Analysis of Genes Controlling Notochord Development in Zebrafish

Kevin Andrew Thomas

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National Institute for Medical Research,
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Abstract

The notochord is a vital and defining organ in vertebrates. Mutagenesis screening in zebrafish identified seven 'dwarf' mutants that lack notochord development; *grumpy, sleepy, bashful, dopey, happy, sneezy* and *doc*. This thesis is concerned with the identification and positional cloning of the *doc* locus as well as the confirmation and characterisation of the *dopey* and *happy* loci. Previous positional cloning efforts identified the *grumpy, sleepy* and *bashful* genes, demonstrating a requirement for the laminin chains β1, γ1 and α1 in formation of the notochord basement membrane (Parsons et al., 2002b; Pollard, 2002) and the mutant *sneezy*, which has been shown to encode the COPI subunit α (Coutinho et al., 2004).

This thesis establishes that the *doc locus* lies within a 0.5Mb region on linkage group 18, containing several genes, including a novel gene encoding a predicted protein with 14 WD40 domains. Antisense morpholino (MO) knock-down of *doc* results in a phenocopy of *doc* and *insitu* expression demonstrates that this gene is expressed specifically within the notochord during development. Expression analysis of *echidna hedgehog* (*ehh*) demonstrated that MO knock-down of this gene results in a lack of notochord differentiation. I therefore expect this novel gene is *doc*.

Analysis of the mutants *dopey* and *happy* has demonstrated that they encode the coatomer subunits COPβ' and COPβ respectively. Expression of these and other COPI subunits demonstrate that the majority of COPI subunits are up-regulated within the notochord during development and maintained abnormally in COPI deficient embryos. I have investigated the mechanism of coatomer gene regulation

and found that loss of coatomer function leads both to up-regulation of coatomer mRNA and activation of the unfolded protein response (UPR). Suggesting that the UPR is the regulator of mRNA expression, functioning to maintain the secretory network during development, though work to provide definitive proof remains.

To my mum, who taught me and to Zoë, who keeps me sane.

It is not enough to have a good mind; the main thing is to use it well.

Rene Descartes 1596-1650

Creationists make it sound as though a “theory” is something you dreamt up after being drunk all night.

Isaac Asimov 1920-1992

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Abbreviations

AP	Antero-posterior
BAC	Bacterial artificial chromosome
bal	*bashful*
BCIP	X-phosphate/5-Bromo-4-chloro-3-indolyl-phosphate
BM	Basement membrane
BSA	Bovine serum albumin
COPI	Coatomer complex
DEPC	Diethylpyrocarbonate
DIG	Digoxygenin
dop	*dopey*
DTT	Dithiothritol
DV	Dorso-ventral
EDTA	Ethylene-diamine-tetra-acetate
EST	Expressed sequence tag
GTP	Guanidine 5'-triphosphate
gup	*grumpy*
hap	*happy*
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
hpf	Hours post-fertilisation

IPTG	Isopropylthio-β-D-galactosidase
LG	Linkage group
MO	Antisense morpholino oligonucleotide
NBT	4-Nitro blue tetrazolium chloride
OD	Optical density
PCR	Polymerase chain reaction
RACE	Rapid amplification of cDNA ends
RAPD	Random amplified polymorphic DNA
RH	Radiation hybrid
SDS	Sodium dodecyl sulphate
sly	*sleepy*
sny	*sneezy*
SSLP	Simple sequence length polymorphism
TAE	Tris, acetate, EDTA
TBE	Tris, borate, EDTA
TE	Tris EDTA
UPR	Unfolded Protein Response
WT	Wild Type
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactosidase
YSL	Yolk Syncytial layer

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