Meeting report

# Chromosomal vectors for gene therapy: castles in the air?

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A report on the ESF/LESC Exploratory Workshop 'Understanding Chromosome Behaviour: Prospects for Constructing Chromosome-Based Vectors for Gene Therapy', Elmau, Germany, 27-30 September 2001.

Approximately 25 scientists from around Europe, and one speaker from an American laboratory, came together in the fairy-tale castle Schloss Elmau, 1,000 meters up in the German Alps for an exploratory workshop, funded by the European Science Foundation (ESF) and organized by Dean Jackson (UMIST, Manchester, UK) and Hans Lipps (University of Witten, Germany). Our aim was to consider what we do, and what we don't, know about how chromatin and chromosomes behave in mammalian cells, and also how this knowledge might be put to good use in the design of vectors, or artificial chromosomes, for use in gene therapy.

A number of practical and ethical issues impinge on the characteristics of vectors designed for delivering genes and gene products into cells. For the purposes of safety, and avoiding immunological rejection, non-viral vectors are preferable. To prevent the possible mutagenic effects of integrating DNA into the host genome, episomal maintenance of the vectors in cells is desirable. If such vectors are to persist and be propagated to daughter cells, some method of ensuring stability and segregation of the vector DNA through DNA replication and cell division is required. Lastly, it is no good having a vector stably maintained in cells if expression of the genes it carries is subsequently extinguished by gene-silencing mechanisms. In the light of these issues, talks at the meeting focused principally on how chromosomes are replicated and segregated in mammalian cells, and how gene expression is maintained or silenced through modulations of chromatin structure.

The stability of endogenous mammalian chromosomes through cell division is mediated by the centromeric DNA and its associated kinetochore (a DNA-protein complex). Unlike the simple short centromeres of budding yeast, human centromeres are large and complex. Although most human centromeres are characterized by the presence of α-satellite DNA, the presence of this repetitive DNA sequence is neither necessary nor sufficient for centromere activity in humans, as is evident from the incidence of dicentric and neocentric chromosomes in the human population. Telomere-mediated chromosome fragmentation has been put to good effect in constructing human mini-chromosomes. It was suggested by William Brown (University of Nottingham, UK) that the variable mitotic stability of these chromosomes in different host cells could be used to screen for *cis*-acting sequences and *trans*-acting factors that contribute to centromere formation and function. Similar fragmentation approaches provided evidence for a fixed position of the kinetochore within arrays of α-satellite DNA (Christine Farr, University of Cambridge, UK). Transfer of mini-chromosomes into cells is inefficient (by micro-cell-mediated transfer), however, so this is not a feasible approach for delivery in a gene therapy context. There was agreement that transfer into stem cells, and expansion of the population of cells that have received the mini-chromosome *ex vivo* before transplantation into the host (stem-cell-mediated gene-therapy) was the way forward on this front.

For the purposes of segregation, an alternative to having a functional centromere on a vector is to allow an episome to piggyback on host chromosomes. This usually requires at least one viral protein for maintenance and segregation, however, because these vectors are based on viral genomes (for example simian virus 40, SV40, or Epstein-Barr virus, EBV). One way around this might be to incorporate an architectural element into the vector that will interact structurally with the host chromosomes. Progress toward this using a S/MAR (scaffold/matrix-associated region) was described by Lipps. Jürgen Bode (German Research Centre for Biotechnology (GBF), Braunschweig, Germany) and Frank Fackelmayer (University of Hamburg, Germany) summarized the structural properties, activities and protein-interaction principles of S/MARs that could explain their properties in segregation.

To be propagated from cell to cell, DNA must be not only segregated accurately but also replicated. It remains unclear whether there are defined sequence requirements for replication origins in mammalian cells. Considerable progress on where and when DNA is replicated in the cell is being made by studying both replicating DNA and the proteins involved in DNA replication in living cells (Daniele Zink, Ludwig Maximilians Universität, München, Germany). During S phase of the cell cycle, not only DNA sequence but also heritable chromatin structures must be copied. The transient acetylated state of heterochromatin immediately after it is replicated, and before it is matured, may provide a window of opportunity during which chromatin structure can be experimentally manipulated by drugs (such as trichostatin A, TSA). The suggestion that RNA may be involved in heterochromatin structure was especially exciting (Geneviève Almouzni, Institut Curie, Paris, France).

What genetic elements and what chromatin structures are involved in maintaining and extinguishing gene expression? DNA methylation has a long association with gene silencing. It was suggested by Francisco Antequera (Instituto de Microbiologia Bioquimica, Salamanca, Spain) that DNA methylation acts to mask most of the mammalian genome from transcription factors. In support of this idea, footprinting experiments showed that protein-DNA interactions at gene promoters are delimited by the 5′ boundary of CpG islands and the transcription-initiation site.

Contrasts between the α and β globin genes were emphasized as well-studied models of the control of gene expression. The levels of α or β globin chains produced from these genes must be exactly matched in red cells. Yet the mechanisms for controlling transcription from these tissue-specific genes seem to be quite different. The CpG island promoter of the human α globin is bound by ubiquitous factors that allow for low-level expression in many cell types. The β globin gene, by contrast, has no CpG island, and its expression level is controlled by the distant locus-control region (LCR), comprised of a cluster of nuclease-hypersensitive sites. The complex relationships between methylation, histone acetylation, sub-nuclear location and rate of gene expression were explored for the β globin LCR by Dirk Schubeler (Fred Hutchison Cancer Research Center, Seattle, USA). Differences between the α and β globin gene loci go beyond their basic chromatin structures. In lymphocytes, which do not express globin genes, the β globin loci and several other inactive genes (such as the c-*fms* proto-oncogene, the *TdT* deoxynucleotidyl transferase gene and the *λ5* gene, which encodes a component of the pre-B cell receptor), associate with pericentric heterochromatin in the nucleus. In contrast, α globin genes do not (Matthias Merkenschlager, MRC Clinical Sciences Centre, London, UK). I showed that the α and β globin genes adopt very different positions within the territories of their host chromosomes in the nucleus. Hence, regulation of α and β globin gene expression occurs in quite different chromatin and nuclear environments but with the same ultimate outcome.

From a gene therapy perspective, it will be important to know whether genes are slaves to their local environment, or whether they can be surrounded by sequences that act as barriers to outside chromatin influences. If a gene had its own domain, would it then matter if it ended up in heterochromatin? In transgenic experiments with the *λ5* locus, Niall Dillon (MRC Clinical Sciences Centre, London, UK) showed that localization of a gene close to heterochromatin is not incompatible with its expression, provided that appropriate regulatory sequences (hypersensitive sites) are intact. These data also provide evidence that transcription-factor binding itself can initiate changes in higher-order chromatin structure.

At the moment, the prospect of being able to introduce genes into humans, in such a way that they will be stably inherited through mitosis and stably expressed in appropriate cell types but their introduction will do no harm, remains a daydream. But we should not worry "if [we] have built castles in the air…; that is where they should be. Now [we must] put the foundations under them." (Henry David Thoreau, in *The Writings of Henry David Thoreau*; Houghton Mifflin, 1906). And those foundations were precisely what were being built at Schloss Elmau.