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

A thesis submitted to the University of Adelaide

for the degree of Doctor of Philosophy

by

Thomas Carl Schulz, B.Sc. (Hons)

Department of Biochemistry

University of Adelaide

Adelaide, South Australia

November 1996

TABLE OF CONTENTS

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*$^{+}$ 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*.