**Christine and T.J. Martin Research Travel Grant (awarded 2016)**

**Audrey Chan**

Throughout the course of my PhD, I have focused on characterising the signalling and trafficking kinetics of parathyroid hormone receptor type 1 (PTH1R) in both HEK293 cells and primary osteoblasts. PTH1R is a class B G protein-coupled receptor (GPCR) and plays crucial roles in regulating skeletal development and mineral ion metabolism, through actions that are mediated through chondrocytes, osteoblasts and osteocytes. PTH1R responds to two endogenous ligands – PTH and PTH-related protein (PTHrP), where ligand-induced receptor activation predominantly leads to the activation of adenylyl cyclase and generation of cyclic AMP (cAMP) at the cell surface. PTH has been shown to induce receptor endocytosis, where unlike most GPCRs, cAMP production continues, an event referred to as ‘non-canonical’ or endosomal signalling. I recently published the requirement of the sorting nexin 27 (SNX27)-retromer trafficking complex as a crucial modulator in terminating non-canonical signalling by directing internalised PTH1R into retromer-mediated recycling tubules that deliver the receptor back to the plasma membrane (Chan et al., 2016).

Though this provided significant contributions to the knowledge of PTH1R signalling and trafficking, the role of SNX27-retromer following PTHrP-stimulation has not yet been investigated. It is known that PTH and PTHrP elicit different cellular responses; PTH functions as a circulating hormone while PTHrP functions as a paracrine/autocrine factor in a variety of tissues, including bone. Furthermore, studies directed at investigating the kinetics of PTHrP-induced PTH1R trafficking have largely focused on the amino-terminal fragment (PTHrP(1-36)) as opposed to the full-length protein found endogenously as PTHrP(1-141). Though PTHrP(1-36) has been shown to be sufficient for ligand binding and eliciting the generation of cAMP, this fragment omits several functional domains of PTHrP that are not shared with PTH and have known functions in placental calcium transfer and nuclear translocation. Therefore, the paracrine/autocrine action in bone is most likely to be driven by full-length PTHrP(1-141). This travel grant gave me the opportunity to work closely with E/Prof TJ (Jack) Martin and A/Prof Natalie Sims and their lab at St Vincent’s Institute in Melbourne to gain invaluable knowledge in the field of PTHrP physiology and action in bone, and to apply my skills in confocal microscopy to thoroughly investigate the differences in PTH1R signalling and trafficking in HEK293 cells and established bone cell lines. This laboratory houses numerous of in-house-generated resources, and is the only possessor of the in-house generated and validated full-length form of PTHrP(1-141) and its truncated variants, as well as numerous anti-PTHrP antibodies and established bone cell lines.

I first began my journey by attending the 42nd Lorne Protein Structure and Function Conference at Lorne, Victoria. I presented the findings from our recent publication that described the role of critical glutamic acidic residues within the carboxy-terminal tail of PTH1R in mediating its interaction with SNX27 and thus, prevent the misrouting of PTH1R for lysosomal degradation (Clairfeuille et al., 2016). By attending this conference, I also gained invaluable insight into the study of protein structure and function and has further enhanced my current knowledge in protein trafficking in the context of the living cell. I was also able to meet current collaborators as well as establish new contacts in varying fields with the potential of new collaborations in the future.

Soon after, I started my placement at St Vincent’s Institute under the guidance of the entire Bone Cell Biology and Disease Unit. Having focused most of my PhD in PTH-mediated PTH1R signalling and trafficking, I investigated any differences that full-length PTHrP or its truncated variants may have in gene expression in the context of bone. Unpublished findings of the SVI group have shown that PTHrP(1-141) but not its truncated variants, can induce the persistent activation of adenylyl cyclase in the osteosarcoma cell line, UMR106. I found that this did not occur in the MC3T3 osteoblastic cell line: there was no significant differences observed in the expression of cAMP-response genes between PTH(1-34), full-length PTHrP or its truncated variants.

Next, I utilised confocal microscopy to visualise whether these apparent cell-limited differences in persistent generation of cAMP were due to any differences in the trafficking of activated PTH1R. For this, I used the HEK293 cell line expressing GFP-tagged PTH1R that I had previously established as a model for visually tracking the trafficking itinerary of PTH1R (Chan et al., 2016). After optimising a series of in-house-generated anti-PTHrP antibodies, I identified one that was capable of detecting PTHrP(1-141) in HEK293 cells. I used this antibody to show that, similar to tetramethylrhodamine-labelled PTH(1-34) (PTH-TMR), PTHrP(1-141) binds PTH1R, they are internalised together into retromer-positive endosomes and remain in complex for up to 30 minutes. However, while PTH-TMR remained in complex with its receptor for up to 60 minutes post-agonist stimulation, PTHrP(1-141) was not detected after 30 minutes following agonist exposure. These findings provide strong new evidence that full length PTHrP-PTH1R does internalise into early endosomes, in contrast to the previous report showing rapid dissociation of PTHrP(1-36) from PTH1R at the cell membrane (Ferrandon et al., 2009). To validate these findings in bone cells, I therefore attempted to visualise these events in MC3T3, UMR106 and the osteoblastic/osteocytic cell line, OCY454. I was able to confirm the time-dependent internalisation of PTH-TMR and PTH1R into early endosomes in these cells, however the PTHrP antibody used in the HEK293 cells was unfortunately incapable of accurately detecting the presence of PTHrP(1-141).

By providing me with the opportunity to work with E/Prof TJ (Jack) Martin, A/Prof Natalie Sims and their experienced laboratory, they have welcomed be as a new member of their laboratory family. Through this experience, my work has contributed to the field of PTHrP, PTH1R and their laboratory, however this is relatively small compared to the amount of invaluable knowledge and experience that they have bestowed upon me during my short time at St Vincent’s. I am forever grateful to the AMGEN Christine and TJ (Jack) Martin Travel Award, ANZBMS and SVI’s Bone Cell Biology and Disease Unit for providing me with this wonderful opportunity to share and discuss with like-minded scientists and clinicians leading the forefront of skeletal biology and research, where I believe I will continue to contribute to the field of PTH1R in bone and how we may continue to elucidate and therapeutically manipulate PTH1R-mediated signalling in different cell types to favour certain cellular outcomes.