12th Stem Cell Club Meeting

Maintenance of ES Cells & Their Directed Differentiation

(Organised by the Stem Cells Research, Singapore, Website Committee, http://www.stemcell.edu.sg)

Date: June 14th 2006 (Wednesday)
Time: 5:30 pm
Venue: Creation theatrette, Matrix building, 4th floor

Host: Paul Robson, ***GIS***

Time	Title	Speakers
5:30-6:00	**Transcription Regulatory Circuitry in Embryonic Stem Cells: Unbiased Mapping of Oct4, Sox2 and Nanog Binding Sites Using ChIP-PET Technology**	Huck Hui Ng *GIS & NUS*
6:00-6:30	**Directed Differentiation of Human Embryonic Stem Cells into the Pancreatic Endocrine Lineage**	N. Ray Dunn *ES Cell International Pte Ltd*

6.30 – Close with cheese and wine

This event is sponsored by invitrogen™

Transcription Regulatory Circuitry in Embryonic Stem Cells: Unbiased Mapping of Oct4, Sox2 and Nanog Binding Sites Using ChIP-PET Technology

Huck Hui Ng, Gene Regulation Laboratory, Genome Institute of Singapore & Dept of Biological Sciences, NUS

Embryonic stem cells are pluripotent cells that can either self-renew or differentiate into many cell-types. Oct4, Sox2 and Nanog are transcription factors essential to the pluripotent and self-renewing phenotypes of ES cells. In this study, we applied a novel mapping technique (known as ChIP-PET) to capture the chromatin immunoprecipitated DNA fragments to achieve unbiased identification of Oct4, Sox2 and Nanog binding sites. We identified about 1000 high confident Oct4 and Sox2 binding sites and over 3000 high confident Nanog binding sites. Informatics analyses revealed a motif responsible for targeting Oct4 and Sox2 in the genome. Depletion of Oct4, Sox2 or Nanog by RNA interference demonstrated that these factors play both positive and negative roles on transcription. Expression microarray analyses provide further evidence for functional associations between the target genes and binding sites on a genome-wide scale. Comparative whole genome location analyses indicated that they share a significant fraction of their targets. We further defined the downstream genes important for pluripotency by RNAi analysis. Apart from identifying novel target genes, we uncovered an intricate network connecting the different key regulators of pluripotency and a mouse-human conserved circuitry for ES cells.

Directed Differentiation of Human Embryonic Stem Cells into the Pancreatic Endocrine Lineage

N. Ray Dunn, Project Manager, Diabetes Group, ES Cell International Pte Ltd

Human embryonic stem (HES) cells represent a potentially unlimited source of transplantable β cells for the treatment of type I diabetes. We have developed a multi-step culture regime that reproducibly directs the differentiation of our proprietary HES-3 line toward the pancreatic endocrine lineage. Differentiation is initiated by removing HES-3 cells from their supporting fibroblast feeder layer and culturing them in a semi-solid 3D matrix and in the presence of the TGFβ-related ligands Activin A and Bmp4. This culture condition induces the formation of definitive endoderm. Next, a series of growth factors known to promote the proliferation and differentiation of pancreatic ductal epithelial cells to the endocrine lineage are added. *Pdx1* expression, which identifies early pancreatic progenitors, is detected after three weeks of differentiation. By day 34, *Insulin* mRNA levels are significantly upregulated, with concomitant release of c-peptide into the conditioned medium. When transplanted into diabetic SCID mice, differentiated cell populations organize into glandular epithelial structures, retain expression of markers of the terminally differentiated β cell, and release c-peptide into the mouse blood stream.