Molecular Mechanisms of Neural Induction and Patterning in the Zebrafish Embryo

Submitted by Carlos Manuel Pereira da Cruz to the University of Exeter

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: …….. Carlos Cruz

Abstract

The brain is our most complex organ, with an estimated 10^{11} neurons. With the spinal cord, it forms the central nervous system which controls our movements and our senses, holds our memories and creates our thoughts. Because of this, neurodegenerative disorders can be extremely distressing and a thorough understanding of how the nervous system develops is essential if progress is to be made in finding ways to treat them. Critically, this includes understanding how the nervous system forms, i.e., the nature of the signals that promote neural identity (neural induction) and determine correct positional information (patterning).

The zebrafish (*Danio rerio*) has become established as a model for embryological studies due to ease of experimental manipulation. Taking advantage of this, the aims of this PhD were to contribute to unravelling the molecular mechanisms of neural induction and patterning, using a variety of embryological and molecular methods. In the first project, functional analyses of the *eve1* gene identified a key factor for posterior neural development. Eve1 was found to be a critical posteriorising factor, with an additional role in posterior neural induction. An outstanding question in neural induction is the relative contribution to this process of two key developmentally important signalling pathways, Bmp and Fgf. In the second project, differential analyses of maternal versus zygotic Bmp and Fgf signalling revealed crucial maternal roles for these two pathways in neural development as neural and epidermal capacitators. The results further suggested that Fgf signalling may be the critical neural inducer. Finally, as a third project, a zebrafish ectodermal explant assay was developed using the organiser-deficient *ichabod* mutant. The aim was to develop a system to analyse how key molecules directly affect ectoderm and neural development, free of mesoderm and endoderm influences, as signalling from these layers can directly or indirectly influence neural development.

Acknowledgements

First of all, I would like to dedicate this thesis to my father, 'Joe' Cruz, who very sadly passed away during the course of my PhD. It is a great shame that he never got to see me get my doctorate. However, he was a great inspiration in my life and for that he will never be forgotten. May he be in a great place.

Secondly, I would like to express my greatest appreciation and gratitude to my supervisor, Dr Tetsuhiro Kudoh, for all the guidance and help that he has provided throughout my PhD. I thank him from the bottom of my heart for the patience he has shown through good times and bad and for always having his door open for me. And thank you for teaching me to focus my mind on what is important.

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Research Papers and Author's Declaration.

Statement: I, Carlos Cruz, was involved in the following parts of the presented papers: In conjunction with my supervisor, Dr Tetsuhiro Kudoh, I planned and carried out most of the experiments and subsequent analyses for both paper 1 and paper 2. In addition, and again together with my supervisor, I helped plan and write both papers. For paper 1, we further received valuable input from Dr Igor Dawid and Professor Steve Wilson who co-authored the paper.

A version of the published paper (research chapter 1) is included in the Appendix.

Descriptions

Terms used

Epiboly – cytoskeleton-dependent process characterised by the thinning and spreading of the blastoderm cell layers over the egg yolk and eventually covers it completely.

Gastrulation – where cells movements, characterised by internalisation of cells from the surface of the embryo, lead to a massive reorganisation of the embryo and formation of the three germ layers (ectoderm, mesoderm and endoderm).

Mesendoderm – embryonic tissue that will differentiate into mesoderm and endoderm.

Neural Induction – in the context of this thesis, neural induction refers to the acquisition of neural identity and specification of the neural tissue during gastrulation. It is characterised by the expression of neural-specific genes.

Neural Patterning – refers to the acquisition and maintenance of anterior or posterior identity within the neural plate and how subsequently cells acquire positional information within the emergent central nervous system along the A-P axis.

Neurogenesis – prospective neuronal cells begin to differentiate into neurons. In zebrafish this process begins at the end of gastrulation and continues throughout somitogenesis and beyond and is characterised by neuron-specific gene expression.

Neurulation – infolding of the neural plate in vertebrates leading to formation of the neural tube. Results in the formation of the spinal cord and the brain. In zebrafish, this process begins at the end of gastrulation and is completed by the end of somitogenesis (~24 hpf).

Posteriorisation – specification of posterior neural fate during gastrulation. It is generally assumed that posterior is 'dominant' to anterior and that acquiring anterior character requires keeping anterior neural cells away from posteriorising signals.

Shield – a tissue in teleost fish (such as zebrafish) that is equivalent in function to Spemann's Organiser.

Somitogenesis – although timing may vary in different vertebrates, it refers to the developmental stage at which the somites (muscle precursors) are formed. The

start of somitogenesis coincides with the end of epiboly and gastrulation in zebrafish and is complete by ~24 hpf. It is therefore a useful staging point for characterising zebrafish development.

List of General Abbreviations.

A-P Antero-Posterior

Bmp Bone morphogenic protein

DM Dorsomorphin

D-V Dorso-Ventral

eve1 *even skipped-like 1* – a zebrafish homologue of the *Drosophila melanogaster even-skipped* gene.

Fgf Fibroblast growth factor

hpf hours post fertilisation

Ich *Ichabod*

RA Retinoic Acid

Wnt Wint – name is derived from the *Drosophila melanogaster wingless* gene and the vertebrate INT genes.

WT Wild Type