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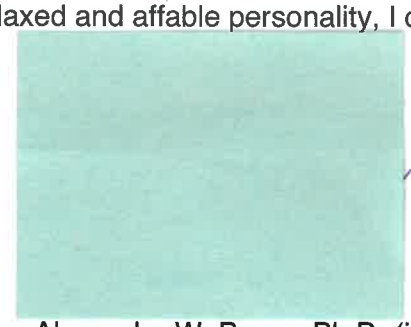
Miriama Pekľanská came to my laboratory, on the recommendation of my post-doc Lenka Gahurová, to begin her Masters degree research project at the turn of 2018/2019. Her project was centred on instigating CRISPR-Cas9 mediated *in situ* genome editing technology in preimplantation stage mouse embryo blastomeres to the lab. Specifically, to introduce recombinant cDNA for the fluorescent mCherry gene to the genomic loci of functionally important early embryonic genes; hence creating C-terminally tagged fluorescent gene fusions that could act as reliable reporters of candidate endogenous temporal and spatial gene expression during preimplantation mouse embryo development. Miriama focused on attempting to tag three candidate genes; a) *Wwc2* – a newly characterised and Hippo-signalling related gene paralog, recently revealed by our group to be involved in acentrosomal cell divisions and spindle assembly in mouse oocyte meiosis and preimplantation stage embryonic cell mitotic cleavages and b) *Mapk13* and *Mapk14*, two isoform/paralogs of the p38-mitogen-activated-kinase, shown by our group to regulate primitive endoderm differentiation in the mouse blastocyst stage embryo inner cell mass. As a starting point for this project, Miriama was using a published CRISPR-Cas9 protocol that had been successfully used to tag other preimplantation mouse embryo lineage marker genes in early embryos (Gu *et al.*, 2018) and was working under my direct supervision.

Consequently, Miriama was embarking on the ambitious project from more or less its very inception (although a previous summer ERASMUS student, had begun to clone sgRNA containing plasmids, specific for the *Wwc2* gene, prior to her arrival in the lab). It involved generating the necessary recombinant plasmid based constructs needed for the CRISPR-Cas9 mediated *in situ* genome editing (*e.g.* mCherry cDNA containing vectors with cloned candidate gene specific homology arms flanking the to be replaced STOP codon of the endogenous chromosomal sequence and the required plasmid template for *in vitro* transcription of both the gene specific and targeting sgRNA and the Cas9 endonuclease itself) and the microinjection of these constructs into single 2-cell stage mouse blastomeres and confocal microscopy based assay for reporter gene expression. I am pleased to say that Miriama was able to successfully conclude all the necessary molecular biology to derive the required constructs and was, with the help of my Ph.D. students (Rebecca Collier and Giorgio Virnicchi), able to microinject them to target the *Wwc2* and *Mapk13* genes. Although, the microinjection phase of the project came towards the near end of her research project schedule, we have in the case of the *Mapk13* gene targeting observed a tantalising potential glimpse of mCherry reporter gene expression. Currently, my Ph.D. students are embarked on empirical optimisation of the microinjection procedure (using Gu *et al.,* 2018 as a guide – *e.g.* construct concentration and exact cell-cycle-related timing of microinjection). I anticipate the molecular biology constructs derived by Miriama, as well as the lessons learnt in their derivation and eventual use in CRISPR-Cas9 mediated *in situ* candidate endogenous genome editing will be invaluable going forward. I would like to place on record my appreciation of Miriama's effort and Master's project work.

I would just like to take a short moment to record a few words about Miriama's professionalism. As referenced above, Miriama was set a 'nose-to-tail' type research project that was initiating an unfamiliar and novel experimental technique to the lab; something she has more or less achieved. This has meant that she has had to tackle each step along the way without any previous personal or lab-based knowledge. It is fair to say that at some points this has meant experiencing some inevitable pitfalls. However, it is a testament to Mirama's professional character that she did not let these get her down (or at least not for long) and was always able to circumvent them. She was keen to discuss any arising problems with myself and her peers but was always proactive and did not simply sit and wait for solutions to be provide to her. She had a deep understanding of what she was trying to achieve and the

underpinning experimental rationale. This was why she was able to offer her own practically based, and often successful, solutions. Moreover, if she was unsure of something, she was not hesitant in asking for clarification. I think this dogged determination and methodical approach, as well as her theoretical understanding, is a great strength. Indeed, it is one that I have recognised and has informed my decision to offer her a Ph.D. position in my group; one that I am delighted she has decided to accept. I also would also like to highlight her dedication, as she was more or less an ever present in the lab. She is also very proactive at extending her own professional horizons and seeking further career development. For example, during her master's studies with us, she also arranged and successfully conducted a three month ERASMUS funded research stay at the Laboratory of Viral Metagenomics at the Rega Institute in Leuven Belgium. Moreover, she prepared an accepted poster abstract (linked to the work she is defending today) and sourced funding to attend the 63rd International Conference for Students of Physics & Natural Science in Vilnius, Lithuania, that was sadly cancelled due to the on-going Covid-19 pandemic.

To summarise, I regard Miriama's time in our laboratory as being very fruitful and I have a high opinion of her as a research scientist. Therefore, for all these reasons and notwithstanding her charming, relaxed and affable personality, I commend her Master's degree thesis to the examining committee.



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