Investigations into the Rab Family of Genes and Their Roles in Signalling During Vertebrate Early Development

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Abstract

The mammalian Rab family consists of between 60-70 members, making it the largest sub family of the Ras superfamily. Rabs are responsible for vesicle trafficking within cells, acting as molecular switches cycling between the GDP inactive and GTP bound active forms. Far from being just cellular housekeeping genes, these genes have been shown to have specific functions which, when disrupted, can lead to clinical disorders and interesting developmental defects.

This thesis therefore seeks to investigate this interesting family of genes and their roles in zebrafish development. Using antisense morpholino oligonuleotides in a loss of function screen, this thesis identifies the function of 13 zebrafish *rabs*. Three of these, *rab1a3, rab3c1* and *rab28* have specific and interesting phenotypes, with pigmentation defects seen in *rab1a3* and *rab3c1* and behavioural defects seen in *rab28*. In particular, the pigmentation defect in *rab3c1* resulted in the discovery that the embryos were blind.

This thesis also shows an essential role for *rab5a2* in zebrafish development and Nodal signalling. Disruption of this *rab* causes a dramatic early phenotype, with 100% mortality in embryos prior to 24 hours post fertilization. *rab5a2* morpholino injected embryos show no visable organizer and have reduced nodal target gene expression. Overexpression of *rab5a2* shows embryos with additional expression of Nodal target genes *no tail* and *goosecoid* in the animal pole of the embryos but not the dorsal marker *chd.* Microarray analysis of *rab5a2* morpholino injected embryos showed reduction and upregulation of expression of many genes involved in dorsal ventral patterning. This suggests a complex role for *rab5a2* in patterning the early embryo, as both dorsalizing and ventralizing genes such as *chd, bmp4* and *wnt8* were down regulated while, ventralizing genes such as *bmp2b* were upregulated.

Contents

Abbreviations

ADMP - anti-dorsalizing morphogenic protein

AP – anterior posterior

Bmp – Bone Morphogenic Protein

Bmpr - Bone Morphogenic Protein Receptor

Boz - Bozozok

COP 1 - Coatomer complex

Cyc – Cyclops

Dkk - Dickkopf

Dpp - decapentaplegic

DV – Dorsal-Ventral

ER – Endoplasmic Reticulum

EVL – Enveloping Layer

Fgf – Fibroblast Growth Factor

FYVE – named after four proteins Fab1, YOTB/ZK632.12, Vac1, and EEA1

GTPase – guanosine triphosphate hydrolyze enzyme

Hh – Hedgehog

Hpf – hours post fertilization

HSPG - heparin sulphate proteoglycans

MBT- Mid Blastula Transition

MO - Morpholino

NSF - *N*-ethylmaleimide Sensitive Fusion protein

Oep – One eyed pinhead

ORF – Open Reading Frame

PtdIns(3)P - phosphatidylinositol-3-phosphate

SFRP - secreted frizzled-related protein

SNARE - Soluble NSF Attachment Protein REceptor

Sqt - Squint

TAE - Tris, acetate, EDTA

TE - Tris EDTA

TGF - Transforming growth factor

VAMP - Vesicle –Associated Membrane Protein

Wg - Wingless

Wnt – combined from the *Drosophila* gene *wingless* and the mouse gene *int*

YSL – Yolk Syncytial Layer

ZMD – Zygotic Maternal Dominant

Glossary

Glypicans - the main cell-surface heparin sulphate proteoglycans,

Mid Blastula Transition – The stage where cell cycles lengthen and become asynchronous this begins at the 512-cell stage

Non- cell-autonomous - genotypically mutant cells cause other cells (regardless of their genotype) to exhibit a mutant phenotype

Prenylation - addition of hydrophobic molecules to facilitate protein attachment to the cell membrane.

Zygotic Maternal Dominant – effect that is expressed when both zygotic and maternal genomes are heterozygous for the mutant locus.

Homophilic cell adhesion - The attachment of an adhesion molecule in one cell to an identical molecule in an adjacent cell

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