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#### Research Focus

#### Epigenetic control of stem cell differentiation and development of germ cells

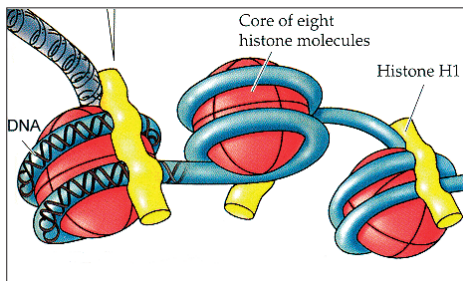
I aim to understand how both adult and embryonic stem cells differentiate into given cell types, how the gene expression patterns required for this are established at an epigenetic level and what mechanism maintains the differentiated states (cellular memory). It has been possible to reverse the epigenotype of a somatic nucleus by transfer into an oocyte and to derive embryonic stem cells using this method. However, this is inefficient and has a low throughput method so I aim to use our growing knowledge of epigenetics to devise strategies for 'reprogramming' human somatic cells towards a stem cell like phenotype. These cells could be used for regenerative therapy without the logistical and ethical problems associated

with 'therapeutic cloning' in humans.

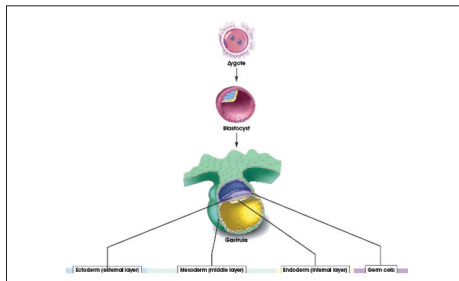
Ovulated human oocytes are limited in availability, so to investigate epigenetic reprogramming, other sources of oocytes must be identified. Mouse embryonic stem cells are capable of differentiating into male and female germ cells in vitro and putative primordial germ cells (PGC's) derived in this way contribute to spermatogenesis when transplanted into the adult testis and are capable of fertilising oocytes in vitro. Oocyte-like cells have been obtained from adherent cultures of differentiating mouse ES cells as indicated by morphology and the expression of germ cell specific genes such as Vasa, Gdf9 and Scp3. Germ cell specific markers are also expressed in embryoid bodies derived from human ES suggesting the presence of PGC's, so I am developing methods to enhance their formation and isolation. Further culture systems are being developed to direct the differentiation and

growth of PGC's towards oocytes.

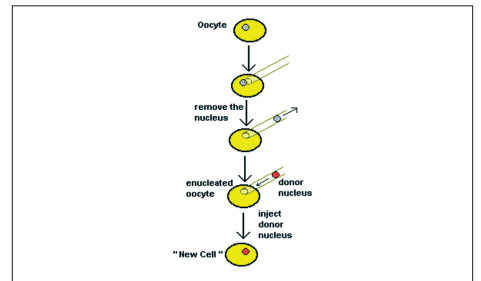
An understanding of the epigenotype of differentiated cells may provide better strategies to direct the differentiation of embryonic stem cells towards the production of tissues of therapeutic value. I am investigating the differentiation of hESC into mesodermal derivatives (eg the haematopoietic system and skeletal muscle). It is also important to determine how the epigenotype of a pluripotent cell such as an hESC is defined and maintained. I am creating 'maps' of modified histones (eg tri- and dimethylation of lysines 4 and 9 of histone H3) in both hESC and their differentiated progeny with particular reference to genomic loci involved in pluripotency. This work is based on microarray studies of similar cells and the observation of high levels of stress defence enzymes and specific signalling pathways unique to hESC and suggestive of a molecular 'signature'.



Structure of the Nucleosome. The DNA macromolecule is wrapped around a core of proteins known as histones which help to package into the cell nucleus. Precise structural modifications of these histones control access to the DNA by the cellular transcription machinery thereby altering the usage of its information content.



Development of early embryo. The single cell stage or zygote can give any of the cell types of the developing organism (totipotency). Cellular differentiation is required to produce such cell types but this results in a progressive restriction of the numbers of cell types that can be produced from such differentiated cells. The key to this is control of the flow of information from the DNA and this in turn is controlled by epigenetic histone modification.



Is cellular differentiation reversible? Nuclear transfer or cloning can reset the program of somatic cells to a state where they support development of the whole organism thus the programming of differentiated cells can be reversed albeit inefficiently. We are seeking ways to improve this reprogramming ability which relies upon altering the epigenetic pattern of the differentiated cell.

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#### Selected publications

**Armstrong L., Hughes O. et al. Lako M. (2006).** The role of PI3K/AKT, MAPK/ERK and NFκB signalling in the maintenance of human embryonic stem cell pluripotency and viability highlighted by transcriptional profiling and functional analysis. *Hum Mol Genet.* Apr27(epub ahead of print)

**Armstrong L., Lako M., Dean W., Stojkovic M. (2005).** Epigenetic Modification is Central to Genome Reprogramming in Somatic Cell Nuclear Transfer. *Stem Cells.* Nov 10; (epub ahead of print)

Stojkovic M., Stojkovic P., Leary C., Hall V.J.,

**Armstrong L., Herbert M., Nesbitt M., Lako M., Murdoch A. (2005).** Derivation of a human blastocyst after heterologous nuclear transfer to donated oocytes. *Reprod Biomed Online.* 11(2):226-31

**Armstrong L., Saretzki G., Peters H., Wappler I., Evans J., Hole N., von Zglinicki T., Lako M. (2005).** Overexpression of telomerase confers growth advantage, stress resistance, and enhanced differentiation of ESCs toward the hematopoietic lineage. *Stem Cells.* 23(4):516-29