



# A System for the Isolation of Markers for Subpopulations of Murine Pluripotent Cells

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by

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# TABLE OF CONTENTS

THESIS SUMMARY .....	i
STATEMENT .....	iv
ACKNOWLEDGMENTS .....	v
<b>CHAPTER 1: GENERAL INTRODUCTION</b>	
1.1 Development.....	1
1.1.1 The Study of Mammalian Development .....	1
1.1.2 The Mouse as a Model for Mammalian Development.....	1
1.2 Preimplantation Development .....	2
1.2.1 Fertilisation and Cleavage Stage Development.....	2
1.2.2 Compaction and Polarisation.....	3
1.2.3 Blastulation and Implantation .....	3
1.3 Peri-implantation Development .....	4
1.4 Postimplantation Development.....	5
1.4.1 Proamniotic Cavity and Primitive Ectoderm Formation .....	5
1.4.2 Gastrulation .....	6
1.4.3 Partitioning of the Primordial Germ Cell Lineage .....	6
1.5 Pluripotent Cell Populations in Early Mouse Development.....	7
1.5.1 The Heterogeneous Nature of Murine Pluripotent Cell Populations.....	8
1.5.2 Gene Expression in Murine Pluripotent Cells.....	10
1.6 Embryonic Stem (ES) Cells as an <i>in vitro</i> Model of Early Development....	12
1.6.1 Isolation of ES cells.....	12
1.6.2 The Relationship of ES Cells to Embryonic Pluripotent Cells: a Model of the Inner Cell Mass .....	13
1.6.3 Maintenance of ES Cells: the Undifferentiated State .....	14
1.6.4 Differentiation of ES Cells and <i>in vitro</i> Models of Development...	14
1.6.5 Spontaneous and Chemical Induction of ES Cell Differentiation...	15
1.6.6 Differentiation of ES Cells as Embryoid Bodies.....	15
1.6.7 Expansion of the Pluripotent Cell Model of Early Development: Lineage-specific Differentiation of ES Cells to X Cells in Response to Soluble Biological Factors .....	16
1.6.8 X Cells as a Model for Primitive Ectoderm .....	17
1.6.9 The Significance of X Cell Formation for <i>in vitro</i> Models of Pluripotent Cell Development.....	18

1.7	Generation and Application of Pluripotent Cell Specific Markers.....	19
1.7.1	Mutational Approaches to Define Markers for Pluripotent Cell Types .....	21
1.7.2	Generating Pluripotent Cell Markers by Homology Based Approaches.....	21
1.7.3	Cloning of Pluripotent Cell Markers from Embryonic Tissue .....	22
1.7.4	Cloning of Pluripotent Cell Markers from Cell Lines <i>in vitro</i> .....	23
1.7.5	Identifying Pluripotent Cell Markers by Gene Traps.....	23
1.7.6	Cloning of Pluripotent Cell Markers by Differential Display Polymerase Chain Reaction .....	24
1.8	Aims and Approaches.....	26

## CHAPTER 2: MATERIALS AND METHODS

2.1	Abbreviations .....	28
2.2	Materials .....	29
2.2.1	Chemicals and Reagents.....	29
2.2.2	Radiochemicals .....	29
2.2.3	Kits.....	29
2.2.4	Enzymes.....	30
2.2.5	Buffers and Solutions.....	30
2.2.6	Plasmid Vectors .....	31
2.2.7	Riboprobe Templates .....	31
2.2.8	Oligonucleotides.....	32
2.2.9	Bacterial Strains .....	33
2.2.10	Bacterial Growth Media .....	34
2.2.11	Tissue Culture Cell Lines and Media.....	34
2.2.12	DNA Markers.....	35
2.2.13	Miscellaneous Materials .....	35
2.3	Molecular Methods .....	35
2.3.1	Restriction Endonuclease Digestion of DNA.....	35
2.3.2	Agarose Gel Electrophoresis.....	35
2.3.3	Polyacrylamide Gel Electrophoresis .....	35
2.3.4	Purification of Linear DNA Fragments .....	36
2.3.5	Agarose Minigels for DNA Southern Blot .....	36
2.3.6	Preparation of Vector DNA .....	36
2.3.7	DNA Ligation Reactions.....	37
2.3.8	Preparation of Competent Cells.....	37
2.3.9	Transformation of Competent Bacterial Cells.....	37
2.3.10	Rapid Small Scale Preparation of DNA (Mini-prep) .....	37

2.3.11	Rapid Large Scale Preparation of DNA (50 ml Midi-prep).....	38
2.3.12	Generation of Sequencing Subclones.....	38
2.3.13	Double Stranded Sequencing of Plasmid DNA.....	39
2.3.14	Library Screening .....	39
2.3.15	Lambda Zapping.....	39
2.3.16	Reverse Transcription.....	40
2.3.17	Reverse Transcription PCR (RT/PCR).....	40
2.3.18	Differential Display PCR.....	40
2.3.19	Rapid Amplification of cDNA Ends (RACE/PCR).....	41
2.3.20	Isolation of Cytoplasmic RNA from Cultured Cells .....	41
2.3.21	Isolation of RNA from Tissue Samples .....	42
2.3.22	Isolation of (A) <sub>n</sub> <sup>+</sup> RNA .....	42
2.3.23	RNA Gels for Northern Blot Analysis.....	42
2.3.24	Northern Blot Transfer .....	43
2.3.25	Radioactive DNA Probes.....	43
2.3.26	Radioactive RNA Probes.....	43
2.3.27	Hybridisation of Radioactive Probes to Nylon and Nitrocellulose Filters.....	43
2.3.28	Ribonuclease Protection Analysis .....	44
2.3.29	Digoxigenin (DIG) Labelled RNA Probes.....	44
2.3.30	<i>in situ</i> Hybridisation of Cell Monolayers.....	44
2.3.31	Embryonic <i>in situ</i> Hybridisation.....	45
2.3.32	Mouse Embryo Powder .....	46
2.3.33	Antibody Preblocking.....	46
2.3.34	Containment Facilities .....	46
2.3.35	Phosphorimager Analysis, Autoradiograph Scanning and Image Manipulation .....	47
2.3.36	Sequencing Software and Database Searches.....	47
2.4	Tissue Culture Methods.....	47
2.4.1	Maintenance of ES Cells.....	47
2.4.2	Differentiation of ES Cells .....	47
2.5	Embryological Methods.....	49
2.5.1	Mouse Strains and Matings .....	49
2.5.2	Embryo Processing Baskets .....	49
2.5.3	Embryo Isolation and Dissection.....	49
2.5.4	Embryo Fixation and Dehydration .....	50
2.5.5	Animal Manipulations.....	50

## CHAPTER 3: A METHOD FOR THE IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES DURING PLURIPOTENT CELL PROGRESSION *IN VITRO*

3.1	Introduction .....	51
3.1.1	<i>In vitro</i> Models of the Inner Cell Mass and Primitive Ectoderm .....	51
3.1.2	Differential Display Polymerase Chain Reaction and Gene Class Enrichment.....	52
3.1.3	Homeobox Genes and Expression in Pluripotent Cell Subpopulations .....	52
3.1.4	Chapter Summary .....	54
3.2	Modification and Trial of Differential Display PCR Conditions .....	54
3.2.1	Differential Display PCR Modifications .....	54
3.2.1i	Amplification Profiles With 3'-hp .....	55
3.2.1ii	Assessment of Template Preparation Methods .....	55
3.2.1iii	3'-hp Display and Detection of Differential Gene Expression .....	56
3.2.2	3'-hp DDPCR Reamplification and Cloning Procedures.....	58
3.2.3	Sequence Analysis of 3'-hp DDPCR Products.....	59
3.2.4	Discussion of 3'-hp DDPCR Modifications.....	60
3.3	DDPCR Screen for ES Cell Restricted Pluripotent Cell Markers .....	62
3.3.1	Identification of Early Pluripotent Cell Specific Markers.....	63
3.3.2	Expression Profile of ES and Early Pluripotent Cell Markers .....	63
3.3.3	Summary of 3'-hp DDPCR Screen for Early Pluripotent Cell Markers.....	65
3.4	Isolation and Cloning of ES and Early Pluripotent Cell Markers .....	66
3.4.1	Validation of A03/360 and B04/400 Expression Patterns .....	67
3.4.2	Sequence of A03/360 and B04/400 Markers.....	69
3.4.3	<i>In situ</i> Analysis of A03/360 and B04/400 Expression in ES Cell Monolayers .....	70
3.5	Discussion.....	71

## CHAPTER 4: EXPRESSION ANALYSIS OF EARLY PLURIPOTENT CELL MARKERS *IN VITRO* AND DURING EARLY EMBRYOGENESIS

4.1	Introduction .....	75
4.2	Expression of A03/360 and B04/400 <i>in vitro</i> .....	76
4.2.1	<i>In situ</i> Hybridisation Analysis of A03/360 and B04/400 Expression During ES Cell Differentiation.....	77

4.2.2	<i>In situ</i> Hybridisation Analysis of A03/360 and B04/400 Expression During the ES to X Cell Transition.....	78
4.3	Analysis of A03/360 and B04/400 Expression During Early Mouse Embryogenesis.....	79
4.3.1	Whole Mount <i>in situ</i> Hybridisation Analysis of <i>Oct-4</i> Expression During Early Mouse Development .....	80
4.3.1i	Specificity of Embryonic <i>in situ</i> Hybridisation Analysis.....	81
4.3.2	<i>In situ</i> Hybridisation Analysis of <i>Icm1</i> (A03/360) Expression During Early Mouse Embryogenesis.....	82
4.3.3	<i>In situ</i> Hybridisation Analysis of <i>Psc1</i> (B04/400) Expression During Early Mouse Embryogenesis.....	83
4.4	Discussion.....	86
4.4.1	The Expression of <i>Icm1</i> and <i>Psc1</i> in Pluripotent Cell Subpopulations <i>in vitro</i> .....	86
4.4.2	<i>Icm1</i> and <i>Psc1</i> Expression and Pluripotent Cell Subpopulations in the Mouse Embryo.....	88
4.4.3	Correlation of <i>Icm1</i> and <i>Psc1</i> Expression to Embryonic Development .....	90
4.4.4	Embryonic Equivalents of ES Cells and X Cells .....	92
4.4.5	Nomenclature of Pluripotent Cell Subpopulations .....	93
4.4.6	Potential for Identifying Pluripotent Cell Subpopulations.....	93

## **CHAPTER 5: ISOLATION OF *Psc1* cDNA CLONES AND ANALYSIS OF *Psc1* EXPRESSION DURING LATER DEVELOPMENT**

5.1	Introduction .....	95
5.2	Isolation and Sequence Analysis of <i>Psc1</i> cDNA Clones.....	96
5.2.1	Isolation of <i>Psc1</i> cDNA Clones .....	96
5.2.2	Sequence Analysis of <i>Psc1</i> cDNA Clones .....	97
5.2.3	Similarity of <i>Psc1</i> with CLEB0336.3 10 and HFBDS04 .....	98
5.3	<i>Psc1</i> Expression in Foetal and Adult Murine Tissues.....	99
5.4	Discussion.....	100
5.4.1	Analysis of <i>Psc1</i> Transcripts.....	100
5.4.2	Cellular Functions of <i>Psc1</i> and Nuclear Speckle Domains.....	100
5.4.4	Function of <i>Psc1</i> During Development .....	102
5.4.5	Future Analysis of the Cellular Function of <i>Psc1</i> .....	103

## CHAPTER 6: FINAL DISCUSSION

6.1	A System for the Isolation of Markers for Subpopulations of Murine Pluripotent Cells.....	104
6.2	Pluripotent Cell Subpopulations or "States" During Early Embryogenesis.....	104
6.2.1	Identification of Pluripotent Cell Subpopulations or "States" During Early Embryogenesis.....	105
6.3	Future Work and Application of <i>Icm1</i> and <i>Psc1</i> to the Analysis of Pluripotent Cell Development.....	105
6.3.1	Analysis of Mutations that Affect Pluripotent Cell Development ...	106
6.3.2	Identifying Developmental Signals During Embryogenesis.....	106
6.3.3	Analysis of Pluripotent Cell Development.....	107
6.3.3i	Functional Analysis of Pluripotent Cell Development <i>in vitro</i> .....	107
6.3.3ii	Mutational Analysis of <i>Icm1</i> and <i>Psc1</i> <i>in vivo</i> and Gene Function During Embryogenesis.....	108
6.3.4	Isolation and Application of <i>Icm1</i> and <i>Psc1</i> Promoters.....	110

APPENDIX: AN UNUSUAL ARRANGEMENT OF 13 ZINC FINGERS IN THE VERTEBRATE GENE <i>Z13</i> .....	111
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REFERENCES.....	117
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## THESIS SUMMARY

The coordinated regulation of pluripotent cell development is critical for the generation of extraembryonic tissues, the differentiated lineages of the embryo, and establishment of the basic body plan during mouse embryogenesis. Accumulating evidence points to considerable heterogeneity within the developing pluripotent cell pool. The generation of specific markers will be critical for the identification and analysis of the implied pluripotent cell subpopulations.

Mouse embryonic stem (ES) cells are a pluripotent cell type derived from the blastocyst inner cell mass and provide a system to investigate pluripotent cell biology *in vitro*. ES cells develop to a distinct pluripotent cell type *in vitro*, termed X cells, in response to MedII conditioned medium. Pluripotent cell types present during the development of ES cells to X cells are a model for the inner cell mass to primitive ectoderm transition *in vivo*, and a source of differentially expressed genes that could be exploited to identify subpopulations of pluripotent cells during early embryogenesis.

The general aim of this thesis was to develop methods for the identification of markers for pluripotent cell subpopulations in the developing mouse embryo.

A screen for ES cell markers was carried out, to identify transcripts that were differentially expressed between ES cells and X cells, to define the embryological equivalents of ES cells, and to investigate pluripotent cell heterogeneity during early development. A modified differential display polymerase chain reaction (DDPCR) system identified nine transcripts that were restricted to ES cells and early pluripotent cell types within the ES to X cell transition. Of these, two novel cDNA markers, A03/360 (*Icm1*) and B04/400 (*Psc1*), were isolated and characterised.

DDPCR analysis identified two types of X cells, an "early" X cell that was closely related to ES cells, and a "late" X cell type with distinctive gene expression. This analysis demonstrated that multiple pluripotent cell subpopulations exist within the ES cell to X cell transition.



*In situ* hybridisation analysis demonstrated that A03/360 (*Icm1*) and B04/400 (*Psc1*) exhibited distinct but overlapping expression profiles during early embryogenesis, subdividing the pool of *Oct-4*<sup>+</sup> pluripotent cells. Inner cell mass 1 (*Icm1*, A03/360) was expressed in pluripotent cells during preimplantation development, in the morula and inner cell mass. *Icm1* expression was downregulated during cellular differentiation to trophectoderm and primitive endoderm and prior to the formation of primitive ectoderm. Expression of *Icm1* therefore identified a pluripotent cell sub-type present during preimplantation development, suggesting potential roles of *Icm1* during the differentiation of trophectoderm and primitive endoderm, or in the maintenance of pluripotency in the inner cell mass.

Peri-implantation stem cell 1 (*Psc1*, B04/400) was expressed in the late stage inner cell mass, in inner cell mass derivatives during peri-implantation development, and in the embryonic ectoderm prior to proamniotic cavitation. *Psc1* expression therefore identified a pluripotent cell subpopulation present during peri-implantation development, suggesting potential roles of *Psc1* in the differentiation of primitive endoderm, proliferation of stem cells, or proamniotic cavitation. *Psc1* was also expressed in the extraembryonic ectoplacental cone, which indicated potential roles during early placental development.

*Icm1* and *Psc1* expression revealed the presence of overlapping subpopulations within the pluripotent cell pool, which highlighted the complexity of pluripotent cell development and regulation. The expression of *Psc1* refined the definition of the embryonic equivalents of ES cells and “early” X cells as pluripotent cells present from approximately 4.0/4.5 days post-coitum (d.p.c.) to 5.0 d.p.c. and the embryonic equivalents of “late” X cells as the primitive ectoderm from approximately 5.25 d.p.c. This verified the *in vitro* model of pluripotent cell development and demonstrated the potential of this system for the identification of pluripotent cell subpopulations from the inner cell mass to the primitive ectoderm stages of embryogenesis.

*Psc1* was selected for additional analysis, and cDNA clones spanning a 3.5 kb *Psc1* sequence were isolated. The 1005 residue *Psc1* open reading frame contained three regions of similarity to the predicted *C. elegans* protein CLEB0336.3 10 and one region to the

human expressed sequence tag HFBDS04, suggesting potential novel protein domains. The presence of potential nuclear and subnuclear localisation sequences suggested that the *Psc1* protein could be localised nuclear “speckle” regions, subnuclear domains that contain pre-mRNA splicing machinery and splicing regulators.

RNase protection analysis demonstrated that *Psc1* was differentially regulated between individual tissues at 16.5 d.p.c. and in the adult. High level *Psc1* expression was detected in embryonic lung and brain, in adult lung, and in the placenta. This suggested that *Psc1* activity could be a component of a recurring developmental function, required at multiple sites during embryogenesis and in the adult, and confirmed a potential role for *Psc1* during placental development.

The approaches described in this thesis demonstrate the potential to identify and characterise molecular heterogeneity within the developing pluripotent cell pool *in vivo*, via the controlled progression and analysis of pluripotent cells *in vitro*.