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Vasanth Thamodaran has the distinction, dubious or otherwise, of being the first of my Ph.D. students; having joined my newly established research group at the end of 2011. I would just like to mention a few words about his application letter. At the time just after my relocation from Cambridge, I was receiving a high volume of emails (mostly from India and China) enquiring about potential Ph.D. positions in my lab. Most often these followed the familiar format of '*Dear Sir, I am extremely motivated to conduct research in your most esteemed research laboratory as I am very excited by molecular biology . . . . etc.*'; however, the thing that made Vasanth's application stand out, other than addressing me by my name, was that his application was carefully thought out and referenced my papers, even making suggestions about potential follow-up experiments. It was this intellectual inquisitiveness that drew me to interviewing and eventually accepting him to my fledgling group and now, at the opposite end of his Ph.D. studies, I still consider it one of his strongest attributes and know that it has served him well during his studies and will continue to do so in the future. I do not have any doubts Vasanth will go on to have a successful career in science.

As I mentioned, Vasanth joined my group more or less from its onset (one could even equate its developmental stage to that akin of the mouse zygote). At this time, we were still very much in the phase of optimising all the day to day things we required to run a pre-implantation mouse embryo research lab, such as setting up the animal house, optimising the *in vitro* culture conditions, embryo microinjection apparatus and for a time lacked important apparatus such as fluorescence illumination on our inverted microscopes and microinjection needle pullers. However, despite these initial hardships (compounded by outside interference) it was fantastic to have someone with Vasanth's patient temperament and methodical nature in the group and surely and steadily we ironed out the most important and limiting faults. It is not an exaggeration to state that the relatively smooth and current day-to-day operation of the lab owes no small debt of gratitude to the groundwork put in by Vasanth. Both myself and those of his subsequent colleagues thank him for this; and his technical contribution in this regard is acknowledged in the middle authorship of the paper of his colleague, Aleksandar Mihajlovic (*Scientific Reports,* 2015) referenced but not discussed in the thesis that Vasanth defends today.

In relation to his research, Vasanth initially begun researching a potential cell-fate influencing role for the Jak/Stat cytokine regulator gene *Socs3*. He actually uncovered some interesting meiotic/ mitotic spindle protein localisation within the MII-arrested oocyte and embryos and some non-penetrant oocyte maturation defects associated with RNAi based strategies to knock-down *Socs3* expression. Additionally, he also identified a putative role for the gene in the embryo, in regulating cell-cycle time/ proliferation in a non-cell autonomous manner, using a siRNA mediated approach to clonally knockdown its expression. However, this phenotype was initially observed whilst he was conducting his mandatory three months foreign research visit (in the laboratory of my post-doc supervisor, Magdalena Zernicka-Goetz in Cambridge UK in 2011) and proved impossible to repeat once he returned to our group (despite all the controls being satisfied). Therefore, owing to the tantalising but inconsistent nature of the results, we decided to switch from an RNAi-based methodology to conditional genetic *Socs3* gene ablation strategy. As the lead in time to obtain the alleles and crosses we needed was anticipated to be long (in fact it has proved to be a lot longer than we expected) it was decided that Vasanth should begin work on a contingency project related to the function of p38-Mapk's in early development and in particular in relation to primitive endoderm (PrE) differentiation and segregation from the epiblast (EPI) during blastocyst inner cell mass maturation, that forms the basis of his defence today (and was published as his first author paper in *Open Biology*, 2016). Before, I mention this work and the considered and pivotal role he has played in carefully designing, executing, interpreting and presenting this work, I would just like to mention that as a result of Vasanth's preliminary work on the *Socs3* gene, the lab was able to

successfully recruit a talented post-doc student from the Babraham Institute in the UK (Lenka Veselovska) who is currently taking forward the genetic ablation based strategy, as a *Marie Curie European Individual Fellow*.

So, during the defended work Vasanth has utilised specific small chemical inhibitors of p38α/β-Mapk14/11 catalytic kinase function to assay its potential involvement in cell-fate maturation within the mouse blastocyst ICM (prompted by earlier work demonstrating a role for these stress kinases in regulating aspects of trophectoderm (TE) differentiation and ES cell studies indicating inhibition of p38 function is associated with naïve ES cell pluripotency). By providing the inhibition at the onset of the blastocyst stage (providing it earlier is associated with arrested embryo development at the morula to blastocyst transition) until the end of the pre-implantation development period (at E4.5), he was able to show a failure to appropriately specify PrE (marked by the expression of the late PrE marker Gata4). Moreover, that the deficient number of 'mature' PrE cells was also associated with an increased number of ICM cells that co-expressed both the early PrE marker Gata6 and the pluripotency transcription factor Nanog (that is normally only restricted to the EPI cells of the ICM by the E4.5 stage assayed) indicative of ICM cells of uncommitted cell fate (similar to those observed at the onset of blastocyst ICM maturation at the E3.5 stage). Results he repeated using an alternative p38α/β-Mapk14/11 chemical inhibitor (controlled for by a known non-biologically active analogue). Hence demonstrating that p38α/β-Mapk14/11 activity is required for entry into a developmental programme of PrE differentiation. He also compared the temporal requirement of p38α/β-Mapk14/11 activity during blastocyst ICM maturation against that of Mek1/2 (activators of Erk1/2), a well known and characterised pathway active during PrE differentiation in the ICM, and found p38α/β-Mapk14/11 acts very early during the process (being dispensable by the E3.75 stage) as opposed to the sensitivity of Mek1/2 pathway to inhibition that extended beyond the E4.0 stage (in agreement with previously reported data).

Therefore, Vasanth's work provides evidence for the novel role of p38α/β-Mapk14/11 being required to 'enable' PrE differentiation at the start of blastocyst ICM differentiation (when ICM cells are initially in an uncommitted state), whereas Mek1/2 (and ergo Erk1/2) activity is required to subsequently 'drive' PrE differentiation to completion. The described ICM maturation experiments also highlighted an increased propensity for cell-death (in the ICM and TE), nevertheless distinct and unrelated to the above described cell-fate phenotypes, that was associated with inhibiting p38α/β-Mapk14/11 activity. However, Vasanth found that supplementing the growth media, during the time of p38α/β-Mapk14/11 inhibition, with the characterised anti-oxidant N-acetyl-cysteine (NAC) could prevent such cell death, indicating a role for p38α/β-Mapk14/11 in protecting cells from increased oxidative damage, possibly caused by the increases in reactive oxygen species (ROS) known to be present during blastocyst maturation. In addition to identifying p38α/β-Mapk14/11 as novel enablers of PrE differentiation, Vasanth experiments have also shown that p38α/β-Mapk14/11 activation is functionally downstream of both activated fibroblast-growth-factor receptor (Fgfr – a known PrE promoting activated pathway, previously studied in the context of Mek1/2 and Erk1/2 activation) and Tak1 kinase (thought to act downstream of activated bone morphogenetic protein receptors/ Bmpr), thus providing, in at least part, the mechanisms by which PrE differentiation can be initiated. He has also conducted extensive experiments trying to elucidate the functionally downstream mechanisms responsible for p38α/β-Mapk14/11 induced initiation of PrE differentiation. Whilst, collectively we still favour a route by which Nanog expression is suppressed the detailed molecular mechanism has not been forthcoming; however, this fact has provided the greater lab with an important platform to investigate the unknown mechanism, using more empirical candidate identifying screen-based approaches, in the future.

In addition to the above published work, Vasanth has discovered (and described in the unpublished section of his thesis) that the severity of p38α/β-Mapk14/11 inhibition induced cell death phenotype can be modulated by the exogenous addition of amino-acids to the *in vitro* culture medium; with embryos cultured in regular commercially available KSOM culture media, that is not supplemented with amino acids, exhibiting a greater levels of p38-inhibition induced cell death, than those culture in amino-acid supplemented media. Although, there is no differential effect on the PrE specification defects caused by p38α/β-Mapk14/11 inhibition in either media. These results are in agreement with previous studies, all be they in non-embryo or ES cell contexts, that suggest p38α/β-Mapk14/11 activity is subject to fluctuations in cellular metabolism, including amino-acid availability and the onset of autophagy (the process by which amino acid and other metabolites can be scavenged by recycling intra-cellular components and organelles). Indeed, although not presented in the thesis, Vasanth has preliminary data suggesting p38α/β-Mapk14/11 inhibition impairs the formation of autophagosomes (still to be validated)

and active autophagy (observed using a GFP-tagged substrate reporter); again supportive of this link. Finally, he has shown that unlike in the situation where p38α/β-Mapk14/11 inhibition mediated cell-death observed in embryos cultured in media supplemented with amino acids could be rescued by addition of the anti-oxidant NAC, inhibited blastocysts cultured in commercial media exhibited greater cell death that was not rescuable. Again reinforcing the hypothesis that p38α/β-Mapk14/11 activity in the embryo is closely tied to metabolic status and can influence cell viability, particularly in respect to the extra-embryonic lineages; although clearly more work is required in this regard (hence the inclusion of the relevant data in the unpublished section of the thesis).

In concluding, I highly recommend Vasanth Thamodaran's thesis for a successful defence. During his stay in my group, he has proved himself an intelligent, diligent, resilient and more than capable scientific researcher. He is not afraid of hard work (one only has to see the appendix section of his thesis in which he has catalogued the results, on a individual embryo basis, of all his experiments, to testify to this statement). Moreover, he is a very affable and likeable chap and I know he will be missed by myself, his colleagues and wider friends throughout the Faculty/ Biology Centre. We all wish him the best in his future and I therefore commend his thesis to the commission.

Alexander W. Bruce Ph.D.

5th December 2016 in České Budějovice