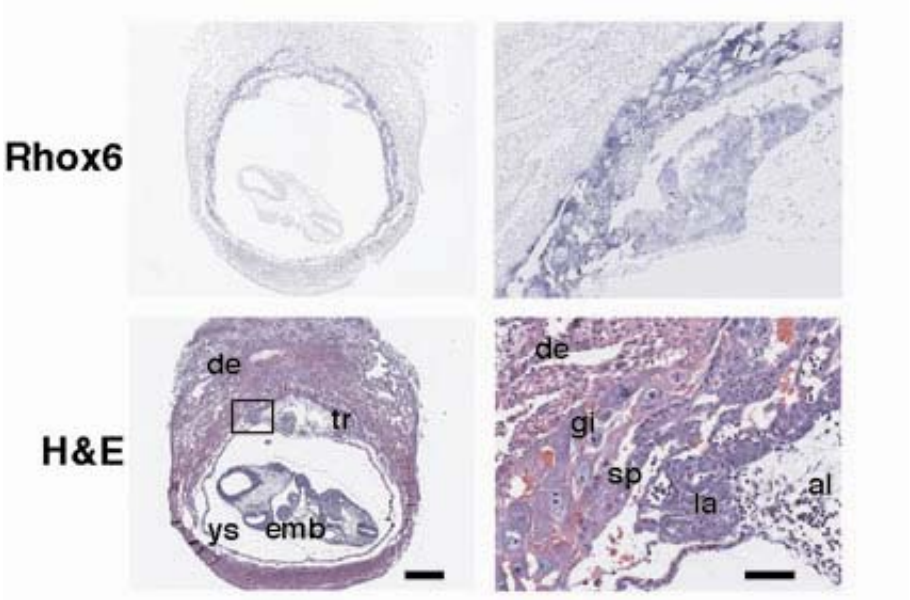
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

Dnmts discriminate: Lineage- and stage-specific Rhox regulation in the early embryo

December 18, 2006 – Essentially every cell in the body contains a full set of the same genetic information, making it critically important for cells to ensure that not all genes are switched on at the same time. In mammals, DNA methylation, which may either block access to DNA binding sites by transcriptional regulators or by attracting repressors to methylated sites, is one of the commonest means of keeping genes under lock and key. This form of regulation is known as epigenetic, as it is both heritable across generations and independent of genomic sequence. DNA methylation involves the activity of a family of enzymes known as DNA methyltransferases (or Dnmts). Dnmt1 is responsible for maintaining methylation, while a pair of factors, Dnmt3a and -3b, work together in the process of establishing methylation *de novo*. The lethality of knockout phenotypes has shown that all of these factors are required for embryonic survival, but little is known of exactly how they operate in genetic regulation during development.



Trophectoderm-specific expression of Homeobox gene, *Rhox6*

Now, in a report published in *Genes and Development*, Masaaki Oda and colleagues from the Laboratory for Mammalian Epigenetic Studies (Masaki Okano; Team Leader) show that DNA methylation regulates the transcription of a cluster of genes expressed in the extraembryonic trophectoderm lineage, but not in the mouse embryo proper. This demonstration of DNA methyltransferase activity in the lineage- and stage-specific silencing of a large genomic region was carried out in collaboration with scientists from the Novartis Institute for Biomedical Research (USA).

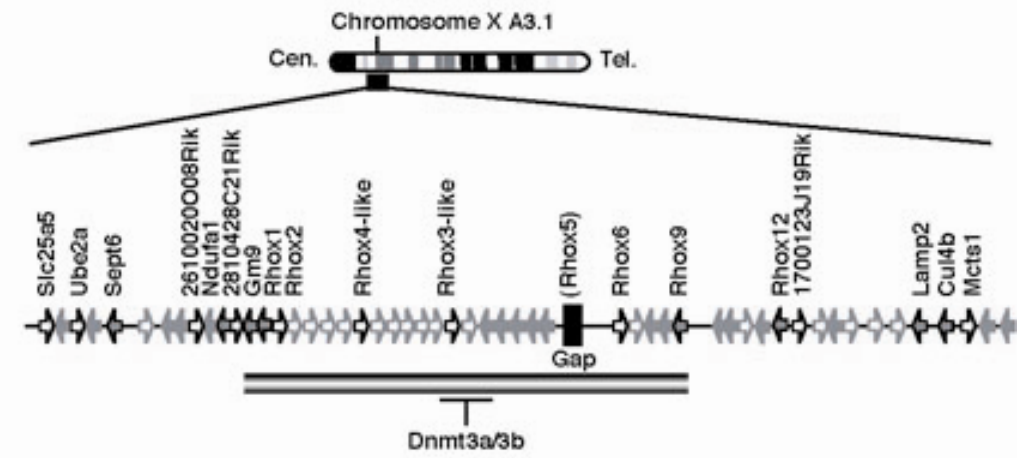
One of the first instances of cellular differentiation in mammalian development takes place in the blastocyst, a hollow ball of a little over 100 cells, which initially comprises primordial embryonic and extraembryonic lineages and subsequently fastens itself to the uterine lining at implantation. The *Rhox* (Reproductive Homeobox) family of genes is known to be repressed in the post-implantation embryonic lineage, leading the team to examine whether DNA methylation might have a role in this silencing process. Oda and colleagues found that two genes,

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs

Rhox6 and *Rhox9*, were both highly methylated in the embryo proper after implantation, but hypomethylated in the trophectoderm (extraembryonic tissue that gives rise to placenta). This high level of DNA methylation was not seen, however, in pre-implantation embryos, indicating that the effect was dependent on both developmental stage and cell lineage.

They next looked at the effects of various combinations of gene deletions involving members of the Dnmt family, and found that while mutations of either *Dnmt3a* and *Dnmt3b* produced loss-of-function phenotypes in the form of reduced methylation of *Rhox* genes in the embryo proper, the result was much more dramatic in the *Dnmt3a*/*3b* double knockout, in which such methylation was lost altogether. Similar effects were seen in the methylation of Oct4 in the trophectoderm, indicating the widespread nature of the activity of these DNA methyltransferases. The loss of methylation of *Rhox6* and *-9* in the embryonic regions of the double knockouts, where their transcription is normally shut down, resulted in their de-repression, an effect that was observed for several other, but importantly not all, trophectoderm-specific genes tested.

The *Rhox* genes occupy a stretch of the mouse genome that includes many genes expressed in trophectoderm but not the embryo proper. In the double knockout mice, a cluster of these *Rhox* and neighboring genes were found to be de-repressed indicating that the regulatory effect of *Dnmt3a* and *-3b* extends over a substantial genomic region, an effect that manifests only after implantation. Using embryonic stem cells as a model of the inner cell mass/epiblast (the embryonic lineage that gives rise to embryo proper), the Okano team confirmed that, *in vitro* as well, the double *Dnmt3a*/*3b* knockout showed loss of methylation for *Rhox6, Rhox9*, and other genes in the vicinity of the *Rhox* cluster, which could be rescued by the expression of exogenous *Dnmt3a*.



Scheme of Rhox cluster region transcriptionally repressed by Dnmt3a/3b in a ICM/epiblast lineage specific manner

The Oda study provides a solid demonstration of a specific regulatory role for DNA methylation in mammalian development. “The large-scale changes in chromatin structure that take place in the germline and early embryo are thought to bear a close relationship to the loss or acquisition of differentiative potency,” says Okano. “The Rhox cluster provides us now with a nice model system for studying one of the molecular bases of chromatin regulation in early embryogenesis, which until now had been quite challenging to study.”

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs