obtain new contacts for experts in the field of spatial transcriptomics, to consult our pilot data

Description of activities: We spent most of our time at EMBL where we met with various people with experience in the field of spatial transcriptomics. We subsequently discussed our current experiments with them in order to get as many tips as possible on how to analyze the data and how to prepare the best possible experiments next time. The meetings were very enriching, as we learned a lot of new things.

Business trip /secondment /conference outcomes: I plan to go back for a short term internship

Useful contacts: Denis Schapiro, Kristy Ou, Vladimir Benes

Future Cooperation with the Institution (not valid for Conferences): EMBL Heidelberg

Date: 17.3.2023

Signature: 

2.6 QIUPEI DU, UEDIN – EDINBURGH, UK

Name, Surname, Title: Qiupei Du, Miss

Date of business trip, No. of Travel Order: 29.01.2023-10.03.2023, CES/7101/0010/23

Name of Institution/Conference: The University of Edinburgh

Address of the Institution/Conference venue: The King's Buildings, Charlotte Auerbach Road, Edinburgh EH9 3FL, UK

Aim of the business trip: Internship

Description of activities: The RNA editing enzymes, ADAR1 and ADAR2 can convert adenosine to inosine in dsRNA. In humans most of the dsRNAs that are edited by ADAR1 are generated by Alu elements embedded in inverse orientations in longer transcripts. Inosine in dsRNA marks the dsRNA as being self and prevents activation of the innate immune response by the dsRNA sensors. We hypothesize that not all dsRNAs formed from inverted Alus are highly immune-inducing as they must be extremely duplex in nature. However, it is difficult to isolate these endogenous, highly immune-inducing dsRNAs using standard experimental approaches based on immunoprecipitation techniques, as the dsRNA sensors MDA5 and PKR are very ‘sticky’, bind many types of dsRNAs and form oligomers, thus decreasing the yield of immunopurified dsRNA. Therefore, by using a tethering approach with the dsRNA sensors fused to ADAR2 deaminase domain, we hope to identify a subclass of highly immunogenic dsRNAs and to determine if they are the same dsRNAs binding to both dsRNA sensors, MDA5 and PKR. I learned and optimized molecular cloning techniques, including PCR, construction of plasmids, transfection, etc.

Business trip /secondment /conference outcomes: The desired target plasmids were made using PCR, gel purification, and Gibson assembly. Fusions were created between the deaminase domain of ADAR2 and the dsRNA sensors MDA5 and PKR.

Future Cooperation with the Institution: Stable cell lines will be generated expressing these fusion proteins under doxycycline promotor control so the level of induction of the fusion proteins can be tightly regulated.

2.7 PEDRO ZENI, EMBL - HEIDELBERG, GERMANY

Name, Surname, Title: Pedro Faria Zeni, Msc.

Date of business trip, No. of Travel Order: 10.10.2023 – 29.10.2023 CES/7101/0320/23

Name of Institution/Conference: European Molecular Biology Laboratory (EMBL)

Address of the Institution/Conference venue: Meyerhofstraße 1, 69117 Heidelberg, Germany

Aim of the business trip: Noncoding Genome symposium, RNA FISH course & internship

Description of activities: I received advanced training in conventional in situ hybridization (ISH), RNA FISH and ISH-HCR and stayed for an internship.

Business trip /secondment /conference outcomes: I acquired overall view of the noncoding genome field which my PhD thesis is placed. Also, I acquired enough knowledge to apply RNA FISH in my lab and my research.
Internship outcomes: Transcription factors (TFs) have been identified as key regulators of RNA regulatory networks in aggressive subtypes of chronic lymphocytic leukemia (CLL) and follicular lymphoma (FL). It has been shown that both diseases harbor complex deregulation of interconnected TFs that leads to aberrant mRNA expression profile together with dysregulated expression of post-transcriptional regulators such as RNA-binding proteins and microRNAs. In malignant B cells, we aimed to describe the regulatory landscape of 3 selected transcription factors. We optimized CHIP-PCR and subsequently conducted the first CEBPB CHIPseq to identify the binding of this transcription factor in B cells. The binding of FOXO1 in the genome has been optimized using Cut&Taq approach (including nuclear extraction). Our preliminary data indicated that each of these TFs is involved in the regulation of essential cellular programs such as survival, proliferation or migration. We also prepared knockout MEC1 cell lines for FOXO1 and HMGA1 (and performed siRNA experiments) and investigate gene expression analysis in these cells to understand the impact of TF modulation on gene expression.

Useful contacts: charlotte.courtney@embl.org

2.8 TOMÁŠ REIGL, EMBL - HEIDELBERG, GERMANY

Name, Surname, Title: Tomas, Reigl, Mgr.

Date of business trip, No. of Travel Order: 2.7.2023 - 29.7.2023, CES/7101/0296/23

Name of Institution/Conference: EMBL Heidelberg

Address of the Institution/Conference venue: Meyerhofstraße 1, 69117 Heidelberg, Germany

Aim of the business trip: Internship with focus on single-cell RNA data analysis

Description of activities: During my internship in EMBL Heidelberg I joined the team of Vladimír Beneš, the Head of Genomics Core Facility. His team of bioinformaticians, mainly Jonathan Landry, helped me to understand single-cell RNA data analysis. Based on data I brought from Brno, I've designed a primary and secondary pipeline for the analysis. I've learned about tools and function libraries used for data analysis of this kind of data and how to chain them to create logical workflow for necessary data transformation, filtering, and inspection. Jonathan also helped me to understand and overcome challenges related to lower quality of the data.

Business trip /secondment /conference outcomes: The internship helped me to understand process of single-cell RNA data analysis. I've created a bioinformatic pipeline tailored for our dataset. The help from Jonathan exceeded my internship. He helps us to understand the results and to change input parameters of the pipeline to fit our needs.

Useful contacts: Jonathan Landry (jonathan.landry@embl.de), Vladimír Beneš (benes@embl.de)

Future Cooperation with the Institution: The cooperation with the team of Vladimír Beneš was very smooth. They are open for any future visits, for single-cell data analysis or any other data analysis. We keep in touch with Jonathan Landry, he helps me to finetune parameters of the analytical pipeline I've created during the internship.

2.9 JANKA MELICHEROVÁ, EMBL – ROME, ITALY

Name, Surname, Title: Janka Melicherová Mgr.

Date of business trip, No. of Travel Order: 1.9.2023-24.9.2023, CES/7101/0314/23

Name of Institution/Conference: EMBL Rome
Address of the Institution/Conference venue:
EMBL Rome
Adriano Buzzati-Traverso Campus
Via Ramarini 32
00015 Monterotondo (RM)
Italy

Aim of the business trip: To acquire understanding and knowledge on how mutant mice are generated by CRISPR/Cas 9 and deepen histology knowledge.

Description of activities: I learned different techniques to generated mutant mice such as CRISPR/Cas9 genome editing and learn more advanced staining and labelling techniques.

Business trip /secondment /conference outcomes: histological samples staining with IB4 isolectin, beta-catenin and cytokeratine, in situ hybridization, CRISPR/Cas9 genome editing.

Useful contacts: Neil Humphreys neil.humphreys@embl.it
Emerald Perlas emerald.perlas@embl.it

Future Cooperation with the Institution: I am contact with both facilities and consult new data and results.

2.10 JIŘÍ RUDOLF, JGU - MAINZ, GERMANY

BUSINESS TRIP REPORT

INTEG – RNA: INTEGRATION OF RNA BIOLOGY FOR NEXT-GENERATION SCIENTISTS

Name, Surname, Title: Mgr. Jiří Rudolf

Date of business trip, No. of Travel Order: 06/10/23-06/11/23, -

Name of Institution/Conference: Johannes Gutenberg University Mainz

Address of the Institution/Conference venue: Staudinger Weg 5, 55128 Mainz, Germany

Aim of the business trip: acquiring a new methodical approach for separation of low-abundant non-coding RNAs,

Description of activities: observation and training in the laboratory, own performance of the method on plant RNA samples, ionex separation of RNAs, optimization of the method for own project

Business trip /secondment /conference outcomes: acquisition of a new methodological approach

Useful contacts: Kevin Kopietz, kkopietz@uni-mainz.de

Future Cooperation with the Institution (not valid for Conferences): follow-up consultations on possible method optimization

Date: 7. 11. 2023

2.11 ANNA RUDOLFOVÁ, IMB - MAINZ, GERMANY

BUSINESS TRIP REPORT

INTEG – RNA: INTEGRATION OF RNA BIOLOGY FOR NEXT-GENERATION SCIENTISTS

Name, Surname, Title: Mgr. Anna Rudolfová

Date of business trip, No. of Travel Order: 06/10/23-06/11/23, -

Name of Institution/Conference: Institute of Molecular Biology Mainz

Address of the Institution/Conference venue: Ackermannweg 4, 55128 Mainz, Germany

Aim of the business trip: learning a new methodical approach (iCLIP method) for studying RNA-protein interactions

Description of activities: observation and training in the laboratory, own performance of the method on proteins related to the telomerase complex, optimization of the method for own project

Business trip /secondment /conference outcomes: acquisition of a new method

Useful contacts: Julian König, J.Koenig@imb-mainz.de

Future Cooperation with the Institution (not valid for Conferences): follow-up consultations on possible method optimization

Date: 7. 11. 2023

2.12 KAROLÍNA TRACHTOVÁ, UEDIN – EDINBURGH, UK

Name, Surname, Title: Karolina, Trachtova, Mgr.

Date of business trip, No. of Travel Order: 18.6.2023 - 25.6.2023, CES/7101/0240/23

Name of Institution/Conference: University of Edinburgh

Address of the Institution/Conference venue:

King's Buildings, Max Born Cres, Edinburgh EH9 3BF, United Kingdom

Aim of the business trip: Internship

Description of activities:

During the stay, I focused mainly on analysing data from next-generation sequencing. Our primary goal was detailed analysis of big data in Python, including state-of-the-art techniques for data manipulation and visualization. I was introduced to advanced Python tools that enables study of DNA methylation and histone modifications.

Business trip /secondment /conference outcomes:

I significantly improved my Python programming skills, especially in the fields of big data manipulation and visualization. This will enable me to create more sophisticated plots for publications as well and communicating research outcomes in a clearer way.

Useful contacts:

Shaun Webb, MR. Bioinformatics Core Facility Manager,

Future Cooperation with the Institution (not valid for Conferences):

We plan to further carry on collaboration in the field of bioinformatics, particularly in NGS data analysis.

2.13 DAGMAR AL TUKMACHI, UEDIN – EDINBURGH, UK

Name, Surname, Title: Dagmar Al Tukmachi

Date of business trip, No. of Travel Order: 18.-25.06.2023; CES/7101/0241/23

Name of Institution/Conference: University of Edinburgh

Address of the Institution/Conference venue: King's Buildings, Max Born Cres, Edinburgh EH9 3BF, United Kingdom

Aim of the business trip: Internship

Description of activities: During the internship at Shaun Webb's central bioinformatics laboratory at the University of Edinburgh, I became deeply involved in the complex processing of next-generation sequencing (NGS) data. This mainly focused on the analysis of the MECP2 gene, which is essential for the normal function of the nerve cells. The internship also included an introduction to advanced bioinformatics tools that specialized in the study of DNA methylation and histone modifications, essential epigenetic factors that significantly influence gene expression. The training provided insights into the complex interplay between these modifications and their potential impact on pathogenesis, paving the way for predictive analysis in genetic disorders and diseases.

Business trip /secondment /conference outcomes: In one week, I significantly improved my Python programming skills, especially in the areas of DNA methylation analysis, big data manipulation, and visualization. This internship provided me with a new skill set and prepared me to better communicate my research results.

Useful contacts: Shaun Webb

Future Cooperation with the Institution: Ongoing collaboration in the field of bioinformatics.

2.14 TERÉZIA KURUCOVÁ, EMBL - HEIDELBERG, GERMANY

Name, Surname, Title: Terézia, Kurucová

Date of business trip, No. of Travel Order: 5.11-25.11.2023, CES/7101/0427/23

Name of Institution/Conference: EMBL Heidelberg

Address of the Institution/Conference venue: Meyerhofstraße 1, 69117 Heidelberg, Germany

Aim of the business trip: Course + two-week intership

Description of activities: I completed a course on preparing the library for the new Avity sequencing platform and subsequently expanded my experience with this platform as part of an internship. During the internship, I converted the libraries prepared for the Illumina platform so that they could be sequenced on the new Avity instrument. Since the conversion rate was very low, this process required extensive troubleshooting, which was ultimately very rewarding as one learned a lot from it. I also had the opportunity to see how samples are prepared for the PacBio sequencer, and how this instrument is operated.

Business trip /secondment /conference outcomes: Avity, a new sequencing platform, was installed in our CFGenomics this month, and thanks to my trip to Heidelberg, I already have experience with it and can use it right away. In addition, thanks to the course, I gained contacts with people from other core facilities, which is very useful and important.

Useful contacts: Vladimír Beneš, Jan Provazník, Daphne Welter, John Baeten, Stefaan Derveaux, Lim De Swert

Future Cooperation with the Institution: I would definitely like to visit GeneCore again, there are many things we can learn from them

2.15 PRAVEENKUMAR RENGARAJ, EMBL - HEIDELBERG, GERMANY

Name, Surname, Title: Praveenkumar Rengaraj

Date of business trip, No. of Travel Order: 3.12.2023 to 16.12.2023

Name of Institution/Conference: EMBL

Address of the Institution/Conference venue: Meyerhofstraße 1 69117 Heidelberg Germany

Aim of the business trip: To advance proficiency in bioinformatics and achieve a deeper understating of RNA-sequencing data.

Description of activities: During the first trip I was able to learn and apply the basic key bioinformatic concepts to analyse the RNA-sequencing data. Then I did the preliminary analysis.
This time, my aim is to validate the parameters I used previously, rectifying any potential inaccuracies on my analysis. Also, to gain the deeper knowledge about the RNA-sequencing data for the several complex downstream analyses.

Business trip /secondment /conference outcomes: After the initial discussion with our collaborator, we noticed the utilization of serval parameters are unnecessary within my analysis pipeline, I repeated the analysis using refined, optimal parameters. Subsequently, a comparative evaluation between the previous and updated analyses revealed noticeable differences. To validate the reproducibility of the new analysis, I did several quality control analyses to ensure pipeline accuracy. Additionally, I acquired new pipelines for analysing high-throughput protein-RNA interaction data.

Future Cooperation with the Institution (not valid for Conferences): We are currently in the preparation of high-throughput protein-RNA interaction data. As part of this study, I am actively working in configuring the pipeline necessary for the analysis of this data. In doing so, we are expanding our collaborative efforts to ensure a comprehensive and robust approach to the analysis.

2.16 KAROLÍNA ČERNOVSKÁ, EMBL - HEIDELBERG, GERMANY

Name, Surname, Title: Karolína Černovská, Mgr.

Date of business trip, No. Of Travel Order: 13.11. - 25.11.2023

Name of Institution/Conference: European Molecular Biology Laboratory (EMBL)

Address of the Institution/Conference venue: EMBL - Meyerhofstraße 1, 69117 Heidelberg, Germany

Aim of the business trip: EMBL GeneCore Genomics Workshop, internship focused on consultation about data analyses.

Description of activities:

- Workshop in several EMBL departments (GeneCore, Imaging center, IT infrastructure).
- Keeping up to date with rapidly progressing areas of cancer genomics during the Cancer Genomics workshop + networking with scientists from across several disciplines connected with cancer research.
- Internship at the EMBL Genomics Core facility to consult WGS and RNA data to learn possible ways to analyze them with expert bioinformaticians from the hosting institution.

Business trip /secondment /conference outcomes:

- Discussion during poster presentation with other researchers oriented in similar fields of work + networking with scientists during the whole conference, which led to novel inspiration for the further heading of Ph.D. project
- Understanding of WGS and RNA results and their usefulness for the analyses of genomic defects and familiarization with bioinformatic tools and databases to analyze sequencing data

Useful contacts:

- Contacts with experts in bioinformatics and advanced sequencing methods from the GeneCore facility and Bioinformatics department

Future Cooperation with the Institution: yes