# *"An understanding of developmental processes provides the basis for regenerative medicine"*

## Persistence of a Hox mediated positional code from development through to adulthood has great implications for regenerative medicine

Regenerative medicine is the relatively new science of developing tissues and organs to replace those damaged by disease or injury. This burgeoning field has seen rapid advances in knowledge since work with embryonic stem cells (ESCs) began in the 1960's (Andrews et al., 2005). Although embryonic stem cells offered a great number of benefits, large disadvantages such as ethical issues and donor-patient compatibility meant that the perceived breakthrough never reached fruition. Newer technologies have now replaced ESCs as the future of regenerative medicine and of great interest is induced pluripotent stem (iPS) cells (Cox and Rizzino, 2010).

Even though iPS cells have been differentiated into a wide range of tissues, a problem of identity still exists. On the gross scale, identity of cells produced from precursors is determined by the lineage restriction which occurs throughout development (Cox and Rizzino, 2010). The interesting problem is that even cells which have been classified as the same identity have many unique characteristics depending on where in the body they are found. Fibroblasts may have up to 1000 alterations in gene expression to ensure they are specific, for example, to the arm, palm, or scalp (Chang et al., 2002). Similar characteristic changes can be observed in bone marrow; Hoxa11 is expressed in the tibia whilst the mandible has no Hox gene expression (Leucht et al., 2008). This is likely to play a large role in determining identity of cells in the adult body. In order to utilise Hox genes during regeneration, we must analyse how Hox genes are expressed and regulated during development.

Hox genes code for transcription factors containing a signature homeodomain, a 60 amino acid structure with DNA binding activity. They were first identified during Wieschaus, Lewis and Nusslein-

Vollhards widespread mutagenesis of drosophila, where the homeotic Antennapedia mutant was documented (Reviewed in Lewis, 1994).

The four human clusters are thought to have duplicated from two clusters in Drosophila. Hox genes are initiated in the epiblast, during the period where cells begin to invaginate to form three germ layers. Before this period, all Hox genes are repressed and this allows ESCs to retain pluripotency. During embryogenesis, highly expressed transcription factors such as Oct4, Nanog and Sox2 prevent the expression of differentiation driving genes. Internally expressed factors such as LIF and BMP also prevent external signals which could lead to differentiation; BMP induces the 'Inhibitor of Differentiation' ID which forms a heterodimer to block transcription of gene targets. It appears that removal of this repression is a process which contributes to differential gene expression. Interactions between non organiser mesoderm and the Spemann organiser cause a temporal and spatial demarcation of cells corresponding to those undergoing involution into the gastrula earlier being given an anterior positional value, while later cells receive a posterior positional value. This pre-specifies cells along the anterior to posterior (A-P) axis which allows a body plan to be laid down, and segmentation to occur.

The most remarkable feature is that gene activation on the A-P axis is recapitulated on the chromosome, with 3' genes being switched on in anterior tissue, and 5' genes in posterior tissue. This is termed temporal co-linearity. The genes are expressed in nested domains and often exhibit posterior prevalence, where 5' genes have a larger effect on the phenotype than co-expressed 3' genes.

Throughout development and adulthood a variable pattern of Hox expression can be observed but no changes DNA sequences occur. Thus expression must be mediated by epigenetic factors. The polycomb group (PcG) proteins have histone methylase function, and cause a selective and highly regulated trimethylation of Lysine 27 at the N-termini of Histone 3 (H3K27me3). This modification appears to effectively prevent any gene translation in the surrounding area. An opposing

modification, H3K4me3 is mediated by Trithorax (or mixed lineage leukaemia, MLL) proteins which cause chromatin rearrangements favouring active transcription. These two modifications are widespread throughout the Hox loci, and seem to be mutually exclusive (Lan et al., 2007), thus preventing any ambiguous signalling. New studies suggest that the specificity is caused by lysine demethylases associated with each protein group which remove the opposing modification (Pasini et al., 2008, Agger et al., 2007). A very recent study (Soshnikova and Duboule, 2009) provided evidence that these histone modifications form a 'transcriptional window' where gene expression is increased, and this window is dependent on the close spatial organisation of the Hox cluster. This process has been well studied during development, but understanding how regulating modifications are maintained over multiple generations will lead to refined utilisation of iPS cells in regenerative medicine (Chang et al., 2002).

The importance of Hox identity was shown during bone grafts in mice (Chang et al., 2002, Leucht et al., 2008). Hox expression is not required for bone growth or repair; no Hox genes are activated in the mandible, while the Tibia has Hoxa11 expressed but both have similar growth and repair ability. Each bone was fractured and a section removed. This section of bone was then inserted either into the same location or into the other bone. When the Hox negative mandible was grafted into the tibia, complete healing occurred. ChIP analysis showed that the inserted cells had taken on the positional identity of the tibia by expressing Hoxa11. When the situation was reversed, the Hoxa11 tibia fragment produced a cartilaginous scar on the Hox negative mandible. The transplanted cells had retained their hox identity, and not integrated with the surrounding tissue. This experiment highlights the fact that although the bone from the mandible and tibia are morphologically identical, the correct Hox code is required for repair and regeneration (Leucht et al., 2008).

The method of how the hox code is retained over multiple generations has yet to be determined. The current hypothesis is that the tightly clustered Hox loci play a role in maintaining chromatin modifications. During cell division, a lone modified nucleosome could be randomly lost due to

replacement with a new un-modified protein (Dodd et al., 2007). In an environment where a large stretch of DNA has associated activating or repressing methylations, it is likely that the daughter cell will maintain approximately half of these modifications. These methylation sites will have a high concentration of either PcG or MLL proteins associated with them, thus replacement of the modifications lost during division could easily occur (Dodd et al., 2007, Leucht et al., 2008).

Another factor which could play a role in maintaining the Hox code is the long non coding RNAs (lncRNA) (Nagano et al., 2008). Micro-RNA regulation can be observed for about 33% of all Drosophila genes, including the Hox clusters. lncRNAs can act *in cis* or *in trans*, and both activate or silence genes (Rinn et al., 2007). The human HoxC locus has an lnc-RNA termed 'hotair' which has both repressive and activating function in adult fibroblasts. The RNA is transcribed from a region in the HoxC locus. This recruits MLL proteins to the area, maintaining RNA polymerase II mediated transcription (Sanchez-Elsner et al., 2006, Rinn et al., 2007). This is the *in cis* activation. The *in trans* repressive function occurs on a 40kb segment of the HoxD locus where PcG proteins including Suz12, EED and EZH2 proteins are recruited (Petruk et al., 2006). The molecular detail of how such a complex interaction occurs in still being explored.

The utilisation of the above described processes for regenerative medicine could yield great advancements in transplant surgery. Skin grafts are a regularly performed surgery to replace damaged tissue after injury and disease. The skin is normally selected on morphological similarity; for example skin from the upper thigh used on the face. A closer look at the molecular expression profile of each fibroblast could reduce the inevitable scarring such an operation would leave. It is conceivable to think that an identical match in hox expression could allow skin grafts where visible scarring is entirely removed. This could be achieved in two ways. Induced pluripotent stem cells could be differentiated into Fibroblasts, and then synthetic RNA molecules could be produced which mimic the action of the endogenous lnc-RNA, allowing selective expression of a Hox code which is appropriate to the region being repaired. Another mechanism would be to utilise a graft from the

patient's own skin, but culture the tissue in an environment where Hox expression could be altered. For the facial areas, where no Hox expression is found, PcG proteins and H3K4-demethylases could be introduced to remove any endogenous expression. In an area where Hox expression is required, Hox-free tissue could be produced as above, and then co-cultured with cells showing the correct Hox code. In the Bone-graft experiments by Leucht et. al., it was shown that a Hox positive cell could induce the same expression pattern in a Hox free cell; co-culturing such cells may produce a large amount of tissue with the correct morphological and molecular characteristics which could then be transplanted.

Understanding of the developmental processes involved in Hox gene expression from embryogenesis to adulthood has identified a number of key mechanisms which require further study in the context of regenerative medicine. Hox genes are a key developmental process and their study has lead to a better understanding of innate repair processes in mammals. Further research into this area is likely to lead to a new generation of transplant and implant surgeries where compatibility between grafted and natural tissue is greatly increased.

**Words: 1445**

**Bibliography**

AGGER, K., CLOOS, P. A. C., CHRISTENSEN, J., PASINI, D., ROSE, S., RAPPSILBER, J., ISSAEVA, I., CANAANI, E., SALCINI, A. E. & HELIN, K. 2007. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature,* 449**,** 731-U10.

ANDREWS, P. W., MATIN, M. M., BAHRAMI, A. R., DAMJANOV, I., GOKHALE, P. & DRAPER, J. S. 2005. Embryonic stem (ES) cells and embryonal carcinoma (EC) cells: Opposite sides of the same coin. *Biochemical Society Transactions,* 33**,** 1526-1530.

CHANG, H. Y., CHI, J. T., DUDOIT, S., BONDRE, C., VAN DE RIJN, M., BOTSTEIN, D. & BROWN, P. O. 2002. Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America,* 99**,** 12877-12882.

COX, J. L. & RIZZINO, A. 2010. Induced pluripotent stem cells: what lies beyond the paradigm shift. *Experimental Biology and Medicine,* 235**,** 148-158.

DODD, I. B., MICHEELSEN, M. A., SNEPPEN, K. & THON, G. 2007. Theoretical analysis of epigenetic cell memory by nucleosome modification. *Cell,* 129**,** 813-822.

LEUCHT, P., KIM, J. B., AMASHA, R., JAMES, A. W., GIROD, S. & HELMS, J. A. 2008. Embryonic origin and Hox status determine progenitor cell fate during adult bone regeneration. *Development,* 135**,** 2845-2854.

LEWIS, E. B. 1994. HOMEOSIS - THE FIRST 100 YEARS. *Trends in Genetics,* 10**,** 341-343.

NAGANO, T., MITCHELL, J. A., SANZ, L. A., PAULER, F. M., FERGUSON-SMITH, A. C., FEIL, R. & FRASER, P. 2008. The Air Noncoding RNA Epigenetically Silences Transcription by Targeting G9a to Chromatin. *Science,* 322**,** 1717-1720.

PASINI, D., HANSEN, K. H., CHRISTENSEN, J., AGGER, K., CLOOS, P. A. C. & HELIN, K. 2008. Coordinated regulation of transcriptional repression by the RBP2 H3K4 demethylase and Polycomb-Repressive Complex 2. *Genes & Development,* 22**,** 1345-1355.

PETRUK, S., SEDKOV, Y., RILEY, K. M., HODGSON, J., SCHWEISGUTH, F., HIROSE, S., JAYNES, J. B., BROCK, H. W. & MAZO, A. 2006. Transcription of bxd noncoding RNAs promoted by trithorax represses Ubx in cis by transcriptional interference. *Cell,* 127**,** 1209-1221.

RINN, J. L., KERTESZ, M., WANG, J. K., SQUAZZO, S. L., XU, X., BRUGMANN, S. A., GOODNOUGH, L. H., HELMS, J. A., FARNHAM, P. J., SEGAL, E. & CHANG, H. Y. 2007. Functional demarcation of active and silent chromatin domains in human HOX loci by Noncoding RNAs. *Cell,* 129**,** 1311-1323.

SANCHEZ-ELSNER, T., GOU, D. W., KREMMER, E. & SAUER, F. 2006. Noncoding RNAs of trithorax response elements recruit Drosophila Ash1 to ultrabithorax. *Science,* 311**,** 1118-1123.

SOSHNIKOVA, N. & DUBOULE, D. 2009. Epigenetic Temporal Control of Mouse Hox Genes in Vivo. *Science,* 324**,** 1320-1323.