

Technology and Development Team for Mammalian Cellular Dynamics

Kuniya ABE

Team Leader, Team for Technology and Development Team for Mammalian Cellular Dynamics

Goal	<p>Our team uses genetic, genomic and transgenic/gene targeting approaches to study mammalian development. Our focus is basically in two areas;</p> <p>1) genome wide analysis of gene expression in totipotent embryonic stem cells and germ cells or other pluripotent stem cells in order to understand gene expression properties and/or genomic reprogramming processes operated in these stem cells, 2) identification and functional characterizations of genes required for mammalian early development through the analysis of <i>t</i>-complex mutant mice, e.g. <i>t^{w5}</i> embryonic lethal, <i>Brachyury</i> and <i>quaking</i>.</p> <p>In order to achieve this goal, we develop novel techniques for stem cell purification or cDNA microcloning, and experimental research resources such as transgenic/knockout mice as well as cell-type specific cDNA libraries or BAC genomic libraries.</p>
Activities	<ol style="list-style-type: none">1. Establishment of methods for visualization, identification and purification of germ-line stem cells from mouse embryos and systematic analyses on gene expression in these cells2. Analysis of mammalian development using mutants mapped in the <i>T/t</i>-complex.3. Establishment of techniques for monitoring dynamic changes in nuclear architectures during development.4. Analysis of developmental gene expression regulation by natural anti-sense RNAs.5. Development of research resources for functional genomic analyses in mice.
Members	<p>Team Leader, Team for Technology and Development Team for Mammalian Cellular Dynamics Kuniya ABE, Ph. D. (2002.1 ~)</p> <p>Research & Development Scientists Kaoru TSUDA, Ph. D. (2002.2 ~) Hidenori KIYOSAWA, Ph. D. (2002.3 ~) Nathan MISE, Ph. D. (2002.9 ~) Michihiko SUGIMOTO, Ph. D. (2003.4 ~)</p> <p>Technical Staffs Misako YUZURIHA (2002. 3 ~) Kaoru NAKANO (2002. 11 ~)</p>

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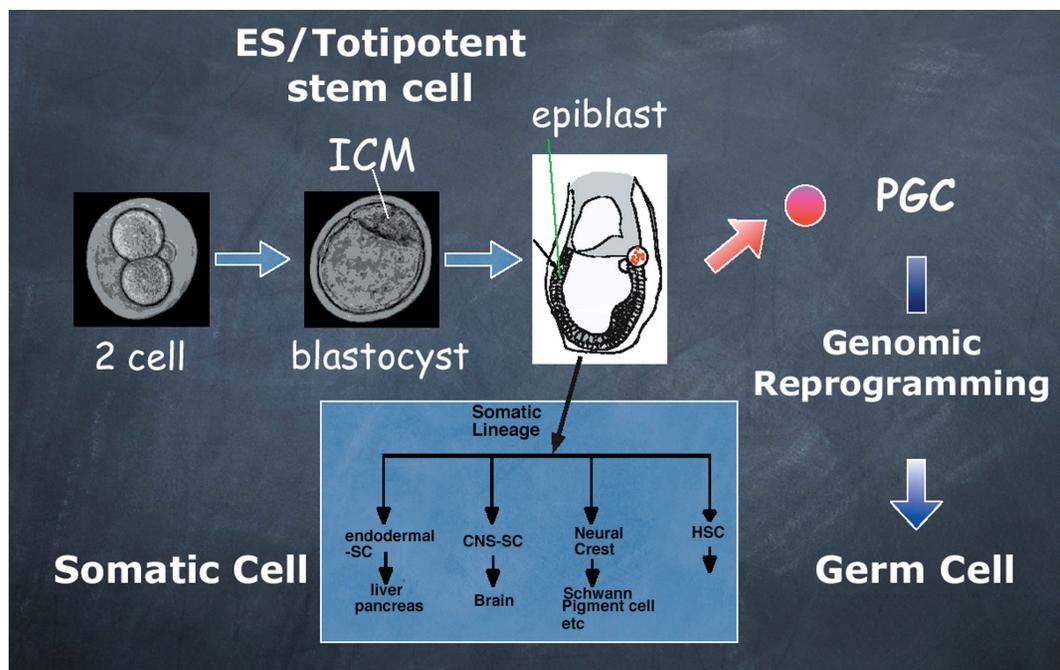


Figure 1. Differentiation of stem cell lineages during early mammalian development.

Specific aim

I. Systematic studies on gene expression in mouse primordial germ cells using large scale cDNA analysis:

In developing embryos of multicellular organisms, distinct sets of differentiated cells are derived from a few stem cell populations. The specification and expansion of diverse cell lineages from pluripotent stem cells are fundamental problems in developmental biology. However, there are technical difficulties to tackle these problems. For example, cells derived from different cell lineages are intermingled within developing embryos, which hampers direct characterization of each differentiating cell type at molecular level. The primordial germ cell (PGC) is a first cell type appeared during germ cell lineage, which gives rise to totipotent embryonic germ cells or gametes capable of making a genetic contribution to the next generation. Epigenetic changes such as genomic imprinting erasure occur only in PGC. PGC is thus considered a key cell type for analysis of germ-soma differentiation as well as totipotency or genomic reprogramming process at the molecular level. However, our knowledge on the molecular events underlying the germ line development is still limited, because, for one reason, attempts to study gene expression in PGC have been confounded by the difficulty in obtaining both sufficient quantities and purity of PGCs. We have overcome this problem using a novel combination of molecular and transgenic approaches. Transgenic mice have been generated in which the cells of the germ lineage express reporter gene. PGCs were purified from these embryos using FACS sorting of the reporter expressing cells. We have used the purified PGCs to construct cDNA libraries. Analysis of these PGC-specific libraries revealed relative abundance and sequence information of ~20,000 ESTs. Clones exhibiting restricted expression pattern were identified in these EST collections. Global gene expression analyses of the PGC-expressed genes are being carried out by large scale *in situ* hybridization as well as high density cDNA array.

Information derived from these 'cell type-specific' developmental analyses will constitute an important resource for future functional studies to understand the biological process of germ line stem cell development.

II. Studies on mammalian early development using t-complex mutants

qkI gene encoding a KH-type RNA binding protein was isolated as a candidate for quaking mutation. *quaking* is a classical neurological mutation mapped in *t*-complex of mouse chromosome 17. Essential roles of the *qkI* in glial cell differentiation was demonstrated by gene targeting and BAC rescue experiments. In addition, we discovered that the *qkI* is indispensable for embryonic development. When homozygous, *qkI*-deficient embryos die at e9.5. This embryonic lethality is due to abnormality in omphalomesenteric artery which connects yolk sac and embryo proper.

tclw5 is a *t*-complex recessive lethal mutation of the t^{w5} -haplotype. Since t^{w5}/t^{w5} embryos die soon after implantation, the *tclw5* gene is thought to play an important role in early embryogenesis. Previous histological studies have demonstrated that t^{w5} homozygotes do

not survive past the gastrulation stage due to extensive death of the embryonic ectoderm, whereas the extraembryonic tissues were less affected. However, according to our chimeric rescue experiments t^{w5} gene is likely to act in extraembryonic tissues and influence embryonic ectoderm development via cell-to-cell interactions. We have narrowed down the t^{w5} critical region to 750 kb by positional cloning strategy, and in-depth characterizations of transcription units within this region are currently underway.

III. Monitoring dynamics of nuclear remodeling during ES cell differentiation.

DNA methylation plays a crucial role for gene expression regulation during mammalian development, and global pattern of DNA methylation can be used as 'marker' for nuclear remodeling. To visualize dynamics of nuclear organization, we have established a novel experimental system, in which methylated DNA binding domain (MBD)-GFP fusion gene was introduced into ES cells thereby enabling us to observe CpG methylation at chromosomal level. Changes in DNA methylation level as well as topological changes in nuclear organization during development can be analyzed using this experimental system.

IV. Identification and expression analyses of natural antisense RNAs found in the mouse transcriptome.

Mouse transcriptome consists of mRNAs encoding protein products, and of some RNAs without protein coding capacity. The latter group of transcripts may act as RNA possibly for regulation of gene expression. On the other hand, there are unexpectedly high number of 'natural antisense RNA' in mice, and majority of the antisense RNAs belong to the non-coding type of RNA. These antisense RNAs are also likely to be involved in various aspects of gene expression regulation. To explore possible roles of the antisense RNAs in developmental regulation, we have identified approximately 2,500 pairs of sense-antisense genes and expression of these genes are currently being studied using 'antisense-gene chip'.

V. Construction of BAC genomic library from MSM/Ms, an inbred strain derived from Japanese wild mouse, *Mus musculus molossinus*.

MSM/Ms is an inbred strain derived from Japanese wild mouse, *Mus musculus molossinus*, and shows high level of genetic polymorphisms against standard inbred strains. Thus, MSM/Ms represents useful resource for genetic analyses of various biological phenomena. We have constructed an arrayed BAC library from male MSM/Ms genomic DNA partially digested with *EcoRI*. The library comprised of 2×10^5 clones with an average insert of 125 kb, covering ~10X genome equivalent. This library can be screened by either colony filter hybridization or PCR of pooled BAC DNA. We have sequenced the ends of the BAC clone inserts and mapped end-sequence pairs onto the C57BL/6J genome (NCBI mouse Build 30). As of July/2003, 41,016 clones were unambiguously mapped, covering 1,977,198 kb or 79.1% of total genome. Comparison of the C57BL/6J and the MSM/Ms sequences revealed 253,840 putative SNPs in 28,820,855 bp sequenced (Phred ≥ 30). Average frequency was estimated to

be 89 SNPs per 10 kb. Distribution of these SNPs along the C57BL/6J genome is not uniform; long stretches of DNA (up to 12 Mb) are nearly devoid of SNPs, while other parts are highly rich in SNPs. The lower SNPs regions occupy ~5% of the C57BL/6J genome, and are likely to derive from the Asian subspecies, probably *M.m. molossinus*. The rate of contribution of Asian subspecies in C57BL/6J genome is much lower than the value of 20~30% reported by other group. The BAC library and the derived SNPs information will constitute valuable experimental resources for functional genomic studies including haplotype analysis.

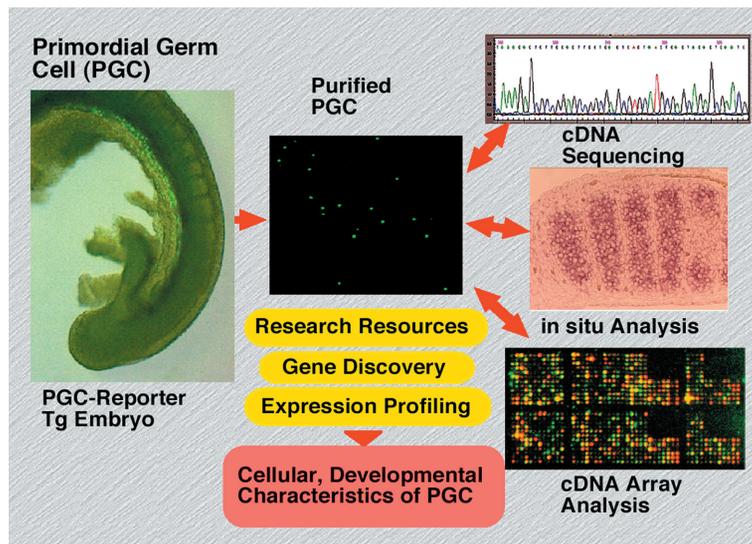


Figure 2. Purification of GFP-expressing PGC (primordial germ cells) and large scale cDNA analysis of gene expression in PGCs.

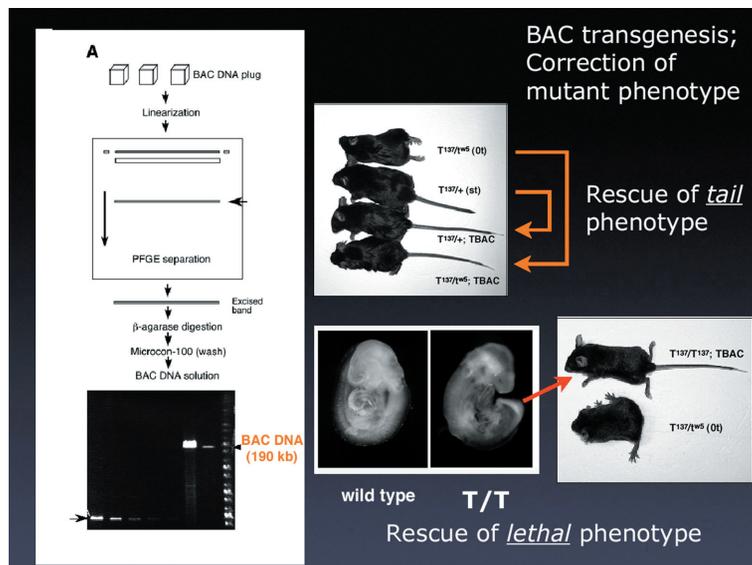


Figure 3. BAC transgenesis and its application for functional rescue of mouse developmental mutation.

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Oral

Presentations

Conference (International)

1. Kuniya Abe: "Studies on gene expression in mouse primordial germ cells using large scale cDNA analysis" International Symposium 'Development and Epigenetics of Mammalian Germ Cells and Pluripotent Stem Cells', Kyoto, Nov. 2001.
2. Kiyosawa H., Kondo S., Yamanaka I., Sakai K., Saito T. and Hayashizaki Y. : "GenoMapper; mapping system of mouse full length cDNA onto genomic sequences", The 15th International Mouse Genome Conference, Edinburgh, Scotland, Oct. 2001.
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