Systematic Analysis of Mouse Early Embryos and Stem Cells

Minoru S. H. Ko, M.D., Ph.D.

Developmental Genomics & Aging Section, Laboratory of Genetics, National Institute on Aging, National Institutes of Health, 333 Cassell Drive, Suite 3000, Baltimore, MD21224-6820, USA
KoM@mail.nih.gov

The long-term goal of my laboratoryis to elucidate the global structure and behavior of gene regulatory network in early embryos and stem cells. To this end, we have been developing tools and resources that make it possible to analyze a large number of genes in various experimental conditions. In our earlier work, we 1) constructed cDNA libraries from early mouse embryos and stem cells and generated a large number of expressed sequence tags (ESTs), 2) developed a glass-slide microarray platform containing *in situ*-synthesized 60-mer oligonucleotide probes representing approximately 44,000 unique mouse transcripts, 3) produced web-based ANOVA-FDR software to provide user-friendly microarray data analysis, and 4) developed an algorithm and a fully-automated computational pipeline for transcript assembly from expressed sequences aligned to the mouse genome. In addition, we recently developed a comprehensive database and web browser of the binding sites of transcription factors (TFs) and *cis*-regulatory modules (CRMs) on the mouse genome (Sharov *et al.*, 2006). These resources and tools are now applied to the systematic analysis of gene regulatory networks in mouse early embryos and embryonic stem cells.

Early Embryos: We previouslycarried out DNA microarray-based global expression profiling of all preimplantation stages in mouse, which revealed and characterized the distinctive patterns of maternal RNA degradation and two major transient waves of *de novo* transcription: zygotic genome activation (ZGA) and mid-preimplantation gene activation (MGA) (Hamatani *et al.*, 2004). Through these analyses, we identified a

number of genes that show unique expression patterns. We have demonstrated that one of them, named Zga1, is expressed exclusively in 2-cell mouse embryos and ES cells and plays a critical role in the progression of preimplantation development. We also found that a gene called Chuk shows constant RNA levels throughout preimplantation development and can be used as aninternal standard suitable for quantitative RT-PCR (Falco *et al.*, 2006). We also developed a technique to do large-scale Whole Mount *In Situ* Hybridization (WISH), which allows us to reveal the spatial expression patterns of 91 genes in mouse preimplantation embryos (Yoshikawa *et al.*, 2006). We are currently studying the functions of these genes in details.

Stem Cells: We previously compared the global expression profiles of mouse ES cells and trophoblast stem (TS) cells by DNA microarrays. We studied Esg1, one of the genes identified as a gene expressed specifically in ES cells, and found that the gene encodes an RNA-binding protein that binds to many RNA targets (Tanaka *et al.*, 2006). We have also compared the expression profiles of mouse ES cells undergoing neural differentiation *in vitro* and those of adult neural stem/ progenitor (NS) cells (Aiba *et al.*, 2006). The results suggested that ES cells undergoing neural differentiation *in vitro* recapitulate the development of neural lineages *in vivo*. We also found a set of ~4,000 genes, the expression of which increased with neural commitment/differentiation and can be used as a scale for the degree of commitment/differentiation in neural differentiation. We are extending these studies by carrying out global gene expression profiling of mouse embryonic germ (EG) and other stem cells.

REFERENCES

Aiba K *et al.*(2005). Defining a developmental path to neural fate by global expression profiling of mouse embryonic stem cells and adult neural stem/progenitor cells. Stem Cells. 2005 Dec 15; [Epub ahead of print]

Carter MG *et al.* (2003). The NIA cDNA Project in mouse stem cells and early embryos. C R Biol. 2003 Oct-Nov;326(10-11):931-40.

Carter MG *et al.* (2005). Transcript copy number estimation using a mouse whole-genome oligonucleotide microarray. Genome Biol 6(7):

R61.

Geppino F (2006). Use of Chuk as an internal standard suitable for quantitative RT-PCR in mouse preimplantation embryos. In press in RBM Online.

Hamatani T *et al.* (2004). Dynamics of global gene expression changes during mouse preimplantation development. Dev Cell 6(1): 117-31.

Ko MSH (2004). Embryogenomics of preimplantation mammalian development: Current Status. Reproduction, Fertility and Development 16: 79-85.

Ko MSH (2006). Expression profiling of the mouse early embryo: Reflections and perspectives. Dev Dyn. 2006 May 31; [PMID: 16739220]

Sharov AA *et al.* (2003). Transcriptome analysis of mouse stem cells and early embryos. PLoS Biol 1(3): E74.

Sharov AA *et al.* (2005). A web-based tool for principal component and significance analysis of microarray data. Bioinformatics. 2005 May 15: 21(10): 2548-9. Epub 2005 Feb 25.

Sharov AA *et al.*(2005). Genome-wide assembly and analysis of alternative transcripts in mouse. Genome Res. 2005 May: 15(5): 748-54.

Tanaka TS(2006). Esg1, expressed exclusively in preimplantation embryos, germline, and embryonic stem cells, is a putative RNA-binding protein with broad RNA targets. Dev Growth Differ. 2006 Aug: 48(6): 381-90. [PMID: 16872451]

Yoshikawa T etal. (2006). High-throughput screen for genes predominantly expressed in the ICM of mouse blastocysts by whole mount in situ hybridization. Gene Expr Patterns. 2006 Jan;6(2):213-24. Epub 2005 Dec 1.