

Establishment and maintenance of gene expression in lymphocytes

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Our group have a longstanding interest in understanding how patterns of gene expression are established, maintained through cell division, and changed in a progressive and ordered fashion during development. In addition to identifying candidate genes involved in lineage choice, we have performed studies to examine the influence of nuclear location for gene expression. Our experiments combine techniques for fluorescence in situ hybridisation (FISH) that preserve nuclear structure with high resolution fluorescence microscopy. These studies have shown that transcriptionally inactive genes are often positioned close to constitutive heterochromatin in the nucleus of dividing lymphocytes¹. This functional 'compartmentalisation' is achieved as cells enter the cell cycle and appears to be important in maintaining the heritable repression of a subset of genes²⁻⁴. We have now applied a PCR-based approach to examine the replication timing of developmentally regulated loci in relation to their expression status and to their position within the nucleus. Contrary to expectations, we regularly observe early replication of genes that are silent and positioned (in trans) close to constitutive heterochromatin. However, late replication is seen for inactive transgenes integrated (in cis) in a heterochromatic environment. Hence, replication timing may indicate whether candidate loci reside in an 'open' or 'closed' chromatin environment. We are using this approach to compare the chromatin status of key genes that determine lineage fate in cells with different lineage potential; totipotent ES cells, multipotent haemopoietic precursors and unipotent lymphocytes. The data so far are consistent with lineage restriction being underpinned by a progressive 'shutting down' of the genome. The implications of this work both for tracing lineage fate-maps and for our understanding of stem cell plasticity are discussed.

- 1 Brown, K.E. et al. Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin. *Cell* 91: 845-54 (1997).
- 2 Brown, K.E. et al. Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division. *Mol. Cell* 2: 207-17 (1999).
- 3 Brown, K.E., Amoils, S. et al. Expression of α - and β -globin genes occurs within different nuclear domains in haemopoietic cells. *Nature Cell Biol.* 3: 602-606 (2001).
- 4 Skok, J.A. et al., Non-equivalent nuclear location of immunoglobulin alleles in B lymphocytes. *Nature Immunology* 2: 848-854 (2001).