Exploring the combinatorial roles for transcription factors in fate decisions in the neural crest

Supervisory team:
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Project description:
How do stem cells choose between different fates? This question remains a crucial one in the fields of developmental and stem cell biology. At the core are transcription factors, which are often envisaged as working singly to determine specific fate choices. The paradigmatic exemplar of such a 'master regulator' is Mitf, vital for the development of melanocytes from neural crest cells. The latter is a key model system here, since these cells generate multiple other pigment cells, apart from melanocytes, but also neurons, glia and many other cell-types. Our recent work has explored the roles for key transcription factors in multiple pigment cell-types in zebrafish, where the pigment cells become an accessible 'model-within-a-model' for how neural crest cells work. Together, this work has been leading us away from the master regulator concept, towards a more combinatorial view of transcription factor function in neural crest development (1-3).

In this project, we propose an explicit test of the concept, looking at combinations of known and suspected players in determining individual pigment cell fate choices from the neural crest. The project will begin by assessing the timing and overlap of expression of these transcription factors, determining how their transcription factor profiles change over time, both via analysis of a spatial transcriptomics data-set, but also through targeted validation and expansion using in situ hybridisation techniques. Meanwhile, single, double and triple mutants will be generated by CRISPR-Cas9 approaches, and their phenotypes analysed using diverse molecular markers, including expression of the mRNAs for the transcription factors themselves to assess transcriptional interdependency. Analysis will also look for cross-repressive interactions, such as are likely to underpin fate choices in the multipotent neural crest cells. Mutant rescue assays will assess the sufficiency of transcription factor combinations. Complementary evaluation of bioinformatic databases will enable proposal of gene regulatory networks, which will be tested and refined using CRISPR-Cas9 targeting of putative regulatory elements. Together these studies will provide an integrated view of the role of transcription factors in determining pigment cell fate choice. This will in turn illuminate how fate choices are made in the neural crest and adult neural crest-derived stem cells.

This interdisciplinary project will give an opportunity to develop numerous specific skillsets, including in zebrafish genetics and husbandry, phenotypic analysis including by in situ hybridisation and immuno-fluorescence, confocal and light sheet microscopy, molecular biology, and bioinformatics. In addition, the student will benefit from collaborative interactions with bioinformaticians.

1) Subkhankulova*, T., Camargo Sosa*, K., et al. (2023). Zebrafish pigment cells develop directly from persistent highly multipotent progenitors. Nature Communications. 14, 1258 (DOI: 10.1038/s41467-023-36876-4)

2) Dawes, J.H.P. and Kelsh, R.N. (2021) Cell fate decisions in the neural crest. Int. J. Mol. Sci. 22(24):13531. https://doi.org/10.3390/ijms222413531

3) Kelsh, R.N., Camargo Sosa, K., Farjami, S., Makeev, V., Dawes, J.H.P., and Rocco, A. (2021) Cyclical Fate Restriction, a new view of neural crest cell fate specification. Development 148, dev176057 (doi:10.1242/dev.176057)

Our aim as the SWBio DTP is to support students from a range of backgrounds and circumstances. Where needed, we will work with you to take into consideration reasonable project adaptations (for example to support caring responsibilities, disabilities, other significant personal circumstances) as well as flexible working and part-time study requests, to enable greater access to a PhD. All our supervisors support us with this aim, so please feel comfortable in discussing further with the listed PhD project supervisor to see what is feasible.