



INSTITUT DE FRANCE  
Académie des sciences

# THERAPIE CELLULAIRE REGENERATIVE *REGENERATIVE CELL THERAPY*

6 - 8 SEPTEMBRE 2006

## RESUMES / ABSTRACTS

COLLOQUE  
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INSTITUT DE FRANCE

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# **THERAPIE CELLULAIRE REGENERATIVE** ***REGENERATIVE CELL THERAPY***

**6 - 8 SEPTEMBRE 2006**

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## Mercredi / Wednesday 6

- 11h **Ouverture du Colloque / Welcome Address**  
**Jules Hoffmann**, Vice-Président de l'Académie des sciences  
**Nicole Le Douarin**, secrétaire perpétuelle honoraire de l'Académie des sciences
- 11h15 **Lecture d'ouverture / Introductory Lecture** : présidée par **Nicole Le Douarin**  
**Normal and Neoplastic Stem Cells; a New Model of Clonal Stem Cell Systems In Vivo**  
**Irving L. Weissman**, Stanford University School of Medicine, USA
- 12h15 **Intervention de François Goulard, Ministre délégué à l'Enseignement Supérieur et à la Recherche**

Session  
1

### CELLULES SOUCHES EMBRYONNAIRES / EMBRYONIC STEM CELLS

President : **FRANÇOIS JACOB**

- 14h15 **Introduction**  
**François Jacob**, professeur honoraire au Collège de France et à l'Institut Pasteur
- 14h30 **Lecture plénière / Plenary Lecture** :  
**Cellules souches et thérapie régénérative : principes, perspectives et problèmes / Stem Cells and Regenerative Medicine : Principles, Prospects and Problems**  
**Sir Richard Gardner**, Edward Penley Abraham Research Professor of the Royal Society, Univ. of Oxford, UK
- 15h15 **Les cellules souches en science et en médecine / Stem Cells in Science and Medicine**  
**Ron McKay**, Laboratory of Molecular Biology, NINDS, Porter Neuroscience Research Center, Bethesda, USA
- 15h45 **Lignée germinale et cellules souches pluripotentes / Germ Line and Pluripotent Stem Cells**  
**Azim Surani**, Research Institute of Cancer and Developmental Biology, The Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK
- 16h15 **Les cellules souches embryonnaires comme modèle cellulaire du développement normal et pathologique de la peau / Embryonic Stem Cells as a Cellular Model for Normal and Pathological Skin Development**  
**Daniel Aberdam**, Inserm U 634, Faculté de médecine Nice-Sophia Antipolis, Nice, France
- 16h45 Pause café

Session  
2

### TRANSFERT NUCLÉAIRE ET CELLULES SOUCHES / NUCLEAR TRANSFER AND STEM CELLS

President : **FRANÇOIS JACOB**

- 17h15 **Reprogrammation du noyau par fusion cellulaire / Nuclear Reprogramming by Cell Fusion**  
**Helen Blau**, Baxter Laboratory in Genetic Pharmacology, Stanford University School of Medicine, USA
- 17h45 **Reprogrammation nucléaire et pluripotence des cellules de l'épiblaste chez les mammifères / Nuclear Reprogramming and Pluripotency of Epiblast Cells**  
**Jean-Paul Renard**, UMR Biologie du développement et reproduction, INRA, Jouy-en-Josas, France

## Jeudi / Thursday 7

Session  
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### RÉGÉNÉRATION ANIMALE ET CELLULES SOUCHES / STEM CELLS AND ANIMAL REGENERATION

President : NICOLE LE DOUARIN

- 9h30 **Identité positionnelle des cellules souches du membre adulte pendant la régénération chez les salamandres** / *Positional Identity of Adult Limb Stem Cells During Regeneration in Salamanders*  
**Jeremy Brockes**, Dept. of Biochemistry and Molecular Biology, University College London, UK
- 10h **L'hydre, une niche pour la plasticité cellulaire et développementale** / *Hydra, a Niche for Cell and Developmental Plasticity*  
**Brigitte Galliot**, Dept. of Zoology and Animal Biology, University of Geneva, Sciences III, Suisse
- 10h30 **La fonction, la régulation et le maintien chez les cellules souches somatiques de l'adulte : un modèle simple pour un problème complexe** / *The Function, Regulation and Maintenance of Adult Somatic Stem Cells: A Simple Model for a Complex Problem*  
**Alejandro Sánchez Alvarado**, Howard Hughes Medical Institute, University of Utah School of Medicine, USA
- 11h Pause café

Session  
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### CELLULES SOUCHES SPÉCIFIQUES DES TISSUS / TISSUE SPECIFIC STEM CELLS

President : RON MCKAY

- 11h30 **Renouvellement des épithéliums stratifiés** / *Renewal of Stratified Epithelia*  
**Yann Barrandon**, Ecole Polytechnique fédérale de Lausanne-CHUV, Laboratoire de dynamique des cellules souches, Suisse
- 12h **Emergence des cellules souches hématopoïétiques pendant le développement** / *Emergence of Hematopoietic Stem Cells During Development*  
**Françoise Dieterlen-Lièvre**, CNRS UPR 2197, Laboratoire DEPSN, Institut de neurobiologie Alfred Fessard, Gif-sur-Yvette, France
- 12h30 Pause déjeuner
- 14h30 **Potentiel accru des cellules souches adultes** / *Greater Potency of Adult Stem Cells*  
**Catherine Verfaillie**, Stem Cell Institute, Katholieke Universiteit Leuven, Belgique
- 15h **Intégrer de nouveaux neurones dans le système olfactif de l'adulte** / *Integrating New Neurons into the Adult Olfactory System*  
**Pierre-Marie Lledo**, Dépt. de Neurosciences, Unité perception et mémoire olfactive, CNRS URA 2182, Institut Pasteur, Paris, France
- 15h30 Pause café
- 16h **Progéniteurs et cellules souches de la crête neurale** / *Neural Crest Progenitors and Stem Cells*  
**Elisabeth Dupin**, CNRS UPR 2197, Laboratoire DEPSN, Institut de neurobiologie Alfred Fessard, Gif-sur-Yvette, France
- 16h30 **Cellules progénitrices du muscle squelettique** / *Skeletal Muscle Progenitor Cells and the Role of Pax Genes*  
**Margaret Buckingham**, de l'Académie des sciences, Dépt. de biologie du développement, Génétique moléculaire du développement, URA CNRS 2578, Institut Pasteur, Paris, France
- 17h **Phylogénèse et ontogénèse du pancréas en relation avec les cellules souches pancréatiques** / *Pancreas Phylogeny and Ontogeny in Relation to a "Pancreatic Stem Cell"*  
**Ole D. Madsen**, Hagedorn Research Institute, Gentofte, Danemark

## Vendredi / Friday 8

Session  
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### THÉRAPIE CELLULAIRE / CELL THERAPY

President : JEAN-FRANÇOIS BACH

- 9h **Application de la thérapie cellulaire aux maladies lymphohématopoïétiques** / *Cell Therapy for Lympho-hematopoietic Diseases*  
**Marina Cavazzana-Calvo**, Inserm U 429, Dépt. de biothérapie, Hôpital Necker-Enfants malades, Paris, France
- 9h30 **Thérapie cellulaire de lésions ischémiques graves du myocarde** / *Cell Therapy of Severe Ischaemic Heart Failure*  
**Radovan Borojevic**, Instituto de Ciências Biomédicas, Dept. de Histologia e Embriologia, Universidade Federal do Rio de Janeiro, Hospital Pró-Cardíaco, Brésil
- 10h **Thérapie cellulaire en cardiologie** / *Cell Therapy in Cardiology*  
**Philippe Ménasché**, Inserm U 633, Dépt. de chirurgie cardiovasculaire, Hôpital Européen G. Pompidou, Paris, France
- 10h30 **Application de la thérapie cellulaire à la maladie de Parkinson** / *Stem Cell Therapy for Parkinson's Disease*  
**Anders Bjorklund**, Wallenberg Neuroscience Center, Division of Neurobiology, Lund University, Suède
- 11h Pause café
- 11h30 **Les cellules souches paraissent exercer une pression homéostatique au niveau du CNS dans le cas de lésions de la moelle épinière par transplantation** / *Stem Cells Appear to Exert Homeostatic Pressure in Degenerative or Injured CNS Environments*  
**Evan Snyder**, Burnham Institute for Medical Research, La Jolla, USA
- 12h **Réparation de la moelle épinière lésée par la transplantation de cellules olfactives engainantes** / *Repair of Spinal Cord Injury by Transplantation of Olfactory Ensheathing Cells*  
**Geoffrey Raisman**, Chair of Neural Regeneration, Institute of Neurology, University College London, UK
- 12h30 **Régulation des cellules épithéliales dans la régénération de la dent** / *Regulation of Epithelial Stem Cells in Tooth Regeneration*  
**Irma Thesleff**, Research Program in Developmental Biology, Viikki Biocenter, Institute of Biotechnology, University of Helsinki, Finlande

Session  
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### 15h-17h - TABLE RONDE : ÉTHIQUE / ROUND TABLE : ETHICS

President : CLAUDE SUREAU

- **Intervention de Didier Houssin, Directeur général de la santé**
- ♦ **Anne Fagot-Largeault**, de l'Académie des sciences, Professeur au Collège de France : **Questions éthiques et anthropologiques** / *Ethical and Anthropological Queries*
  - ♦ **Jean-Claude Ameisen**, Université de Paris, CHU Bichat-Claude Bernard, Paris : **Recherches sur l'embryon : jusqu'où « je » peut-il instrumentaliser « un autre » ?** / *"The End is Where We Start From..." on Beginnings, Means, and Ends*
  - ♦ **Claude Huriot**, Président de l'Institut Curie, Sénateur honoraire, Vice-Président du Comité international de Bioéthique de l'Unesco, Paris : **Que valent les interrogations éthiques face aux espoirs thérapeutiques et aux enjeux financiers ?** / *What About Ethical Considerations Facing Therapeutic Hopes and Economic Stakes ?*
  - ♦ **Anne McLaren**, The Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, UK : **Est-il éthique de créer des embryons pour la recherche ?** / *Is it Ethically Acceptable to Make Embryos for Research ?*
  - ♦ **René Frydman**, Université Paris-Sud 11, Service de Gynécologie-Obstétrique et médecine de la reproduction, CHU Antoine Béclère, Clamart : **La production de gamètes à partir de cellules souches est-elle éthiquement envisageable ?** / *Differentiation of Animal ES Cells into Gametes : Ethical Comments*
  - ♦ **Frédérique Dreifuss-Netter**, Université de Paris V, Faculté de droit, Paris : **1994-2004 : le législateur face à l'embryon in vitro** / *1994-2004 : The In Vitro Embryo Facing the Law*
  - ♦ **Conclusions par Claude Sureau : Vers une médecine de l'embryon ?** / *Towards a Specific Medical Care of the Human Embryo ?*

## Lecture Plénière / Plenary Lecture

### **CELLULES SOUCHES ET THERAPIE REGENERATIVE : PRINCIPES, PERSPECTIVES ET PROBLEMES** *STEM CELLS AND REGENERATIVE MEDICINE : PRINCIPLES, PROSPECTS AND PROBLEMS*

**Richard L. GARDNER**  
University of Oxford, UK

Stem cells have been used routinely for more than three decades to repair tissues and organs damaged by injury or disease, most notably haematopoietic stem cells taken from bone marrow, umbilical cord or, increasingly, from peripheral blood. Other examples, such as grafts of skin to treat severe burns, entail transplantation of stem cells within organized tissue rather than following isolation. The prospect of exploiting stem cells more widely in regenerative medicine was encouraged by two more recent developments. The first, championed initially more than two decades ago by Robert Edwards, was to use early human conceptuses as a source of stem cells. This was prompted by two considerations. One was the expectation that such stem cells would retain the potential to form all types of somatic cells, and the other that human assisted conception in vitro would ensure the availability of ample material for their derivation. By the time the first human embryonic stem (ES) cell lines had been produced, reports of unexpected versatility of adult cells started to emerge. Most now agree that simultaneous investigation of the potential of stem cells from both sources offers the best prospect of securing rapid progress towards more general application of regenerative medicine.

The aim is to employ stem cells as a source of appropriately differentiated cells to replace those lost through physical, chemical or ischaemic injury, or as a result of degenerative disease. This may entail transplantation of just a single type of cell or, more challengingly, require a complex of several different types of cells possessing a defined architecture. Cardiomyocytes, hepatocytes or neuronal cells producing specific transmitters offer promising examples of the former, although whether transplanted healthy cells will function any better than native ones in a perturbed tissue environment remains to be established. Recent success in repairing urinary bladder defects with grafts of urothelial and muscle cells seeded on a biodegradable collagen scaffold is an encouraging step towards assembling organs in vitro. Nevertheless, this is still far removed from the level of sophistication required to counter the ever increasing shortfall in supply of kidneys.

Various problems must be addressed if recent advances in the laboratory are to be translated into clinical practice. In many cases it has yet to be established that cells derived from adults that retain plasticity are actually stem cells. There is also a pressing need for appropriate assays to ensure that, regardless of source, stem cells maintained in vitro are safe to transplant. Assays currently available for human ES cells are far from ideal. It is, in addition, important to ensure that differentiated cultures are pure and, depending on whether cell renewal is required or to be avoided, retain or lack appropriate stem cells. Neither autografts nor those obtained by so-called 'therapeutic cloning' are options for treating condition with an obvious genetic basis. Moreover, claims that some stem cells are more likely than others to yield successful allografts has both to be confirmed and explained.

**LES CELLULES SOUCHES EN SCIENCE ET EN MEDECINE**  
*STEM CELLS IN SCIENCE AND MEDICINE*

**Ron MCKAY**

NINDS, Porter Neuroscience Research Center, Bethesda, USA

The interest in stem cells revolves around the idea that development of tissues can be understood and controlled. The work in our group is focused on the development and function of the central nervous system. We have identified stem cells and made progress showing that our understanding of the basic aspects of development has strong links to tissue function and dysfunction. Very often in the public debate on the clinical potential of stem cells in medicine a detailed analysis of restoration of function is not provided. However, this information will be necessary for regenerative medicine to succeed. Our increasing ability to control the origin of neural architecture identifies key regulators controlling the development of neural networks. If the same basic mechanisms regulate early and mature neuronal functions, understanding the biology of neural stem cells may lead rapidly to fundamental new insights into problems as diverse as cancer and psychological plasticity.

Androutsellis-Theotokis A, Leker RR, Soldner F, Hoepfner DJ, Ravin R, Poser SW, Rueger MA, Bae S-K, Kittappa T, McKay R. Notch signalling regulates stem cell numbers *in vitro* and *in vivo*. *Nature* (in press).

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Tsai R., McKay RDG. "A nucleolar mechanism controlling proliferation in stem cells and cancer cells", *Genes and Dev.* vol. 16, pp. 2991-3003, 2002.

**LIGNEE GERMINALE ET CELLULES SOUCHES PLURIPOTENTES**  
*GERM LINE AND PLURIPOTENT STEM CELLS*

**Azim SURANI**

Wellcome Trust Cancer Research UK Gurdon Institute,  
University of Cambridge, UK

Pluripotent epiblast cells in the early postimplantation embryo give rise to both the somatic cells and primordial germ cells. However, only a few cells that are destined to establish the germ cell lineage escape from the somatic fate and retain an underlying pluripotent state. Recent evidence shows that Blimp1, a known transcriptional repressor with a SET/PR domain is crucial for the repression of somatic program in germ cell precursors and the specification of primordial germ cells (PGCs). Following specification of PGCs, there is expression of *stella*. There are further dynamic epigenetic changes that occur after the establishment of the germ cell-specific chromatin signature. These are significant changes since this results in re-expression of *nanog* but only in the germ cell lineage. Note that *nanog* and *stella* are closely linked genes together with other pluripotency-specific genes in this cluster in mouse and man. Some of these genes may share some common control elements.

Recent evidence also shows the involvement of a novel Blimp1/Prmt5 complex in the germ cell lineage following specification of PGCs. Prmt5 is an arginine methylase, and a number of targets of Blimp1/Prmt5 have been identified, including Dhx38. Blimp1/Prmt5 may have a significant role in the maintenance of the germ cell lineage, which may prevent these cells from acquiring an overtly pluripotent state. Derivation of pluripotent embryonic germ cells (EG) from PGCs results in a loss of Blimp1 from PGCs, and re-expression of Dhx38 and c-Myc, another putative target of Blimp1, which contribute to the generation of self-renewing pluripotent stem cells from PGCs. These studies provide further insights into the mechanism involved in the specification of PGCs and the maintenance of the germ cell lineage. They also provide an insight into the mechanism underlying the reversible phenotypic changes involving PGCs and pluripotent embryonic germ cells.

**LES CELLULES SOUCHES EMBRYONNAIRES COMME MODELE CELLULAIRE DU DEVELOPPEMENT NORMAL  
ET PATHOLOGIQUE DE LA PEAU**  
*EMBRYONIC STEM CELLS AS A CELLULAR MODEL FOR NORMAL AND PATHOLOGICAL SKIN DEVELOPMENT*

**Daniel ABERDAM**  
INSERM U634, Faculty of Medicine, Nice, France

Embryonic stem (ES) cells can be cultured indefinitely, differentiated into many cell types *in vitro*, thus providing a potential unlimited supply of cells for cognitive *in vitro* studies and cell-based therapy. We recently reported the efficient derivation of ectodermal and epidermal cells from murine ES cells. These differentiated ES cells were able to form, in culture, a multilayered epidermis coupled with an underlying dermal compartment, similar to native skin. Thus, ES cells have the potential to recapitulate the main events characteristics of embryonic skin formation, including the reciprocal instructive ectodermal-mesodermal commitments. We clarified the function of BMP-4 in the binary neuroectodermal choice by stimulating sox-1<sup>+</sup> neural precursors to undergo specific apoptosis while inducing epidermal differentiation through  $\Delta$ Np63 gene activation and we demonstrated that TAp63 seems necessary to commit ES-derived ectodermal cells to epidermal fate. This unique cellular model further provides a powerful tool for identifying the molecular mechanisms controlling normal skin development, the role of each p63 isoform and their miRNA and target genes in epidermal commitment, proliferation and differentiation and in p63-ectodermal dysplasia human congenital pathologies.

**Keywords:** stem cells, p.63, ectodermal dysplasia.

**Category:** Embryonic stem cells.

**REPROGRAMMATION DU NOYAU PAR FUSION CELLULAIRE**  
*NUCLEAR REPROGRAMMING BY CELL FUSION*

**Helen BLAU**

Baxter Laboratory in Genetic Pharmacology,  
Stanford University School of Medicine, USA

Once a cell becomes specialized for function in a particular tissue, that differentiated state is stable, yet the molecular mechanisms that control the expression of its characteristic repertoire of genes are largely dynamic. Our research is directed at understanding this apparent paradox and elucidating the nature of cell memory and cell plasticity. By perturbing the intracellular or extracellular milieu, we are probing the regulatory network that determines cell fate and how it can be altered. Such knowledge is key to our understanding of stem cell quiescence, self-renewal, differentiation, and how cancer arises and to the use of somatic cells or stem cells for therapeutic purposes.

**REPROGRAMMATION NUCLEAIRE ET PLURIPOTENCE DES CELLULES DE L'ÉPIBLASTE  
CHEZ LES MAMMIFÈRES**  
*NUCLEAR REPROGRAMMING AND PLURIPOTENCY OF EPIBLAST CELLS*

**Jean-Paul RENARD**

National Institute of Agronomical Research (INRA),  
UMR 1198, Developmental Biology and Reproduction, Jouy-en-Josas, France

Nuclear reprogramming through nuclear transfer (NT) refers to the ability of the nucleus from a differentiated somatic cell to acquire again the functional properties of an undifferentiated embryonic nucleus when placed into the cytoplasm of an enucleated oocyte. While the *in vivo* development of NT-embryos is most frequently compromised, their *in vitro* explantation from the inner cell mass cells (ICM) of the embryo at the blastocyst stage can be used to derive pluripotent embryonic stem cell (ES) lines with the same functional properties as those obtained from fertilised embryos (1). This is considered to be the consequence of the erasure of some epigenetic memory of the donor nucleus during the process of ES cell derivation while this memory is retained *in vivo* with detrimental consequences on foetal development (2).

By combining embryological observations, gene expression analysis during gastrulation and generation of chimeric embryos, we recently have shown that the differentiation potential of the ICM is not altered *in vivo* in mouse NT-blastocysts (3). Epiblast pluripotency which encompasses the ability of this derivative of the ICM to differentiate into various cell lineages and to establish the embryonic axes and body plan of the conceptus is retained. It is not the early patterning of NT-embryos which is affected rather than their growth which is frequently altered in a trophoblast dependent manner. Since in the mouse the early patterning of the embryo is concomitant to the establishment of close relationships between the conceptus and the maternal environment, we extended our analysis to cattle embryos where gastrulation is completed before implantation. Using NT-blastocysts of different genetic background but with a pattern of gene expression close to fertilised blastocysts (4), we observed few abnormal phenotypes during early embryonic patterning rather than the diverse abnormalities that would have been expected if epigenetic memory of the donor nucleus had been maintained. A high proportion of cattle NT-embryos implanted and their development became compromised only at various and often late foetal stages. The spectrum of abnormalities observed upon the recovery of fetuses appeared, as in the mouse, to be largely linked to a growth-mediated perturbation of placental functions leading to fetal adjustments in response to adverse changes in the biological environment of the developing foetus and to frequent severe postnatal physiological disorders.

Taken together these data indicate that nuclear reprogramming does not affect the differentiation potential of epiblast cells neither *in vitro* nor *in vivo*. Rather they suggest that the frequent compromised development of NT-embryos *in vivo* stems from some early perturbations in the cross-talk between the epiblast and extra embryonic tissue that regulates their growth and differentiation.

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- 1-Wakayama et al., (2006) Stem Cell online. May 11[Epub ahead of print]
- 2-Brambrick et al., (2006) Proc.Natl.Acad.Sci.USA, 103 : 933–8
- 3-Jouneau et al., (2006) Development, 133:1597-607.
- 4-Smith et al. (2005) Proc.Natl.Acad.Sci.USA, 102:17582-7.

**IDENTITE POSITIONNELLE DES CELLULES SOUCHES DU MEMBRE ADULTE PENDANT LA REGENERATION  
CHEZ LES SALAMANDRES**  
*POSITIONAL IDENTITY OF ADULT LIMB STEM CELLS DURING REGENERATION IN SALAMANDERS*

**Jeremy P. BROCKES**

Department of Biochemistry, University College London,  
London, UK

Limb regeneration in salamanders proceeds by formation of a blastema, a growth zone of mesenchymal stem cells at the end of the stump. Blastemal cells are derived in part by de-differentiation from mesenchymal cells, including myofibres, at the plane of amputation, and they derive critical cues about their identity and potentiality from their precursors. The cells derived after amputation at any level on the proximodistal axis give rise precisely to the structures distal to their level of origin; wrist cells regenerate a hand, shoulder cells an arm. This property is stably expressed by blastemas transplanted to the fin or eye and is sometimes called positional memory. An understanding of its molecular basis is important for our appreciation of how stem cells are specified to give rise to different structures, as opposed to different cell types. A variety of experiments in which blastemal cells of different PD level are confronted have led to the view that positional identity is encoded at the cell surface, possibly as a graded level of expression along the axis. Retinoic acid (RA) as well as precursor or synthetic retinoids are able to respecify distal cells to a more proximal identity, and this respecification occurs continuously over a 2.5 fold range of retinoid concentration, suggesting that the differences in gene expression that underly PD identity may be relatively small.

These considerations led to the identification of Prod1, a gene whose expression in normal and regenerating newt limbs is regulated by PD location and by RA, such that RA upregulates and P>D (both in a blastema and normal limb, and both at RNA and protein levels). Prod1 is a small protein linked to the cell surface by a GPI glycolipid anchor and is apparently the newt ortholog of the mammalian protein CD59, as evidenced by the recent determination of its 3D structure by NMR. Compelling evidence for its relevance to PD identity has come from the laboratory of E. Tanaka (MPI, Dresden) by electroporating a Prod1/CD59 expression vector into distal cells of the blastema in a larval axolotl and converting them to proximal cells. We have identified a secreted protein which is apparently a ligand for Prod1/CD59 and I will discuss recent evidence about how this system operates to regulate positional identity.

**References**

- 1) S Morais da Silva, PB Gates & JP Brockes, The newt ortholog of CD59 is implicated in proximodistal identity during amphibian limb regeneration, *Dev Cell* 3, 547-555 (2002)
- 2) K Echeverri & EM Tanaka, Proximodistal patterning during limb regeneration, *Dev Biol.* 279, 391-401 (2005)
- 3) JP Brockes & A Kumar, Appendage regeneration in adult vertebrates and implications for regenerative medicine, *Science* 310, 1919-1923 (2005)

**L'HYDRE, UNE NICHE POUR LA PLASTICITE CELLULAIRE ET DEVELOPPEMENTALE**  
*HYDRA, A NICHE FOR CELL AND DEVELOPMENTAL PLASTICITY*

**Brigitte GALLIOT, Marijana MILJKOVIC-LICINA and Simona CHERA**  
Department of Zoology and Animal Biology, University of Geneva,  
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The freshwater hydra polyp is a classical model system to investigate the cellular and molecular basis of regeneration. Like all cnidarian polyps, hydra displays a tube shape with a single opening circled by a ring of tentacles, which has a mouth-anus function, whereas the basal disk attaches to the substratum. Hence cnidarian polyps, which are formed of two cell layers, display an oral-aboral polarity, with differentiated tissues and/or structures at the extremities but no organs as recognized in bilaterians. The ectodermal myoepithelial cell lineage provides the outer cell layer, while the endodermal myoepithelial cells together with the gland cells line up the gastric cavity. A third distinct pool of cells, the interstitial cells, are common stem cells for the somatic and germinal cell lineages including neurons, mechanoreceptor cells (nematocytes), gland cells and gametes. As a result, hydra tissues differentiate from three distinct stem cell populations: the interstitial stem cells, which are multipotent fast-cycling stem cells, uniformly distributed along the body column but absent from the head and foot regions, and the epithelial cells, either ectodermal or endodermal, also continuously cycling in the body column, although at a slower pace, and getting displaced towards the extremities where they terminally differentiate (see in ref.1). Beside this dynamic maintenance of patterning, hydra polyps display budding and regenerative capacities whatever their age. Surprisingly, hydra can regenerate their head in the absence of cell proliferation as well as in the absence of neurogenesis. These observations led to the assumption that hydra regeneration occurs through cellular processes that are distinct from those characterized in bilaterian species, as planarians and urodeles.

The recent establishment of gene silencing through RNA interference upon feeding opens avenues to decipher the molecular control of such plasticity in hydra (2). Recent studies identified cell-specific and stage-specific genes involved in the molecular control of head regeneration after amputation. Immediately post-amputation, the serine protease inhibitor *Kazal1* gene produced by the gland cells prevents an excessive autophagy in regenerating tips (2). This cytoprotective function, or *self-preservation*, is similar to that played by *Kazal*-type genes in the mammalian exocrine pancreas, either during development or after injury, likely reflecting an evolutionarily conserved mechanism linking cell survival to cell plasticity (2, 3). Indeed, in wild-type hydra, within the first hours post-amputation, the endodermal epithelial cells located in the head-regenerating tips, which normally carry out the digestive function, transiently dedifferentiate into blastema-like cells that phagocytose surrounding apoptotic cells. Soon after, adjacent interstitial stem cells enter mitosis. The activation of the MAPK pathway, which leads to the RSK-dependent CREB phosphorylation, is required for these early cellular events (4, SC et al., submitted). Later, at the early-late stage, prior any apical morphological change can be detected, the induction of the *Gsx/cnox-2* ParaHox gene in the ectodermal layer leads to the proliferation of apical neuronal progenitors, which is required for head patterning (MM-L et al., submitted).

Hence head regeneration in wild-type hydra relies on spatially restricted and timely orchestrated cellular modifications, i.e. cell dedifferentiation, cell proliferation and neurogenesis. These modifications display obvious similarities with those reported during vertebrate epimorphic regeneration, raising the possibility that evolutionarily distant animals share some conserved genetic circuitry to achieve the post-amputation reactivation of their developmental programs.

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**LA FONCTION, LA REGULATION ET LE MAINTIEN CHEZ LES CELLULES SOUCHES SOMATIQUES DE  
L'ADULTE : UN MODELE SIMPLE POUR UN PROBLEME COMPLEXE**  
*THE FUNCTION, REGULATION AND MAINTENANCE OF ADULT SOMATIC STEM CELLS:  
A SIMPLE MODEL FOR A COMPLEX PROBLEM*

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It is paradoxical that for many organisms (including humans), the apparent anatomical stability of their adult bodies is maintained by constant change. Under normal physiological conditions, the functions of many organs depend on the continuous destruction and renewal of their cells. For example, it has been estimated that in a year each of us produce and, in parallel, eradicate a mass of cells that is equal to almost our entire body weight. Equally remarkable is the fact that the adult tissues and organs of many organisms can be fully restored after being subjected to traumatic situations such as amputation. Hence, metazoans have evolved a series of renewal and repair mechanisms to respond to both trauma and normal wear and tear, and these mechanisms are under tight regulatory control to maintain the form and function of metazoans. As important as these mechanisms are to the survival of multicellular organisms and the obvious relevance to regenerative medicine, we know very little about how these processes are effected and regulated at the cellular and molecular levels. What is becoming exceedingly clear is that populations of adult somatic stem cells appear to play key roles in these processes. Here, I will discuss how the study of a simple metazoan, the planarian *Schmidtea mediterranea*, is beginning to shed light on the way adult somatic stem cells are regulated in animals to maintain tissue homeostasis and to replace missing body parts lost to injury.

**RENOUVELLEMENT DES EPITHELIUMS STRATIFIES**  
*RENEWAL OF STRATIFIED EPITHELIA*

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Stratified epithelia protect the body from environmental hazards. They self renew and contain stem cells that are distributed throughout the basal layer of the epithelium. Each stem cell is normally renewing a limited portion of the epithelium, which appears as an assembly of functionally independent columnar units. It is thought that stem cells of stratified epithelia divide infrequently and asymmetrically to generate daughter stem cells and transient amplifying cells with limited potential. The latter cells then divide actively to compensate for the differentiated cells that are sloughed off from the epithelial surface. Among stratified epithelia, the corneal epithelium appears as an exception because it is supposedly renewed by migration of transient amplifying cells generated by stem cells located at the limbus, i.e. at the junction of the conjunctiva and the cornea. Using clonal and functional analysis, we demonstrate that mammalian cornea can self renew in complete absence of limbal stem cells and that the cornea epithelium can be serially transplanted for months. Moreover we demonstrate that corneal stem cells are multipotent and can generate conjunctival cells if provided with a proper microenvironment. We propose that the fate of ocular stem cells, i.e. corneal or conjunctival, depends on strict interactions with the underlying stroma.

## ÉMERGENCE DES CELLULES SOUCHES HEMATOPOÏÉTIQUES PENDANT LE DEVELOPPEMENT

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Des cellules souches hématopoïétiques (CSH) auto-renouvelables sont supposées se déposer pendant dans le développement dans une réserve, d'où elles se mobilisent au fur et à mesure des besoins physiologiques. Les organes, où a lieu l'hématopoïèse définitive, doivent être colonisés par des CSH extrinsèques émanant d'une source centrale. L'émission de CSH se produit plus ou moins continûment pendant une certaine période du développement dans des sites parallèles ou successifs.

Le plus récemment découvert de ces sites est le placenta. L'allantoïde (qui contribue au placenta), testé avant vascularisation, se révèle un site de détermination de progéniteurs clonogéniques. Le placenta est donc peut-être un site d'hématopoïèse. Jusqu'à présent l'aorte et les tissus périaortiques sont considérés comme le site de détermination des CSH définitives.

Les relations de développement entre lignages hématopoïétique et endothélial et les expressions moléculaires spécifiques ont été analysées dans le modèle Oiseau. L'échange de somites entre caille et poulet a révélé l'existence de deux lignages distincts ; l'un est dorsal et purement endothélial ; l'autre, ventral, constitue le revêtement endothélial primitif du plancher de l'aorte, puis émet des cellules hématopoïétiques et disparaît, étant remplacé par des cellules d'origine somitique qui s'installent à sa place. Le processus hématopoïétique et la disparition des cellules endothéliales impliquées sont précédés d'une évolution moléculaire mettant notamment en jeu le facteur de transcription Runx1 et le récepteur VEGF-R2.

Un dernier aspect intéressant est que la colonisation de la moelle osseuse est accomplie, au moins en partie, par des CSH circulantes.

### ***EMERGENCE OF HEMATOPOIETIC STEM CELLS DURING DEVELOPMENT***

Self renewable hematopoietic stem cells (HSC) are supposed to become deposited during development into a finite pool, from which they are mobilized upon physiological requirement. A central feature is that the organs, where definitive hematopoiesis occurs become colonized by HSC originating from a central source. Various experimental schemes uncovered that the emission of HSC occurs more or less continuously during a protracted period in parallel or successive sites. The most recently discovered of these sites is the placenta. The allantois (part of the placenta), probed before it becomes vascularized, turns out to be a location where clonogenic progenitors become committed. The placenta may thus be a site of intrinsic hematopoiesis. Until this finding the aorta and periaortic tissues were held to be the site of definitive HSC commitment. In the chicken embryo, the hematopoietic process in the aorta is prominent and displays striking anatomical features. Together with the experimental approaches possible in the avian embryo model, this feature has made it possible to investigate the cytological and molecular relationship between the endothelial and hematopoietic cells. Somite exchanges between quail and chicken have disclosed two distinct lineages: a dorsal one, which is purely endothelial; a ventral one, which builds at first the floor of the aorta, then emits hematopoietic cells and disappear, being replaced by endothelial cells of somitic origin which migrate around the aortic wall. The hematopoietic process and the disappearance of the endothelial cells involve a changing molecular pattern, within which the expressions of transcription factor Runx1 and receptor VEGF-R2 are faithful markers of the lineage transformation.

Another noticeable feature of the hematopoietic system in development is that colonization of the bone marrow niche is achieved, at least partly, by circulating HSC.

Thus the analysis of developmental hematopoiesis uncovers a number of features that can still be observed later in life and may bring about ways of dissecting the mechanisms of HSC commitment.

**INTEGRER DE NOUVEAUX NEURONES DANS LE SYSTEME OLFACTIF DE L'ADULTE**  
*INTEGRATING NEW NEURONS INTO THE ADULT OLFACTORY SYSTEM*

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In the adult olfactory bulb, newly born neurons are constitutively generated throughout life and form an integral part of normal functional circuitry. This process of late neurogenesis is subject, at various stages, to modulation and control by external influences, suggesting strongly that it represents a plastic mechanism by which the brain's performance can be optimized according to the environment in which it finds itself. But optimized how? And why?

This presentation will concentrate on such functional questions regarding neurogenic plasticity. After outlining the processes of adult neurogenesis in the olfactory system, and after discussing their regulation by internal and environmental influences, we shall ask how existing neuronal circuits can continue to work in the face of constant cell arrivals and departures, and explore the possible functional roles that newborn neurons might subserve in the adult olfactory system. In particular, we shall report the degree of sensitivity of the bulbar neurogenesis to the level of sensory inputs and, in turn, how the adult neurogenesis adjusts the neural network functioning to optimize sensory information processing. We will bring together recently described properties and emerging principles of adult neurogenesis that support a much more complex role for the adult-generated cells than just providers of replaceable units. Throughout, and concentrating exclusively on mammalian systems, we shall stress that adult neurogenesis constitutes another weapon in the brain's armory for dealing with a constantly changing world.

**PROGENITEURS ET CELLULES SOUCHES DE LA CRETE NEURALE**  
*NEURAL CREST PROGENITORS AND STEM CELLS*

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In the vertebrate embryo, multiple cell types originate from a common structure, the neural crest, which forms at the dorsal tips of the neuroepithelium. The neural crest gives rise to migratory cells that colonise a wide range of embryonic tissues and later differentiate into neurones and glial cells of the peripheral nervous system, pigment cells (melanocytes) in the skin and endocrine cells in the adrenal and thyroid glands. In the head and neck, the neural crest also yields mesenchymal cells that form craniofacial cartilages, bones, dermis, adipose tissue and vascular smooth muscle cells. The neural crest is therefore a model system to study cell diversification during embryogenesis and phenotype maintenance in the adult.

By analysing the developmental potentials of quail neural crest cells in clonal cultures, we have shown that the migratory neural crest is a collection of heterogeneous progenitors, including various types of intermediate precursors and highly multipotent cells, some of which being endowed of self-renewal capacity. We also have identified common progenitors for mesenchymal derivatives and neural/melanocytic cells in the cephalic neural crest. These results are consistent with a hierarchical model of lineage segregation wherein environmental cytokines control the fate of progenitors and stem cells. One of these cytokines, endothelin-3 peptide, promotes the survival, proliferation and self-renewal capacity of common progenitors for glial cells and melanocytes. At post-migratory stages, when they have already differentiated, neural crest-derived cells can exhibit phenotypic plasticity. Epidermal pigment cells and Schwann cells from peripheral nerves in single cell culture are able to reverse into multipotent neural crest-like progenitors endowed with self-renewal.

Therefore, stem cell properties are expressed by a variety of neural crest progenitors and can be reacquired by differentiated cells of neural crest origin, suggesting potential function for repair.

**CELLULES PROGENITRICES DU MUSCLE SQUELETTIQUE**  
*SKELETAL MUSCLE PROGENITOR CELLS AND THE ROLE OF PAX GENES*

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Satellite cells, which are defined by their position under the basal lamina of muscle fibres, participate in the repair of adult skeletal muscle. However the extent to which they constitute the major stem cell population for muscle regeneration has been challenged by claims that cells from other sources, such as bone marrow, play this role. Satellite cells are marked by the expression of *Pax7* and also, in many skeletal muscles, by transcription of the *Pax7* paralogue, *Pax3* (1). The development of a *Pax3<sup>GFP/+</sup>* mouse line permitted the isolation by flow cytometry of satellite cells, which could then be examined as a pure population, both in tissue culture and after grafting into injured skeletal muscle *in vivo*. These cells are myogenic; they contribute very efficiently to skeletal muscle regeneration and also self-renew, thus demonstrating their role as muscle stem cells (2). *Pax3/7* play a critical role in the designation of muscle stem cells (1). These *Pax* genes regulate the entry of a cell into the programme of skeletal muscle differentiation. In the adult, they are required for the activation of the myogenic determination gene, *MyoD*. *Pax7* is also essential for the survival of satellite cells. This dual role underlines the importance of ensuring that a tissue stem cell that has lost its myogenic instruction, should not be left to run amok, with the potential risk of tissue deregulation and cancer.

Earlier embryological experiments had suggested that satellite cells derive from the paraxial mesoderm of the somites. This view is now reinforced by the demonstration that a somite-derived population of *Pax3/Pax7* positive cells is responsible for muscle growth during development and gives rise to the satellite cells of postnatal muscles. In the absence of both *Pax3* and *Pax7*, these cells die or assume other cell fates, leading to a major muscle deficit by early fetal stages (3). In this muscle progenitor cell population *Pax3/7* lie genetically upstream of both *MyoD* and *Myf5* and it is the activation of these myogenic determination genes that decides the skeletal muscle fate of these cells.

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**PHYLOGENESE ET ONTOGENESE DU PANCREAS**  
**EN RELATION AVEC LES CELLULES SOUCHES PANCREATIQUES**  
*PANCREAS PHYLOGENY AND ONTOGENY IN RELATION TO A "PANCREATIC STEM CELL"*

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Blood glucose regulation has likely evolved hand in hand with vertebrate evolution to allow complex brain and nervous system evolution: "an inner milieu of constant blood glucose levels through millions of years has allowed the brain an extra degree of freedom to evolve without having to *think* of getting energy supply".

The pancreatic islet beta cell is uniquely specified to produce and administer insulin to the blood circulation in response to glucose levels. The islet beta cell thus continuously monitors glucose levels and a glucose-increase following food intake is quickly counter-acted by increased insulin release – and consequently insulin-induced glucose uptake in peripheral tissues. Functional beta-cell deficiency (and thereby insulin deficiency) is the hallmark of diabetes (T1 vs. T2 diabetes are characterized by a complete vs relative deficiency of a functional beta cell mass). Insulin deficiency cause hyperglycemia and diabetes. Long term elevations of blood glucose leads to damaging glycosylation reactions eventually causing devastating diabetes-late-complications. During fasting, glucose levels are maintained via glucagon action where low glucose stimulates glucagon release from the pancreatic islet alpha cell which in turn stimulates glucose production from the liver. Brain tissue cannot survive prolonged hypoglycaemia and unregulated excess insulin release from e.g. even a small benign insulinoma may cause lethal hyperinsulinemia-induced hypoglycaemia.

Interestingly, work pioneered by Falkmer and others indicate that pancreas phylogeny was pioneered by the insulin producing beta cell. In the hagfish and lampreys (our most primitive vertebrate species of today) the first sign of 'a new organ' is found as collections of endocrine cells around the area of the bile duct connection with the duodenum. These endocrine organs are composed of 99% beta cells and 1 % somatostatin producing delta-cells. Compared to the more primitive protochordates (e.g. amphioxus) this represents a stage where all previously scattered insulin producing cells of the intestinal tissue have now quantitatively migrated to found a new organ involved in sensing blood-glucose rather than gut-glucose. Only later in evolution the beta-cells are joined by exocrine tissue and alpha-cells (exemplified by the rat-, rabbit- and elephant-fishes). Finally, from sharks and on we have also the islet PP-cell entering. Hagfish and lampreys may have one or more endocrine buds – and later the vertebrate pancreas develop as independent ventral and dorsal buds which eventually fuse to become one organ. In the bony fish, a giant islet – known as the 'Brockman-body' is derived of dorsal origin, while ventral bud may give rise to acinar cells, ducts and smaller islets.

Interestingly, amphioxus Pdx-1 expression is already narrowed to a confined region of the gut. The presence of Pdx-1 may have been instrumental for the subsequent evolutionary accumulation of beta cells in this region – as well as for the elaborate involvement of Pdx-1 as a beta-cell specific transcriptional regulator of glucose responsive genes. Lack of Pdx-1 in vertebrates cause pancreas agenesis. Pdx-1 and Nkx6.1 are both transcription factors with a restricted expression within the mature pancreatic beta-cell. In fact, the co-expression of those two markers in adult islets specifically identifies the beta-cell subpopulation. During pancreas ontogeny Pdx-1 is expressed within the early budding tissue as well as in the duodenum and antral stomach (albeit at lower levels). Again the co-expression of Pdx-1 and Nkx6.1 selectively specifies all of the early pancreatic progenitors found in the buds of ventral and dorsal origin.

It may thus be speculated that the early Pdx-1+/Nkx6.1+ cells of the ontogenic pancreas reflects the phylogenetically first appearing beta-cells in this region – and that these progenitors subsequently have adopted a wider differentiation potential to cover additional pancreatic cell types (reflecting their subsequent appearance during phylogeny). It may be deduced that the early ontogenic Pdx-1+/Nkx6.1+ pancreatic epithelial cells may constitute a source of progenitor cells carrying a phylogenetically imprinted pre-programming favouring beta-cell formation.

## APPLICATION DE LA THERAPIE CELLULAIRE AUX MALADIES LYMPHOHEMATOPOÏÉTIQUES

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Le traitement de référence des pathologies héréditaires du système lymphohématopoïétique et de certaines maladies en métaboliques consistent en la greffe des cellules souches hématopoïétiques (CSH) dont la première a été réalisée en 1968. Actuellement, une greffe de CSH réalisée à partir d'un donneur HLA génoidentique confère une probabilité de survie à long terme autour de 80% pour l'ensemble de ces pathologies. En l'absence d'un tel donneur le recours à un donneur familial HLA partiellement compatible confère une probabilité de survie à 5 ans aux alentours de 50%. La mortalité est dans ce cas fortement influencée par l'âge au moment de la greffe, le diagnostic, la présence de complications infectieuses et le degré de disparité entre donneur et receveur. Pour essayer d'améliorer ces résultats nous essayons de mettre au point des nouveaux protocoles, d'une part, d'immunothérapie adoptive et d'autre part de thérapie génique. Les protocoles innovants d'immunothérapie adoptive visent à accélérer la reconstitution immunitaire. En particulier, la description récente des étapes précoces de la différenciation lymphocytaire T laisse entrevoir des nouvelles pistes thérapeutiques. L'utilisation des ligands des Notch, capables d'engager un précurseur immature dans la voie T, semble très intéressant pour ce type de greffe HLA partiellement compatible.

La thérapie génique constitue un moyen puissant d'introduire une copie normale du gène déficitaire dans les CSH de type adulte qui peuvent être, après transduction, réinjectées sous forme d'une autogreffe aux patients. L'efficacité de cet approche de thérapie génique a été récemment documenté pour deux formes différentes de Déficit Immunitaires Combinés Sévères (DICS-X1 et Déficit en Adénosine Désaminase) et pour la Granulomatose Chronique. Un certain nombre d'obstacles reste cependant à résoudre ; la mutagenèse insertionnelle et le ciblage de la CSH sont deux obstacles majeurs. La résolution de ces obstacles pourraient permettre une plus large application clinique de cet approche qui concentre les efforts de nombreux chercheurs et cliniciens. Les résultats cliniques obtenus et les effets secondaires dus à la mutagenèse insertionnelle seront discutés en détail pendant la présentation.

### ***APPLICATIONS OF CELL THERAPY TO THE DISEASES OF THE LYMPHOHAEMATOPOIETIC SYSTEM***

The treatment of choice of the inherited diseases of the lymphohaematopoietic system and of certain metabolic diseases consist in the transplantation of haematopoietic stem cells (HSC), for which the first one was done in 1968. Presently, an allogenic HSC transplantation from an HLA-genoidentical donor confers to a long-term survival probability of around 80% for the whole of these diseases. In the absence of such a donor, the recourse towards a HLA partially compatible familial donor confers to a survival probability of 50%. The mortality is in this case strongly influenced by the age at the time of the transplantation, the diagnosis, the presence of infectious complications and the degree of HLA disparity between the donor and the recipient. In order to try to improve these clinical results, we are trying to develop protocols of adoptive immunotherapy, on one hand, and of gene therapy, on the other. The protocols of adoptive immunotherapy are aimed to accelerate the immune reconstitution. In particular, the recent description of the precise steps characterising the lymphoid T differentiation pathway allows one to foresee new therapeutic leads. The use of Notch ligands, capable of committing an immature precursor into the T cell pathway, seems very interesting for this type of HLA partially compatible transplantation.

Gene therapy constitutes a powerful way to introduce a normal copy of the deficient gene in the adult haematopoietic stem cells which can, after transduction, be re-injected in the form of auto-transplantation to the patients. The effectiveness of this gene therapy approach was recently documented for two different forms of Severe Combined Immune Deficiency (SCID-X1 and Adenosine Deaminase Deficiency) and for Chronic Granulomatosis Disease. However, a certain number of obstacles remain to be resolved: insertional mutagenesis and the targeting of the haematopoietic stem cells, representing two very important issues. The resolution of these issues could allow a larger clinical application to this approach which concentrates the efforts of numerous researchers and clinicians. The observed clinical results and the severe adverse events due to insertional mutagenesis will be discussed in detail during the presentation.

**THERAPIE CELLULAIRE DE LESIONS ISCHEMIQUES GRAVES DU MYOCARDE**  
*CELL THERAPY OF SEVERE ISCHAEMIC HEART FAILURE*

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Patients with severe heart failure consequent to extensive chronic ischaemic lesions are at high risk. Traditional therapy is only palliative, in many cases the coronary vascular bed has already suffered surgical interventions, and patients can not be submitted to further angioplasty or vascular surgery. The heart transplantation, which is often the only therapeutic proposal, is hampered by shortage of donors, high risk, and cost of complex surgical procedures and of post-surgical complications. Cell therapies have opened new proposals, since in several experimental models improvements of the damaged heart function and of the clinical state have been observed and documented. From the beginning of this decade on, clinical studies were done by several groups, introducing bone marrow mononuclear cells by transepiscardic injection during surgery for revascularization of myocardium, or using the intracoronary route in patients with chronic ischaemic cardiomyopathy. Improvements of heart functions, of global and segmental ventricular contractibility and of the general patients' clinical state have been reported.

In the same time, we have undertaken a phase I-II clinical trial using transendocardial injection of autologous bone marrow-derived mononuclear cells (14 patients, 7 controls). After two months, the treated patients had significantly less heart failure and angina, and after four months they had a sustained significant improvement of pumping power, exercise capacity, cardiac muscle irrigation and blood supply to the body. The patients continued improving for twelve months, and the overall maintenance of their state was confirmed 24 and 48 months after the therapy. Five of the studied patients had been listed for cardiac transplantation. After 6 months, their clinical improvement was such that they were no longer eligible for transplantation. Since the transendocardial cell injection was preceded by electrical and mechanical mapping of the heart muscle, which was repeated 4 months after the therapy, we could locate precisely the improvement of heart muscle functions. We found an increase of the unipolar voltage in regions surrounding the cell injections region, potentially indicating an increased number of viable cardiomyocytes, as well as an increase of segments with normal function as observed by echocardiography. One of the patients deceased 11 months after treatment of a stroke, and anatomopathological study could be done. It showed a significant increase in blood vessel density in the cell-injected regions, hyperplasia of pericapillary pericytes and mural cells, their migration into the adjacent tissue and progressive induction of the synthesis of cardiomyocyte-specific cytoskeletal proteins in the vicinity of pre-existing cardiomyocytes. These findings are suggestive of neoangiogenesis, and of a possible trans-differentiation of perivascular cells into cardiomyocytes.

In a further series of 5 patients, we found that the total number of cells correlated with the overall heart function improvement. We also found the upper limits of the number of injected cells and of transendocardial injections that can be safely done. Therefore, we examined the possibility to proceed to positive and/or negative selection of cells, by a back-cross study of the relative content of different cell phenotypes in the injected cell populations and its correlation with different parameters of the patients' cardiac improvements. We found a negative correlation of several parameters with CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes, CD19<sup>+</sup> B-lymphocytes and CD56<sup>+</sup> NK cells, and positive correlation with CD14<sup>+</sup> monocytes, CD34<sup>+</sup> cells, and mesenchymal progenitor cells (CFU-F). While this information may bring practical improvements in cardiac muscle cell therapies, we can at present only propose hypotheses on the cellular and molecular mechanisms potentially involved in the repair and regeneration of the heart muscle severely damaged by chronic ischaemia.

## **THERAPIE CELLULAIRE EN CARDIOLOGIE**

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Visant à redonner une fonctionnalité à des territoires myocardiques devenus akinétiques à la suite d'un infarctus, la thérapie cellulaire en cardiologie est déjà une réalité clinique comme l'atteste le nombre d'essais actuellement en cours. Ces études concernent soit les cellules musculaires squelettiques chez des patients présentant une dysfonction ventriculaire gauche ischémique sévère, soit des cellules médullaires plus électivement réservées à des patients vus au stade aigu de l'infarctus, et bénéficiant dans le territoire greffé d'une revascularisation complémentaire par une technique interventionnelle. Les procédés de préparation, expansion et conservation des cellules musculaires sont aujourd'hui parfaitement opérationnels et permettent d'obtenir à partir d'une petite biopsie musculaire des quantités importantes de cellules myogéniques viables. Le problème est un peu différent dans le cas de la moelle car dans la plupart des procédures, le geste est limité à une biopsie de la crête iliaque suivie d'une réinjection presque immédiate de la moelle non fractionnée, la technique utilisée étant alors celle qui a été validée par des années d'expérience clinique en hématologie. Il est toutefois également possible de sélectionner plus spécifiquement des populations de progéniteurs CD 34+ ou CD 133+ à l'aide de dispositifs approuvés par les autorités réglementaires pour un usage humain. Si, globalement, ces techniques apparaissent bien tolérées, la question de leur efficacité reste incertaine. En effet, l'enthousiasme généré par les premiers essais de phase I doivent aujourd'hui être relativisés à la lumière des résultats plus mitigés des études randomisées récemment publiées. Ces études ont cependant déjà le mérite d'avoir permis d'identifier au moins deux problèmes essentiels (l'efficacité modeste du transfert des cellules dans le tissu cardiaque et le taux élevé de leur mortalité post-greffe) dont la solution paraît un pré-requis pour que le bénéfice potentiel de la thérapie cellulaire soit optimisé. Par ailleurs, il est maintenant acquis que la plasticité des cellules adultes est sans doute beaucoup plus limitée que ce que l'on pensait et que leur capacité à donner naissance à de nouvelles cellules cardiaques permettant une véritable régénération myocardique reste à démontrer. En fait, cela a toujours été clair pour les myoblastes dont la différenciation purement myogénique est établie de longue date. Le débat est plus controversé en matière de cellules de la moelle mais l'utilisation de techniques d'identification rigoureuses reposant principalement sur des marqueurs génétiques, a permis de constater que les événements interprétés comme transdifférenciation en cardiomyocytes correspondaient en réalité à des fusions voire à des artefacts méthodologiques. Ces remarques n'impliquent par pour autant que la thérapie cellulaire n'ait pas d'effets fonctionnels qui peuvent procéder de mécanismes alternatifs tels la limitation du remodelage ventriculaire ou des effets paracrines conduisant à une modification de la composition de la matrice extra-cellulaire, à une stimulation de l'angiogenèse et peut-être même au recrutement de cellules souches cardiaques. Toutefois, ni les cellules de la moelle ni les cellules musculaires ne remplissent le pré-requis à une véritable régénération cardiaque : un couplage électrique des cellules greffées avec les cardiomyocytes du receveur aboutissant à la formation d'un syncytium et permettant au greffon de se contracter de façon synchrone avec le cœur receveur et donc de contribuer efficacement à améliorer sa fonction contractile. Il est donc important de continuer à explorer d'autres pistes au sein desquelles les cellules souches embryonnaires tiennent une place dominante. Il est en effet bien établi que ces cellules, correctement pré-programmées vers une lignée cardiomyogénique, peuvent se différencier en véritables cardiomyocytes après implantation dans des zones d'infarctus et améliorer la fonction ventriculaire gauche. Bien que les problèmes à régler avant d'éventuelles utilisations cliniques restent nombreux, les cellules souches embryonnaires offrent aujourd'hui de réels espoirs en matière de régénération du myocarde.

### ***CELL THERAPY IN CARDIOLOGY***

Targeted at restoring function of postinfarction myocardial akinetic scars, cell therapy is already a clinical reality as demonstrated by the number of ongoing trials. These trials involve either skeletal myoblasts in patients with chronic left ventricular dysfunction or bone marrow-derived cells which have been more electively used in patients with acute myocardial infarction undergoing concomitant percutaneous revascularization by angioplasty and stenting. Techniques of culture, expansion and storage of muscular cells are now well established and allow to grow large numbers of viable myogenic cells from a small muscular biopsy. The problem is slightly different in the case of bone marrow cells which are usually

harvested by an iliac crest biopsy and almost immediately reinjected in the coronary artery, without any processing or purification (except for centrifugation to remove red blood cells), according to procedures validated in clinical haematology. However, it is also possible to select purified populations of progenitors (CD34+, CD133+) by using devices which are approved by the regulatory authorities for human use. If, overall, the safety of these procedures seems established, their efficacy remains uncertain. Indeed, the enthusiasm generated by the earliest reports of the phase I studies has now been tempered by the less successful outcomes of the recently published randomised controlled phase II trials. At least, these studies have the merit to have highlighted two major issues (the modest efficiency of cell transfer and the high rate of posttransplantation cell death) which need definitely to be addressed if the functional benefits of cell therapy are to be optimally exploited. Furthermore, it is now increasingly recognized that the plasticity of adult somatic cells is likely to be much more limited than initially thought and that their capacity to give rise to new cardiomyocytes allowing a true myocardial regeneration is still elusive. Indeed, this has been demonstrated from the onset in the case of myogenic cells which are known to remain strictly lineage-restricted. The debate is more controversial in the case of bone marrow-derived cells although techniques of cell tracking based on reliable genetic tags have provided growing evidence that most of the so-called transdifferentiation events have probably been mistakenly interpreted and actually corresponded to fusion or methodological artefacts. These observations do not imply that cell therapy cannot be functionally effective through alternate mechanisms like limitation of remodelling or paracrine signalling acting on the extracellular matrix, angiogenesis or even recruitment of endogenous cardiac stem cells. Whatsoever, neither skeletal myoblasts nor bone marrow-derived cells fulfill the criteria required for a true myocardial regeneration, i.e., an electrical coupling between donor and recipient cells leading to the formation of a syncytium and allowing the graft to beat in synchrony with the remainder of the heart and, thus, to effectively contribute to improve its pump function. It is therefore mandatory to continue to explore other paths among which embryonic stem cells play a key role. It is now well established that if appropriately precommitted towards a cardiac lineage, these cells can differentiate into cardiomyocytes following engraftment into postinfarction scars and improve left ventricular function. Although the clinical use of these cells is still associated with several hurdles, they really raise serious hopes that their use might provide an effective means of repairing diseased myocardial tissue.

**APPLICATION DE LA THERAPIE CELLULAIRE A LA MALADIE DE PARKINSON**  
*STEM CELL THERAPY FOR PARKINSON'S DISEASE*

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Clinical trials in patients with Parkinson's disease (PD) have provided proof-of-principle that fetal dopamine neurons can survive and function in the brains of PD patients, restore striatal dopamine release, and ameliorate impairments in motor behavior. The clinical outcome, however, has so far been highly variable and further developments are needed in order to turn the cell replacement approach into a clinically useful and competitive therapy. Until now, the cells used for transplantation has been obtained from developing mesencephalic tissue obtained from aborted fetuses at the stage of dopamine neuron development that is optimal for survival and growth of the grafted cells. The ethical and practical problems associated with the use of human fetal tissue, and the very limited access to this tissue material, is today the most serious obstacle to further developments of the cell replacement approach. For further progress in this field, therefore, it is essential to find alternative sources of dopamine neurons or dopamine neuron progenitors for transplantation based on stem cell technology and in vitro cell expansion techniques. The most promising results so far have been obtained using embryonic stem (ES) cells as starting material: cell engineering, combined with in vitro expansion and cell enrichment techniques, have made it possible to generate transplantable dopamine neurons in large numbers, and the recent identification of genes that serve as intrinsic determinants of midbrain dopamine neuron development (Andersson et al, Cell 124:393, 2006) have opened new possibilities to drive the development of undifferentiated ES cells toward a mesencephalic dopamine neuron phenotype that can survive, grow and function after transplantation to the striatum in animals with experimental PD.

**LES CELLULES SOUCHES PARAISSENT EXERCER UNE PRESSION HOMEOSTATIQUE AU NIVEAU DU CNS  
DANS LE CAS DE LÉSIONS DE LA MOELLE ÉPINIÈRE PAR TRANSPLANTATION**  
*STEM CELLS APPEAR TO EXERT HOMEOSTATIC PRESSURE  
IN DEGENERATIVE OR INJURED CNS ENVIRONMENTS*

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An intriguing phenomena with possible therapeutic dividends has begun to emerge from our observations of the behavior of neural stem cell (NSC) clones in various mouse and primate models of CNS injury & degeneration. During phases of active neurodegeneration, factors seem to be transiently elaborated to which NSCs may respond by migrating (even long distances) to degenerating regions & attempting to restore homeostasis. This may include differentiating towards the replacement of degenerating neural cells of multiple types, not only neurons but also requisite non-neuronal "chaperone" cells, all of which are essential for the proper development and reconstitution of function. These "repair mechanism" may reflect the re-expression of basic developmental programs (particularly during temporal "windows" following injury) that may be harnessed for therapeutic ends. There is an enormous amount of "programmed" cross-talk between stem cells and the milieu that add complexity but also enrich therapeutic promise to the system. In addition, NSCs in their native state (as well as following genetic-engineering) may serve as vehicles for protein delivery allowing for the possibility of simultaneous cell replacement & gene therapy (e.g., with factors that might enhance differentiation, neurite outgrowth, connectivity, neuroprotection, anti-inflammation, anti-scarring, and angiogenesis). For example, multiple model approaches to most neurological conditions are likely required. The stem cell may serve as the "glue" for these. When combined with certain synthetic biomaterials, NSCs may be even more effective in "engineering" the damaged CNS towards reconstitution. Not only gene expression programs, but also an epigenetic chromatin modification programs seem critical for dictating plasticity and potency.

**REPARATION DE LA MOELLE EPINIÈRE LESEE PAR LA TRANSPLANTATION  
DE CELLULES OLFACTIVES ENGAINANTES**  
*REPAIR OF SPINAL CORD INJURY BY TRANSPLANTATION OF OLFACTORY ENSHEATHING CELLS*

**Geoffrey RAISMAN**  
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Transplantation of cultured adult olfactory ensheathing cells has been shown to induce anatomical and functional repair of lesions of the adult spinal cord and spinal roots. Histological analysis of olfactory ensheathing cells, both in their normal location in the olfactory nerves and also after transplantation into spinal cord lesions, shows that they provide channels for the growth of regenerating nerve fibres. These channels have an outer, basal lamina-lined, surface apposed by fibroblasts, and an inner, naked surface in contact with the nerve fibres. A crucial property of olfactory ensheathing cells, in which they differ from Schwann cells, is their superior ability to interact with astrocytes. When confronted with olfactory ensheathing cells the superficial astrocytic processes, which form the glial scar after lesions, change their configuration so that their outer pial surfaces are reflected in continuity with the outer surfaces of the olfactory ensheathing cells. The effect is to open a door into the central nervous system. We propose that this formation of a bridging pathway may be the crucial event by which transplanted olfactory ensheathing cells allow the innate growth capacity of severed adult axons to be translated into regeneration across a lesion so that functionally valuable connections can be established.

**REGULATION DES CELLULES EPITHELIALES DANS LA REGENERATION DE LA DENT**  
*REGULATION OF EPITHELIAL STEM CELLS IN TOOTH REGENERATION*

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Epithelial stem cells reside in specific niches that regulate their self-renewal and differentiation, and they are responsible for the continuous regeneration of tissues such as hair, skin and gut. The regenerative potential of mammalian teeth is generally rather limited, but rodent incisors grow continuously throughout life. We have identified a stem cell niche in mouse incisors at their proximal ends in the so called cervical loops (Harada et al., J.Cell Biol.147,105,1999). This stem cell niche bears anatomical similarities to other epithelial organs, in particular to hair and intestine. In addition, the incisor stem cell niche has an additional uniquely powerful property: the stem cell compartments in the labial and lingual cervical loops vary greatly in size and proliferative capacity, and this contributes to the characteristic asymmetric growth and enamel distribution which maintain the sharpness of the continuously growing incisor. In the labial cervical loop, the epithelial stem cells proliferate and migrate along the labial surface, differentiating into enamel-forming ameloblasts. In contrast, the lingual cervical loop contains fewer proliferating stem cells, and the lingual incisor surface lacks ameloblasts and enamel. We have shown that epithelial stem cell proliferation in the cervical loops is controlled by signals from the adjacent mesenchyme. There is a complex integrated signal network consisting of Activin, BMP, FGF and Follistatin within the incisor stem cell niche. Mesenchymal FGF3 stimulates epithelial stem cell proliferation while BMP4 represses *Fgf3* expression. In turn, Activin, which is strongly expressed in labial mesenchyme, inhibits the repressive effect of BMP4 and restricts *Fgf3* expression to labial dental mesenchyme, resulting in a large labial stem cell niche. Follistatin antagonizes Activin to limit the number of lingual stem cells, further contributing to the characteristic asymmetry of mouse incisors. Interestingly, while BMP inhibits the proliferation of stem cells it induces their differentiation into ameloblasts, and on the lingual side of the incisor Follistatin acts as a BMP antagonist and prevents enamel formation. These results show how the spatially restricted and balanced effects of specific components of a signaling network can regulate stem cell proliferation and differentiation. Subtle variations in this or related regulatory networks may explain the different regenerative capacities of various organs and animal species.

**TABLE RONDE**  
Présidée par **Claude Sureau**

**QUESTIONS ETHIQUES ET ANTHROPOLOGIQUES**

**Anne FAGOT-LARGEAULT**  
de l'Académie des sciences, Professeur au Collège de France

Les traités « *De l'homme* », ou « *De la nature humaine* » abondent dans la littérature : de Thomas Hobbes, René Descartes, David Hume, etc., jusqu'à Edward O. Wilson, et bien d'autres. Kant disait que la question *Was ist der Mensch?* résume toute la quête philosophique.

La recherche sur les thérapies cellulaires régénératives pose un problème anthropologique dans la mesure où elle est perçue comme faisant peser une menace sur l'intégrité de la 'nature' humaine. La possibilité de créer des êtres vivants chimériques ou hybrides, dont le génome mélange ce que la nature ne mélange pas : éléments issus de génotypes variés, voire d'espèces différentes (porcs et souris humanisés, êtres humains transgéniques), remet directement en question ce que l'Unesco considère comme un héritage humain à protéger : « *Le génome humain sous-tend l'unité fondamentale de tous les membres de la famille humaine, ainsi que la reconnaissance de leur dignité intrinsèque et de leur diversité. Dans un sens symbolique, il est le patrimoine de l'humanité* ». La question est de savoir si ce que la « nature » a fait de l'homme doit être respecté dans tous les cas, ou si l'homme peut s'autoriser à corriger l'œuvre de la nature ('amélioration génétique', perspectives de la 'post-humanité').

Dans la seconde hypothèse, les problèmes éthiques ont trait aux limites que nous assignons ou devons assigner à nos entreprises régénératives. Est-il moralement acceptable (pour quelles raisons, au regard de quelle éthique ?) de vouer des embryons humains à la recherche, même avec une visée thérapeutique ; de mettre en culture des lignées cellulaires dérivées de la masse interne de blastocystes humains, et de développer une ingénierie de ce matériel humain que sont les cellules souches embryonnaires (CSE), aux fins de rendre obsolète l'artisanat héroïque de la greffe (de moelle, de fragments épidermiques, d'os, de pancréas, etc.), au profit d'une exploitation industrielle des cellules souches (hématopoïétiques, épidermiques, mésenchymateuses, sécrétrices d'insulines, etc.). Les législations existantes posent différemment les limites à ne pas franchir, les intuitions éthiques sur ces sujets sont encore floues, et largement discutées.

**ETHICAL AND ANTHROPOLOGICAL QUERIES**

Treatises « *Of man* », or « *On human nature* » abound in the literature : from Thomas Hobbes, René Descartes, David Hume, etc., up to Edward O. Wilson, and many others. Kant said that the question *Was ist der Mensch?* summarizes the entire philosophical quest.

The research on regenerative cell therapies raises an anthropological problem, to the extent that it is perceived as a potential menace on the integrity of human nature. Having made it possible to create hybrid or chimerical living beings, the genome of which mixes what nature kept separate : genes issued from a variety of genotypes, or of different species (humanized pigs or mice, transgenic human beings), directly questions the UNESCO declaration on the human genome and human rights (1997), which states that « *The human genome underlies the fundamental unity of all members of the human family, as well as the recognition of their inherent dignity and diversity. In a symbolic sense, it is the heritage of humanity* ». The point is, should the work of nature, and the human genome in particular, be preserved in all cases, or should the human race feel responsible for introducing proper corrections (*genetic enhancement*, and our « post-human » future).

If we grant the latter hypothesis, then ethical problems pertain to the limits that we should assign to our *regenerative* endeavours. Is it morally acceptable (for what reasons, *wrt.* what ethics ?) to instrumentalize human embryos for research, on account of a therapeutic purpose ; to derive embryonic cell lines from human blastocysts, and develop a sophisticated engineering of that human material, meant to render obsolete and replace the previous transplantation techniques (resecting a part of a human being, such as blood or skin tissue, and graft it on another human being). Existing legislations diverge on the thresholds that should not be crossed, ethical intuitions are hesitant and widely discussed.

**“THE END IS WHERE WE START FROM...” ON BEGINNINGS, MEANS, AND ENDS**

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President of Inserm Ethics Committee,  
Member of National Consultative Ethics Committee

*In my beginning is my end [...]*

*What might have been and what has been  
Point to one end, which is always present [...]*

*What we call the beginning is often the end  
And to make an end is to make a beginning [...]*

*The end is where we start from.*

T.S. Eliot. *Four Quartets*.

Research on human pluripotent embryonic stem cells currently requires the prior destruction of in vitro created, non-implanted embryos at the early developmental stage of blastocyte.

There are at least two different procedures by which such research may be initiated.

The first one uses an end as a means for a beginning.

Upon destruction of an embryo as a consequence of an abandonment of the parental project, its cells can be isolated and used for research. The recent French Bioethics Law allows such a procedure as a derogatory measure, for a five years period, provided that the parents have given informed consent, that another couple has not express a will to “adopt” the embryo, and that the research project has been approved by the *Agence de Biomédecine*, according to several criteria, including that the project has “*important therapeutic implications*”. When considered in the broad context of the ethical regulations concerning the potential use for research of cells isolated from a dead foetus, a dead child or a dead adult, an exceptional status seems to have been attributed to a dead embryo (as exemplified by the specific restriction that the research will only be authorized if it has “*important therapeutic implications*”).

The other procedure involves creating a beginning as a means for an end.

It is the in vitro creation of an embryo (either by in vitro fertilization, or nuclear transfer into an oocyte) for the sole purpose of research. This procedure is currently forbidden as a crime in France, while it is authorized, and can be publicly funded in some European neighbour countries, such as Great Britain. Which approaches of the ethical problems involved may account for such a radical difference? The complexity of the matter has been further exemplified by the international position of France, refusing at UNO an international ban on the creation of embryos for research by nuclear transfer, while banning it at a national level.

“*One enters into ethics*” wrote Paul Ricoeur, “*by expressing the will that the freedom of the other be*”. In such a context, the ethical question becomes: is the freedom of *others* affected by the in vitro creation of an embryo for research? In addition to the patients, the diseased foetuses (and even the embryos) that might one day benefit from such research, there are at least two radically different kinds of *others* that might be considered. One is the embryo created for research: should such an early stage of development of a human being be viewed as that of a future person or rather as a mere differentiation stage of the progeny of a cell? One of the main arguments for not considering an early non-implanted embryo as an *other* is its developmental stage, namely the absence of nervous system. Accordingly, because the cessation of any detectable cerebral activity – cerebral death – is currently a legal definition for a human person’s death, the absence of emergence of a brain will define the absence of a human person. But might there be more in a beginning than the sole mirror image of the end?

A second *other* involved in the in vitro creation of embryos for research is the woman who will be the donor of the egg cells required either for in vitro fertilization or nuclear transfer. Because oocyte donation implies

potential risks for the donor health, the possibility and ethical conditions of egg cell donation for research is an important topic that should be discussed. Recent data, however, suggest the possibility that oocytes might be derived in vitro from embryonic stem cells (and even from somatic cells). If this were confirmed, embryonic stem cells derived from oocytes differentiated from embryonic stem cells derived from embryos (destroyed after abandonment of parental project, and after parental informed consent) could be obtained without any use of adult oocytes. Might this in vitro derivation of early stage embryos from other early stages embryos allow us to further dissociate a process of cell differentiation from the first stages of development of a future human being?

Two additional remarks. The first one is that restricting research on embryonic stem cells to their sole potential therapeutic use raises ethical problems by conferring to such a research an intrinsically derogatory status (that of a transgression), by establishing a clear hierarchy between the respective importance of basic and applied research, and by implicitly linking an acceptance of such a research by society to an unrealistic promise of direct therapeutic benefit. Beyond any foreseeable application, this novel research aims at exploring the epigenetic mechanisms of cell differentiation, plasticity, renewal, aging, and the pathogenesis of many diseases, including neurodegenerative disorders and cancer. Rather than being considered as a specific branch of research – *using* embryonic stem cells –, should it not be discussed as an important part of a much broader enterprise, aimed at understanding what a stem cell might be, what makes a cell a stem cell, and how to make a stem cell?

Finally, while ethical reflection concerning embryonic stem cell research is important, focalisation on this topic in our rich countries should not divert us from considering it in the global context of the major ethical problems worldwide. As expressed in the recent *UNESCO Universal Declaration on Bioethics and Human Rights*, ethics in life sciences aims at reduction of human suffering, refusal of abandonment, and global promotion of human rights. We should not forget that the major ethical problems worldwide today do not mainly concern the earliest stages of the beginnings of future human beings, but rather the tragic premature death or suffering of children and adults through famine, infectious diseases, massacres, inhuman treatments, and the denial of health, freedom and dignity.

This is *the end where we should start from*, in order to prevent it.

**QUE VALENT LES INTERROGATIONS ETHIQUES FACE AUX ESPOIRS THERAPEUTIQUES ET  
AUX ENJEUX FINANCIERS ?**

*WHAT ABOUT ETHICAL CONSIDERATIONS FACING THERAPEUTIC HOPES AND ECONOMIC STAKES ?*

**Claude HURIET**

Sénateur honoraire

Vice-président du Comité International de Bioéthique de l'UNESCO

France

Dans un contexte de compétition internationale où tous les coups semblent permis, les interrogations éthiques concernant l'utilisation « des éléments et produits du corps humain » apparaissent aux yeux de certains, comme une entrave à la liberté du chercheur.

Le débat est d'autant plus nécessaire - et difficile - que des espoirs thérapeutiques constituent à eux seuls « une fin » qui justifie « les moyens ».

Encore faut-il raison garder et ne pas faire miroiter, face à une opinion attentive, des perspectives aujourd'hui incertaines et souvent lointaines.

Malgré ces incertitudes, des investissements considérables sont engagés tant le marché potentiel des biotechnologies apparaît prometteur.

Toutes ces considérations rendent encore plus indispensables une réflexion sur le sens du progrès et sur cette question lancinante qui ne pourrait être escamotée : « tout ce qui est désormais possible est-il permis ? »

**EST-IL ETHIQUE DE CREER DES EMBRYONS POUR LA RECHERCHE ?**  
*IS IT ETHICALLY ACCEPTABLE TO MAKE EMBRYOS FOR RESEARCH ?*

**Anne MCLAREN**

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The issue of making embryos for research, by fertilization, somatic cell nuclear transfer or any other way, will be discussed.

**LA PRODUCTION DE GAMÈTES A PARTIR DE CELLULES SOUCHES  
EST-ELLE ETHIQUEMENT ENVISAGEABLE ?**  
*DIFFERENTIATION OF ANIMAL ES CELLS INTO GAMETES : ETHICAL COMMENTS*

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Les récents travaux chez l'animal ayant montré la possibilité d'obtention de gamètes mâles et femelles à partir de cellules souches ouvrent la question de la transposition, spécialisation des cellules ES chez l'Homme.

Bien en amont des possibilités réelles, le temps est néanmoins venu de réfléchir sur une telle éventualité. La faisabilité semble pouvoir être du même ordre que la faisabilité de toute différenciation fonctionnelle à partir des cellules souches.

Reste à préciser si les gamètes ainsi obtenus seront fonctionnels tel n'est pas encore le cas chez l'animal où une descendance n'a pas été réalisée à partir de tels constituants.

Il faut donc attendre les résultats des travaux scientifiques tout en abordant dès maintenant les problèmes éthiques.

La production de gamètes à partir de cellules somatiques abolirait le statut de personne infertile, bouscule le temps de la filiation et évoque des scénarii de science fiction où la fertilité serait indéfiniment maintenue du fait de renouvellement d'individus depuis longtemps décédés mais dont les cellules germinales seraient constamment renouvelées.

La création de banques de sperme anonymes sans besoin de donneurs, création de banques d'ovocytes sans besoin de donneuses, la seule limite étant les risques de consanguinité de la descendance.

Les préoccupations médicales qui sous-tendent ces recherches sont le bon résultat des traitements anti-cancéreux qui sauvent la vie tout en détruisant la fertilité, de telles avancées pourraient dépasser cette contradiction mais soulèvent un certain nombre d'interrogations.

**1994-2004 : LE LEGISLATEUR FACE A L'EMBRYON IN VITRO**  
*1994-2004 : THE IN VITRO EMBRYO FACING THE LAW*

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En France, les lois du 29 juillet 1994 ont été précédées d'un débat d'idées, à l'issue duquel a prévalu le principe du respect de la dignité de la personne humaine, interdisant toute instrumentalisation de l'être humain, même au stade embryonnaire. L'embryon in vitro, facilement manipulable, a été protégé par la loi du risque d'être considéré comme un chose. C'est pourquoi toute recherche était interdite, y compris sur les embryons n'ayant aucune chance d'implantation ou sur des embryons « morts ». La découverte postérieure des propriétés des cellules souches embryonnaires ainsi que la naissance de Dolly ont fait naître des interrogations sur les conséquences de ce choix, qui aboutissait en pratique à faire obstacle à des progrès thérapeutiques majeurs.

Le réexamen de la loi en 2004 a été l'occasion d'une nouvelle réflexion. Mais le Parlement n'a pas voulu revenir sur les principes. C'est pourquoi la nouvelle loi réaffirme l'interdiction de la recherche sur l'embryon tout en y apportant exception, à titre temporaire, pour permettre la recherche sur les cellules souches embryonnaires, tandis que la recherche en vue du clonage thérapeutique demeure prohibée.

Si le dispositif actuel constitue une option politique délibérée, on peut s'interroger sur sa cohérence, tant au regard de ses conséquences (qu'en est-il par exemple du régime des cellules différenciées obtenues à partir de cellules souches embryonnaires ?) qu'au regard des principes. Si l'on considère que le respect de la dignité de la personne humaine, dans la version retenue par la jurisprudence française, est intangible, alors la loi nouvelle est conceptuellement critiquable et donne l'impression que l'éthique ne résiste pas à la pression des pratiques. En revanche, il nous semble que la société aurait tout à gagner à une réflexion sur les principes eux-mêmes, y compris le principe de dignité dont la présentation nous paraît aujourd'hui un peu manichéenne. Cette réflexion ne saurait être le domaine réservé d'aucune discipline mais devrait avoir une dimension pluridisciplinaire et publique.