Development and application of novel cloning strategies for analysis of genes controlling embryo development

Richard Tamme

Submitted for partial fulfillment of the requirements for the degree of Doctor of Philosophy

The University of Adelaide
School of Molecular and Biomedical Science
Discipline of Genetics
Adelaide, Australia

January 2004

CONTENTS

List of publications and declaration of contributions to each publication 4

LITERATURE REVIEW 6

1. Early development of the vertebrate CNS 7

1.1. Mechanisms of neural induction 9

1.1.1. Spemann's organiser and BMP antagonists as putative neural inducers in *Xenopus* 9
1.1.2. Requirement for BMP antagonists in neural induction in other vertebrates 10
1.1.3. Genes linking neural induction and neurogenesis 10

1.2. Genetic control of neurogenesis 12

1.2.1. Acquisition of neural cell fate is achieved by the interplay between extrinsic and intrinsic cell fate regulators 12
1.2.2. Proneural and neurogenic genes - positive and negative regulators of neural cell fate 14
1.2.3. Mechanisms of Delta/Notch signalling 17
1.2.4. Evolution of vertebrate *Notch* genes 18

2. T-box genes in animal development 21

2.1. The T-box gene family in vertebrates and invertebrates 22
2.2. Developmental roles of the T-box genes 22
2.3. T-box genes in the control of mesoderm development 23
2.4. Upstream regulators and downstream targets of T-box genes 25

3. Genetic screens for discovering novel developmental control genes 28

3.1. Recessive mutation screens 28
3.2. Modifier screens and screens with sensitised backgrounds 30

3.3. Forced expression screens 30
3.4. Screens for genes with restricted expression patterns
(*in situ* transcript hybridisation screens) 31
3.5. Screens for genes with differential expression patterns 32

4. Basic steps of cDNA library construction 33

5. Isolation of unknown flanking DNA sequences 36

6. Zebrafish as a model system for vertebrate developmental biology 38

6.1. Embryological characteristics of zebrafish 38
6.2. Genetic characteristics of zebrafish 39
6.3. Neuronal classes of the zebrafish developing spinal cord 41

7. Amphioxus as a model organism in evolutionary developmental biology 44

SUMMARY OF PAPERS I-IV, AND CONTEXTUAL LINKAGES BETWEEN THEM

Paper I: Simple, directional cDNA cloning for *in situ* transcript hybridisation screens 46

Paper II: The identity and distribution of the neural cells expressing the mesodermal determinant *spadetail* 48

Paper III: Nonspecific, nested suppression PCR method for isolation of unknown flanking DNA 49

Paper IV: Characterisation and developmental expression of the amphioxus homolog of *Notch* (*AmphiNotch*): evolutionary conservation of multiple expression domains in amphioxus 50

PAPERS I-IV

CONCLUDING REMARKS 52

REFERENCES 56

'Development and Application of Novel Cloning Strategies for Analysis of Genes Controlling Embryo Development.'

Richard Tamme -PhD Thesis, The University of Adelaide 2004

ABSTRACT

Initially, we aimed to identify novel genes regulating vertebrate neurogenesis and somitogenesis by screening cDNAs derived from gastrulation/neurulation stage zebrafish embryos for clones revealing corresponding genes with expression patterns suggestive of roles in these processes. The lack of suitable cDNA libraries prompted us to devise a simplified method for producing randomly-primed, directionally cloned cDNA libraries from small amounts of embryonic tissue. To achieve this, several techniques were combined, including cDNA synthesis on a solid carrier, random priming of 1st cDNA strand synthesis, non-specific priming of 2nd cDNA strand synthesis and amplification of initially small amounts of cDNAs by suppression-PCR.

A pilot-scale *in situ* screen using a cDNA library produced by the above method identified a gene, *spadetail*, that is expressed in presomitic mesoderm and in unidentified, apparently irregularly distributed cells of the spinal cord. *spt* functions in mesodermal development, yet its role in neural tissue remains unknown. Analysis of the *spadetail*-expressing neural cells' gene co-expression profile and dorsoventral location implied that they are Dorsal Longitudinal Ascending interneurons. Quantitative analysis of these cells' rostrocaudal distribution showed that there is a tendency to higher cell numbers in rostral spinal segments. The observation that *spadetail*-expressing neurons are frequently juxtaposed to somitic cells expressing *spadetail* at low levels suggests that the distribution of *spadetail*-expressing neurons may be 'inefficiently' patterned by *spadetail*-expressing somitic cells or that the expression of *spadetail* in both tissues is induced by a common positional cue.

The strategy for non-specific priming was then extended to develop a simple technique for cloning unknown DNA sequences flanking known DNA. An initial non-specific PCR amplification was performed with a single primer that binds specifically within known sequence and non-specifically in the unknown DNA region. In a second reaction, the sequences of interest were amplified from the primary reaction mixture (that also contains undesired sequences) with nested PCR using a primer that had been extended further downstream from the primer used in the initial PCR. This enabled isolation of a 0.5 kb region of amphioxus *Notch* cDNA, that, in turn, contributed to the subsequent analysis of the evolution of vertebrate *Notch* genes.