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Michaela Kubíčková came to my lab, on the recommendation of my post-doc Lenka Gahurová, to begin her Masters degree research project towards the end of 2016. Her project was centred on characterising the potential role of the *Dynactin-2* (*Dctn2*) gene in regulating the generation of the first spatially distinct cells in the mouse preimplantation embryo. Specifically, the first population of completely encapsulated inner cells, generated as a consequence of the 8- to 16-cell stage cleavage cell divisions, that give rise to the so-called inner cell mass and are distinct from the outer population that go on to form the trophectoderm (and eventually the placenta). The importance of the generation of this initial founder population of inner cell mass cells is provided extra significance by reports that suggest these cells preferentially contribute to the epiblast population of cells (i.e. progenitors for the eventual foetal tissues) over a second extraembryonic inner cell mass lineage represented by the primitive endoderm (the progenitors of which are thought to be internalised in a second wave consequent to the 16- to 32-cell stage transition), by the implanting blastocyst embryo stage.

The decision to focus on the potential role of the *Dctn2* gene in regulating this important process of segregating early embryonic cells within both developmental space and time was made due to previous observations made in the laboratory by Lenka Gahurová. Namely, that a short pharmacological pulse of mTOR signalling pathway inhibition, just prior to the 8- to 16-cell stage transition, is sufficient to negatively affect the number of primary inner cell mass founder cells, in a manner potentially related to impaired translation of a sub-class of mRNA transcripts containing a so-called TOP-sequence motif in their 5' untranslated regions. As we had identified the Dctn2 transcript as a candidate TOP-motif containing mRNA and the encoded protein, as part of a greater macro-molecular complex, had in other contexts been implicated in nuclear positioning and mitotic spindle formation and alignment, we decided to focus Michaela's project on assaying the number of primary inner cell mass founder cells generated by the 16-cell stage in embryos in which we functionally impaired Dctn2.

Consequently, Michaela embarked on first confirming the expression of the *Dctn2* gene during the mouse embryo preimplantation developmental period, and specifically at the 8- to 16-cell stage transition, and then designed, synthesised and clonally microinjected long dsRNA construct specific to the Dctn2 transcript to elicit RNAi mediated knock-down in Dcnt2 gene expression (*i.e.* at the functional protein level). She revealed that such *Dctn2*-specific loss-of-function experiments, caused a statistically significant and cell/ clone autonomous reduction in the number of primary inner cell mass founder cells generated by the mid-16-cell stage; thus confirming a functional role for Dctn2 in regulating cell division orientations that preferentially give rise to the generation of the earliest possible population of inner cells during mouse embryo development. Moreover, she also successfully cloned the cDNA for the full-length *Dctn2* gene and created a recombinant fluorescent fusion gene construct that when expressed in the early mouse embryo, was used, in conjunction with immuno-fluorescence targeting the endogenous protein, to probe the sub-cellular localisation of Dctn2 protein at this critical developmental time-point. Lastly, she investigated the expression level of endogenous Dctn2 protein, using both immuno-fluorescent confocal microscopy and western blotting, in response to similar mTOR inhibition regimes as described above; uncovering a potential sensitivity of Dctn2 protein synthesis to mTOR inhibition that is consistent with our original hypothesis that TOP-motif containing mRNA transcripts require an active mTOR pathway to be translated and thus drive cell divisions favouring the generation of primary inner cell mass founder cells at the 8- to 16-cell stage transition.

As I hope one can gather from the above descriptions, Michaela has in her relatively short time with us generated a very large amount of high quality and extremely relevant data that we hope will, together with work from Lenka Gahurová, be shortly incorporated into a manuscript; indeed she has

generated data of a quality and quantity that reflects a person who might well be mistaken as being more advanced in terms of her research science based career path! Not only this, she is also remarkable in that she has been able to quickly integrate into the laboratory, learn and perfect a diverse range of new and often demanding techniques (for example, delicate embryo micromanipulations such as dsRNA/mRNA microinjections), often with minimal supervision (although one must also acknowledge her day-to-day supervisor, Lenka Gahurová, for her considerable input in this regard). I have also been particularly impressed that she has a clear and focussed grasp on her research, not only on the practical level but also the theoretical level; she knows why she is doing her experiments and is able to design and modify her approaches appropriately. Moreover, when we discussed something she was able to quickly assimilate the premise at stake and retain this information. Added to this, is her careful, neat and methodical approach to both her practical work but also, and I may say importantly, to her record keeping; a fact that helped her to write, what I regard as a very good thesis, in good time and with minimal fuss.

I would also like to highlight her dedication. As with many developmental model systems, the hours of work can often be very unsociable; developmental processes will not adapt to the researchers needs and preimplantation mouse embryo development is no exception. Indeed, working with mouse embryos at or around the 16-cell stage, involves being in the laboratory during the early hours of the morning. Notwithstanding this inconvenience, Michaela discharged her responsibilities without complaint (at least to me) and honourably; this is to be highly commended and speaks to her professional dedication.

To summarise, I regard Michaela's time in our laboratory as being extremely fruitful and I have a very high opinion of her as a research scientist; so much so, that I have been frantically trying to persuade her to pursue a Ph.D. in our group too (*and I want to make it clear to her, even now, that it's not too late for her to change her mind and stay with us, indeed our door will always be open to her!*). I have no doubt that she has all the attributes to have a very successful future career in research science. I know she is in the process of considering a number of different future career options (which highlights her desire to try new things and gain a diversity of experiences) but I would stress that she has a frankly rare talent for practical scientific research and it would be a great shame if this were allowed to lapse. Therefore, for all these reasons and notwithstanding her charming, relaxed and affable personality, I commend her Master's degree thesis to the examining committee.

20/5/18
E. Brno

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Date & place