so they are likely to have driven adaptive change. We have recently located new circadian QTL in *Arabidopsis*, several which are specific for particular temperature regimes (K. Edwards and A.J. Millar, unpublished). QTL analysis in mice might thus reveal pleiotropic genes that are involved in the mouse clock. These might well include 'central' clock genes that are otherwise difficult to identify. Phenotypic testing under various environmental conditions can increase the number of genes recovered. The method can be efficient: testing 196 F2 individuals [1] is not such a large number, compared to a conventional mouse screen after mutagenesis. Strain collections with detailed behavioural and habitat data are still required to complete the causal chain from temporal ecology to the molecular mechanism of the clock, but the supporting tools are being moved into place [12].

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Meeting Report

Five years of vector service for gene therapy

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The Gene Vector Production Network Conference (http://www.genethon.fr/gvpnconf.html) was held at Genocentre, Evry, France, from 4 to 5 October 2001.

The Gene Vector Production Network (GVPN; http://www.genethon.fr/gvpn) is a coordinated service for the production of reagents for gene transfer experiments (viral vectors, plasmids and cell lines) for the scientific community. It started in 1997, following an initiative of the Association Française contre les Myopathies (AFM) to encourage the development of gene therapy for genetic diseases. The objectives of the meeting were to assess progress, and to allow GVPN users to meet, develop collaborations and plan future strategies. Abstracts are available on the conference website.

GVPN material has been used in a wide range of projects aimed at the treatment of genetic, degenerative, auto-immune and virally induced diseases, and cancer. In addition, there has been further development of gene transfer technology; scaling up vector production, purification and quality control, routes of administration, transgene expression and immune response.

The first gene therapy successes

The first success was achieved at the Necker Hospital in Paris, with treatment of four children suffering from X-linked Severe Combined Immune Deficiency (SCID-X). Patients were auto-grafted with hematopoietic stem cells (HSC) transduced with a retrovirus carrying the wild-type γC gene. This restored normal immune function, all clinical symptoms disappeared, and the children are healthy to date [1]. Marina Cavazzana-Calvo (Necker Hospital, Paris, France) envisages performing additional trials using a similar protocol with other SCID children presenting alterations in the *RAG-1* and *RAG-2* genes, which encode two other factors involved in the immune disorder.

Naomi Taylor (IGM-CNRS, Montpellier, France) is developing a similar protocol for SCID patients with altered *ZAP-70*, another factor involved in the disease. Re-population with $CD4^+$ and $CD8^+$ differentiated cells occurred normally in *zap-70*$^{-/-}$ mice grafted with HSC transduced with the *zap-70* gene, although the characterization of the resulting phenotype is not yet complete. Thus it seems that retroviral vectors are efficient vehicles for the transfer of therapeutic factors into hematopoietic cells and could be used to treat other immune and hematological disorders.

Other approaches to treatment of genetic diseases

Guerrino Meneguzzi (INSERM-CHU, Nice, France) was able to produce laminin-β3 (LB3) in LB3-deficient keratinocytes from junctional epidermolysis bullosa (JEB) patients [2]. The keratinocytes were transduced with the wild-type *LB3* gene using retroviral vectors, which transduced almost 100% of cells, confirming the efficiency of this vector system for epidermal stem cells. The feasibility of treating genetic skin diseases by grafting engineered epidermal stem cells on to human patients is currently under investigation in dog models of JEB.

Marc Peschanski (INSERM, Creteil, France) reported on a Phase I–II trial in which he implanted

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intracerebroventricularly incapsulated BHK cells genetically engineered, via plasmid transduction, to synthesize and release the neuroprotective cyliar neurotrophic factor (CNTF) in six patients with Huntington's disease [3]. Although the approach was shown to be safe (i.e. without side effects), survival of implanted cells was heterogeneous. A more reliable approach could be to deliver the neuroprotective factor using newly developed canine adenoviruses, which are powerful vehicles for gene transfer into the brain, since such vector system has been proven to produce a widespread and long-lasting protection to the entire striatum in rats.

The treatment of β-thalassemia in mice by inducing erythropoietin (Epo) secretion from muscles was discussed by Jean-Michel Heard (Institut Pasteur, Paris). Transferring mouse Epo cDNA with adeno-associated virus (AAV) vectors or with naked DNA electrotransfer obtained similar results, except that the correction was permanent with AAV and transient with naked DNA [4]. Results indicated that the subsequent correction of anemia was the consequence of an intense stimulation of fetal-like erythropoiesis.

Another pre-clinical protocol to treat the familial hypercholesterolemia was discussed by Anne Weber (INSERM, Clamart, France). She presented data on the intra-portal transplantation of hepatocytes transduced with MLV-LacZ vectors in non-human primates [5]. Transplanted hepatocytes expressing the β-galactosidase were widely distributed in the host liver (portal tracts, sinusoids, and hepatocyte plates), demonstrating the feasibility of the approach for future clinical trials in patients affected with this hepatic metabolic disorder.

Fetal gene therapy

Charles Coutelle (Imperial College, London, UK) discussed fetal somatic gene therapy as a preventive approach to the management of human genetic diseases, one which could potentially avoid early onset disease manifestation. A large population of actively dividing cells are present during fetal life, and the fetus has increased immune tolerance to transgenic proteins. Thus, this approach could lead to permanent somatic gene supplementation, and facilitate repeated postnatal treatments. The major problem is to find adequate vector systems; that is, those allowing high-titer preparations, such as adenovirus and AAV. Preliminary data indicate that this is the case by using mice as small animal system. Furthermore, because of their immunogenic potential, adenovirus vectors are good tools for investigation on immune reactions against vector and transgene product after *in utero* application, and data indicate a postnatal tolerance to *in utero* transgenic protein could be possible [6].

The developments in gene transfer technology

Despite the tremendous progress in research and the initial clinical achievements, many problems remain unresolved, due to the absence of vector standardization with validated procedures of production, purification and quality control. Estuardo Aguilar-Cordova (Harvard University, Cambridge, MA, USA) stressed the requirement for reference standard vector preparations with normalization of their physical and functional characteristics (titer, purity, etc.) [7]. These normalized parameters are necessary to obtain reproducible results, establish safety and efficacy profiles of the vectors and facilitate regulatory approval, when they are used in clinical trials (Xavier Chenevisse, Agence Française de Sécurité Sanitaire des Produits de Santé, Vendargue, France). Furthermore, it is necessary to scale up vector production for *in vivo* transfer into large animals and for clinical trials. New bio-processes to scale up AAV, HIV and MLV production, and to assess the quality and bio-safety parameters are currently in progress, namely, the development of novel cell lines engineered to produce AAV vectors, and of fluidized bed reactor biotechnology. This will be carried out on a multidisciplinary platform involving academic and industrial institutions and regulatory agencies to integrate expertise of each partner to transform bench-manufactured vectors into new medicines.

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