The aim of this collaborative project is to determine the roles of Cherpgrip, the only orthologue of Rpgrip1 and Rpgrip1l, in Clytia hemisphaerica during embryogenesis and to assay its potential conserved contribution to the Wnt-PCP pathway. In mammals, RGRIP1 and RGRIP1L both localize at cilia transition zone, but the expression of Rpgrip1 is limited to the retina, whereas Rpgrip1l is broadly expressed. Defects in these genes lead to Leber congenital amaurosis or Meckel and Joubert syndromes respectively. Many of the reported defects found in Rpgrip1l mutants can be accounted for by abnormal hedgehog signaling. Indeed, cilia have been shown to play a critical role in this signaling pathway, and transition zone proteins such as RGRIP1L are required for its proper regulation.

In addition, RGRIP1L, as well as other ciliary proteins, have been shown to play a role in the cellular planar polarization (PCP) of various epithelia, which often translates into an asymmetric positioning of centrosomes or basal bodies (modified centriole at the base of cilia). Our lab has previously shown that in zebrafish neural tube floor plate, this role depends on RGRIP1L-mediated Dishevelled stabilization, but the molecular details that link ciliary proteins and planar polarization such as asymmetric centrosome positioning remain largely unknown.

Like many marine organism, Clytia hemisphaerica (Cnidarian, Hydrozoan) have free swimming embryos, whose movements rely on the coordinated directional beating of ectodermal cilia. The planar polarization of the ciliated ectoderm has been shown to be regulated by core PCP proteins such as Vang Gogh/Strabismus (Momose et al. 2008). The main goal of this project is to determine if Cherpgrip subcellular localization and functions are conserved in Clytia.

During the two weeks I spent in Evelyn Houlston’s laboratory I was able, with the help of Tsuyoshi Momose, to inject 2 different Cherpgrip targeting morpholinos in Clytia’s eggs. The disruption of motility at 2 days post-fertilization suggests that there could be a defect in ciliogenesis and/or in the planar polarization of motile ectodermal cilia. The analysis of PCP at earlier developmental stages (early gastrula and 1 day post-fertilization) is still ongoing.

We also expressed our myc-tagged version of Cherpgrip in gastrula and planula embryos. The immuno localization of Cherpgrip is consistent with a localization at the transition zone of ectodermal cilia. We also detected Cherpgrip-myc below or next to the gamma-tubulin staining at the base of ectodermal cilia, which suggests that Cherpgrip does not only localizes at the transition zone, but maybe in a more proximal position close to rootlets. Finally Cherpgrip-myc labeled both the spindle poles and the mitotic spindles in dividing cells, which suggest that Cherpgrip could play a role in cell division, for example by regulating their orientation, which is the case of IFT20, another ciliary protein, in mammalian kidney. We will confirm this data in vertebrates, using the same cytoskeleton stabilization buffer during fixation.

In order to obtain a Cherpgrip mutant, we also injected a gRNA along with Cas9 protein in Clytia eggs, extracted genomic DNA and assessed the proportion of different indels with the TIDE website. The results show that DNA reparation leads to a frameshift in
44% of cases. There was also no indels in about 20% of cases and a -3 deletion in about 9% of the cases. We will soon start growing CRISPR-injected colonies and will check the genotype within 2 months (time needed to grow female and male polyps after metamorphosis, that are sufficiently mature to produce small medusa that will in turn grow and reach sexual maturity).

To sum-up, I hope I will be able to assess (thanks to morpholinos injections) if the transition zone protein Rpgrip1L has a conserved function in metazoans to position the basal body on the elongating side of gastrulation embryos, results that will be confirmed or not by the obtention of mutant colonies by Crisper-Cas9.

The novel protocole I followed for immunofluorescence allowed to reveal novel sub-cellular localisations for Rpgrip1L, at the base of the centrioles and on the mitotic spindles. This opens interesting hypothesis that may better explain its PCP function via direct links with rootlets for exemple and potential non-ciliary function during cell division, if the use of the same protocole confirms this finding in vertebrates.