Research

Cilia and Ciliopathies

Cilia and flagella are hair-like filamentous organelles that have been conserved evolutionarily in the eukaryotes. Our high school lectures have taught us how protozoans, like *Paramecium*, use beating cilia to swim around in water. We have also learnt that certain tissues in our own bodies have motile cilia. For instance, motile cilia that line the length of our respiratory tract beat to clear mucus that entangles pathogens and pollutants which enter through the nose as we breathe. Cilia also perform sensory functions. Besides locomotion, cilia in the protozoans are used for phototactic and chemotactic behavior. In the metazoans, many sense organs have cilia that have lost the motility apparatus and have become dedicated sensory organelles. Photoreceptors in the eye and olfactory neurons in the nose have such highly specialized sensory cilia. Several decades ago it was discovered that in the vertebrates, not just the sense organs, but almost every cell of the body differentiates a single immotile cilium at the end of cell division or after differentiation. Although regarded as vestigial structures for a long time, it has now become apparent that these so called primary cilia also function as hubs for a large number of signaling pathways that operate during embryonic development and in adult physiology.

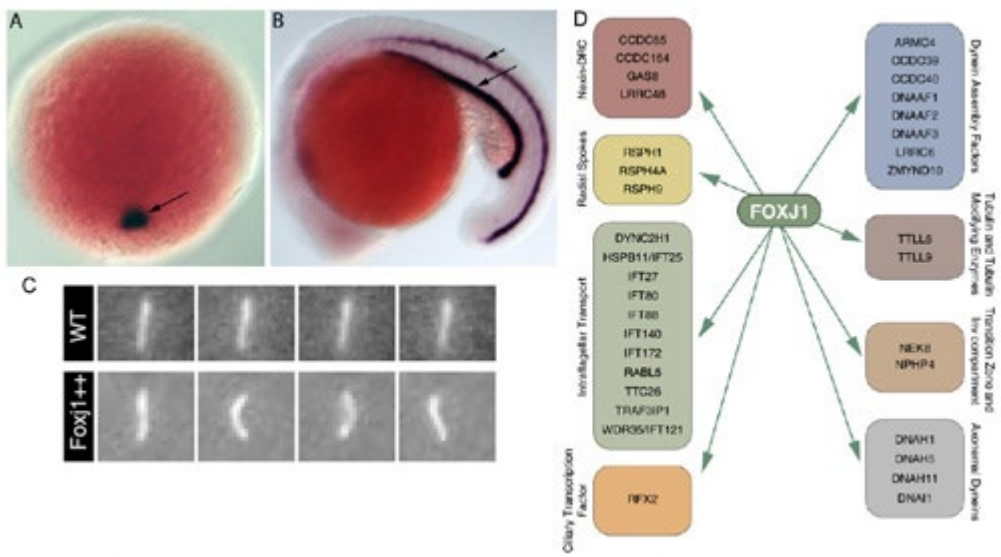

Figure 1: Foxj1 is the master regulator of motile cilia biogenesis. (A) Expression of *foxj1* in Kupffer's vesicle (arrow) of a gastrulating zebrafish embryo. Kupffer's vesicle functions like the mammalian node in specifying left-right asymmetry. (B) *foxj1* expression in the spinal canal (short arrow) and kidney duct (long arrow) of an 18 hour post-fertilization zebrafish embryo. C) Over-expression of Foxj1 can induce ectopic motile cilia. D) A large cohort of known ciliary genes are transcriptionally regulated by Foxj1.

The filamentous part of the cilium that extends out of the cell surface is called the axoneme, and consists of a microtubule scaffold enveloped by an extension of the cell membrane. The axoneme remains anchored to the basal body that is derived from the mother centriole. The ultrastructure of cilia can vary, depending on their function. Sensory or primary cilia typically have 9 radially arranged microtubule doublets (9+0 pattern), whereas the motile cilia, in addition, usually have a central pair of singlet microtubules (9+2 pattern). Furthermore, the motile cilia have dynein arms on the peripheral microtubule doublets that confer motility. A wide spectrum of human diseases arises from defects in cilia formation and function. Abnormalities of the primary cilia result in syndromes like Alstrom, Bardet-Biedl, Joubert, Meckel-Gruber, Senior-Loken as well as other conditions like polycystic kidney disease and nephronophthisis. Symptoms of these diseases range from orofacial deformities, abnormalities in limb development, retinal degeneration, as well as formation of kidney cysts. On the other hand, dysfunctional motile cilia can also instigate pathological consequences, exemplified by disorders such as Primary Ciliary Dyskinesia (PCD) and Kartagener syndrome. Individuals afflicted with PCD have immotile or dyskinetic cilia and flagella, and, consequently, defective mucociliary clearance, chronic pulmonary infections and infertility (in males). Many individuals with PCD show perturbations in left-right asymmetry of internal organs (Kartagener syndrome) that ensue from defective motility of cilia in the embryonic node. Fluid flow over the node, driven by rotary beating of motile cilia, is thought to trigger a signaling cascade that breaks the initial bilateral symmetry of the embryo. Thus, elucidating the developmental basis of ciliary biogenesis will not only further our understanding of key events in embryogenesis , but will also broaden our insights into the etiology of ciliopathies. Such a profound impact of the cilium on human health is indeed the motivation behind the intensive research being carried out world-wide on the biology of this organelle. We are using genetic and cell biological analysis in the zebrafish embryo and the mouse to understand the regulatory pathways that direct ciliogenesis, the activities of many of the individual proteins that constitute structural and functional components of cilia, and how different developmental and physiological pathways use cilia as platforms for their signaling activity. Over the past few years, we have made important contributions to the field of ciliary biology, some of which are highlighted below. In 2004, through positional cloning in zebrafish, we identified a new zinc finger and coiled-coil containing protein, Dzip1, that we subsequently showed to be associated with the ciliary basal bodies, at the transition zone. In 2017, we discovered that mutations in the paralogous gene *DZIP1L* cause autosomal recessive polycystic kidney disease (ARPKD), by compromising the transition zone, and thereby the trafficking of ciliary membrane proteins PC1 and PC2. In 2005, we published our work on the identification of Kif7, the first vertebrate orthologue of Costal2, a kinesin-like protein in *Drosophila*, involved in Hedgehog signaling. Several groups have followed up on our findings and have now shown that Kif7 is a ciliary kinesin that is mutated in fetal hydrolethalus, acrocallosal and Joubert syndromes. In a seminal piece of work

published in 2008, our group made the pioneering discovery that the forkhead transcription factor FoxJ1 is the master regulator of motile cilia biogenesis. We found that FoxJ1 is not just necessary for motile cilia to form, but is also sufficient to reprogram cells that normally do not make motile cilia to ectopically differentiate this organelle. Inspired by this striking attribute of FoxJ1, we now have undertaken a genome-wide search for the targets genes that are regulated by this transcription factor. We have found that the expression of more than 600 genes is activated by FoxJ1 during ciliogenesis, giving us the first global view of the vertebrate ciliary transcriptome. Many of these genes encode previously described components of the ciliary apparatus; more importantly, the list is replete with many completely novel genes that have not been implicated in cilia formation or function in previous studies. Our current efforts are directed at understanding the contributions of the proteins encoded by these genes in cilium differentiation and function, and their possible roles in the pathogenesis of ciliopathies. In 2015, we showed that mutation of one such target gene, *CCDC11*, causes heterotaxy and congenital heart disease in humans, by affecting the motility of cilia within the left-right organizer. In the same year, we showed that another FoxJ1 target gene, *gmnc*, which encodes a coiled-coil domain containing transcription regulator, programs the differentiation of multiciliated cells.

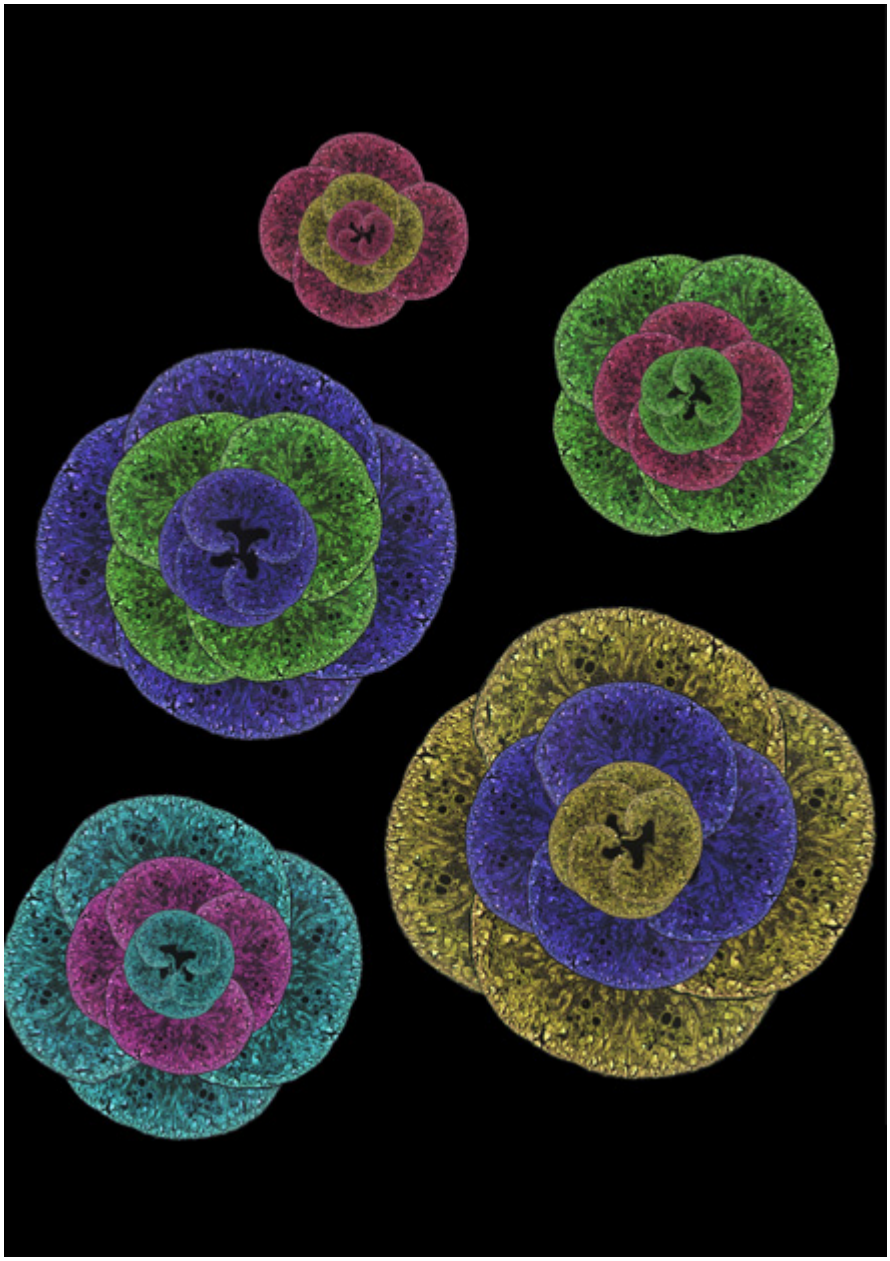

Figure 2: Transverse sections of a *Dzip1l* mutant mouse kidney, false coloured and arranged in a spiral form. Note the presence of cysts in the cortical region. (Courtesy M. C. Rondón Galeano).

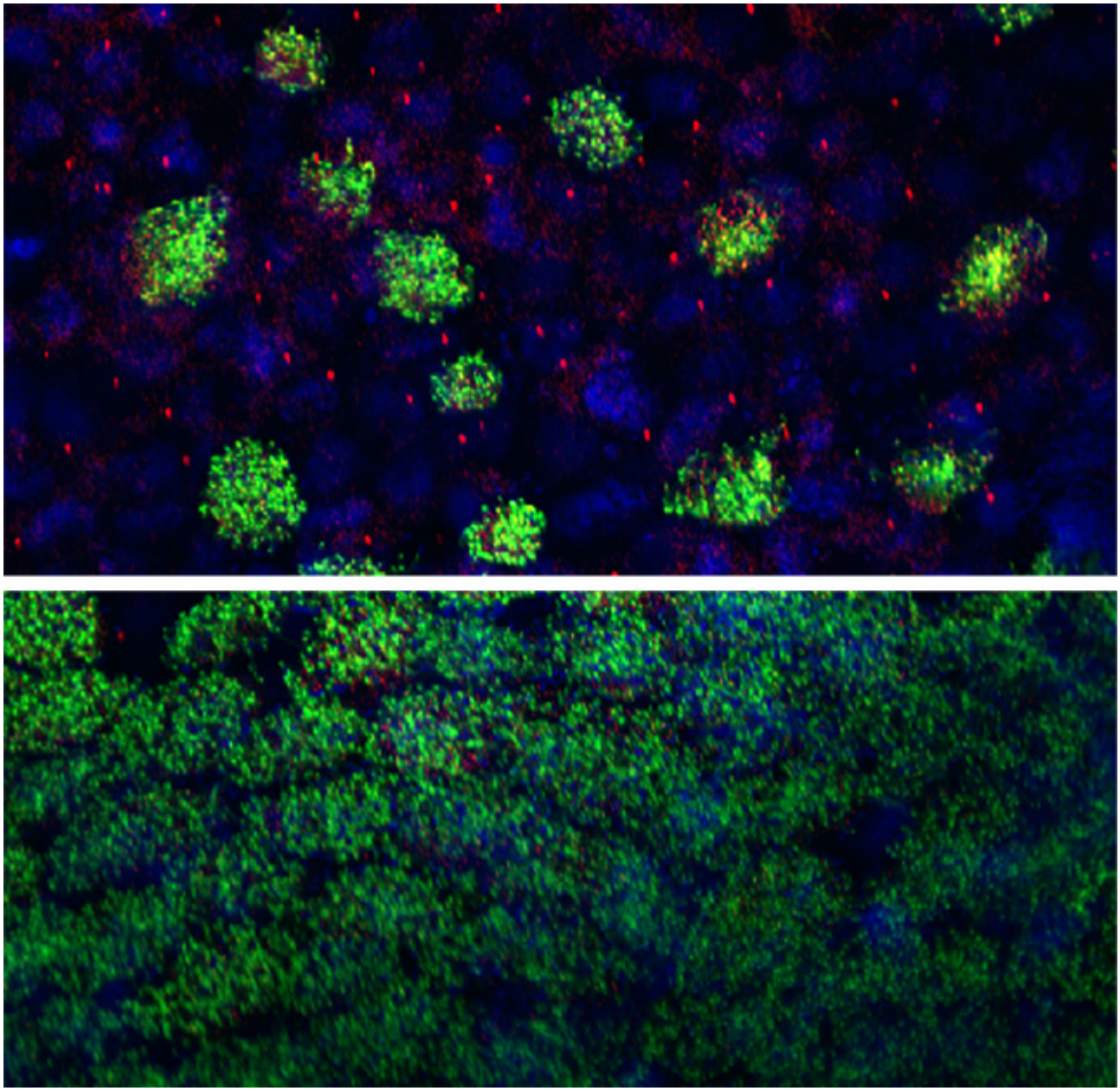

Figure 3. Skin of a *Xenopus* tadpole showing scattered multiciliated cells (upper panel). Skin of a tadpole over-expressing Gmnc, showing a lawn of multiciliated cells (lower panel). Cilia were stained with anti-acetylated tubulin antibodies (green), centrioles/basal bodies with g-tubulin antibodies (red) and DNA with DAPI (blue). (Courtesy F. Zhou, B. Reversade and S. Roy).