Huntingtin function during zebrafish (*Danio rerio)* development

A thesis submitted in requirement for the degree of Doctor of Philosophy, December 2009

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Statement of Originality

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* Henshall, T. L., Tucker, B., Lumsden, A. L., Nornes, S., Lardelli, M. T. and Richards, R. I., Selective neuronal requirement for huntingtin in the developing zebrafish. *Hum Mol Genet* 2009 18: 4830-4842.

Tanya Lynn Henshall

Acknowledgements

Firstly, I would like to thank my supervisors, Professor Robert Richards and Dr Michael Lardelli for the support and encouragement throughout this PhD.

Many thanks to members of the Richards and Lardelli labs for all of their friendship and continued support. In particular I would like to thank Amanda Lumsden, Morgan Newman, Sonia Dayan and Saumya Samaraweera. I know we will always be great friends!

I would also like to acknowledge the help of my friends and family (especially my Mum and Dad) for all of their support. Without your love, I would most certainly not have achieved as much as I have. Thank you all so much.

Tanya xox

Abbreviations

aa	amino acid
acridine orange	acridine orange hemi (zinc chloride) salt
amp	ampicillin
BCIP	5-bromo-4-chloro-3-indolyl phosphate
bh	basihyal (cartilage)
bp	base pairs
BDNF	brain derived neurotrophic factor
cDNA	complementary DNA
ch	ceratohyal (cartilage)
*c*MO	standard control morpholino
Ct	cycle threshold
DASPEI	2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide
DEPC	diethylpyrocarbonate
DF	degrees of freedom
DiI	1,1'-dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate ($DiIC_{18}(3)$)
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
dpf	days post fertilization
EDTA	ethylenediamine tetra-acetic acid
ef1a	elongation factor 1a
EGFP	enhanced green fluorescent protein
emx3	empty spiracles homeobox 3
ES	embryonic stem (as in ES cells)
EtBr	ethidium bromide
fgf8	fibroblast growth factor 8
GABA	γ-aminobutyric acid
HAP	huntingtin associated protein
HD	Huntington's disease

*hd*MO	morpholino antisense to zebrafish htt mRNA (as in *hd*MO1 and *hd*MO2)
HIP	huntingtin interacting protein
htt	huntingtin
hpf	hours post fertilization
hs	hyosymplectic cartilage
Kb	kilobase pairs
kDa	kilodalton
m	Meckel's cartilage
*mc*MO1	5 base mismatch of the *hd*MO1 antisense sequence
μM	micromolar
ml	millilitre
MLK2	mixed lineage kinase 2
mM	millimolar
morpholino/MO	morpholino oligonucleotide
MQ	milli-Q
mRNA	messenger RNA
NBT	nitro blue tetrazolium chloride
ng	nanogram
nl	nanolitre
NMDA	*N*-methyl-D-aspartic acid
nM	nanomolar
ntl	no tail
oligo	oligonucleotide primer
omp	olfactory marker protein
ORF	open reading frame
OSN	olfactory sensory neuron
otx2	orthodenticle homolog 2
p(3-7)	pharyngeal arch (3-7)
PBS	phosphate buffered saline
PBS-T	PBS with 0.1% tween-20
pbx2	pre-B-cell leukemia transcription factor 2
PCR	polymerase chain reaction
pmol	picomoles
polyQ htt	huntingtin with a pathogenic number of glutamine repeats

pq	palatoquadrate (cartilage)
PTU	1-phenyl-2-thiourea
qPCR	quantitative real-time PCR
RA	retinoic acid
REST/NRSF	RE-1 silencing transcription factor/neuron-restrictive silencer factor
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
six1	sine oculis homeobox homologue
SSC	sodium chloride/sodium citrate buffer
TBS-T	tris-buffered saline with 0.1% Tween-20
TUNEL	terminal deoxynucleotide transferase (TdT)-mediated dUTP nick-end labeling
UTR	untranslated region

Abstract

Huntington's disease shares a common molecular basis with eight other neurodegenerative diseases: expansion of an existing polyglutamine tract. In each case, this repeat tract occurs within otherwise unrelated proteins. These proteins show widespread and overlapping patterns of expression in the brain and yet the diseases are distinguished by neurodegeneration in a specific subset of neurons that are most sensitive to the mutation. It has therefore been proposed that expansion of the polyglutamine region in these genes may result in perturbation of the normal function of the respective proteins, and that this perturbation in some way contributes to the neuronal specificity of these diseases. The normal functions of these proteins have therefore become a focus of investigation as potential pathogenic pathways. Here, synthetic antisense morpholinos have been used to inhibit the translation of huntingtin protein during early zebrafish development. The results obtained show the effects of huntingtin loss-of-function on the developing nervous system, including distinct defects in morphology of the lateral line neuromasts, olfactory placode and branchial arches. The potential common origins of these defects were explored, revealing impaired formation of the anterior-most region of the neural plate as indicated by reduced pre-placodal and telencephalic gene expression with no effect on mid- or hindbrain formation. These investigations demonstrate a specific 'rate-limiting' role for huntingtin in formation of the telencephalon and the pre-placodal region, and differing levels of requirement for huntingtin function in specific nerve cell types.