

Understanding early mammalian embryogenesis: a focus on muscle and blood.

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Early mammalian development is characterized by the presence of a lineage of pluripotent cells; cells with the ability to differentiate to form all the cell types of the developing embryo and adult. First distinguishable within the inner cell mass (ICM) of the blastocyst, the pluripotent lineage persists within the primitive ectoderm before being confined to the germ lineage. During development the primitive ectoderm acts as the substrate for the generation of the somatic lineages through the formation of the three primary germ layers, the ectoderm, mesoderm and endoderm, at gastrulation. The signals that induce cell differentiation and lineage specification at gastrulation, and during the subsequent development of the embryo, are tightly regulated in both time and space. This regulation underlies the generation of the body plan, ensuring correct cellular location and appropriate cell fate. One of the major goals of this laboratory is to understand the molecular nature of the signals that direct differentiation in the early mammalian embryo.

Stable pluripotent embryonic stem (ES) cell lines were isolated from the ICM of the early mouse embryo over 20 years ago. The ability to contribute to all the tissues of the embryo and adult after reintroduction into the embryo, coupled with the ability to differentiate extensively in vitro, has led to the widespread use of these cells as vectors for transmission of genetic alterations into the mouse germline, for modelling embryonic development and as a source of cell populations for experimental analysis. With the isolation of embryonic stem cells from human embryos in 1998, interest in the scientific and commercial application of ES cell technology has increased dramatically. The potential to exploit human ES cells as a source of cell populations with therapeutic applications has led to enormous interest in processes that regulate and control the differentiation of ES cells in vitro. We have been working with mouse ES cells for more than 15 years, developing in vitro differentiation technologies that promote the differentiation of ES cells to specific cell lineages, recapitulating the developmental processes of gastrulation in the early mammalian embryo. These models provide a unique opportunity to identify and characterize differentiation and developmental processes at a cellular and molecular level. Moreover, these differentiation methodologies and the understanding at a molecular level of the regulatory mechanisms will have application to the production of therapeutic agents from human ES cells.

The research in our lab is two-fold:

- To understand the molecular mechanisms driving the generation of cellular complexity during mammalian embryogenesis through the development and analysis of *in vitro* models of pluripotent cell differentiation.
- To use this information to direct the differentiation of pluripotent cells in culture to specific cell types that can be used in the treatment of human disease.

We have been able to recapitulate in culture the differentiation of the pluripotent cells of the ICM to the primitive ectoderm by differentiating ES cells to an early primitive ectoderm-like, or EPL, cell. Subsequent differentiation using EPL cells as a starting point results in the formation of highly enriched or homogenous populations of mesendoderm (forerunner of many tissues including muscle, blood, gut and bone) or ectoderm/neuroectoderm (forerunner of many tissues including neurons, glia and skin). This contrasts to the mixed populations that result from the differentiation of ES cells in culture (Figure 1).

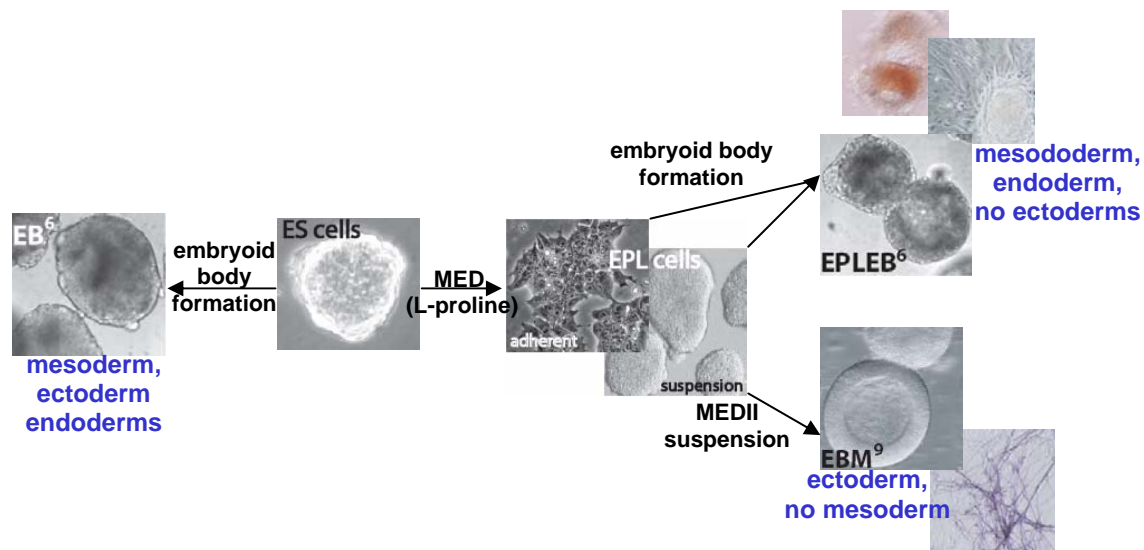


Figure 2: Differentiation from ES and EPL cells results in distinct cellular outcomes.

The differentiation of ES cells in embryoid bodies results in a disorganised cellular mass that comprises cells of all three germ lineages and the extraembryonic endoderm. In contrast, the differentiation of EPL cells in embryoid bodies results in the formation of only mesoderm (including muscle and blood) and embryonic endoderm, whereas differentiation of EPL cell aggregates in MEDII results in the formation of only ectoderm (neurons).

We have been investigating the signaling requirements and biological mechanisms involved in the formation of mesendoderm from EPL cells. We have demonstrated a requirement for disruption of cell:cell contact and an alteration in the culture environment. In addition, preliminary evidence suggests the

involvement of a number of signaling pathways that are activated endogenously during the differentiation process. The roles of antagonists and agonists of these pathways in mesendoderm formation are currently under investigation, providing assays to identify activities that could be exploited to drive mesendoderm formation *in vitro*. Furthermore, we wish to understand the hierarchy of signaling pathways in mesendoderm formation and to intergrate these pathways with the functional manifestations of the process. This is a very fast-moving and exciting area of science and although it is anticipated that honours students in 2007 will work within this area it is not possible to delineate specific honours projects at this time.