Research

Philip INGHAM Research

The powerful genetics and exquisite embryology of the zebrafish has established this organism as an outstanding non-mammalian model for the analysis of vertebrate embryonic development. The unique experimental advantages of the zebrafish include the optical clarity, accessibility and rapid development of its embryo, the availability of large collections of mutations disrupting essential genes and the relative simplicity of its organ systems. Despite this simplicity, the zebrafish shares many fundamental similarities with other vertebrates; for instance, the patterning of the neural tube, the control of neural and glial differentiation, the specification and differentiation of blood cell lineages and the development and function of the heart. Thus insights from studies in zebrafish can readily be applied directly to higher vertebrates, including humans.

Our research group uses the zebrafish Danio rerio as a model system to study a number of related processes in vertebrate development. In particular, we focus on the role of signaling pathways and the gene regulatory networks (GRNs) that they control. We also use the fish to model human disease related processes such as the inflammatory response and tumour angiogenesis and metastasis. Our approach is based on understanding complex biological processes in the context of the whole organism: we use a range of techniques that take advantage of the properties of the zebrafish, including in vivo imaging, transgenesis, antisense mediated gene knockdown, Zinc finger nuclease mediated targeted gene knock-out, Tandem Affinity Purification of protein complexes and Chromatin Immuno Precipitation (ChIP).

Hedgehog Signalling

Hedgehog (Hh) proteins constitute one of the handful of families of signaling molecules that regulate animal development. Dysfunction of the Hh signaling pathway results in severe developmental defects and is associated with a number of different types of tumour in human. Although the pathway has been highly conserved through evolution, there are some important differences, particularly between Drosophila, the species in which most is know about the mechanism of Hh signaling, and vertebrates. We are using a combination of genetic and proteomic analyses in the zebrafish to probe both the conservation and divergence of Hh pathway mechanisms and function.

Collaborators: Dr. F van Eeden, University of Sheffield, UK; Dr. W. Blackstock, IMC

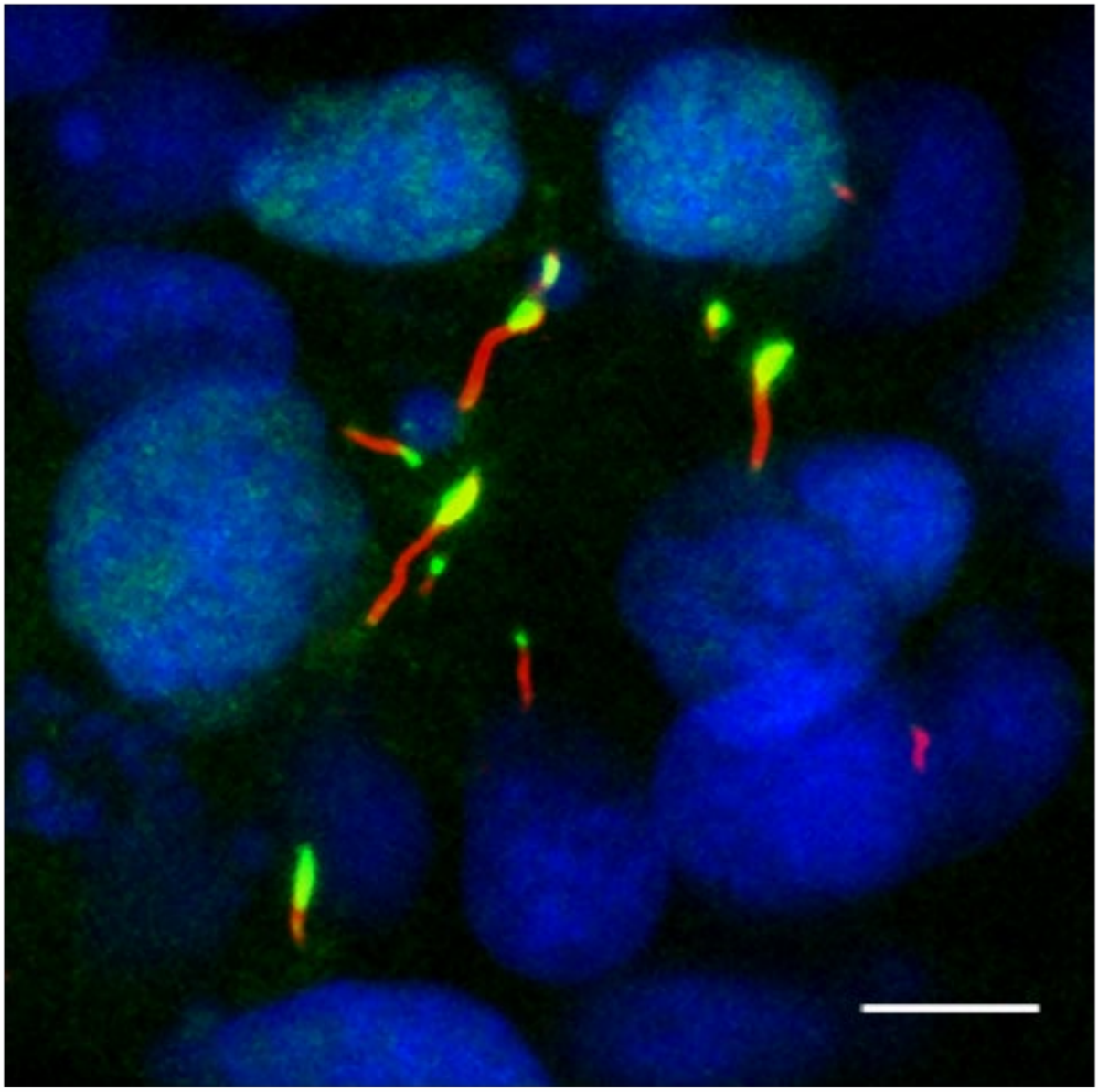

Figure 1: Somitic cells in a zebrafish embryo showing accumulation of GFP tagged Gli2 protein at the tips of primary cilia in response to Hedgehog pathway activation (from Kim et al., 2010).

Myogenic Gene Regulatory Networks (GRNs)

Skeletal muscle is a major component of vertebrate anatomy, making up around 50% of the body mass of a human and around 80% of that of a fish. A number of transcription factors are known to commit cells to the myogenic lineage, but how myoblasts differentiate into different types of muscle is rather less well understood. We are use a combination of genetics, in vivo promoter analysis and ChIP to elucidate the GRNs that underlie the commitment and differentiation of myoblasts into different muscle cell type. A particular focus of our research is the transcription factor Sox6, which plays a key role in regulating the choice between slow-twitch and fast-twitch fibre type. We are studying the targets of this protein and also the transcriptional and post-transcriptional regulation of the gene that it is encoded by.

Collaborators: Prof. Y-J Ruan, Genome Institute of Singapore; Dr. J. von Hofsten, Umea University, Sweden; Dr. V. Cunliffe, University of Sheffield; Dr. N. Hagiwara, Univeristy of California, Davis

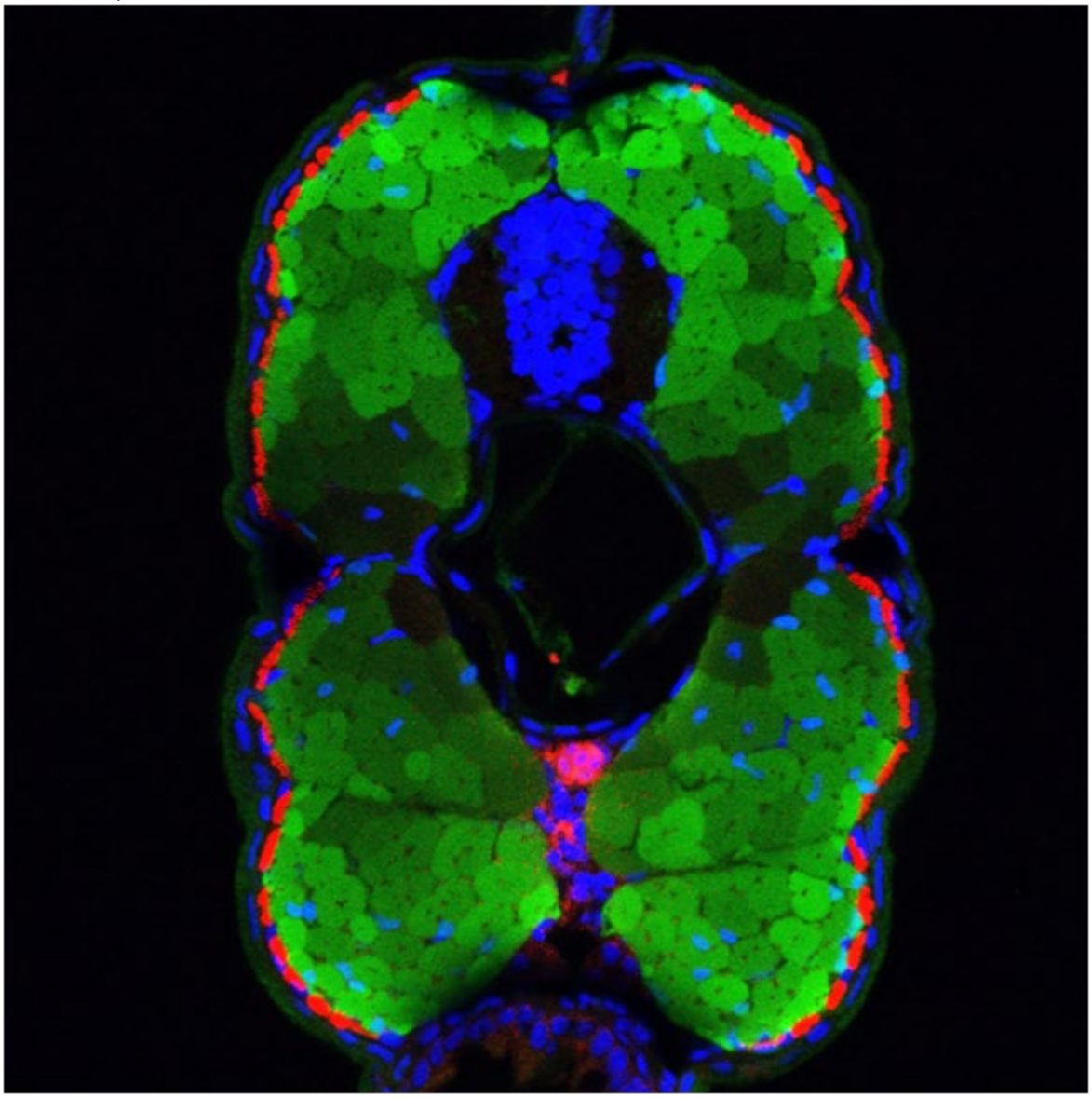

Figure 2: Cross section through the trunk region of a 6 day old zebrafish embryo showing the superficial slow twitch muscle fibres (labeled red) and expression of a sox6:gfp reporter gene (green) restricted to the fast twitch muscle fibres.

Haematopoietic Stem Cell Factors

All vertebrates have primitive and definitive waves of hematopoiesis, the latter process producing the self-renewing pluripotent hematopoietic stem cells (HSCs). Elucidating the GRNs that underlie the formation and maintenance of HSCs will facilitate the generation and manipulation of these cells for therapeutic use and at the same time lead to a better understanding of the molecular pathways underlying leukemia. Many of the transcription factor

known to play a critical role during mammalian hematopoiesis have analogous roles in zebrafish, making the fish an attractive model system for studying HSC biology. We are conducting a large-scale functional screen of candidate HSC regulators, using antisense oligonucleotide mediated gene knockdown.

Collaborators: Prof. R Patient, University of Oxford, Prof T Enver, University College London

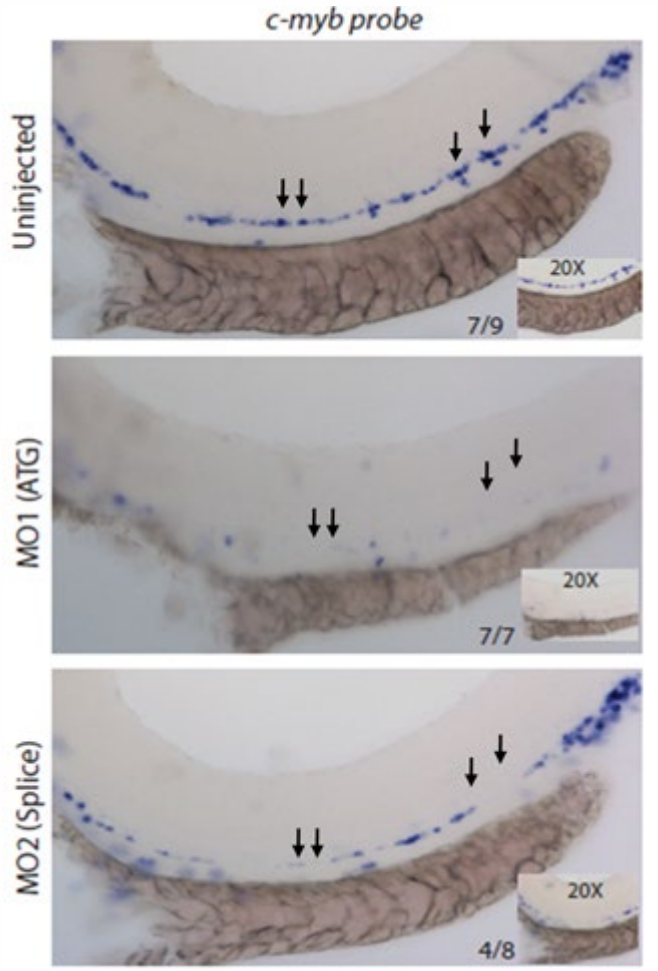

Figure 3: Depletion of Haematopoietic Stem Cells (HSCs) in the floor of the dorsal aorta, reveled by ISH for the c-myb transcript, following Morpholino mediated knockdown of a candidate HSC regulatory gene.

Disease Models

The zebrafish is increasingly being used to model human disease-related processes, its easily accessible and transparent embryos and relatively simple and low-cost husbandry making it an attractive alternative to the expensive conventional mammalian model organisms. We have developed paradigms for the analysis of two different pathological conditions: first, using a transgenic line expressing GFP under the control of a neutrophil specific promoter, we study neutrophil behaviour in response to trauma or chronic inflammatory stimuli. In a second line

of research, we have developed a tumour xenograft model to study the interaction of tumour cells with the vascular system and the dispersal of cells away from the primary tumour site.

Collaborators: Dr. S Renshaw, Prof. M. Whyte, University of Sheffield; Prof. Y Cao, Karolinska Institute, Stockholm

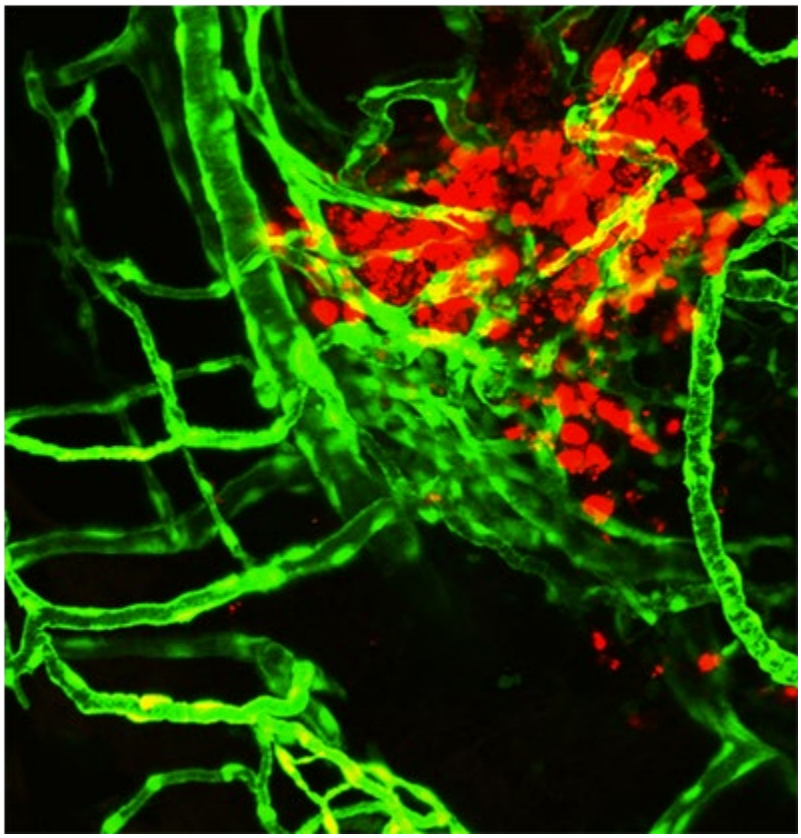

Figure 4: A cluster of implanted murine fibrosarcoma tumour cells (red) in a three day old fli1:gfp transgenic zebrafish embryo showing recruitment of blood vessels (green) by the xenograft.

Links

- http://www.fp7-healing.eu/network/advisors.html
- ZFIN: Ingham Singapore Lab
- http://f1000.com/thefaculty/devbiol
- http://royalsociety.org/
- http://www.imm.ox.ac.uk/wimm-research/molhaem/roger-patient
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