
Analysis of Genes Controlling Notochord Development in Zebrafish

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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 $\beta 1$, $\gamma 1$ and $\alpha 1$ 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 COP β ' and COP β 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
<i>bal</i>	<i>bashful</i>
BCIP	X-phosphate/5-Bromo-4-chloro-3-indolyl-phosphate
BM	Basement membrane
BSA	Bovine serum albumin
COPI	Coatomer complex
DEPC	Diethylpyrocarbonate
DIG	Digoxygenin
<i>dop</i>	<i>dopey</i>
DTT	Dithiothritol
DV	Dorso-ventral
EDTA	Ethylene-diamine-tetra-acetate
EST	Expressed sequence tag
GTP	Guanidine 5'-triphosphate
<i>gup</i>	<i>grumpy</i>
<i>hap</i>	<i>happy</i>
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
<i>sly</i>	<i>sleepy</i>
<i>sny</i>	<i>sneezy</i>
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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