Technology and Development Team for Mammalian Cellular Dynamics

Team Leader, Kuniya ABE

Goal

We develop technologies to monitor and to analyze dynamic nature of mammalian cells, which will be useful tools for phenotyping biological resources collected at the BioResource Center. In order to achieve this goal, our team uses genetic, genomic and transgenic/gene targeting approaches. Our focus is in the following areas.

Activities

1. Visualization of pluripotent embryonic stem cells and germ cells, and genome wide analysis of gene expression to understand genomic reprogramming processes.
2. Functional genomic analysis using wild-derived mouse strains collected at BRC.
3. Identification and functional characterizations of genes required for mammalian early development through the analysis of t-complex mutant mice.
4. Development of technologies to monitor nuclear reorganizations and epigenetic changes during stem cell differentiation.
5. Novel research tools to analyze expression and functions of non-coding RNAs during development.

Members

Team Leader

Kuniya ABE, Ph.D. (2002.1 ∽)

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 ∽)
Takuya FUCHIKAMI, Ph.D. (2004.4 ∽)

Technical Staffs

Misako YUZURIHA (2002. 3 ∽)
Kaoru NAKANO (2002. 11 ∽)
Masayo KONDO (2005. 4 ∽)
Rieko KOBAYASHI (2005. 7 ∽)

Assistant

Miwako KUSAYAMA (2002. 4 ∽)

Student Trainees

Satoru KOBAYAKAWA (2002. 4 ∽)
Chiaki TASHIRO (2004. 4 ∽)
Machiko NAKASHIMA (2005. 4 ∽)

Tashiro, Kondo, Nakano, Kiyosawa, Sugimoto, Fuchikami, Mise, Tsuda, Nakashima, Kobayashi
Kobayakawa, Yuzuriha, Abe, Kusayama

Specific aim

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

In developing mammalian early embryos, there exist pluripotent stem cells, giving rise to all somatic cells as well as germ line cells. Primordial germ cell (PGC) is the cell-type appeared first in the germ cell lineage, sharing many features with the embryonic stem cells (Figure 1). Unlike differentiated somatic cells, the PGCs possess ability to erase epigenetic modifications on the genome accumulated during development. Despite of this biological importance, molecular nature of the PGCs remains largely unknown. We have established systematic methodologies to analyze PGCs and related embryonic cells: PGCs were purified from transgenic mouse embryos, in which the PGCs were marked by GFP-reporter expression, and cDNA libraries were made with the purified PGCs; transcriptome of the PGCs were explored by EST analyses and microarray (Figure 2).

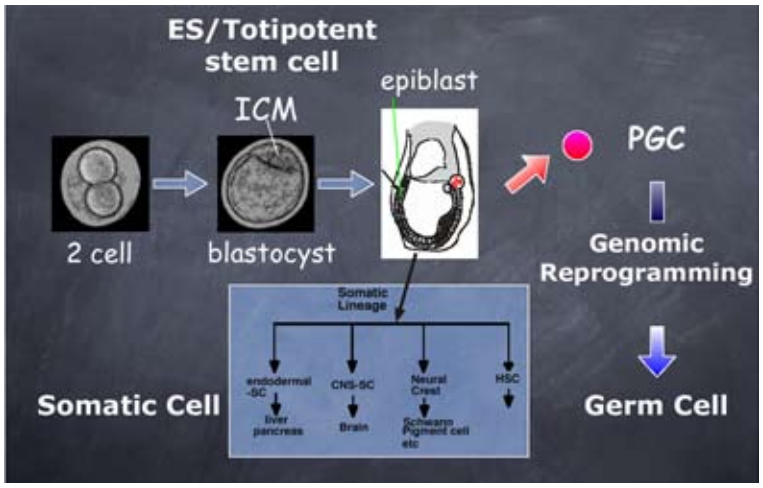

Figure 1.
Differentiation of stem cell lineages during early mammalian development.

We compared gene expression profiles of ES, EG, PGC, and PGC-like cells derived from ES cells in vitro (Toyooka et al., 2003). EG cells have an expression profile quite similar to that of ES cells (only 1-2% of about 20,000 genes showed significant differences), although EG cells are different from ES cells in terms of genome-reprogramming activity (Tada et al., 1997). In contrast, PGCs have an expression program distinct from ES cells: for example, about 17% of genes showed differences between ES and E13.5 female PGCs, suggesting that

dynamic changes in gene expression occur during establishment of germ cell lineage from undifferentiated stem cells. Comparisons of PGCs with the in vitro-formed PGCs identified a set of genes that characterize PGC development.

Knowledge and resources obtained in this study should facilitate a wide range of research in germ cell and stem cell biology.

II. 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 the Japanese wild mouse, *Mus musculus molossinus.* It is believed that subspecies *molossinus* has contributed substantially to the genome constitution of common laboratory strains of mice, although the majority of their genome is derived from the west European *M. m. domesticus.* Information on the *molossinus* genome is thus essential not only for genetic studies involving *molossinus* but also for characterization of common laboratory strains. Here, we report the construction of an arrayed bacterial artificial chromosome (BAC) library from male MSM/Ms genomic DNA, covering <11 × genome equivalent. Both ends of 176,256 BAC clone inserts were sequenced, and 62,988 BAC-end sequence (BES) pairs were mapped onto the C57BL/6J genome (NCBI mouse Build 30), covering 2,228,164 kbp or 89% of the total genome. Taking advantage of the BES map data, we established a computer-based clone screening system. Comparison of the MSM/Ms and C57BL/6J sequences revealed 489,200 candidate single nucleotide polymorphisms (SNPs) in 51,137,941 bp sequenced. The overall nucleotide substitution rate was as high as 0.0096. The distribution of SNPs along the C57BL/6J genome was not uniform: The majority of the genome showed a high SNP rate, and only 5.2% of the genome showed an extremely low SNP rate ; these sequences are likely derived from the molossinus genome (Figure 3).

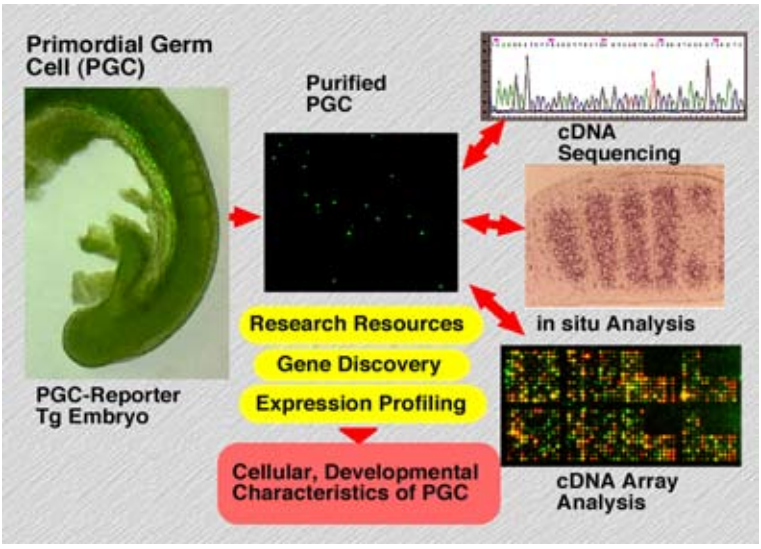

Figure 2.
Purification of GFP-expressing PGC (primordial germ cells) and large scale cDNA analysis of gene expression in PGCs.

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

*tclw*5 is a t-complex recessive lethal mutation of the t^{w5}-haplotype. Since t^{w5}/t^{w5} embryos die soon after implantation, the *tclw*5 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 including BAC transgenic rescue.

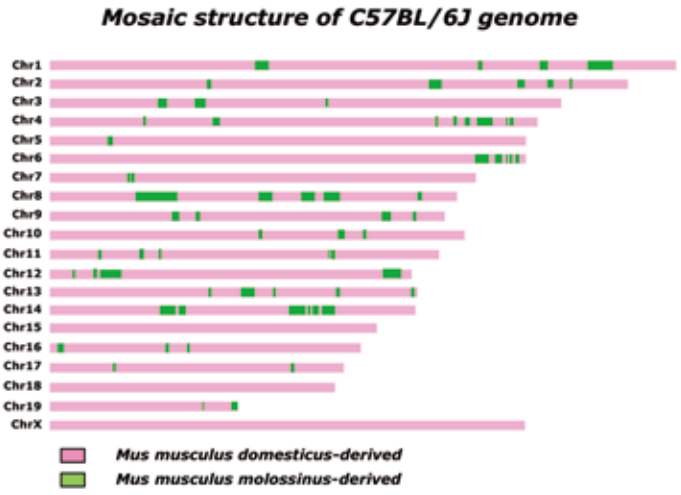

Figure 3. SNP haplotype map of C57BL/6 (B6) genome.
(Pink) Genomic regions where frequency of SNPs between B6 and MSM/Ms is high. These regions are probably derived from Mus musculus domesticus.
(Green) Genomic regions of low SNP frequency; These are possibly derived from Asian mouse subspecies, Mus musculus molossinus.

IV. 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. Furthermore, transgenic mouse lines carrying similar constructs were established, and changes in DNA methylation and chromosomal positioning are being analyzed.

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

Genome-wide *in silico* analysis identified thousands of natural sense–antisense transcript (SAT) pairs in the mouse transcriptome. We investigated their expression using strand-specific oligo-microarray that distinguishes expression of sense and antisense RNA from 1947 SAT pairs. The majority of the predicted SATs are expressed at various steady-state levels in various tissues, and cluster analysis of the array data demonstrated that the ratio of sense and antisense expression for some of the SATs fluctuated markedly among these tissues, while the rest was unchanged. Surprisingly, further analyses indicated that vast amounts of multiple-sized transcripts are expressed from the SAT loci, which tended to be poly(A) negative, and nuclear localized. The tendency that the SATs are often not polyadenylated is conserved, even in the randomly chosen SAT genes in the plant Arabidopsis thaliana. Such common characteristics imply general roles of the SATs in regulation of gene expression.

Publications

Original Papers (*Peer reviewed Journal)

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Oral Presentations

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