Congratulations to IMCB's latest PhD graduate – Dale Wallace Maxwell

Tuesday, 8 March 2022

Thesis Title: Motile Cilia Dysfunction: Characterising Zebrafish *prpf8* and *pierce1/pierce2* Mutants with Left/Right Axis Defects

The vast majority of multicellular eukaryotes show some form of symmetry, whether this be radial or bilateral. In vertebrates, bilateral symmetry is apparent at the whole organism level, however internal organs have a defined left and right sidedness in placement and internal symmetry. In mammals, the left/right body axis is defined early in development in an organ called the Embryonic Node. The analogous laterality organ is the Kupffer's Vesicle in teleost fish. In this project I characterise two zebrafish mutants which exhibit defects in laterality establishment; one which contains a premature stop codon in the spliceosomal gene *prpf8* and another which contains null mutations in *pierce1* and *pierce2*, the encoded proteins being microtubule inner proteins of the ciliary axoneme.

Preliminary investigations into these *prpf8* and *pierce1*/2 mutants observed a high percentage of unlooped hearts, indicative of problems in left/right axis formation. I investigated Kupffer's Vesicle to determine whether the cilia in this structure are present and functional. Subsequently, I examined key laterality marker genes to elucidate the downstream effects on cardiac laterality caused by the KV cilia perturbation. Additionally, for the *prpf8* mutant fish I investigated why mutation of a protein involved in the key processes of splicing would have such detrimental effects on cilia morphology and function by examining prpf8 protein localisation in both *prpf8* mutant and prpf8 knockdown fish models. Our findings indicate that prpf8 plays a significant role in ciliogenesis, with mutants and knockdowns exhibiting global cilia defects. The reason for this, as of yet, is still unclear. However, I present strong preliminary data that show prpf8 localises to cilia axonemes, suggesting a direct mechanism for prpf8-cilia protein interaction. In contrast, I also present data showing an increase in mis-splicing of *arl13b*, a critical cilia gene, in multiple prpf8 mutant models, suggesting a more indirect, spliceosomal role for prpf8 in causing cilia dysfunction.

In regards to the pierce1/pierce2 double mutant, I show that loss of both these proteins is critical for cilia motility in the KV, and that rescue of either one of the genes rescues cilia motility and restores correct heart looping directionality.

This project has wide reaching implications on the understanding and possible diagnosis of a range of conditions including heterotaxy related congenital heart disease as well as primary cilia dyskinesia and Retinitis Pigmentosa.

I joined SR lab in October 2018 having completed one year of my PhD at the University of Manchester. My project involved investigating the function of a spliceosomal protein in ciliogenisis and its relation to the establishment of the left/right axis using zebrafish as a model. Additionally, I took on another project investigating the role of two uncharacterised proteins called Pierce 1 and Pierce2 in which zebrafish mutants also displayed defects in left/right axis formation. During this time Sudipto constantly pushed and supported not only my research but my development as a scientist. This mentorship was invaluable during my PhD with the Pierce1/2 work resulting in a publication in Cell. I have now started a 4 year postdoctoral position at the University of Oxford, which I can categorically say would not have been achievable without the skills and mindset fostered in SR lab.

Dale Wallace Maxwell
Supervisor: Prof Sudipto Roy

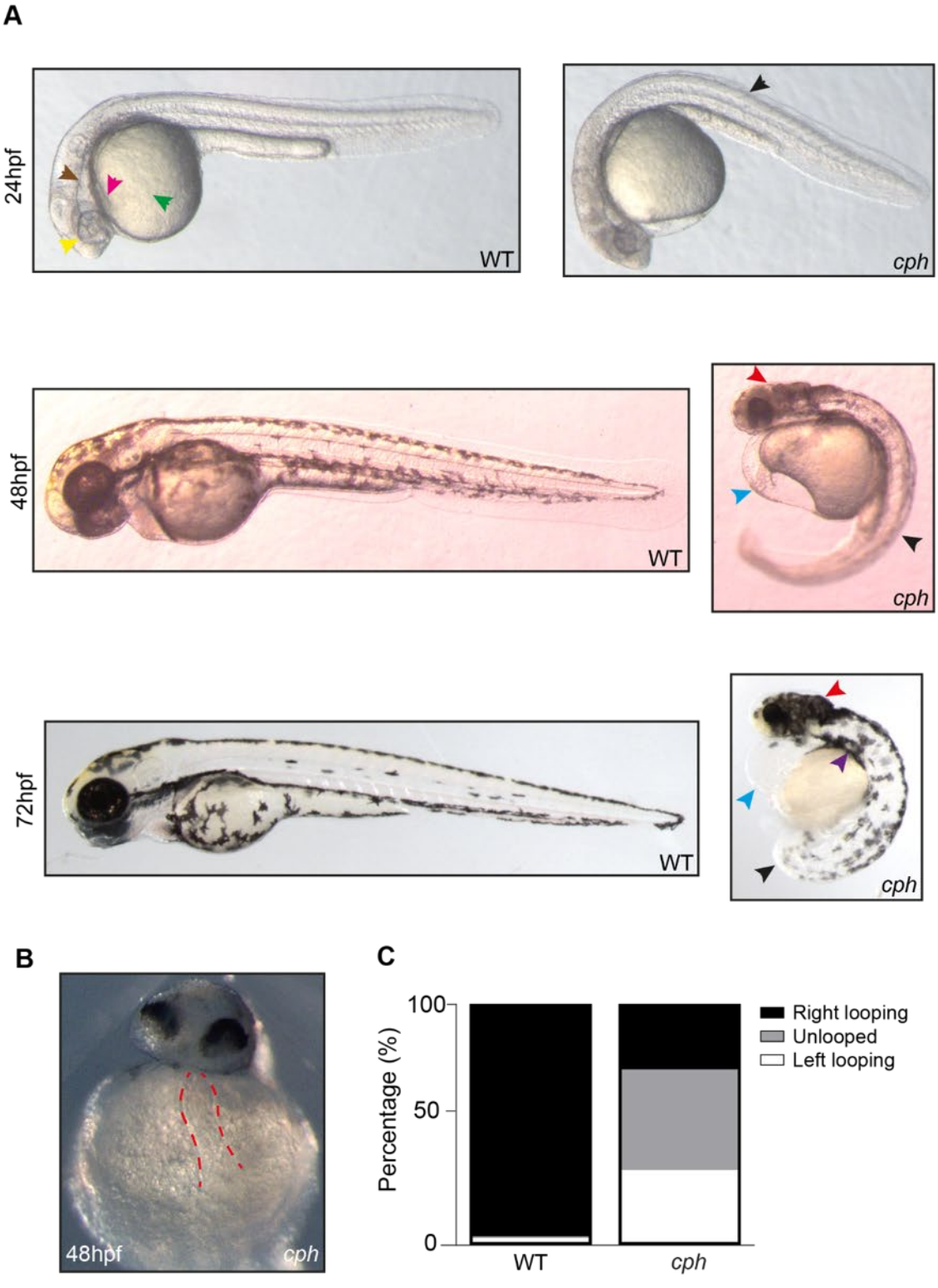

Figure 3.3: Morphological phenotype of *cph* zebrafish. (A) Morphological characteristics of WT and *cph* zebrafish embryos at 24hpf, 48hpf and 72hpf. Black arrows indicate curled down A-P body axis. Blue arrow indicates pericardial oedema. Red arrow indicates neural cell death. Purple arrow indicated kidney cyst. Green and pink arrow indicated yolk sac and heart respectively **(B)** Unlooped heart of a *cph* embryo at 48hpf and graph depicting frequency of each looping direction. n=134 WT and n=104 *cph*