ecopa european consensus-platform for alternatives

Exploring Stem Cells

Alicante 29 September 2006

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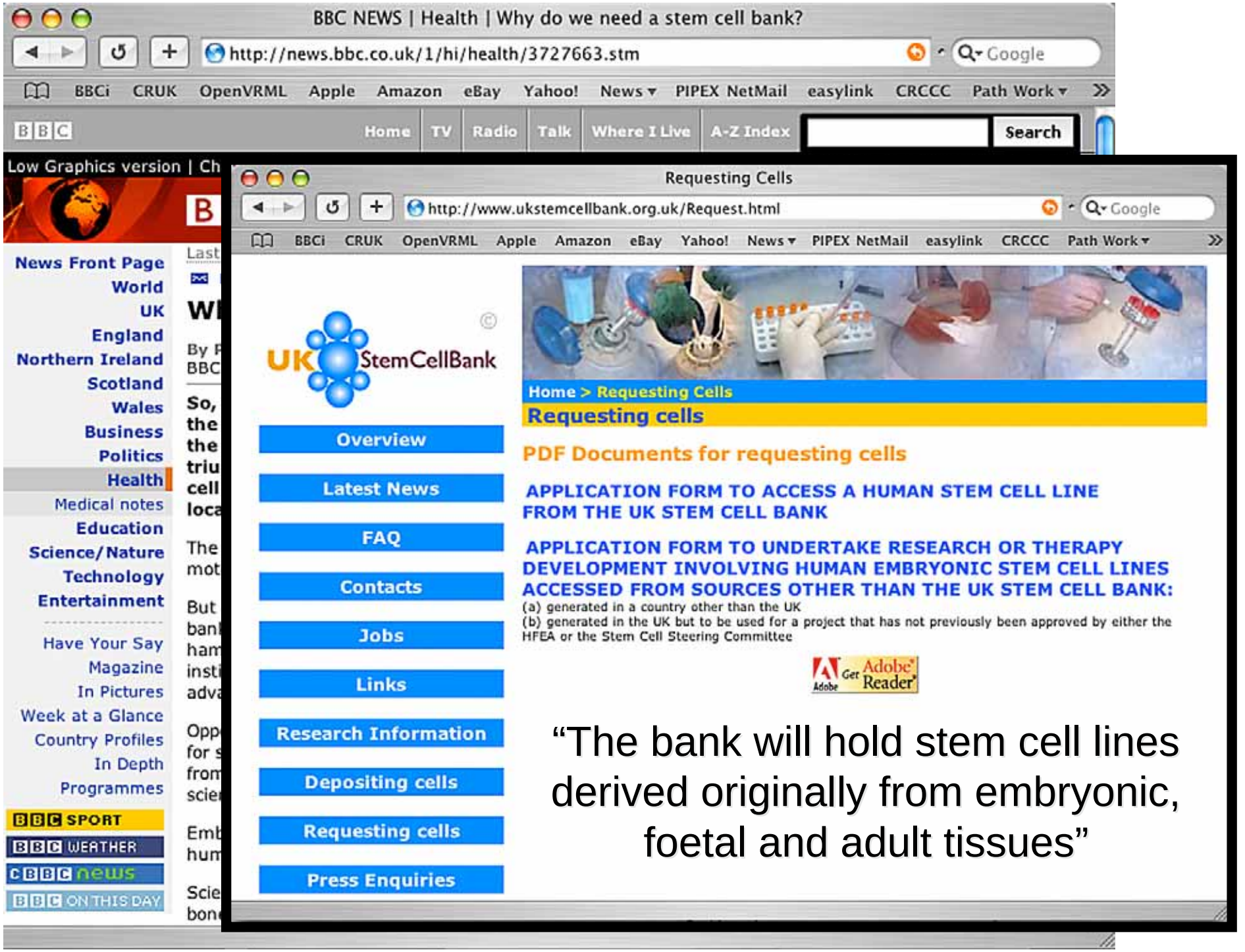
Institute of Cell & Molecular Science
Queen Mary's School of Medicine & Dentistry
m.alison@qmul.ac.uk

Stem Cells: a hierarchy of potential

- Totipotent - fertilized oocyte
- Pluripotent - capable of forming many cell types, contributing to all 3 germ layers
- Multipotent - gives rise to a limited number of cell types
- Unipotent - gives rise to a single cell type


BBC NEWS | Health | Why do we need a stem cell bank?
http://news.bbc.co.uk/1/hi/health/3727663.stm
Google
BBCi CRUK OpenVRML Apple Amazon eBay Yahoo! News PIPEX NetMail easylink CRCCC Path Work
BBC
Home TV Radio Talk Where I Live A-Z Index
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World
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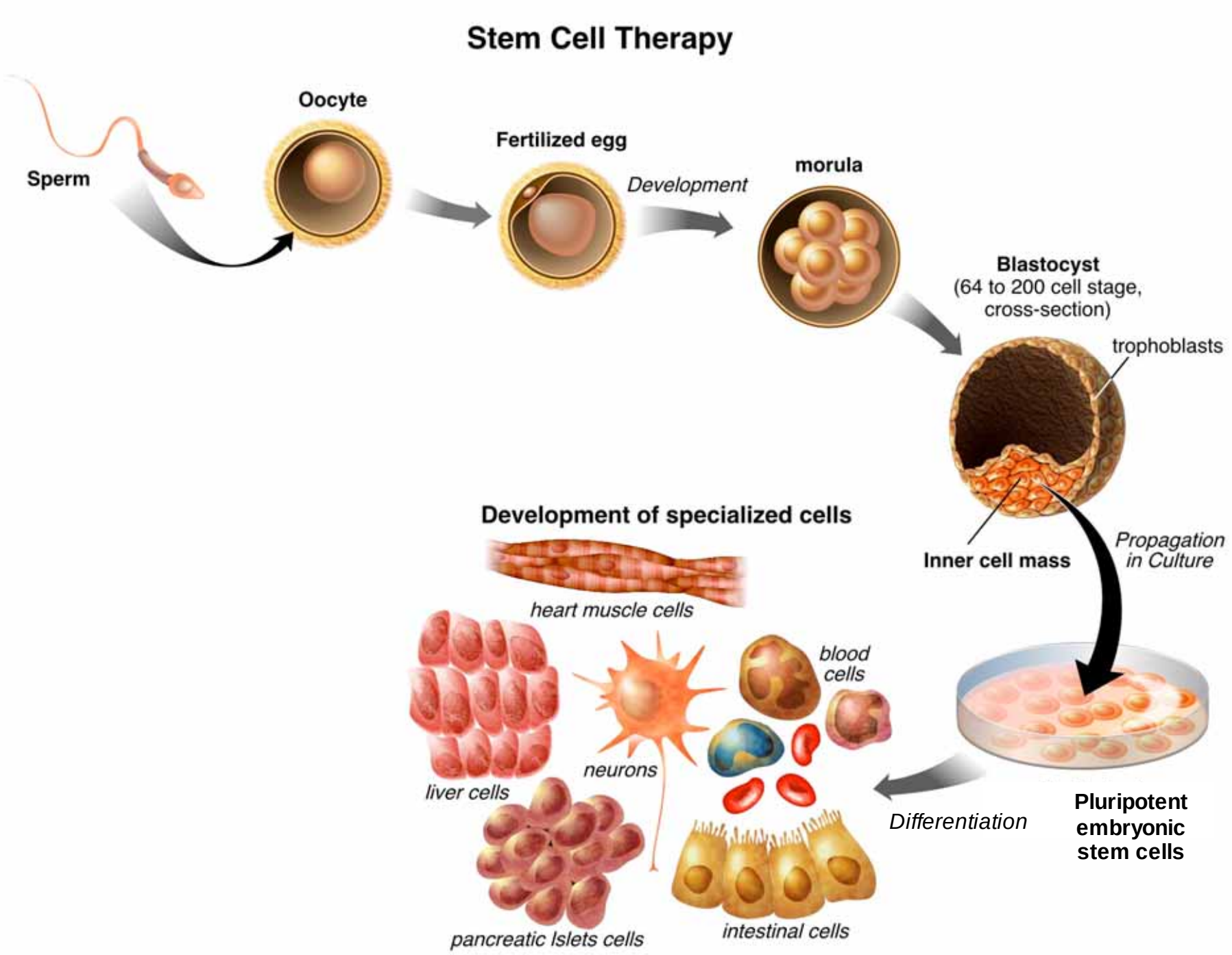
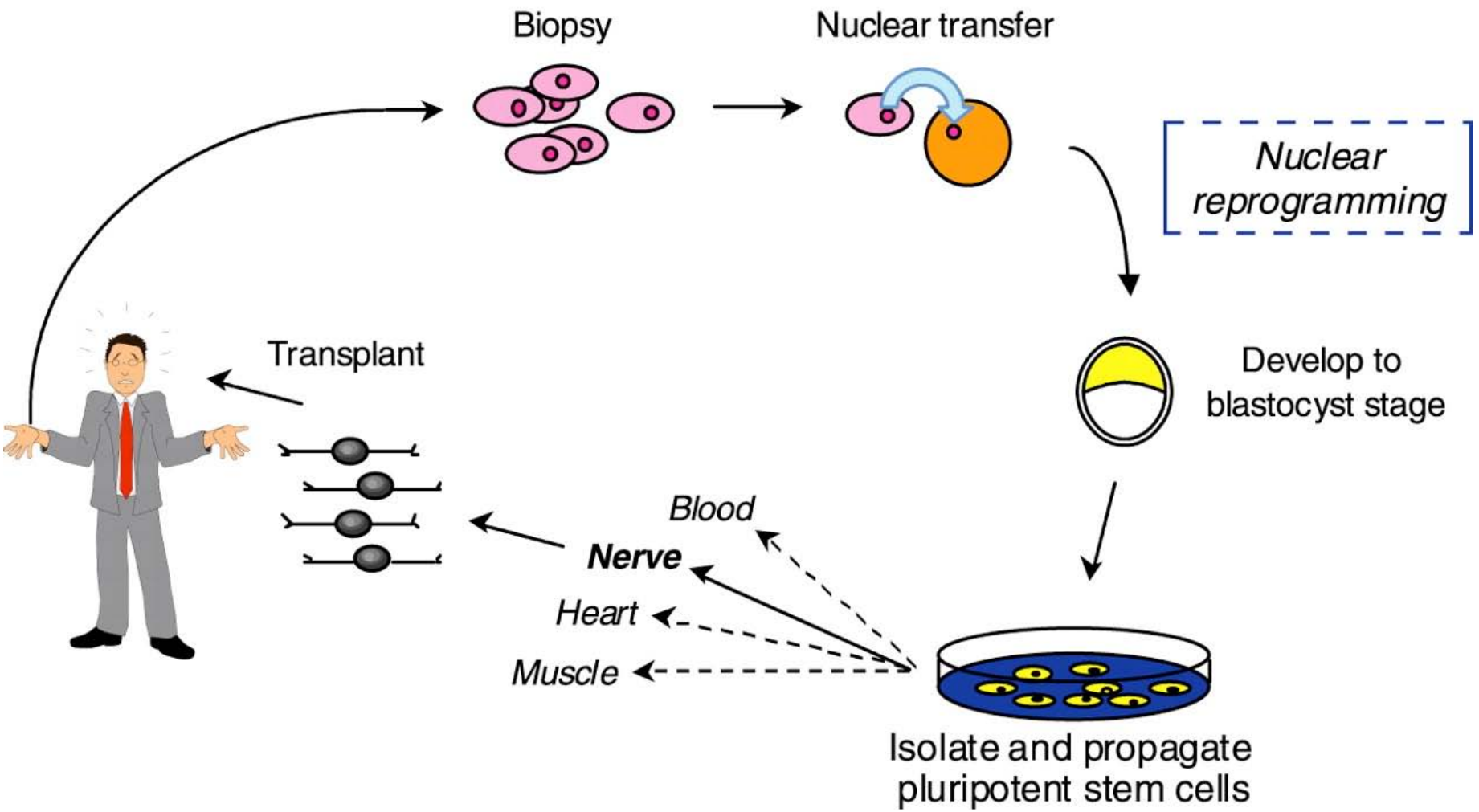
Have Your Say
Magazine
In Pictures
Week at a Glance
Country Profiles
In Depth
Programmes
BBC SPORT
BBC WEATHER
CBBC news
BBC ON THIS DAY
Requesting Cells
http://www.ukstemcellbank.org.uk/Request.html
Google
BBCi CRUK OpenVRML Apple Amazon eBay Yahoo! News PIPEX NetMail easylink CRCCC Path Work
UK StemCellBank
Overview
Latest News
FAQ
Contacts
Jobs
Links
Research Information
Depositing cells
Requesting cells
Press Enquiries
Home > Requesting Cells
Requesting cells
PDF Documents for requesting cells
APPLICATION FORM TO ACCESS A HUMAN STEM CELL LINE FROM THE UK STEM CELL BANK
APPLICATION FORM TO UNDERTAKE RESEARCH OR THERAPY DEVELOPMENT INVOLVING HUMAN EMBRYONIC STEM CELL LINES ACCESSED FROM SOURCES OTHER THAN THE UK STEM CELL BANK:
(a) generated in a country other than the UK
(b) generated in the UK but to be used for a project that has not previously been approved by either the HFEA or the Stem Cell Steering Committee
Get Adobe Reader
“The bank will hold stem cell lines derived originally from embryonic, foetal and adult tissues”


Stem Cell Therapy
Sperm
Oocyte
Fertilized egg
Development
morula
Blastocyst
(64 to 200 cell stage,
cross-section)
trophoblasts
Inner cell mass
Propagation
in Culture
Development of specialized cells
heart muscle cells
blood
cells
neurons
liver cells
pancreatic Islets cells
intestinal cells
Differentiation
Pluripotent
embryonic
stem cells

Patient-Specific Stem Cell Therapy



‘Therapeutic cloning’

Last Updated: Tuesday, 10 January 2006, 05:39 GMT

Scientist's embryo cloning faked

An investigation into the work of discredited South Korean cloning scientist Hwang Woo-suk has found further fabrications in his research.


The investigation's findings have stunned many South Koreans

Dr Hwang's landmark claim to have cloned human embryonic stem cells was false, a university panel concluded.

But the panel, which last month rejected other research by Dr Hwang, has accepted that he did create the world's first cloned dog.

Dr Hwang has admitted errors, but claims his work was sabotaged.

State prosecutors are now expected to look into the case.

BBC Seoul correspondent Charles Scanlon says the conclusion of the university's investigation completes the disgrace of Dr Hwang, who was South Korea's most celebrated scientist.

No proof

Dr Hwang claimed in a 2004 paper published in the US journal Science that his team had produced a line of stem cells from a cloned human embryo.

The achievement was judged to be a major scientific breakthrough in the search for cures for a range of degenerative diseases including diabetes and Parkinson's.

But the nine-member Seoul University panel, which spent a month examining Dr Hwang's

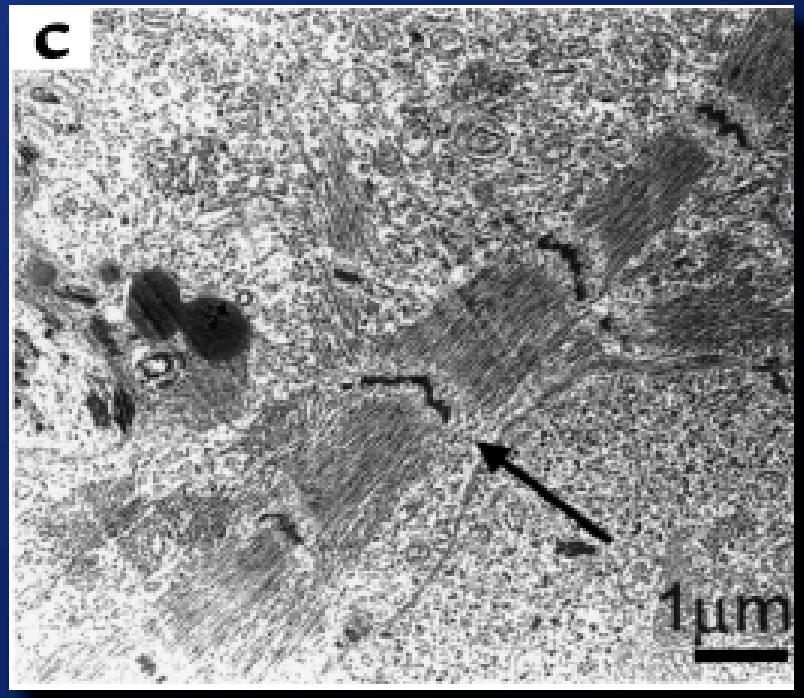
Stem cells for the broken heart

- Embryonic stem cells (ECs)
- Skeletal Myoblasts
- Endothelial Progenitor Cells (EPCs)
- Mesenchymal Stem Cells (MSCs)
- Haematopoietic Stem Cells (HSCs)
- Cardiac Stem Cells (CSCs)
- What cell to use?

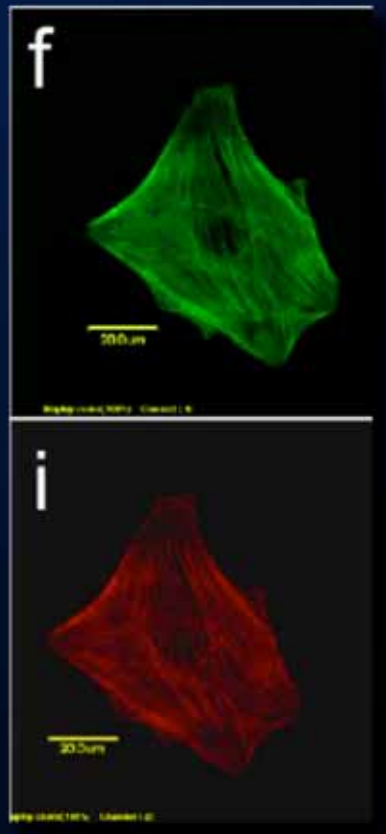
Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes

See related Commentary on pages 363–364.

Izhak Kehat,[1] Dorit Kenyagin-Karsenti,[2] Mirit Snir,[1] Hana Segev,[2] Michal Amit,[2] Amira Gepstein,[1,3] Erella Livne,[3] Ofer Binah,[4] Joseph Itskovitz-Eldor,[2] and Lior Gepstein[1]



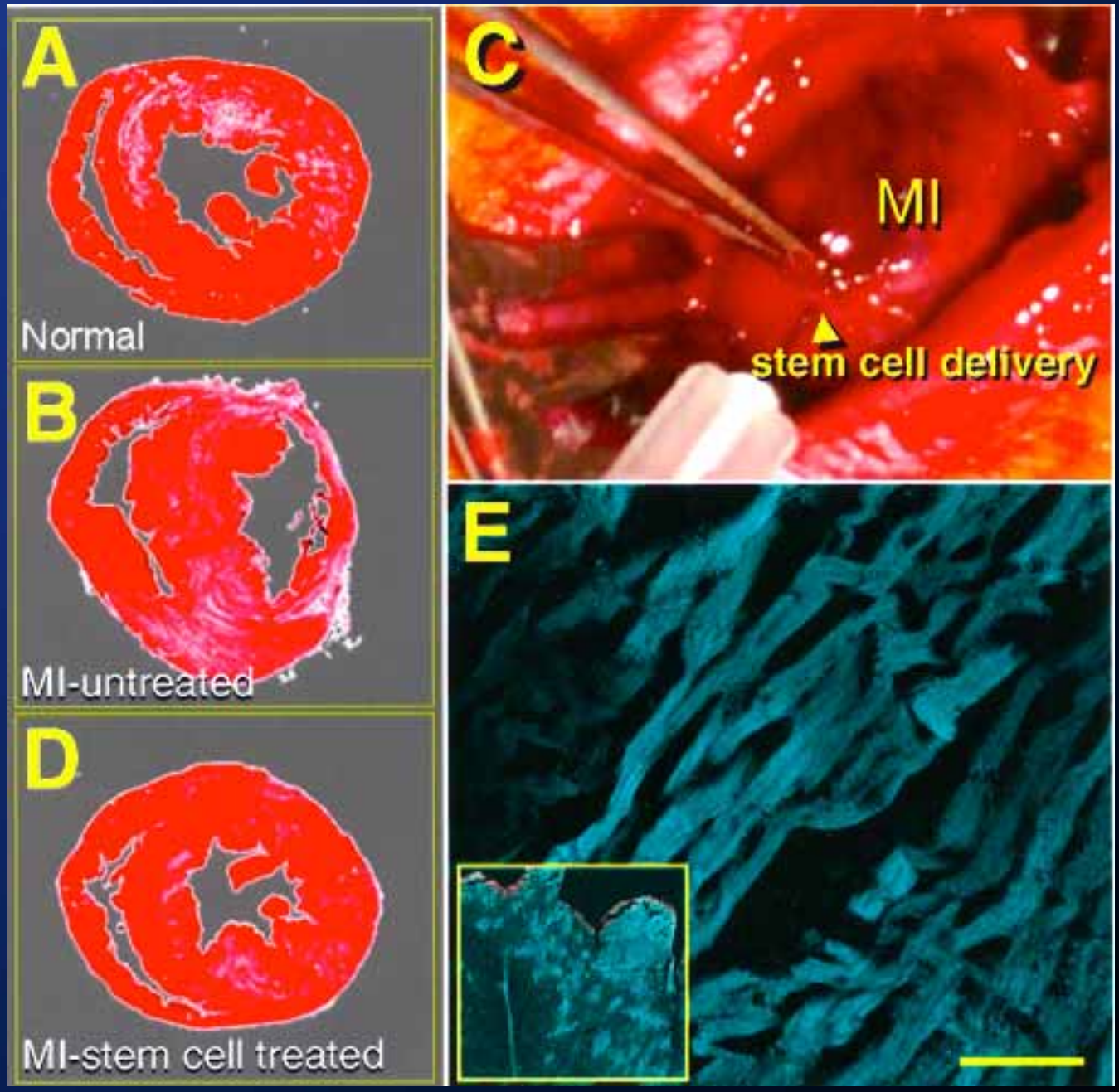
Morphology



Immunohistochemistry

ES cells: In vivo

- A Behfar et al, Ann N Y Acad Sci, May 2005 1049:189-198.
- mES cells used to repair MI in rats
- Conclusion: ES cells useful and effective promoting sustained repair

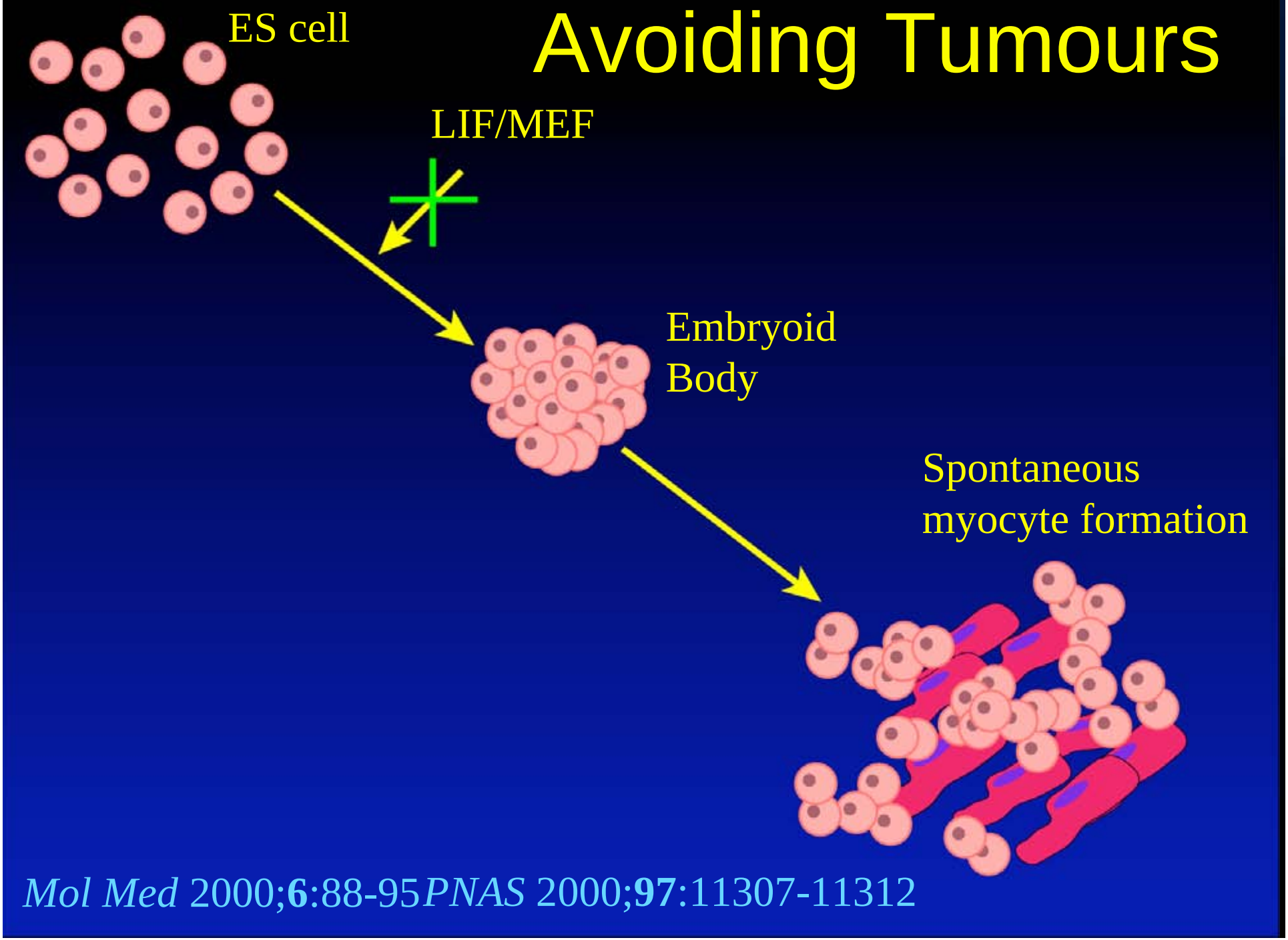


A Behfar et al, Ann N Y Acad Sci, May 2005 1049:189-198.

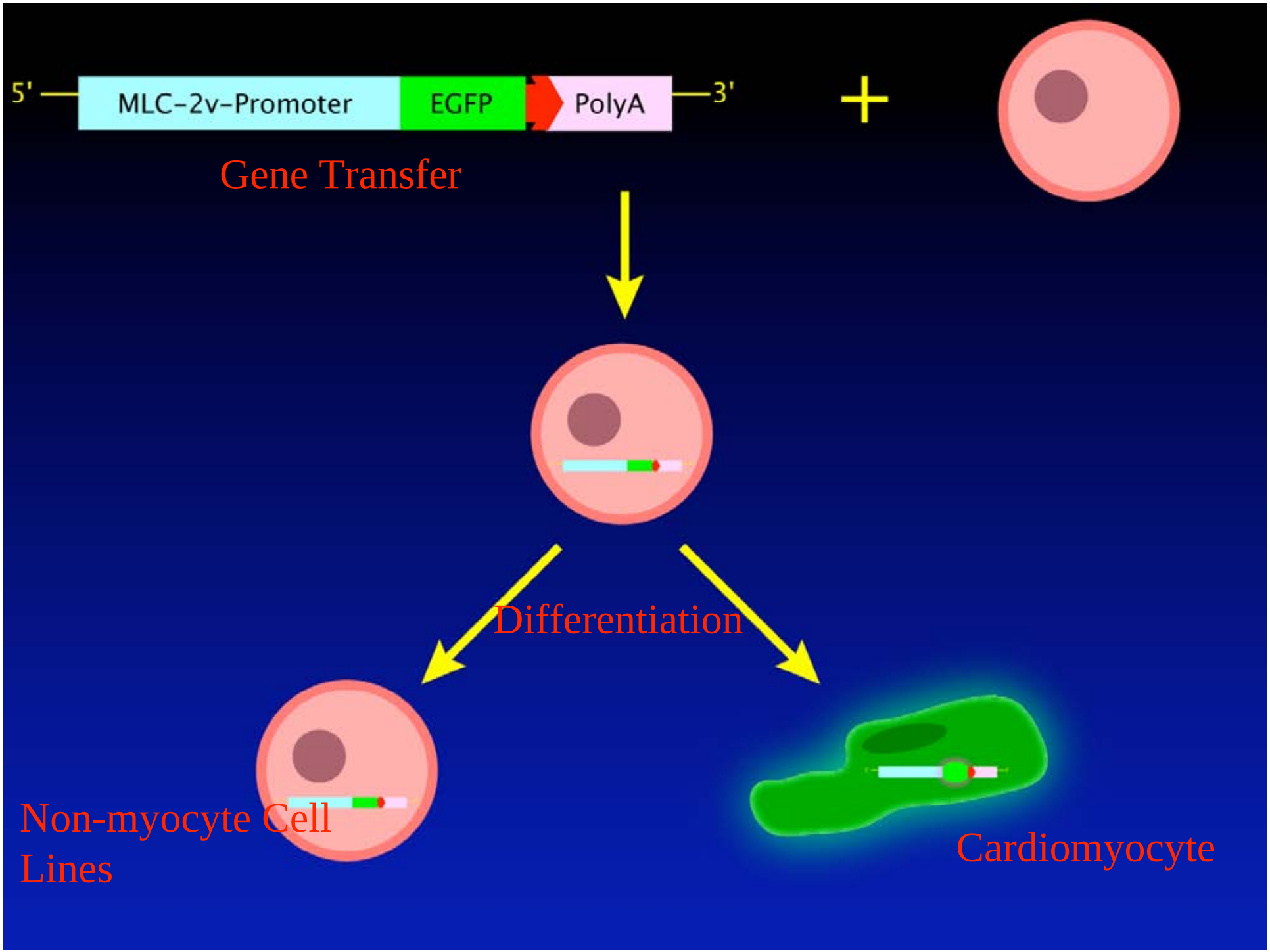
ES Cells - problems

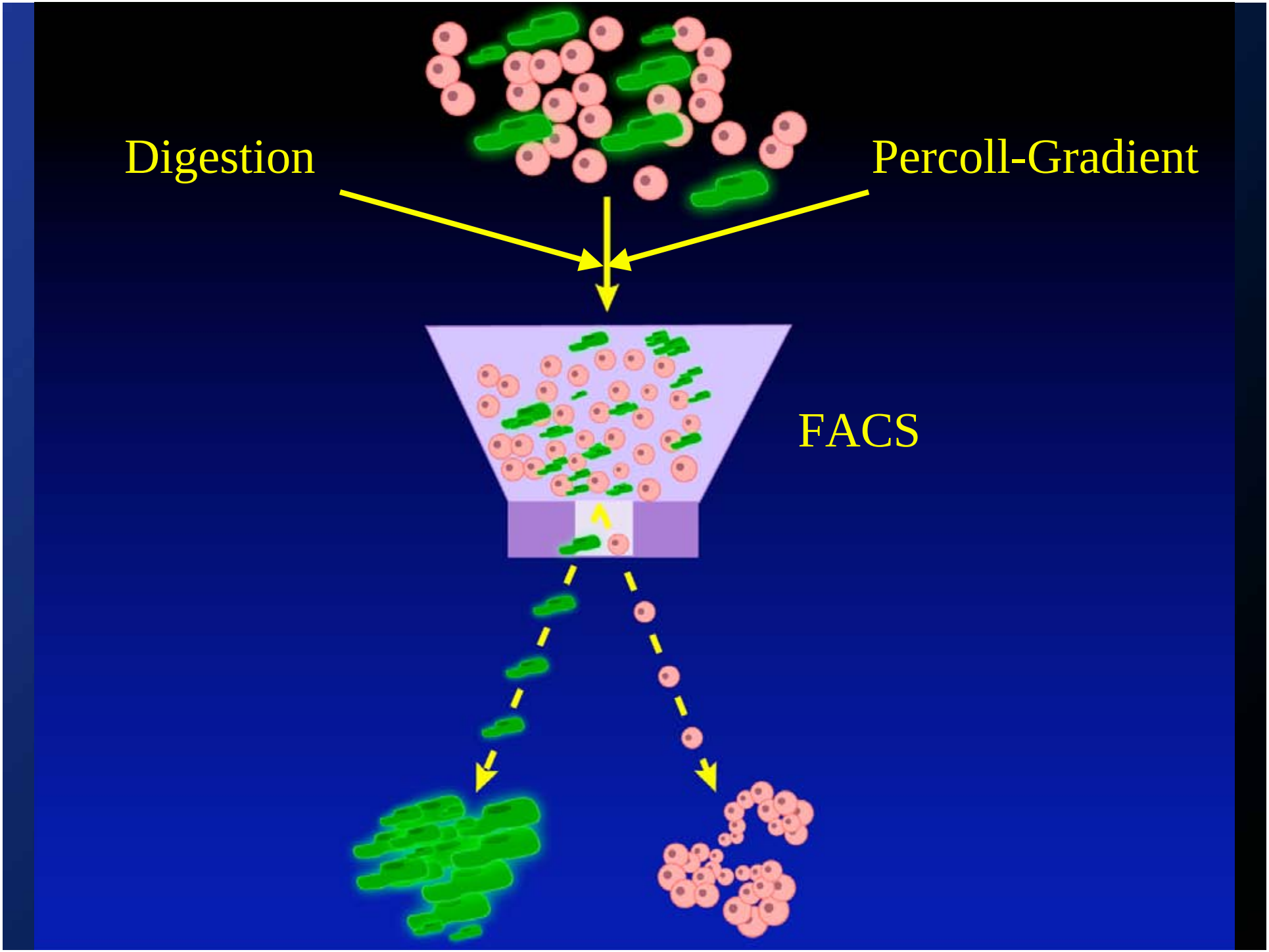
- Transmit Infection (HIV, Hepatitis)
- Tumorigenic potential
- Cultured under non-GMP conditions
  - Xenoproducts
- Immunology (Allogenic)
- Ethical

Avoiding Tumours



Mol Med 2000;**6**:88-95 *PNAS* 2000;**97**:11307-11312


5'
MLC-2v-Promoter
EGFP
PolyA
3'
Gene Transfer
Differentiation
Non-myocyte Cell Lines
Cardiomyocyte


Digestion
Percoll-Gradient
FACS

Xenoproducts

- R Passier et al
- Increased cardiomyocyte differentiation from human embryonic stem cells in serum-free cultures.
- Stem Cells 2005, 23(6): p772

- Xu C et al
- Feeder-free growth of un-differentiated human embryonic stem cells.
- Nat. Biotechnol 2001, 19: p971

The Allogenic Problem

- Somatic cell nuclear transfer (SCNT)
- Therapeutic cloning
- Adult cell nucleus from the recipient into an enucleated oocyte
- Generates pluripotent ES cells
- Shown to form cardiomyocytes in vitro and in vivo

Genesis 2000,28:156 Nat Biotech 2002;20:689 NEJM 2003,349:275

Sources of adult stem cells for regenerative medicine

Human Stem Cells for Therapeutic Use

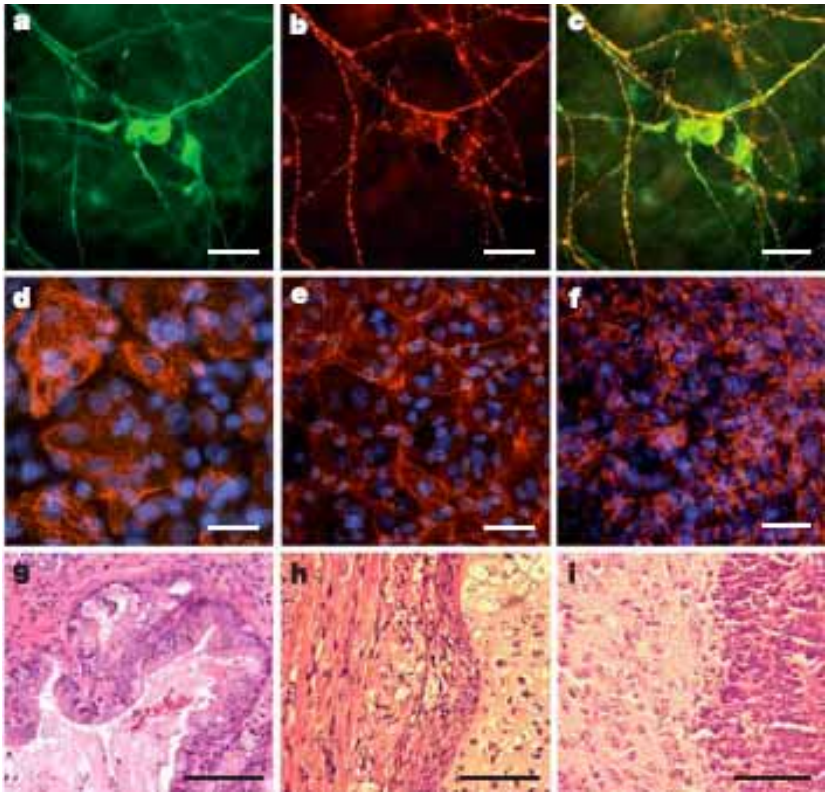
The beaker contains unprocessed liposuction waste material. Artecel Sciences uses

Vol 440|27 April 2006|doi:10.1038/nature04697 nature

LETTERS

Pluripotency of spermatogonial stem cells from adult mouse testis

Kaomei Guan[1]*, Karim Nayernia[2]*, Lars S. Maier[1], Stefan Wagner[1], Ralf Dressel[3], Jae Ho Lee[2], Jessica Nolte[2], Frieder Wolf[1], Manyu Li[2], Wolfgang Engel[2] & Gerd Hasenfuss[1]



Neurones

Hepatocytes

Teratomas

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The Company

The Service

The Science

The Choice

Welcome to Cells4Life

The experts in stem cell storage

Cells4Life are the first UK based private umbilical cord blood stem cell storage facility, offering parents access to expert medical professional advice and information on stem cell storage techniques. ***Please visit "the Company" section of our site to find out more...***

Cells4Life offer a unique and quality service providing a dedicated medical courier service, 24 hours a day, 365 days year to ensure the quick and safe arrival of your cord blood sample to our laboratory for immediate storage. Cells4Life are the only company to store the whole umbilical cord blood, rather than just extracting the stem cells, offering parents the a ability to take advantage of all future developments that may take place in this rapidly developing and exciting field of medicine. ***Please visit "the Service" section of our site to find out more...***

Stem cells are found in the blood of the umbilical cord and the placenta, which is normally discarded after birth. Stem cells found in the umbilical cord blood can be used for the treatment of many blood disorders. The collection and storage of umbilical cord blood immediately after birth provides a perfectly matched source of stem cells that are available without delay should the need for treatment arise. ***Please visit "the Science" section of our site to find out more...***

Cells4Life are setting the standards in umbilical cord blood stem cell storage, offering the best possible support, service and technology to parents. ***Please visit the "the Choice" section of our site to find out more...***

Please take time to discuss stem cell storage with your consultant or midwife. If you require more detailed information please contact our customer support team on ***0870 049 3360***

Today's Science - Tomorrow's Possibilities

Why Choose Cells4Life>>

Frequently Asked Questions>>

Healthcare Professionals>>

Stem Cell News>>

The Portland Hospital for Women and Children

midwivesonline.com

bbc.co.uk Health

DH Department of Health

Mitchell, et al. Stem Cells 2003;21:50-60

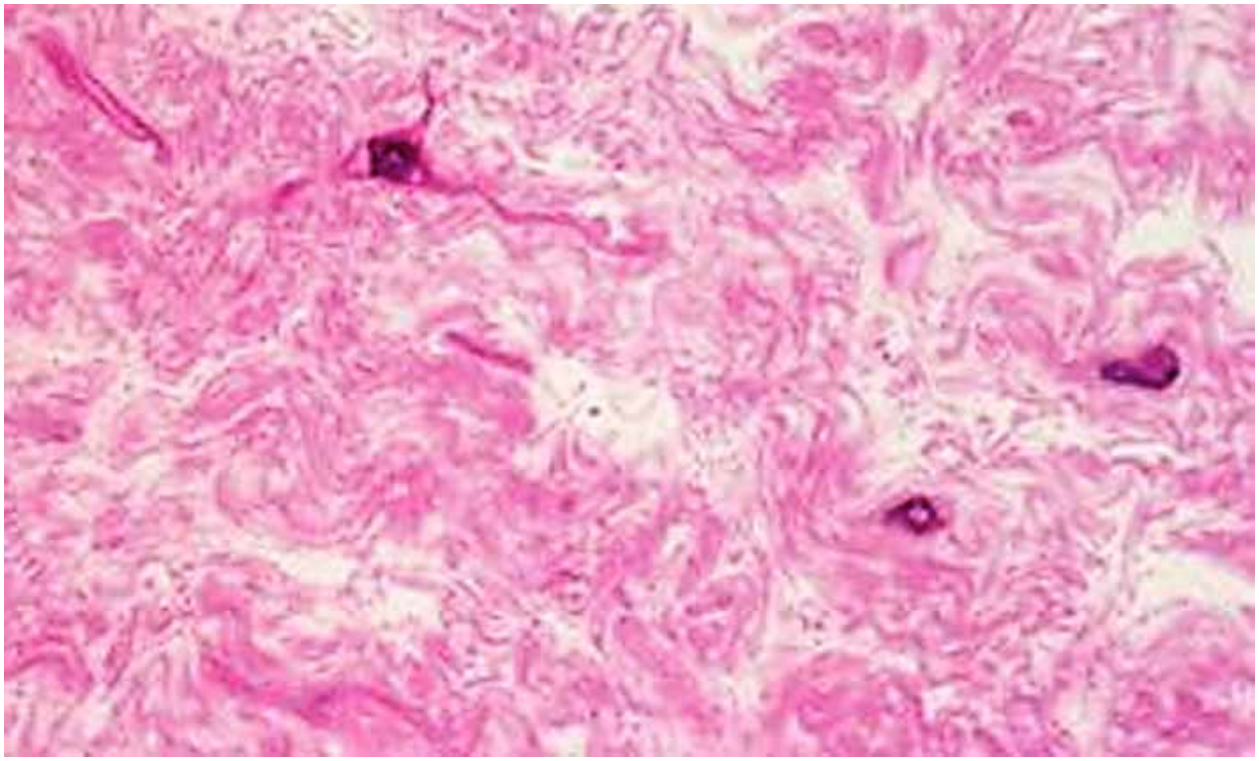
We have identified an easily attainable source of primitive, potentially multipotent stem cells from Wharton's jelly, the matrix of umbilical cord.

Stem Cells Galore

"Umbilical cord matrix cells could provide the scientific and medical research community with a non-controversial and easily attainable source of stem cells for developing treatments for Parkinson's disease, stroke, spinal cord injuries, cancers and other conditions."-- **Deryl Troyer**, Ph.D. Veterinary Medicine.

Wharton's Jelly

a soft connective tissue that occurs in the *umbilical cord* and consists of large stellate fibroblasts and a few wandering cells and macrophages embedded in a homogeneous jelly-like intercellular substance

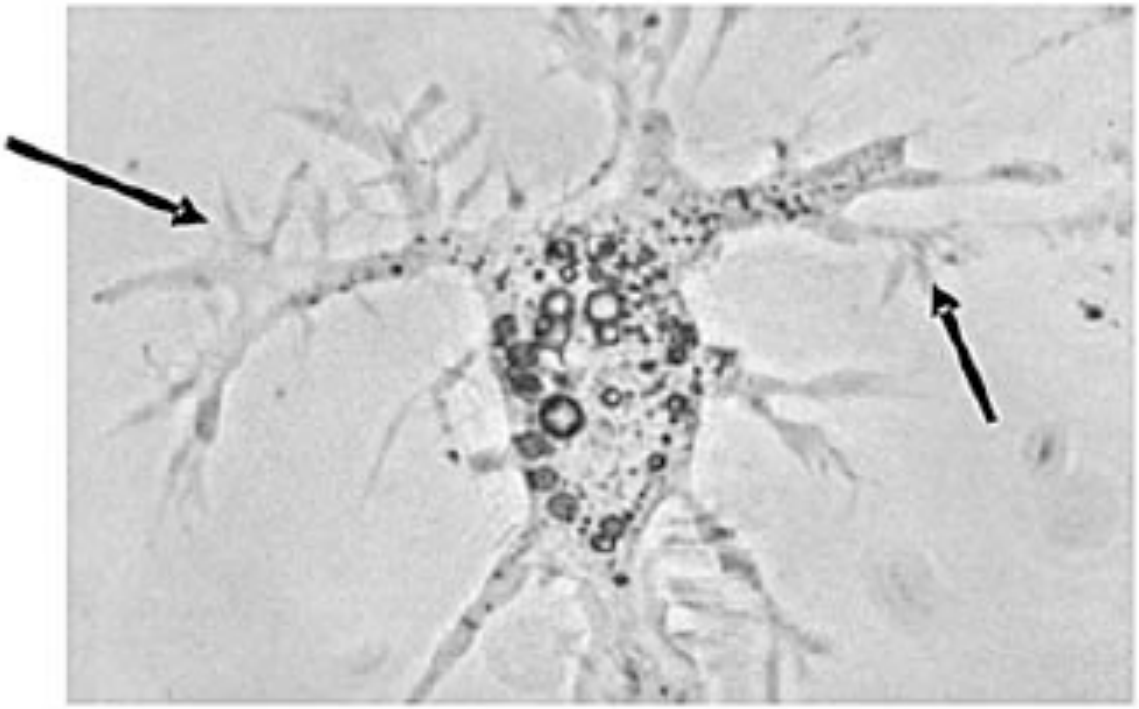


Thomas Wharton:
Adenographia: sive glandularum totius corporis descriptio.
London, 1656, pp. 243-244.

Mitchell, et al. Stem Cells 2003;21:50-60

After 3 days the neuron-like cells in these cultures stained positively for several neuronal proteins, including neuron-specific class III β-tubulin, neurofilament M, an axonal growth cone-associated protein, and tyrosine hydroxylase.

Note the multiple neurites with primary and secondary processes (arrows).



ORIGINAL ARTICLE

Induction of stem cell-like plasticity in mononuclear cells derived from unmobilised adult human peripheral blood

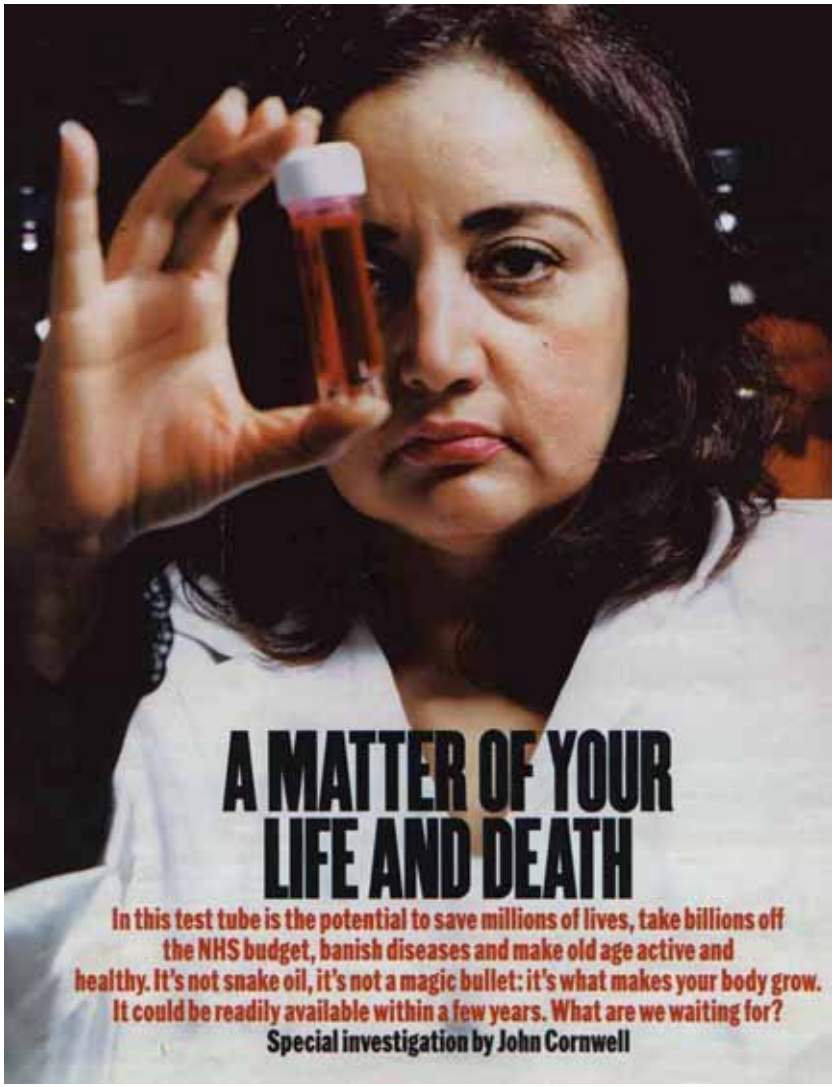
Ilham Saleh Abuljadayel

TriStem UK Limited, London, UK

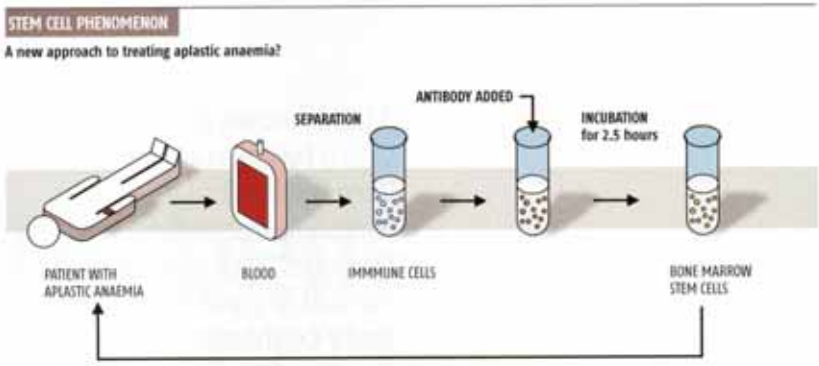
CURRENT MEDICAL RESEARCH AND OPINION®
VOL. 19, NO. 5, 2003, 355-375

“Retrodifferentiation”

Sunday Times 1.2.04




WEEKLY
NEWS IDEAS INNOVATION
THE BEST JOBS IN SCIENCE
NewScientist
9 October 2004
CANCER
08 OCT 2004
RESEARCH UK
Are you listening, America?
Global issues a president can't ignore
Not to be taken from the Library
BEAST WITH NO NAME
Is it a chimp, a gorilla or something entirely new?
INDIA'S STEM CELL 'MIRACLE'


STEM CELL PHENOMENON
A new approach to treating aplastic anaemia?
SEPARATION
ANTIBODY ADDED
INCUBATION
for 2.5 hours
PATIENT WITH APLASTIC ANAEMIA
BLOOD
IMMUNE CELLS
BONE MARROW STEM CELLS
TREATED CELLS INJECTED BACK INTO PATIENT'S BLOODSTREAM

CR3/43 (Dako)

Organ-specific stem cells

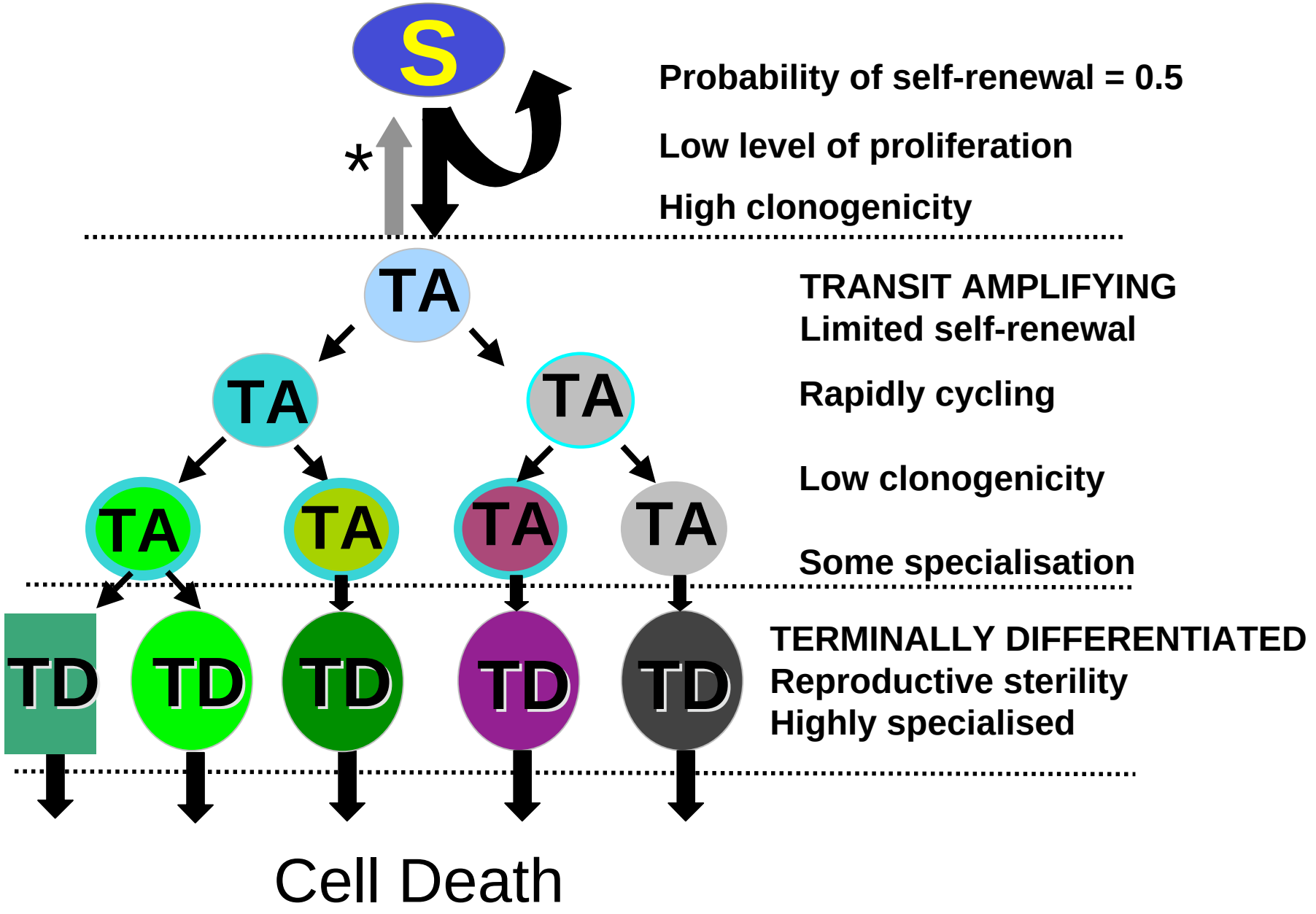
Journal of Pathology
J Pathol 2002; **197**: 419–423.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1187

Review Article

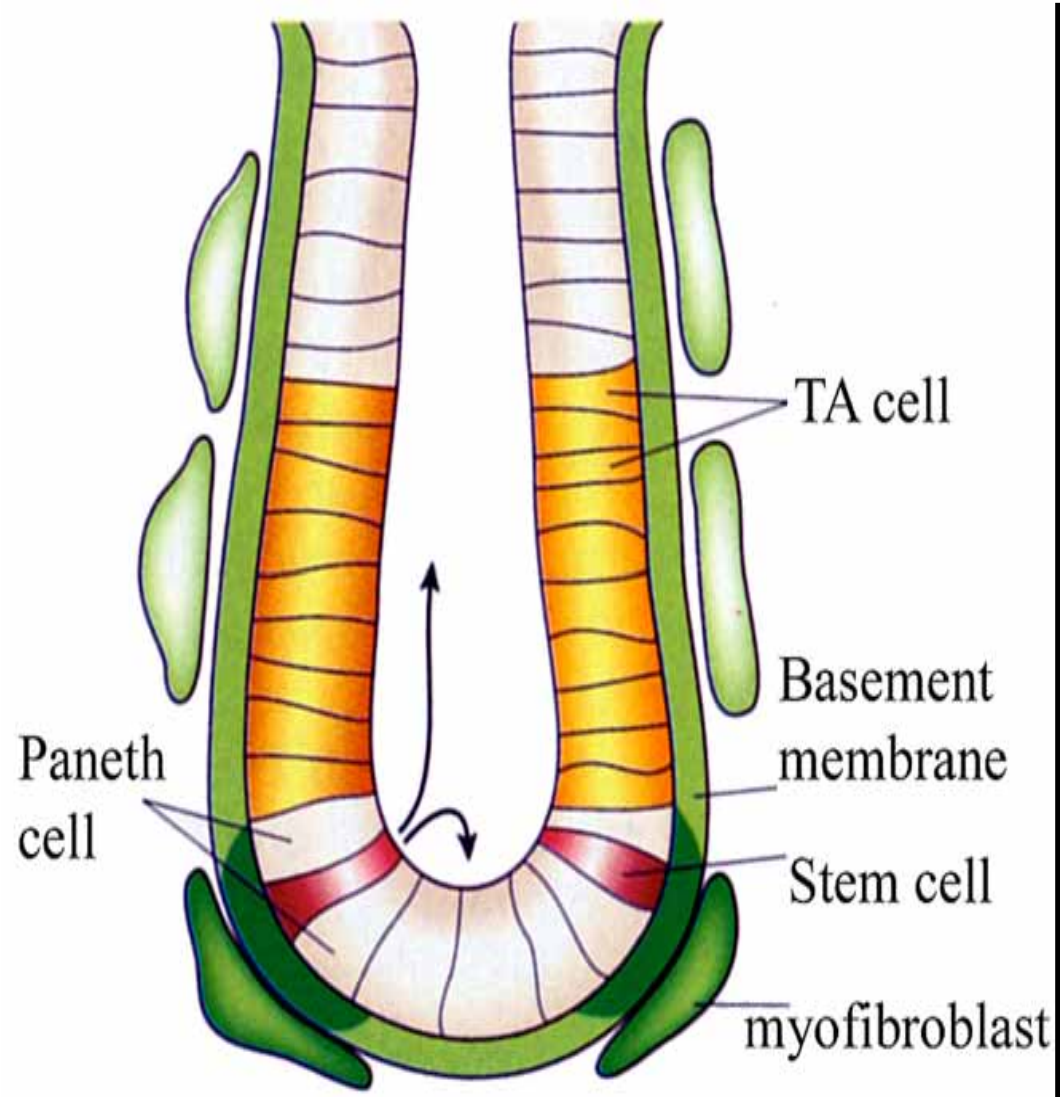
An introduction to stem cells

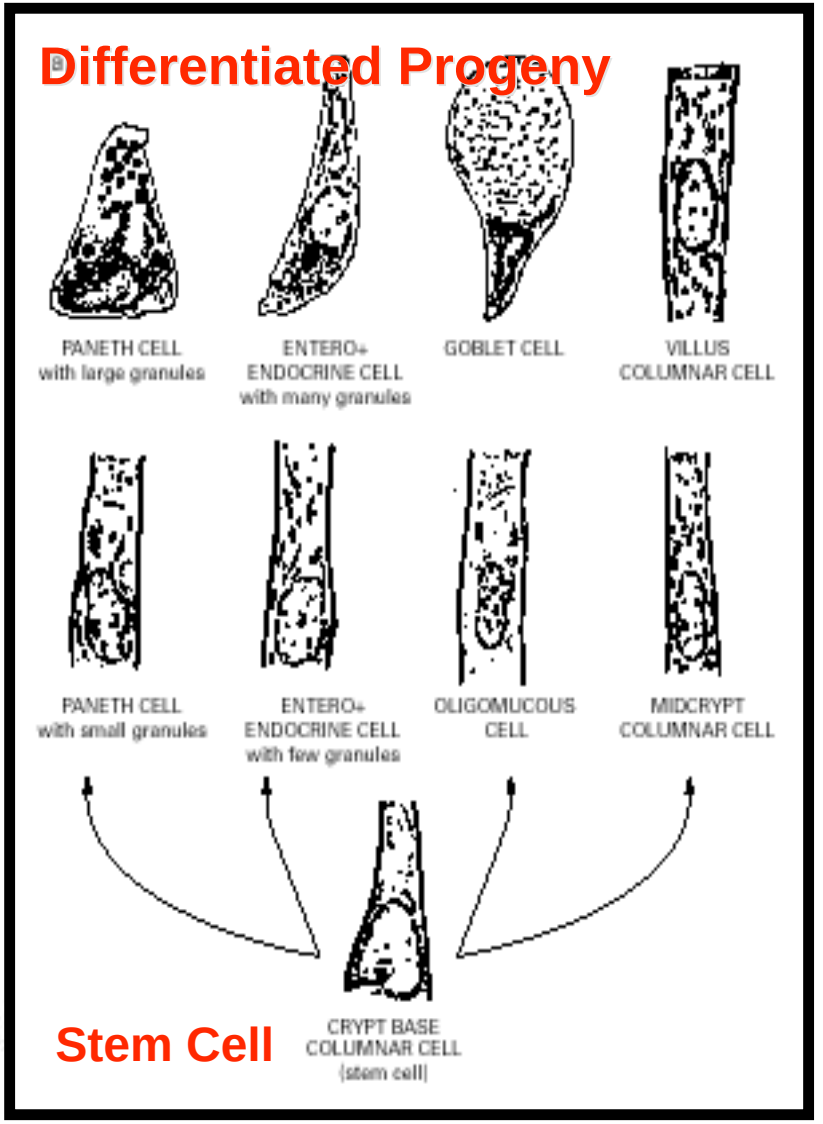
Malcolm R. Alison[1,2]*, Richard Poulsom[1], Stuart Forbes[3] and Nicholas A. Wright[1]

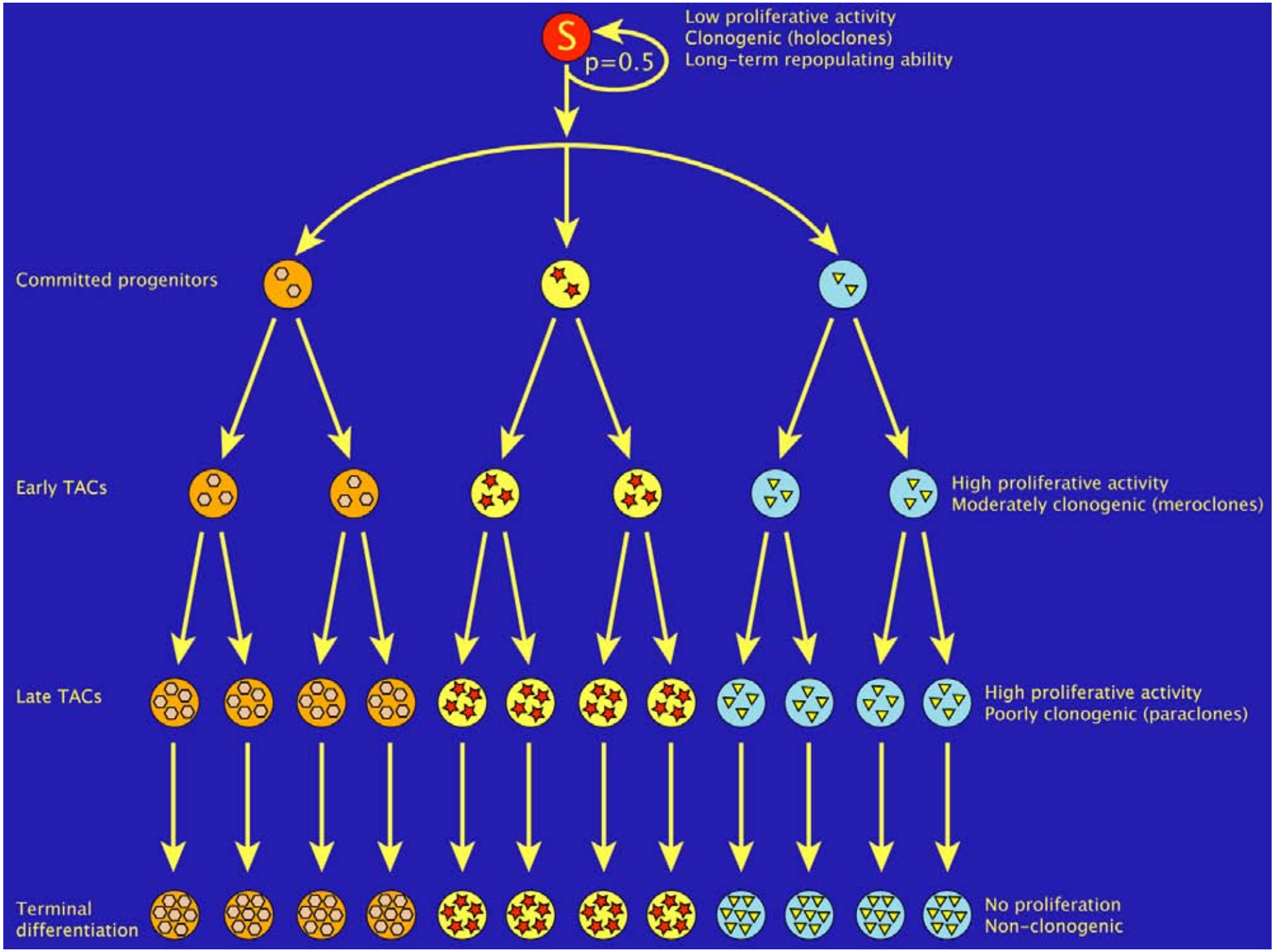
Adult tissue-specific stem cells



Adult tissue-specific stem cells: small intestine



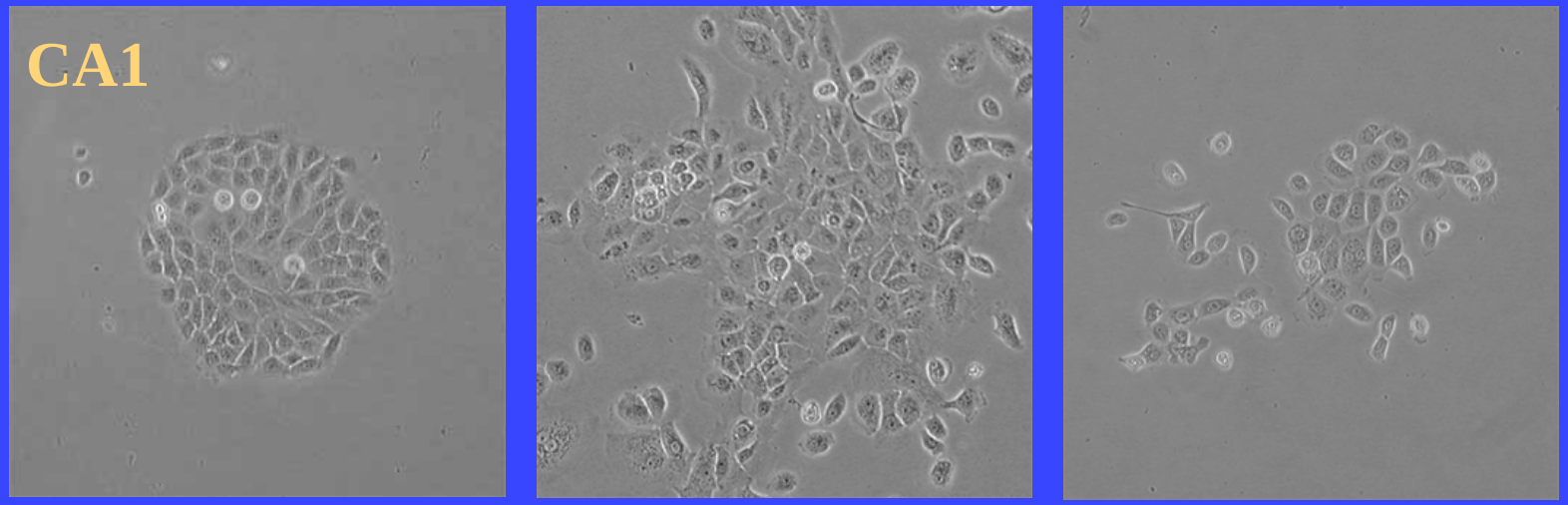



S
p=0.5
Low proliferative activity
Clonogenic (holoclones)
Long-term repopulating ability
Committed progenitors
Early TACs
High proliferative activity
Moderately clonogenic (meroclones)
Late TACs
High proliferative activity
Poorly clonogenic (paraclones)
Terminal differentiation
No proliferation
Non-clonogenic

Normal epithelial cells: ***in vitro*** **colonies can be classified by properties of the founder cells and by their abilities to be passaged further (Barrandon and Green).**

Holoclones (Stem)	→	**Meroclones** (Amplifying)	→	**Paraclones** (Differentiating)
Expansive growth		**Growth**		**Little growth**
Large and smooth (30%)		**Wrinkled (50%)**		**Small and irregular (20%)**

Malignant epithelial cells: **patterns of colony morphology are found similar to those of normal epithelia.**



Adult Stem Cell Properties

Self-renewal

Relatively undifferentiated

Multilineage differentiation

Slow cycling, but clonogenic

Have immortal template DNA strands (LRCs)?

Slowly cycling

Slowly cycling - so label retaining

Research article Development and disease 5241

Manipulation of stem cell proliferation and lineage commitment: visualisation of label-retaining cells in wholemounts of mouse epidermis

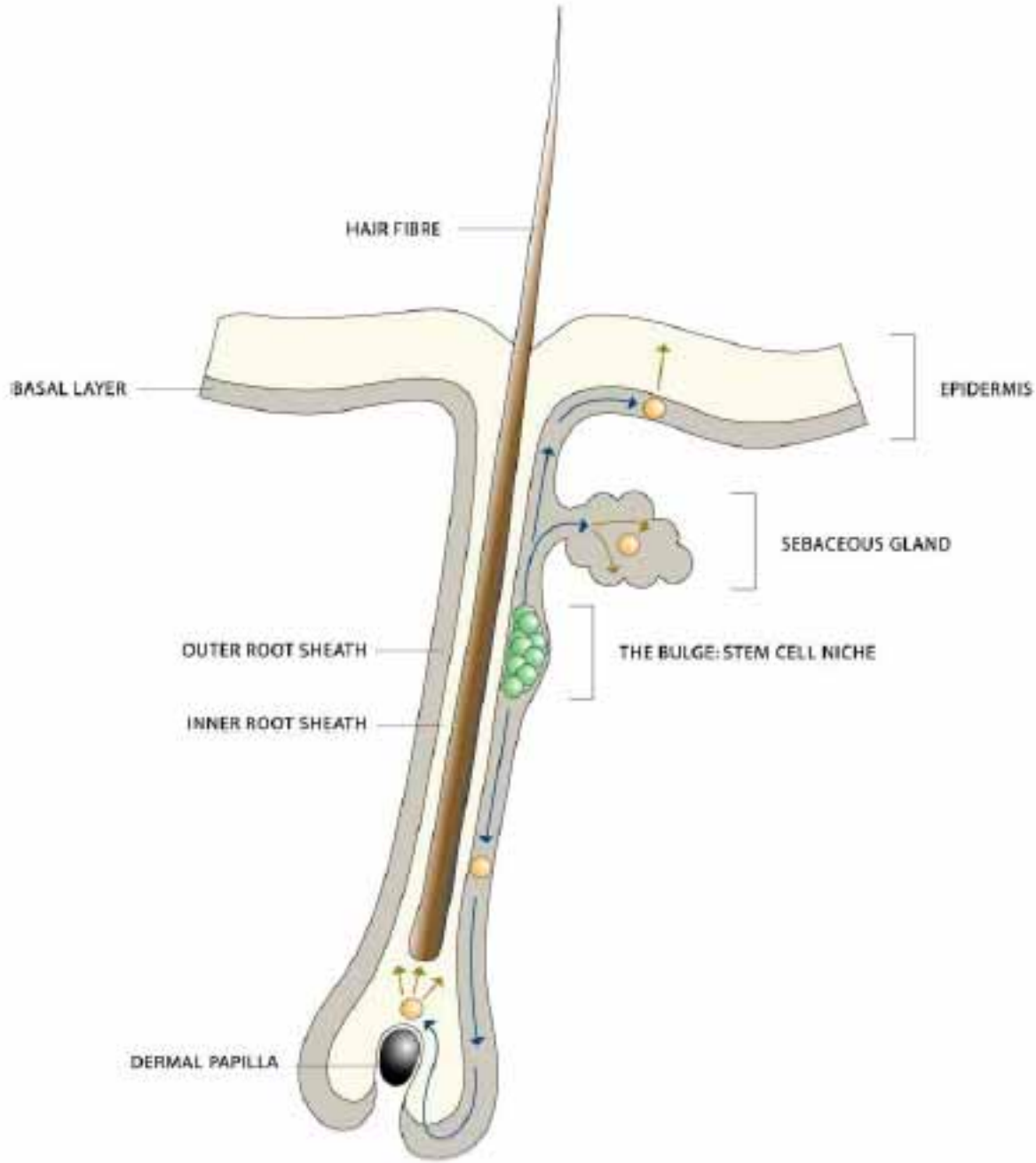
Kristin M. Braun[1], Catherin Niemann[1], Uffe B. Jensen[2], John P. Sundberg[3], Violeta Silva-Vargas[1] and Fiona M. Watt[1,*]

[1]Keratinocyte Laboratory, Cancer Research UK London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3PX, UK
[2]Department of Human Genetics, The Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark
[3]The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609-1500, USA
*Author for correspondence (e-mail: fiona.watt@cancer.org.uk)

Accepted 2 July 2003

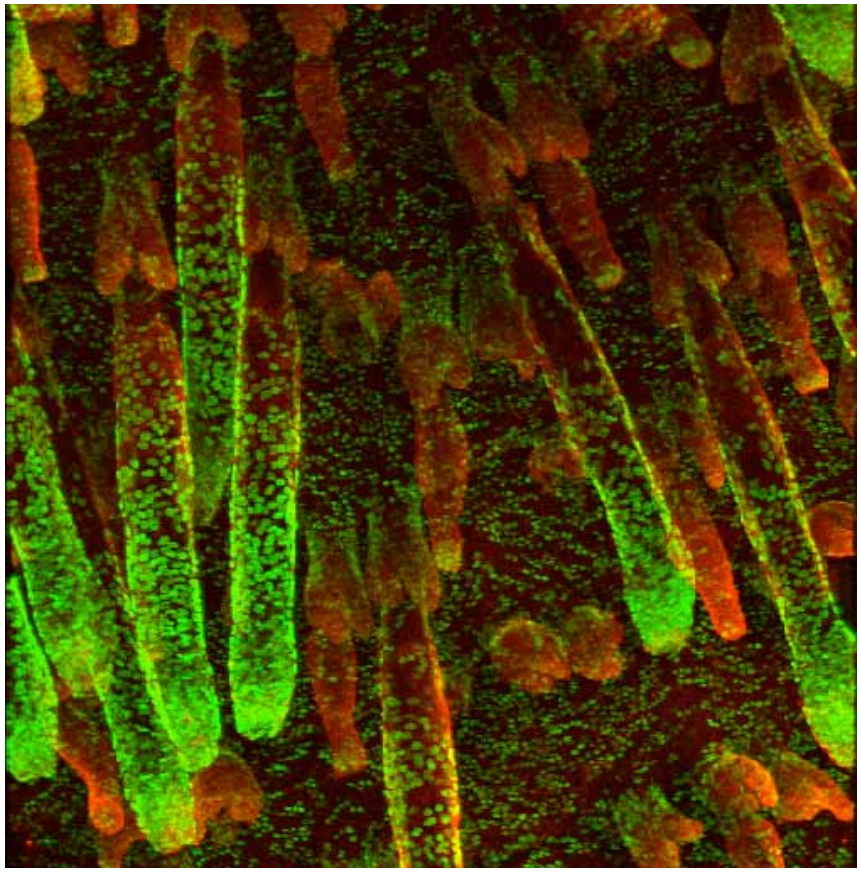
Development 130, 5241-5255

doi:10.1242/dev.00703

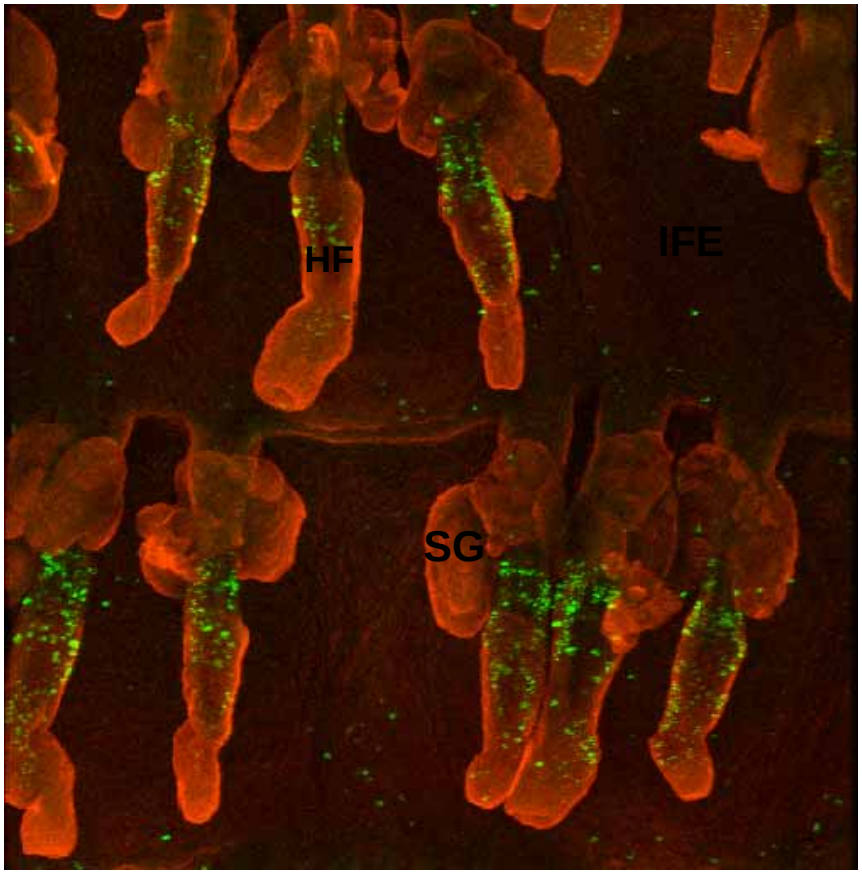

HAIR FIBRE
BASAL LAYER
EPIDERMIS
SEBACEOUS GLAND
OUTER ROOT SHEATH
THE BULGE: STEM CELL NICHE
INNER ROOT SHEATH
DERMAL PAPILLA

BrdU LRC

Keratin 14



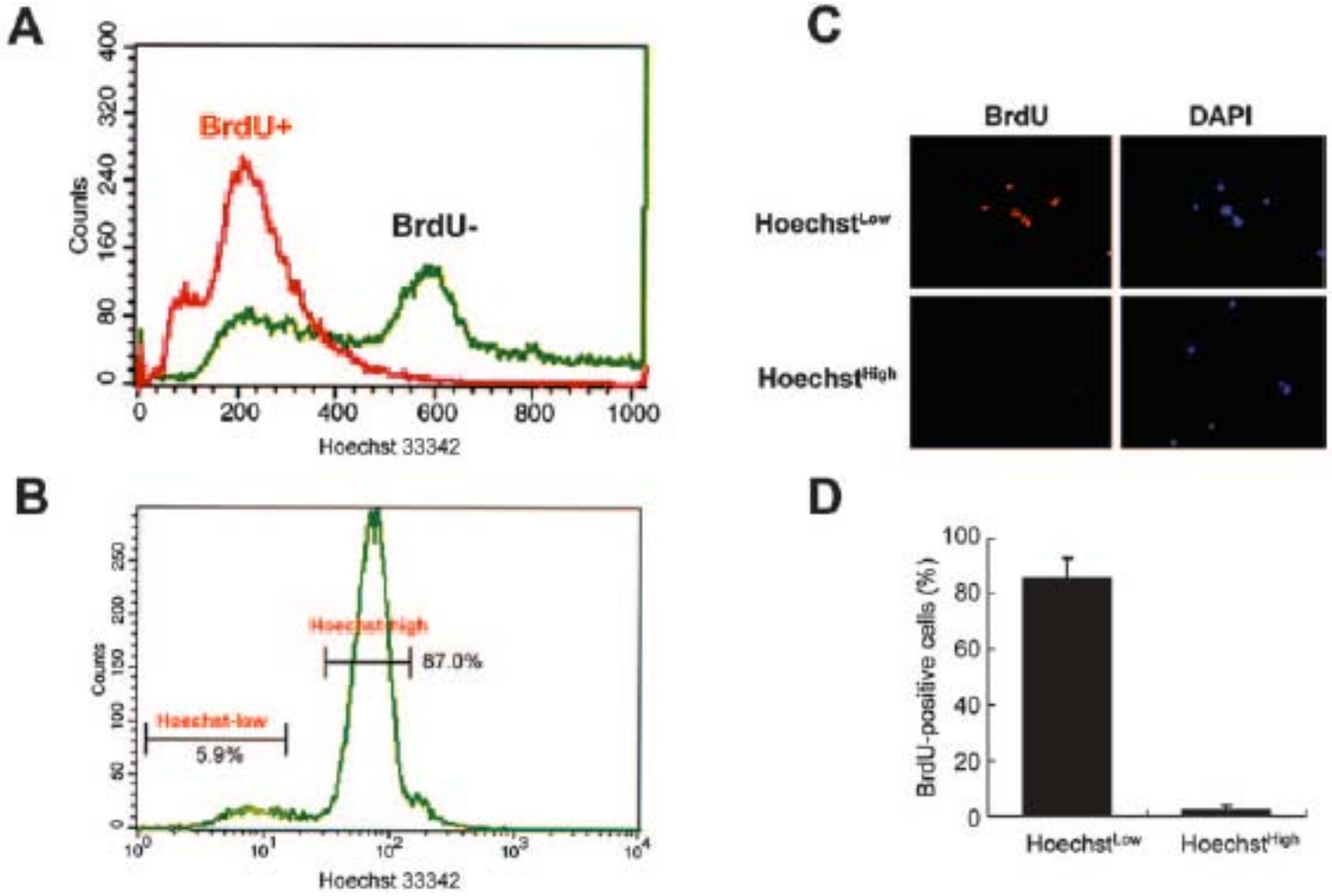
2 days post-BrdU



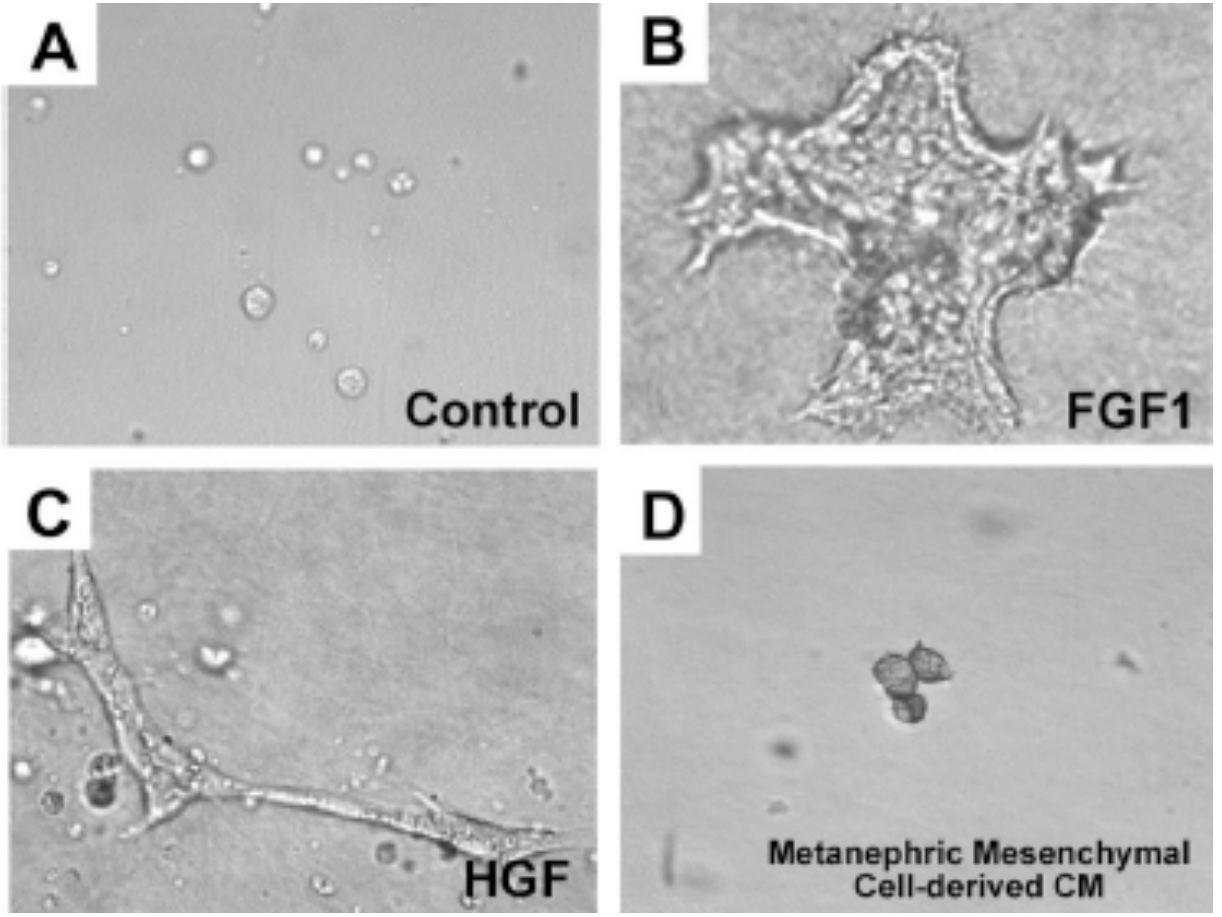
140 days post-BrdU

Adult Kidney Tubular Cell Population Showing Phenotypic Plasticity, Tubulogenic Capacity, and Integration Capability into Developing Kidney

J Am Soc Nephrol 17: 188–198, 2006.



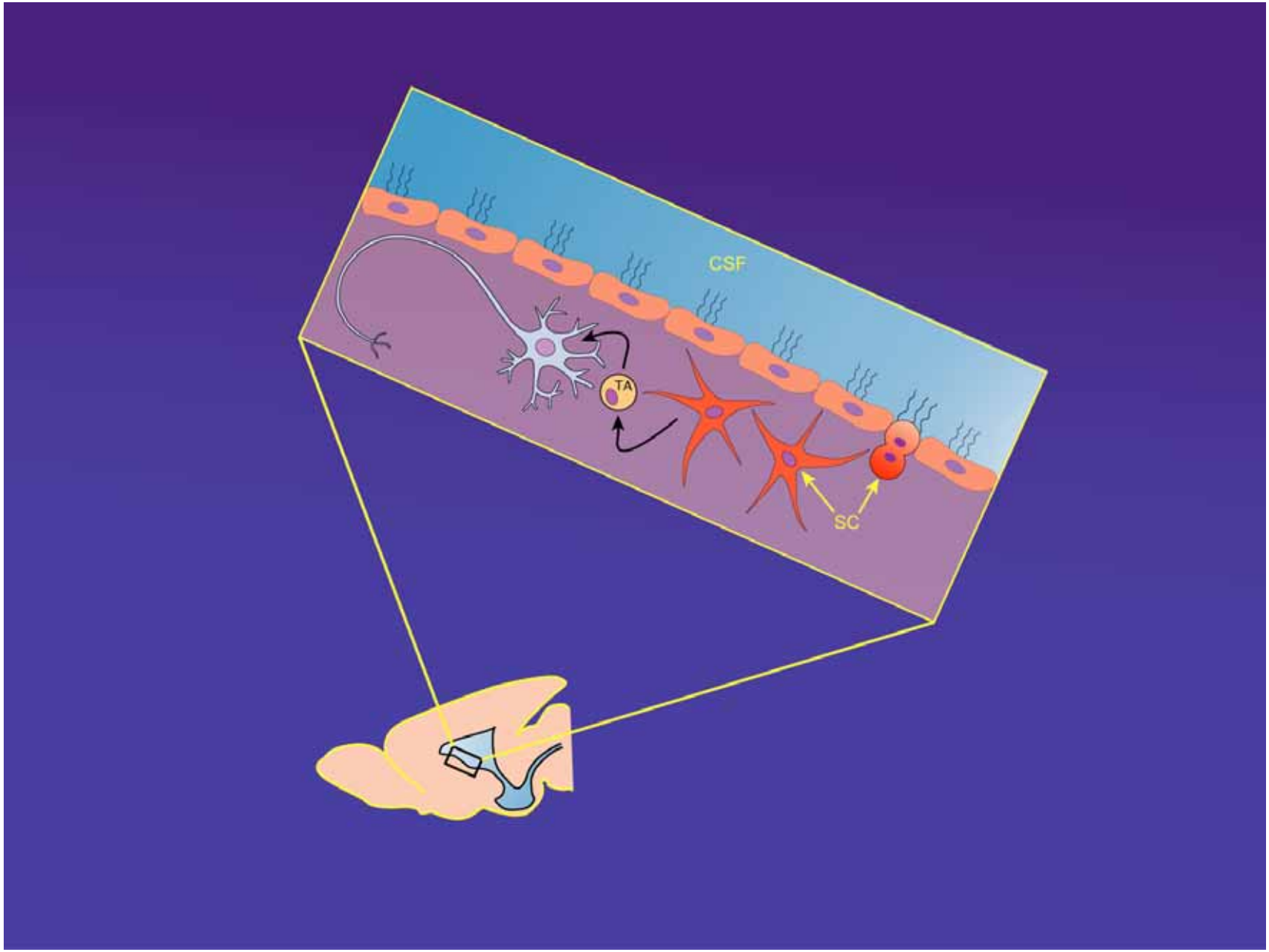
Properties of Hoechst^{low} LRTCs

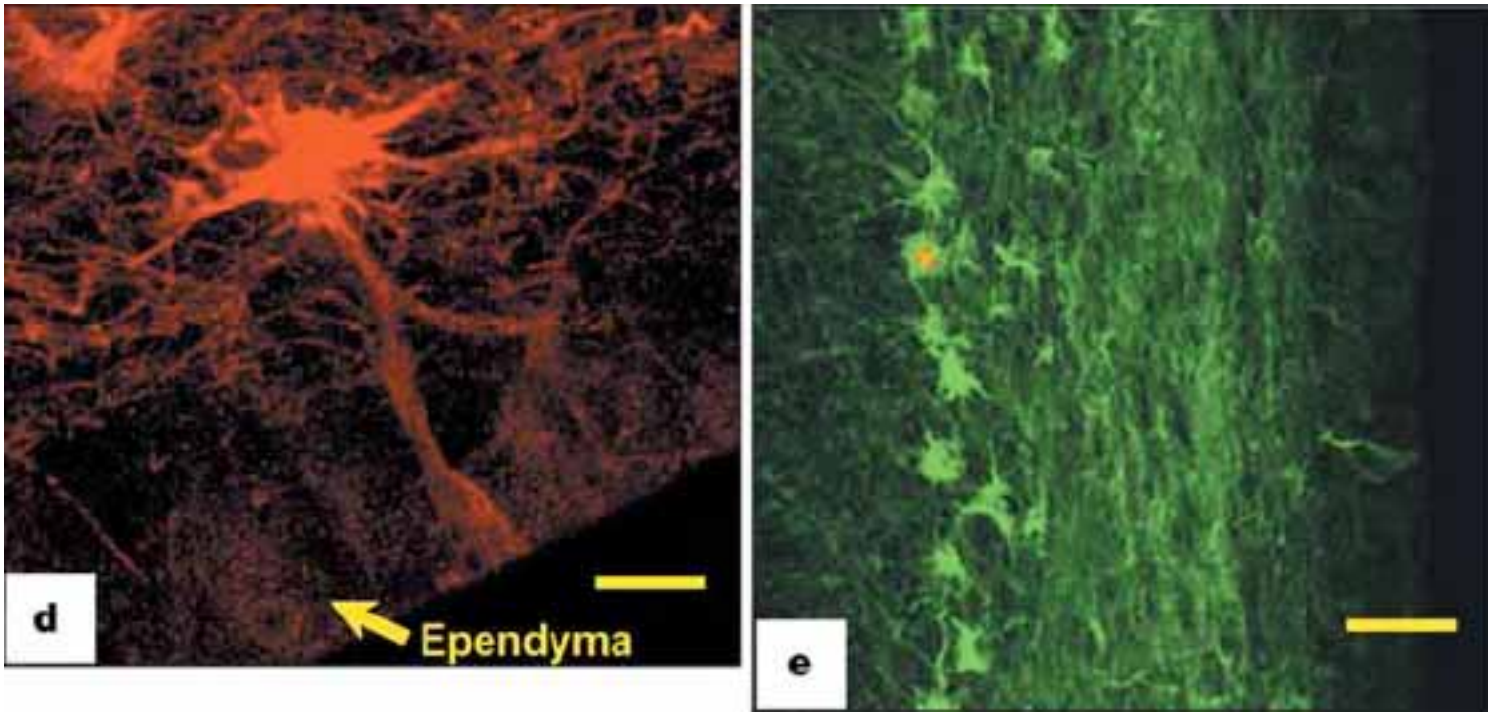


Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration

Nader Sanai[1,2], Anthony D. Tramontin[1,2], Alfredo Quiñones-Hinojosa[1], Nicholas M. Barbaro[1], Nalin Gupta[1], Sandeep Kunwar[1], Michael T. Lawton[1], Michael W. McDermott[1], Andrew T. Parsa[1], José Manuel-García Verdugo[3], Mitchel S. Berger[1] & Arturo Alvarez-Buylla[1,2]

NATURE | VOL 427 | 19 FEBRUARY 2004 | www.nature.com/nature

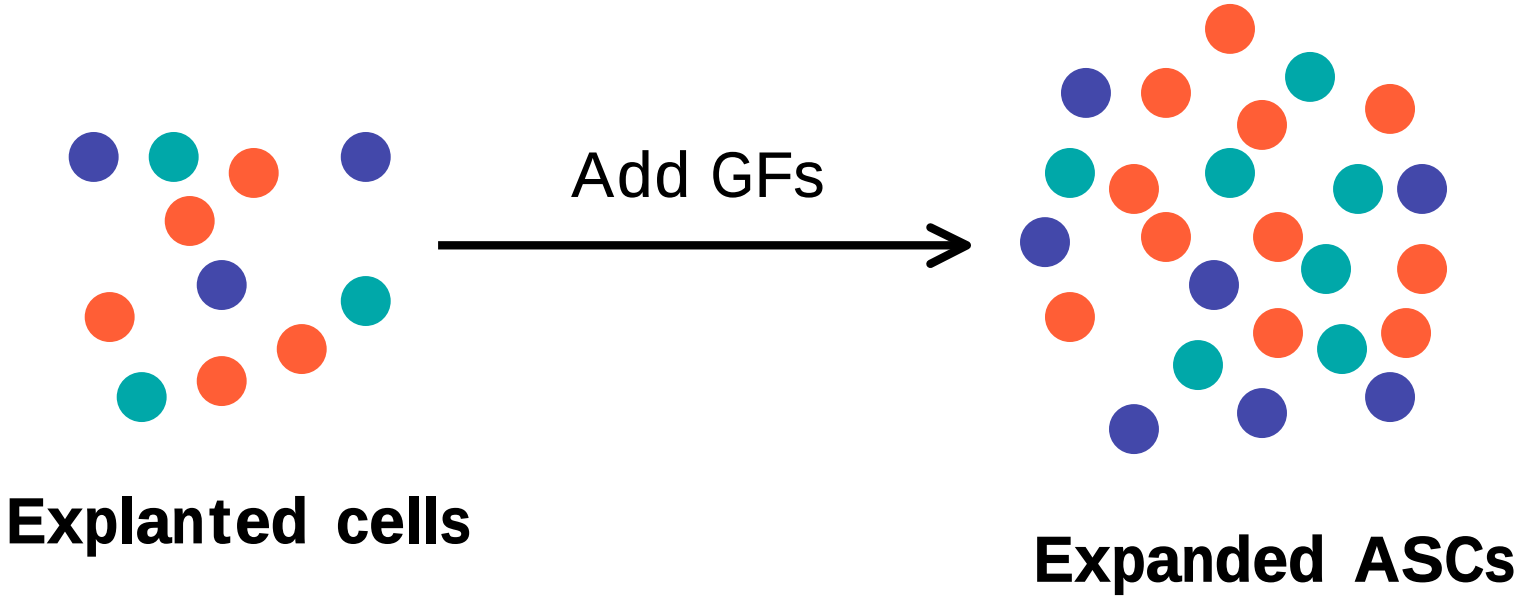

CSF
TA
SC

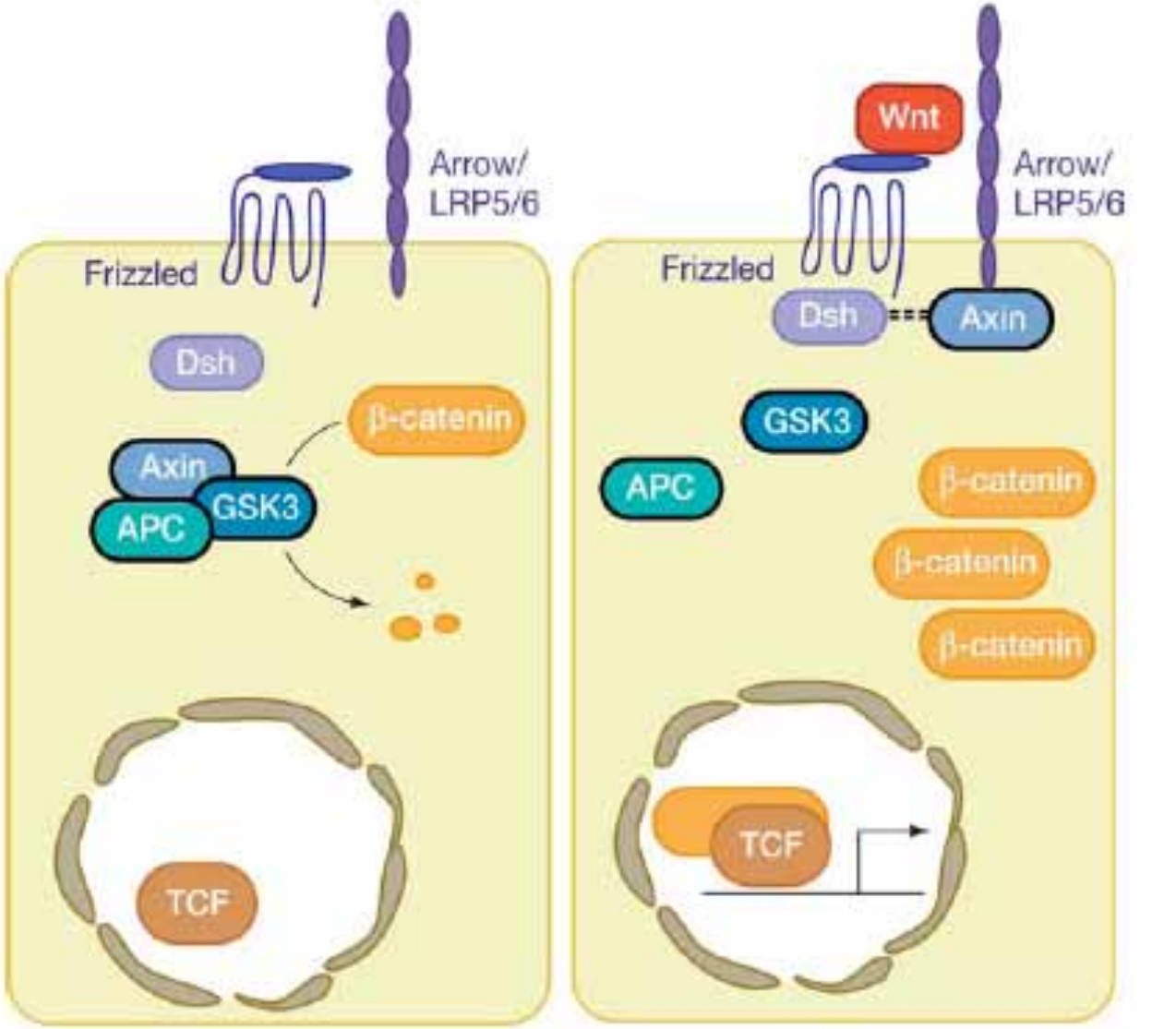


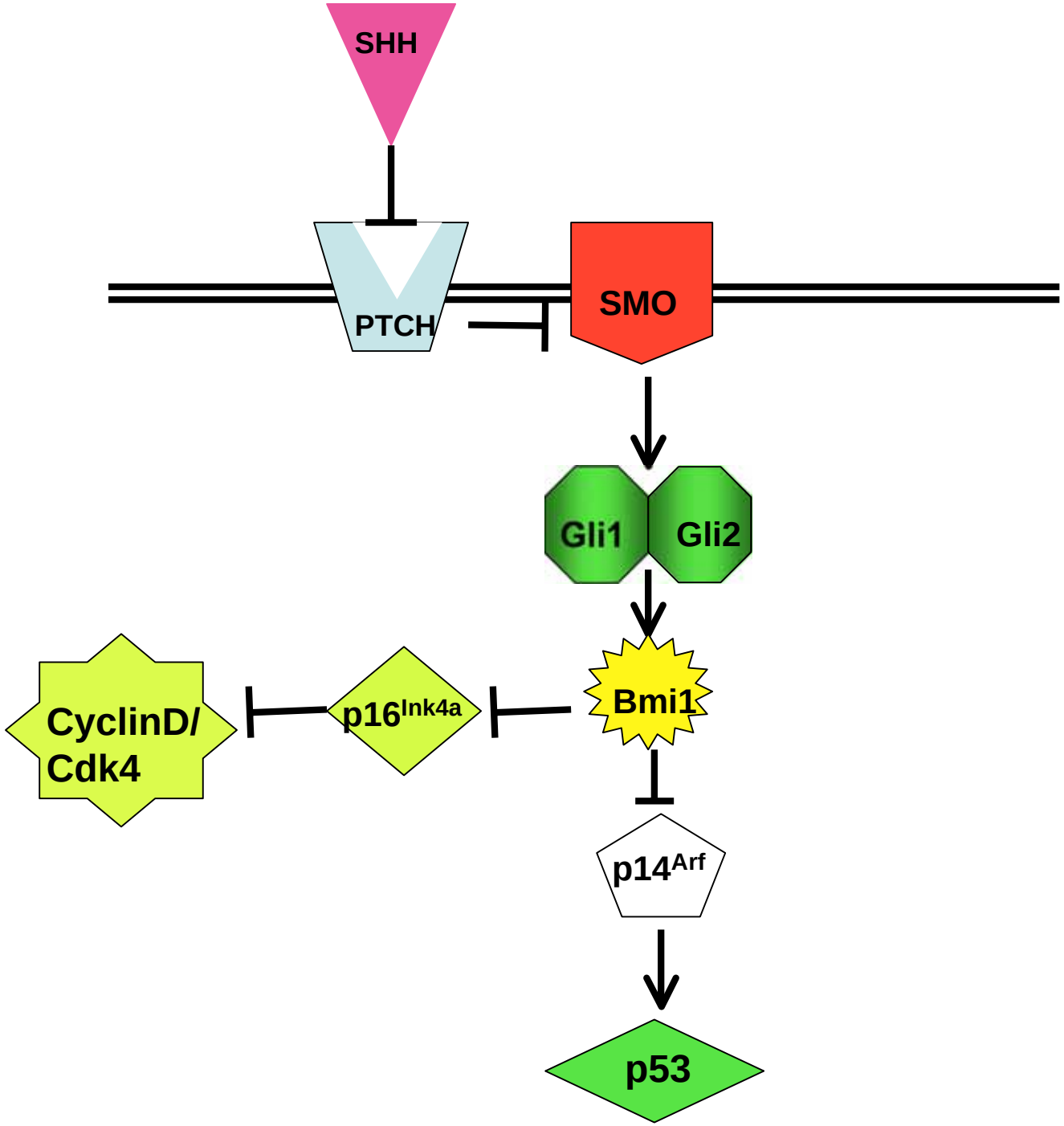
GFAP **Ki-67**

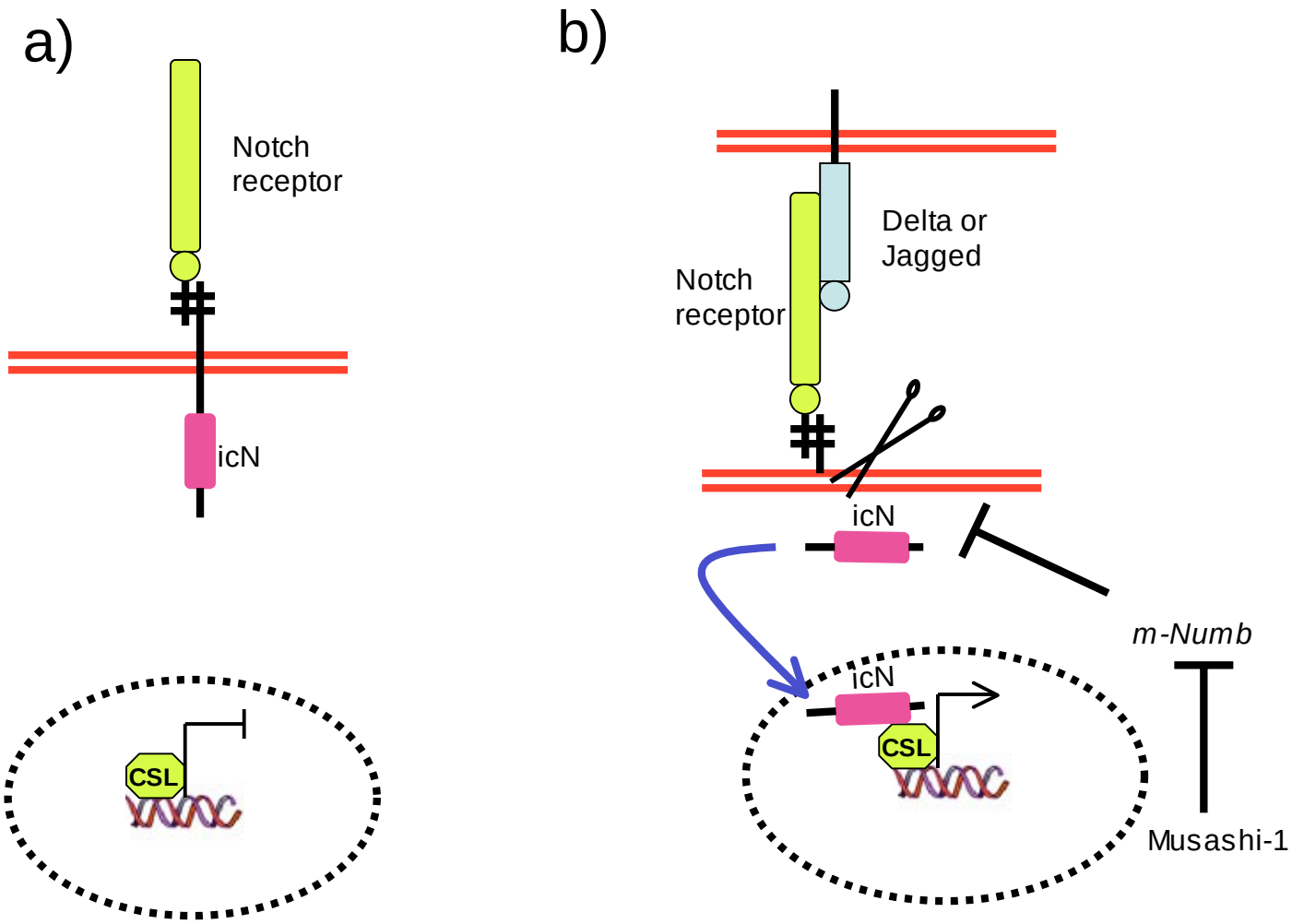
Approaches to the expansion of adult stem cells

1. Growth factor supplementation

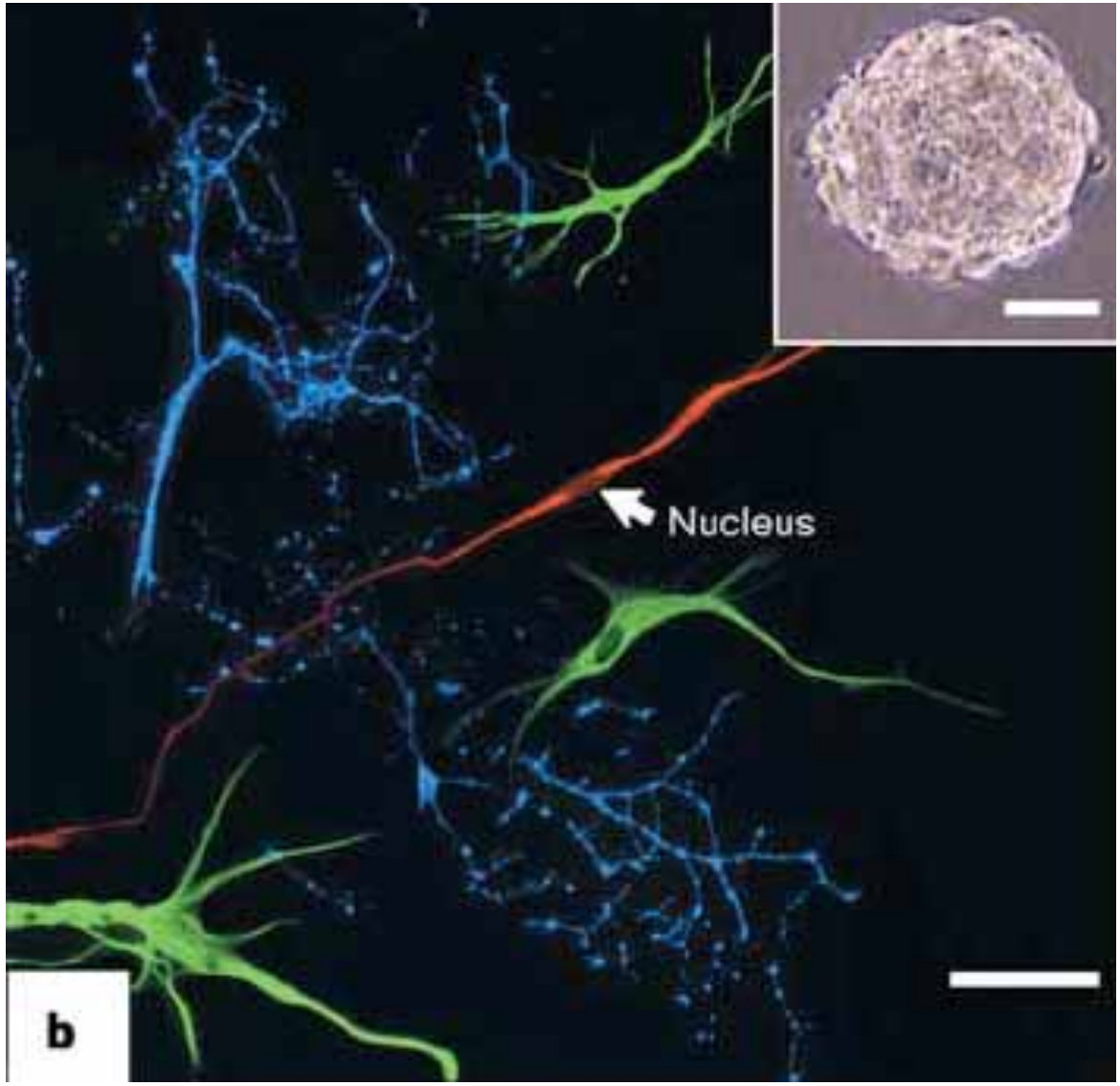



Arrow/
LRP5/6
Frizzled
Dsh
β-catenin
Axin
APC
GSK3
TCF
Wnt
Arrow/
LRP5/6
Frizzled
Dsh
Axin
GSK3
APC
β-catenin
β-catenin
β-catenin
TCF


SHH
PTCH
SMO
Gli1
Gli2
CyclinD/
Cdk4
p16Ink4a
Bmi1
p14Arf
p53

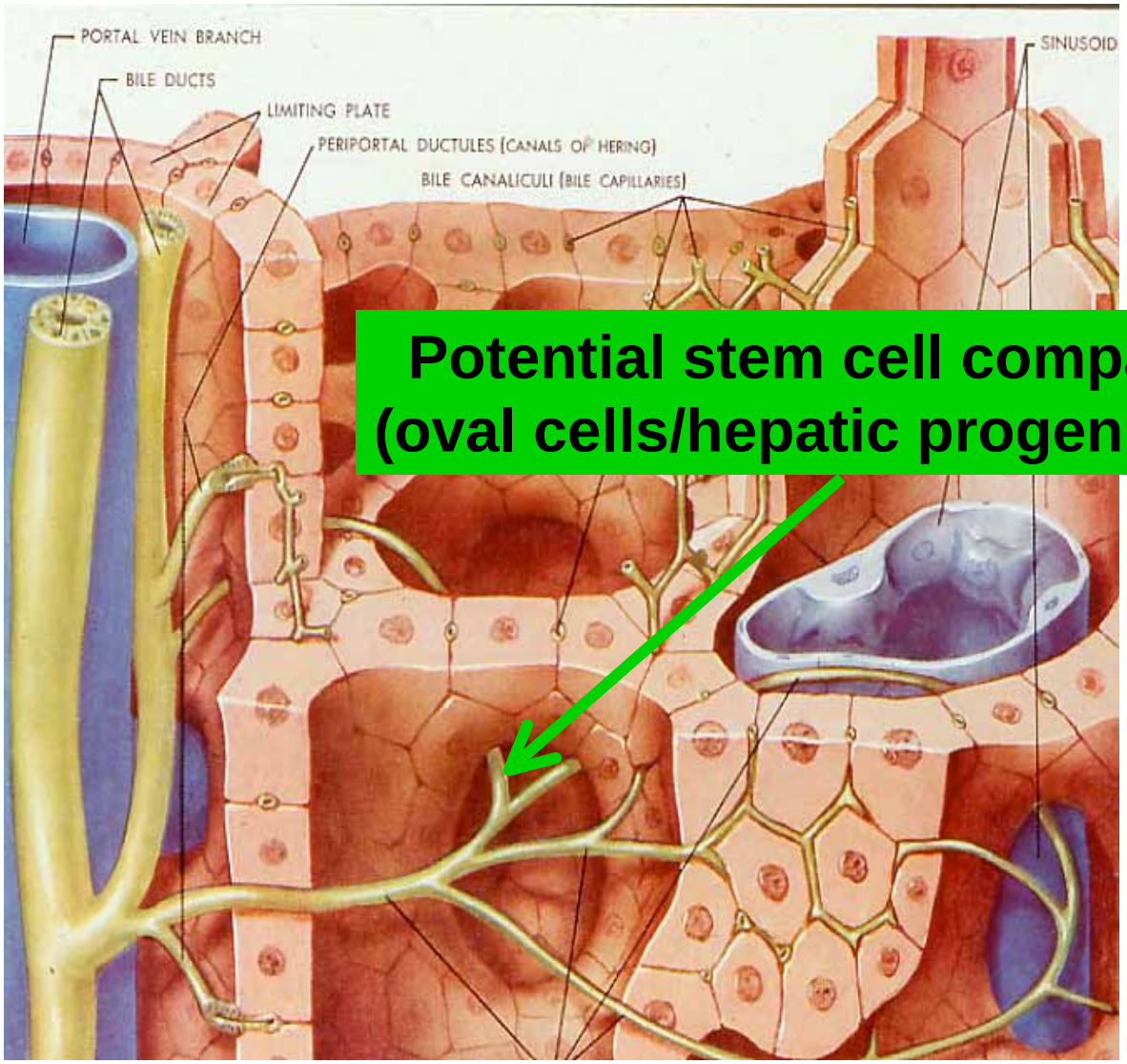

a)
Notch
receptor
icN
CSL
b)
Delta or
Jagged
Notch
receptor
icN
icN
CSL
m-Numb
Musashi-1

Trilineage potential in vitro

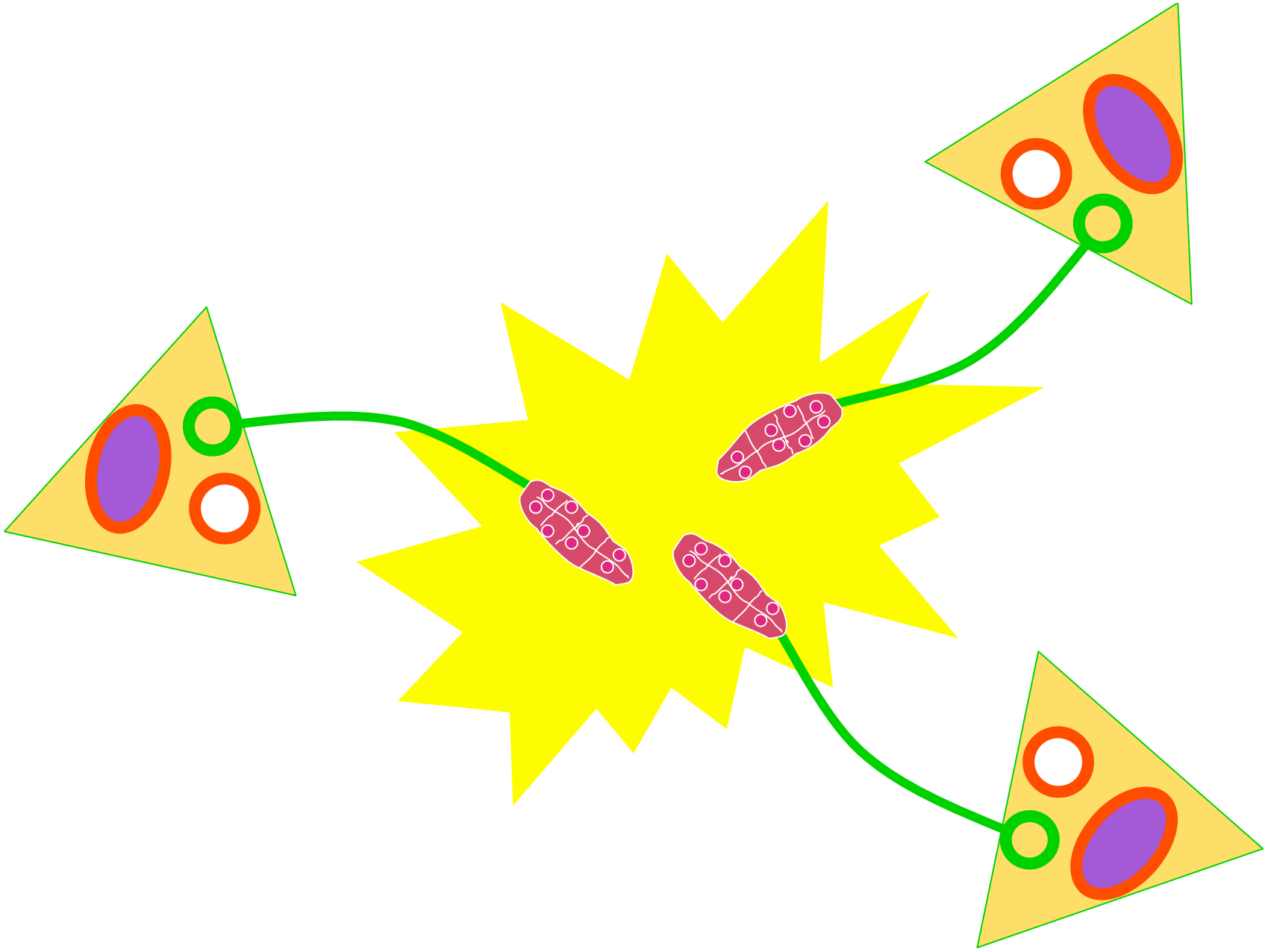


Are clonogenic

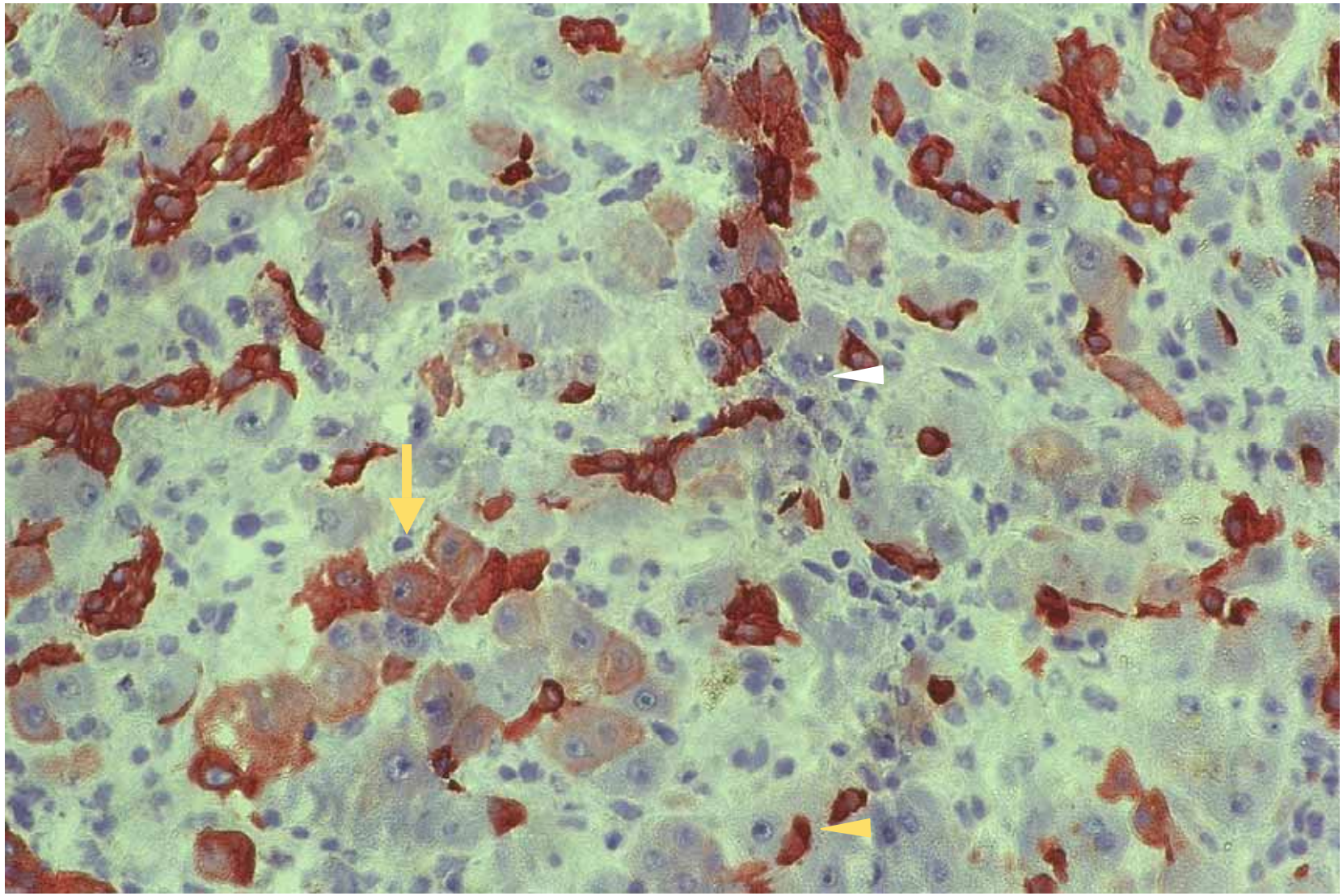
The biology of cholangiocytes (in humans)



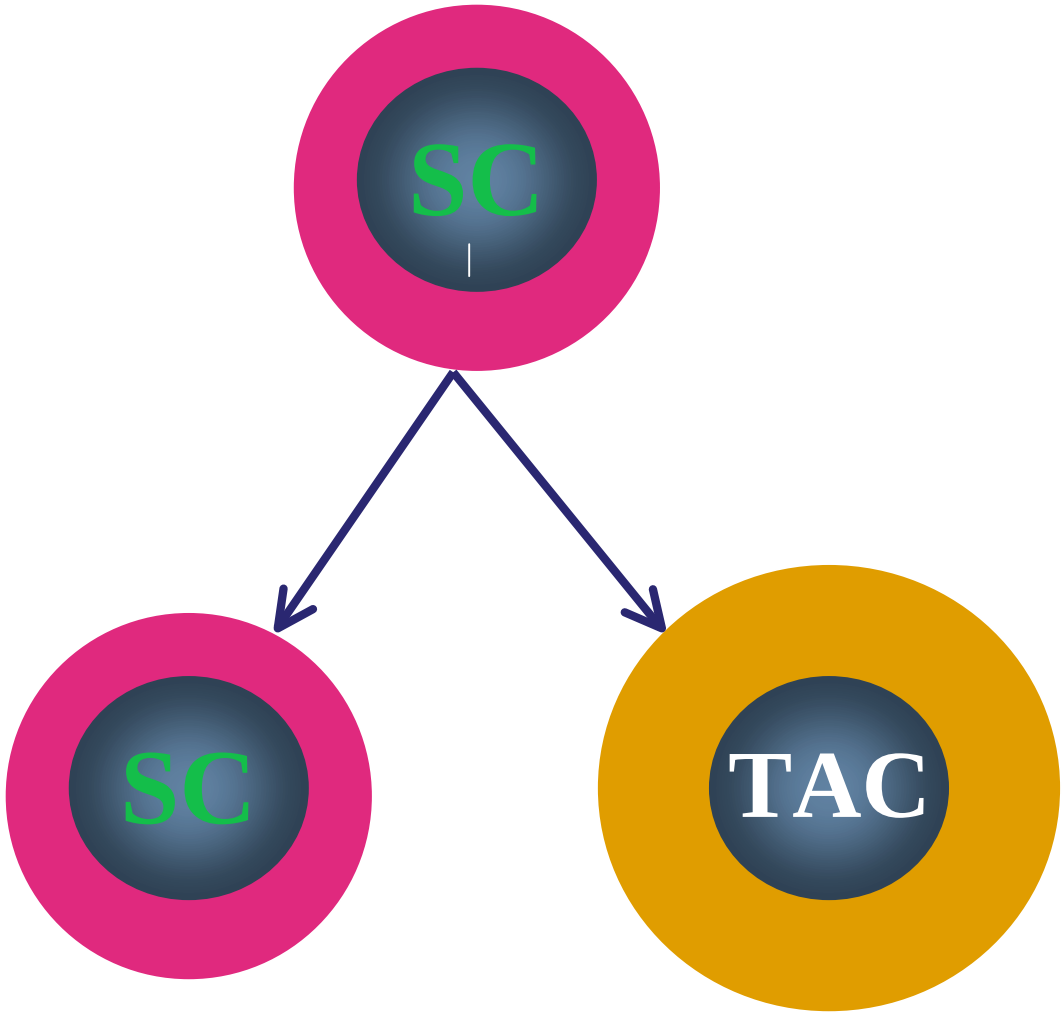
Potential stem cell compartment (oval cells/hepatic progenitor cells)



CK 7: progenitor cells/intermediate hepatocytes in regeneration

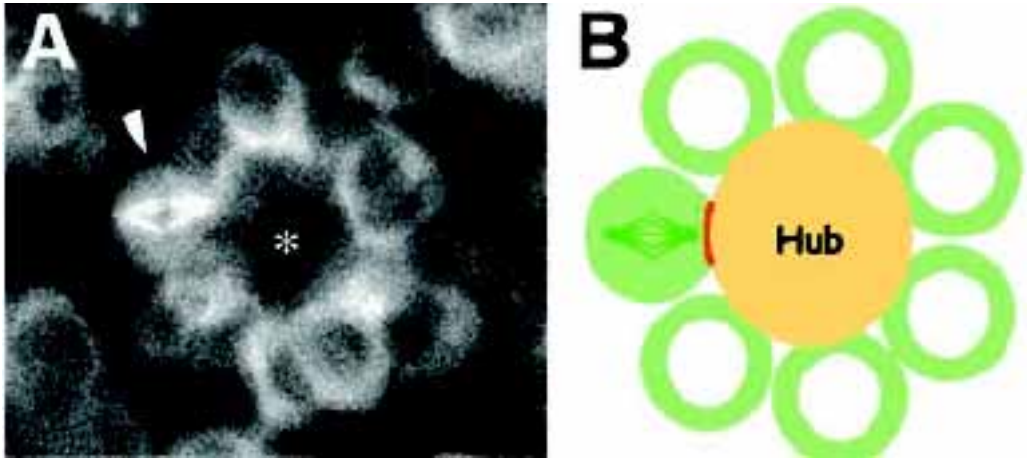


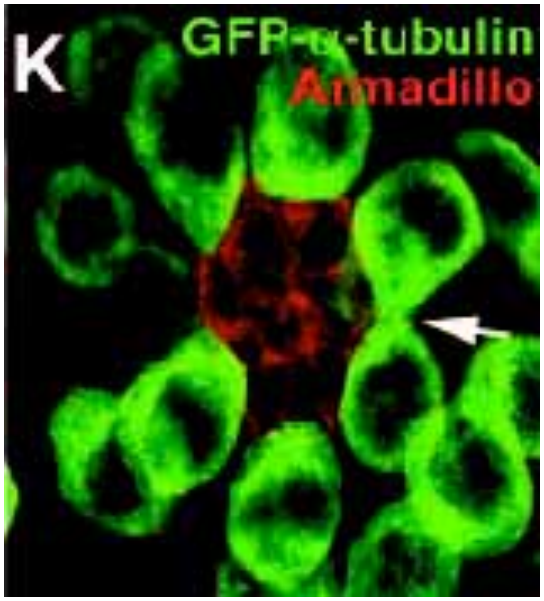
Asymmetric cell division



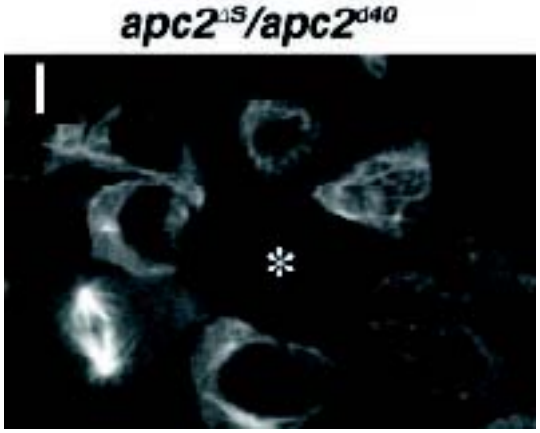
Orientation of Asymmetric Stem Cell Division by the APC Tumor Suppressor and Centrosome

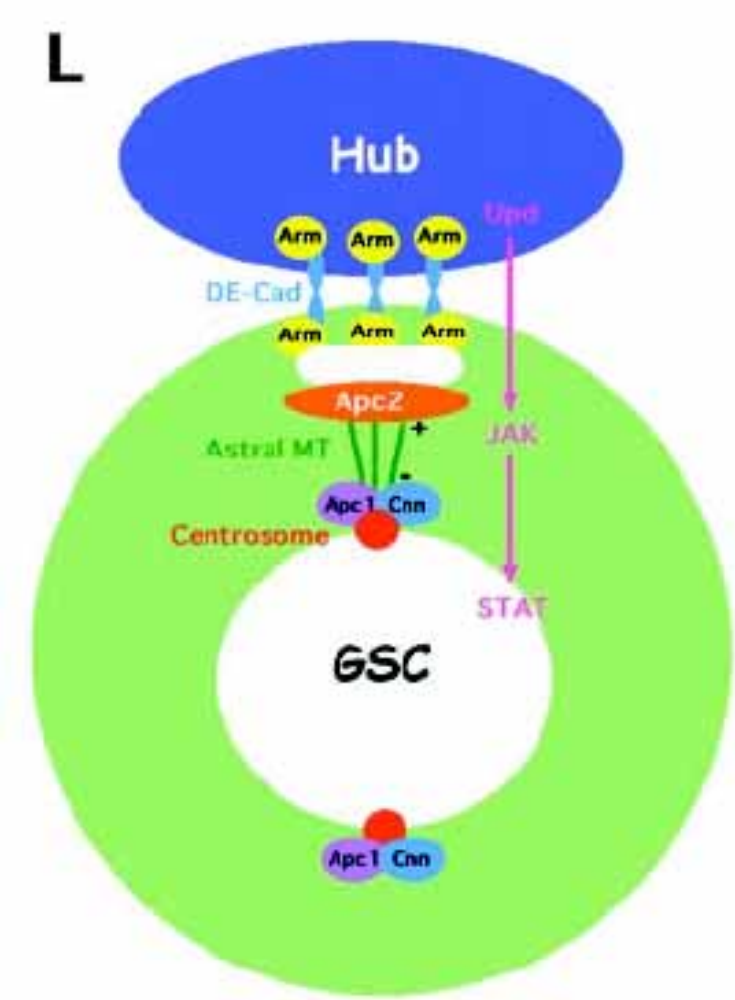
Yukiko M. Yamashita,[1] D. Leanne Jones,[1] Margaret T. Fuller[1,2]*



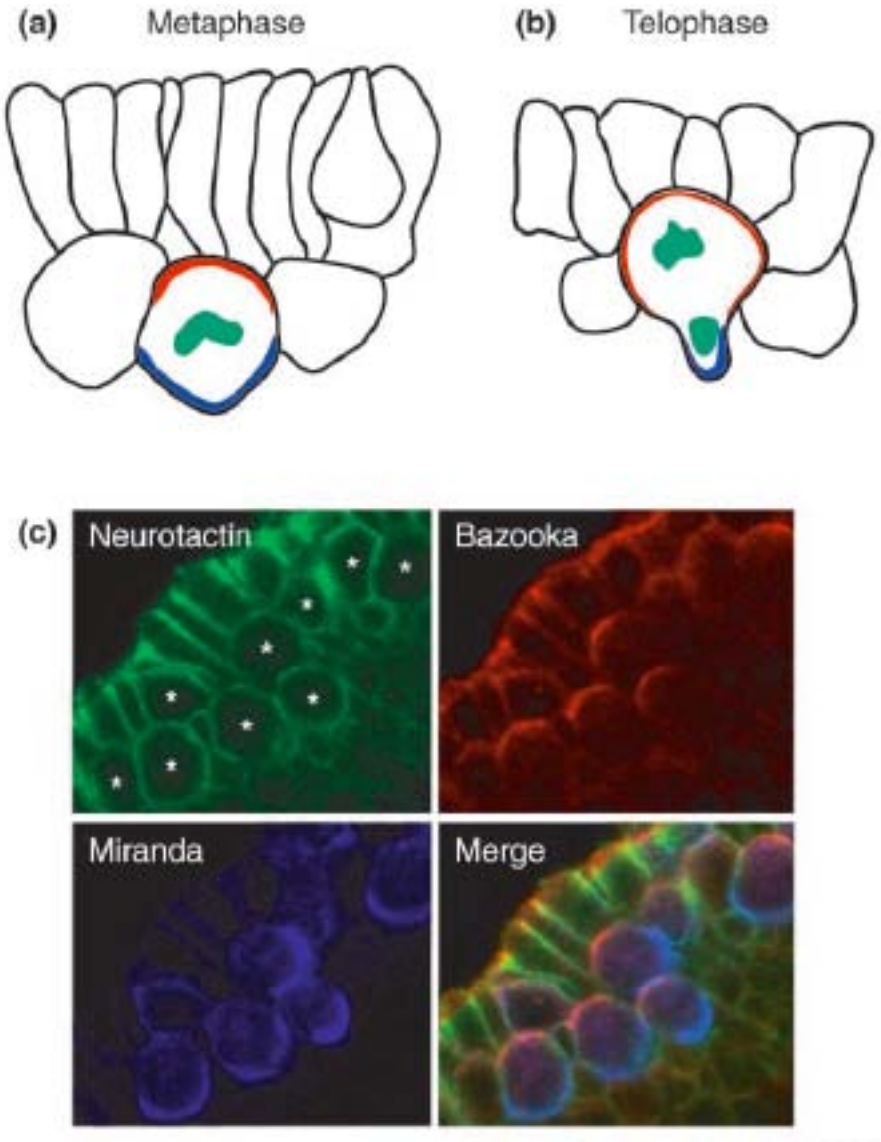


Centrosomin mutant

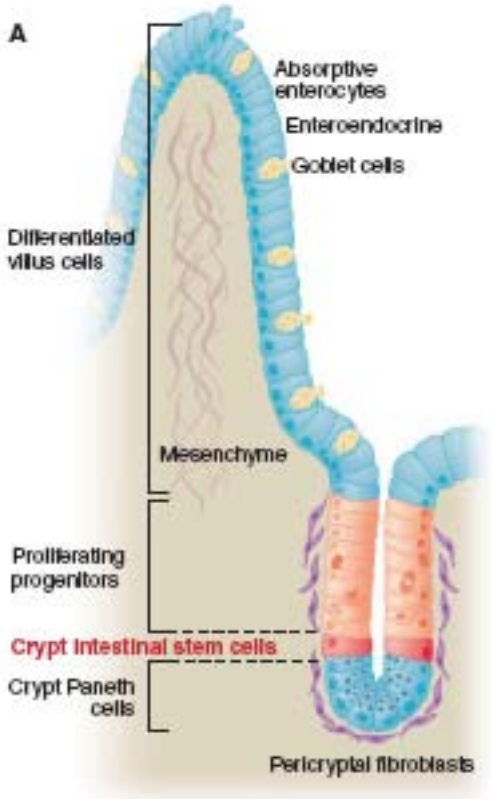


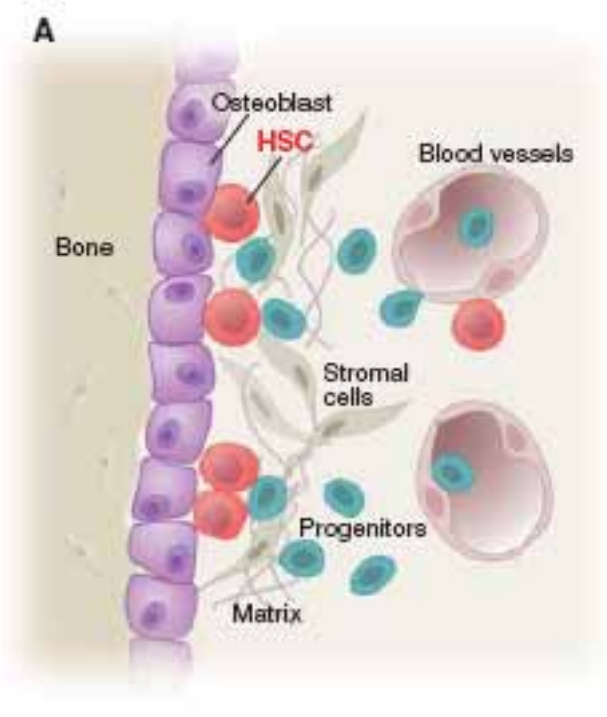


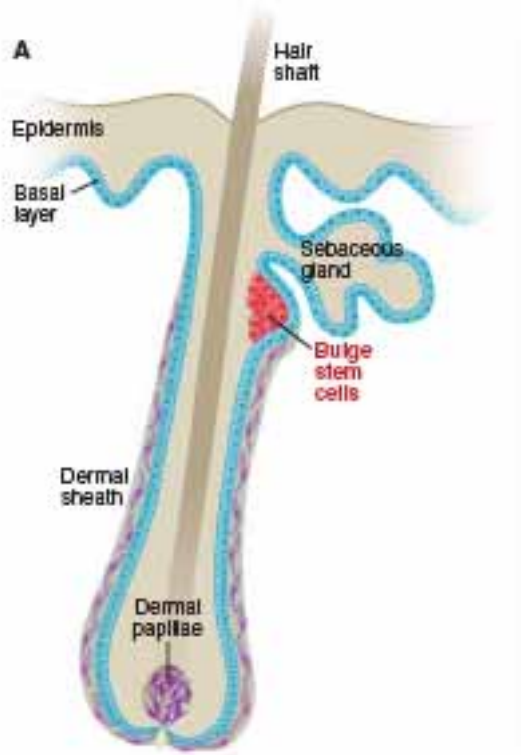
Drosophila neuroblasts



There's no place like home: the stem cell niche

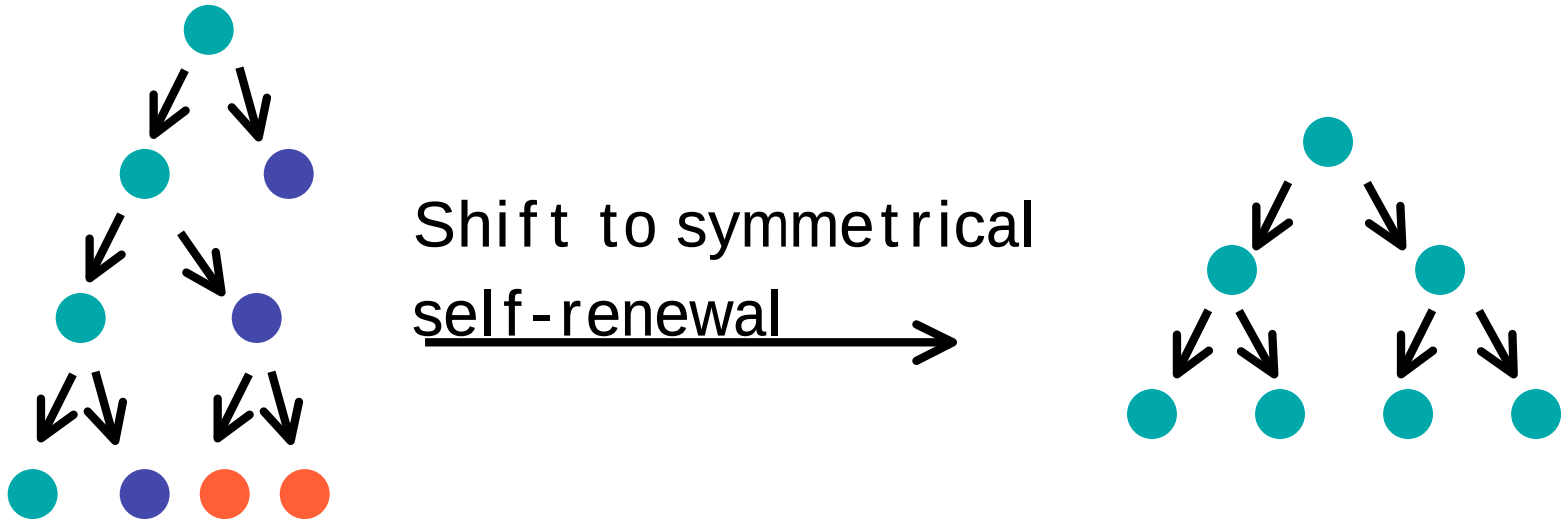




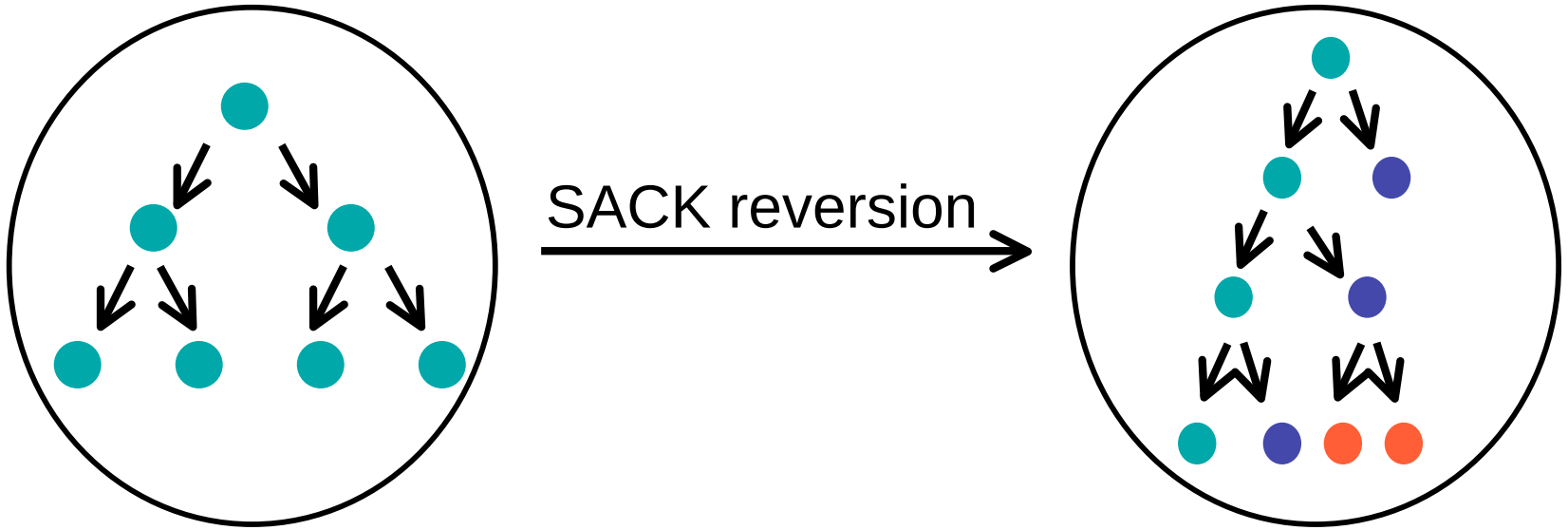


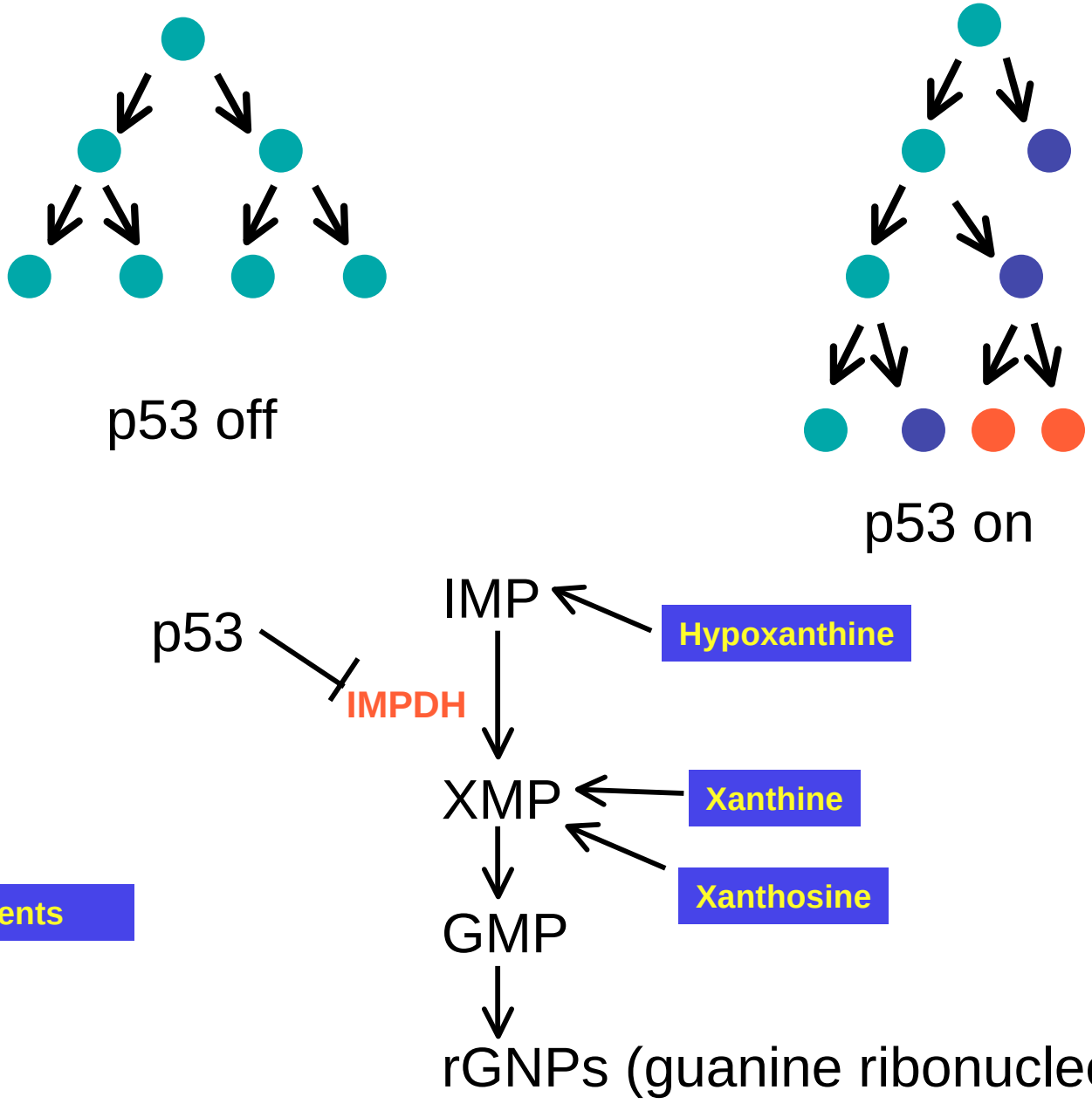
Approaches to the expansion of adult stem cells

2. Suppression of asymmetric cell kinetics (SACK)



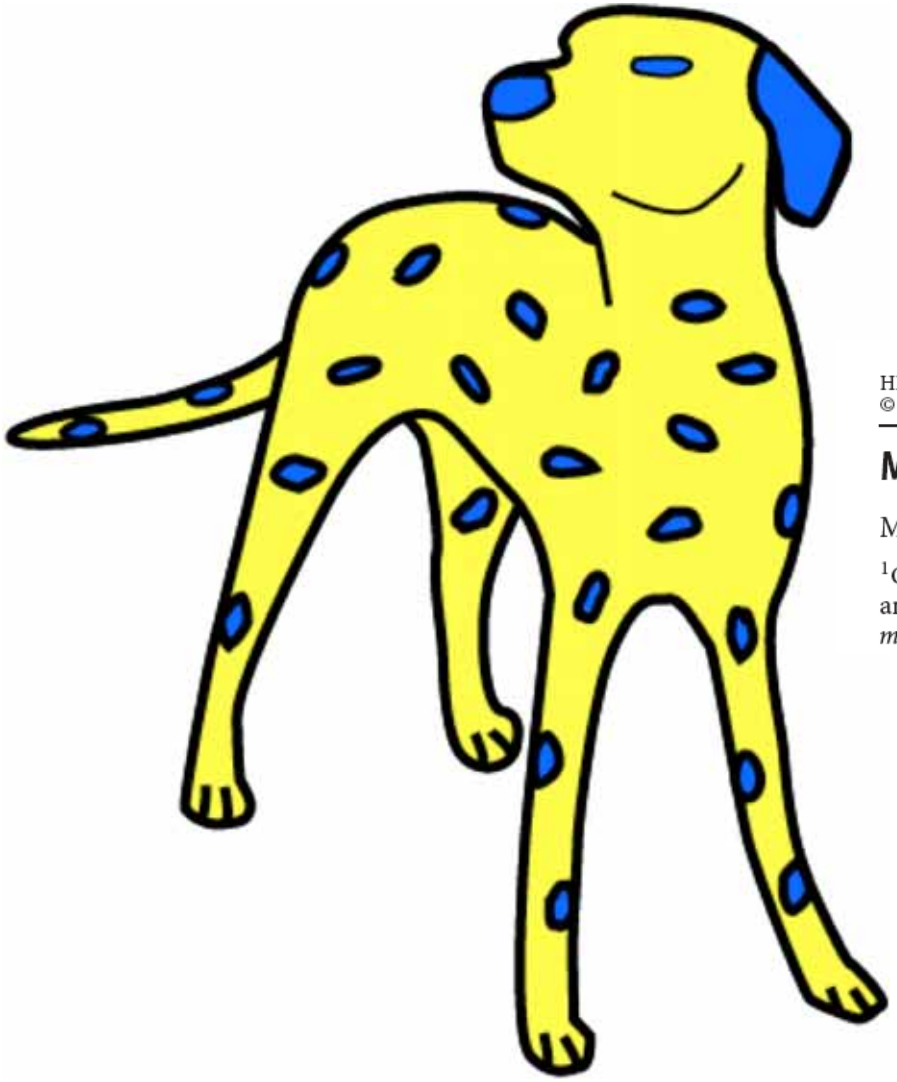
Asymmetric self-renewal


SACK reversion



Adapted from Pare and Sherley. Biological principles for ex vivo adult stem cell expansion. *Current Topics in Developmental Biology* 2006; **73**: 141-171

Markers?



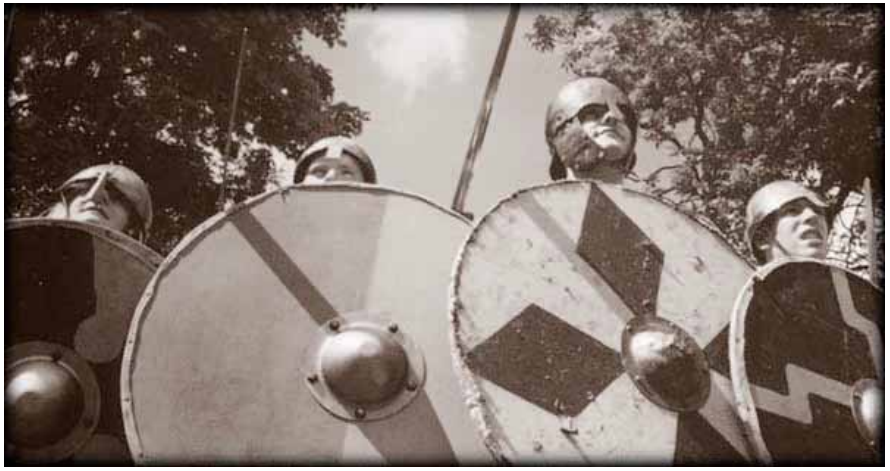
HEP (2006) 174:185–227

Markers of Adult Tissue-Based Stem Cells

M.R. Alison[1] (✉) · M. Brittan[2] · M. Lovell[2] · N.A. Wright[2]

[1]Centre for Diabetes and Metabolic Medicine, Queen Mary's School of Medicine and Dentistry, Royal London Hospital, Whitechapel, London E1 1BB, UK
m.alison@qmul.ac.uk

Cytoprotection



Survival kit:

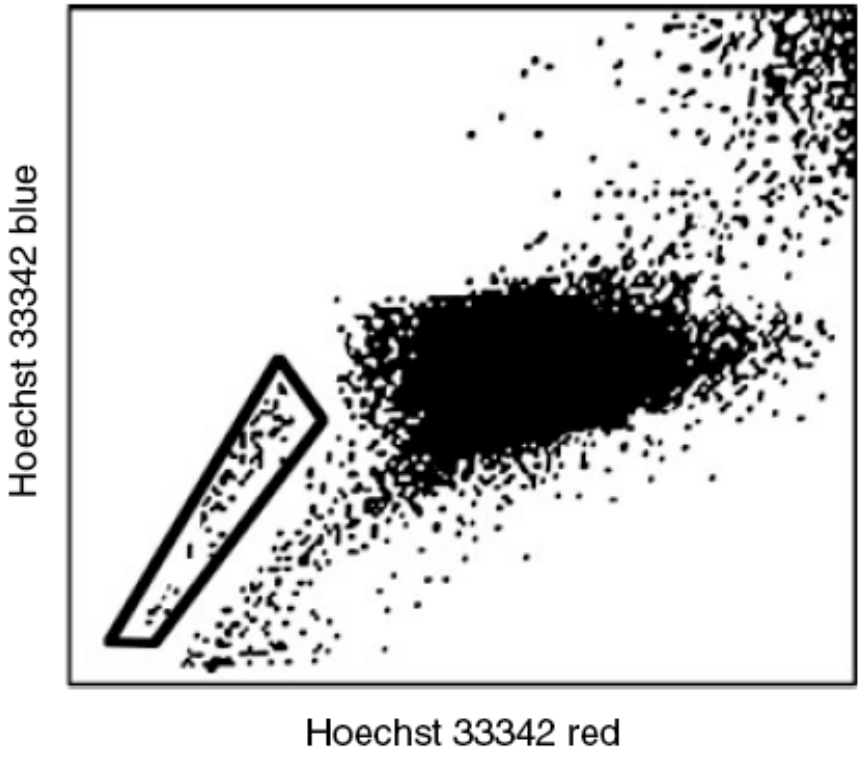
- **ABC Transporter proteins**
- **Aldehyde Dehydrogenase activity (ALDH)**
- **Low Cytochrome P450 activity**

Journal of Pathology
J Pathol 2003; **200**: 547–550.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1411

Commentary

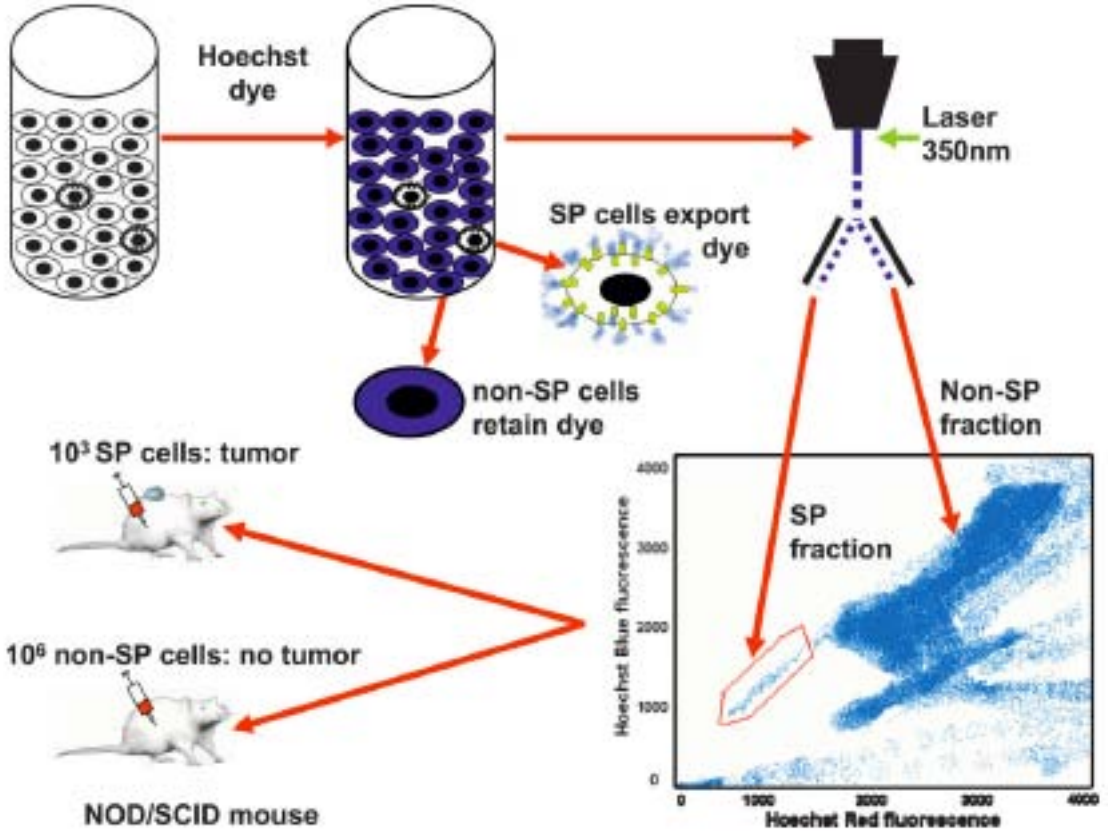
Tissue-based stem cells: ABC transporter proteins take centre stage

Malcolm R Alison*
Department of Histopathology, Imperial College London at the Hammersmith Hospital, Du Cane Road, London W12 0NN, UK



The side population (SP)

Side Population (SP) Cells: Taking Center Stage in Regeneration and Liver Cancer?



Adhesion

Differentiation (2004) 72:387–395

ORIGINAL ARTICLE

Angela Webb · Amy Li · Pritinder Kaur

Location and phenotype of human adult keratinocyte stem cells of the skin

α6 CD71^{dim}

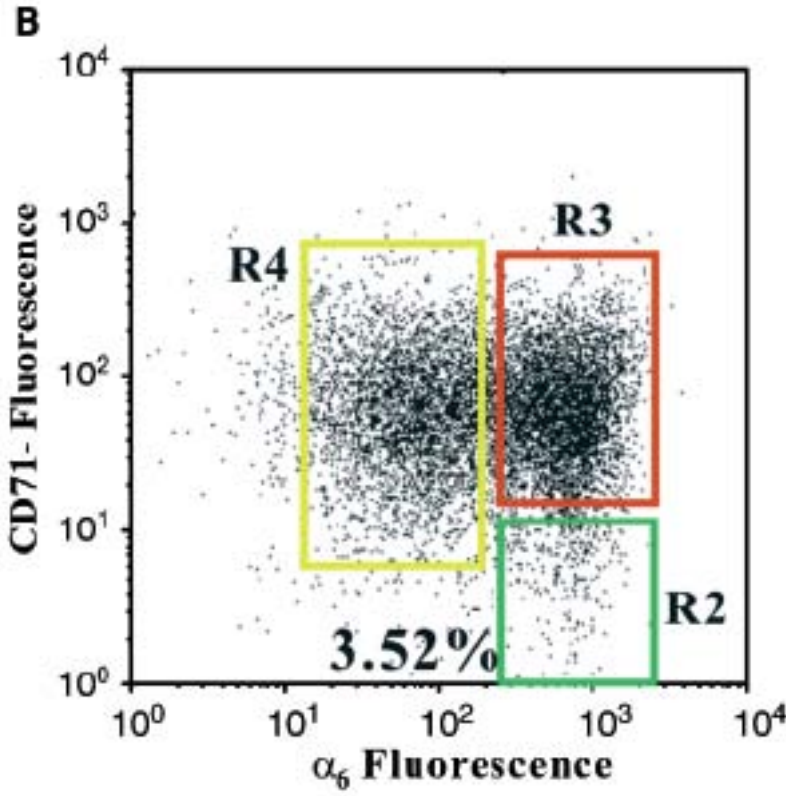
- **2-5% of CK14^{+} basal cells**
- **blast-like morphology**
- **lack CK10**
- **regenerates full thickness epidermis**

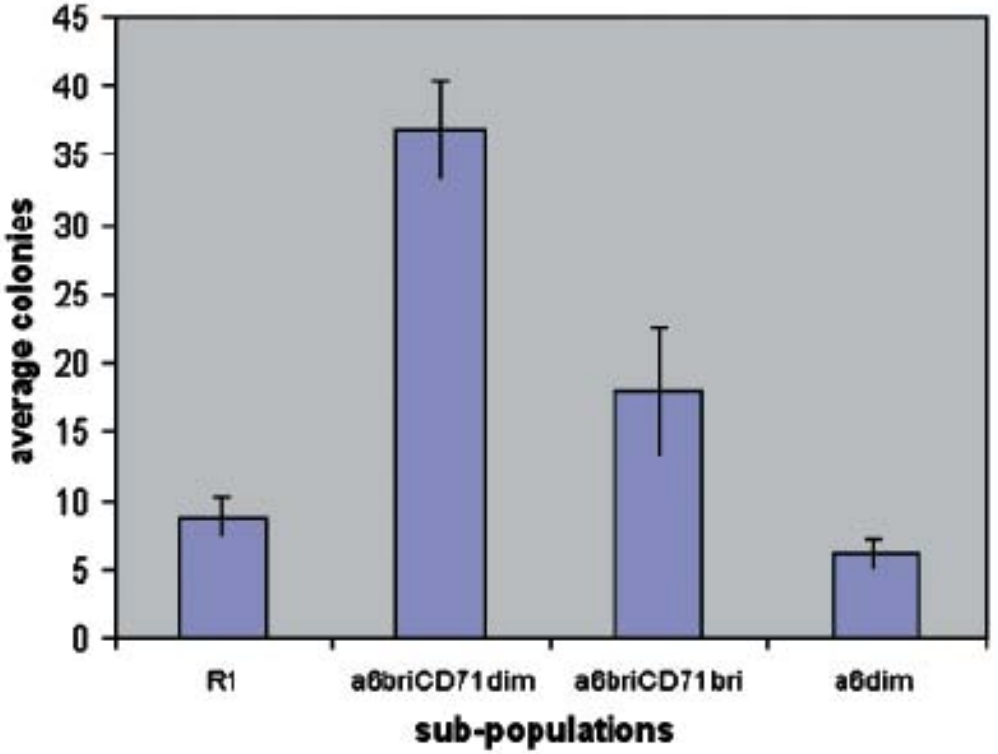
α6 (partners β4 in binding to laminin)
CD71 (transferrin receptor)

Angela Webb · Amy Li · Pritinder Kaur

Differentiation (2004) 72:387–395

Location and phenotype of human adult keratinocyte stem cells of the skin



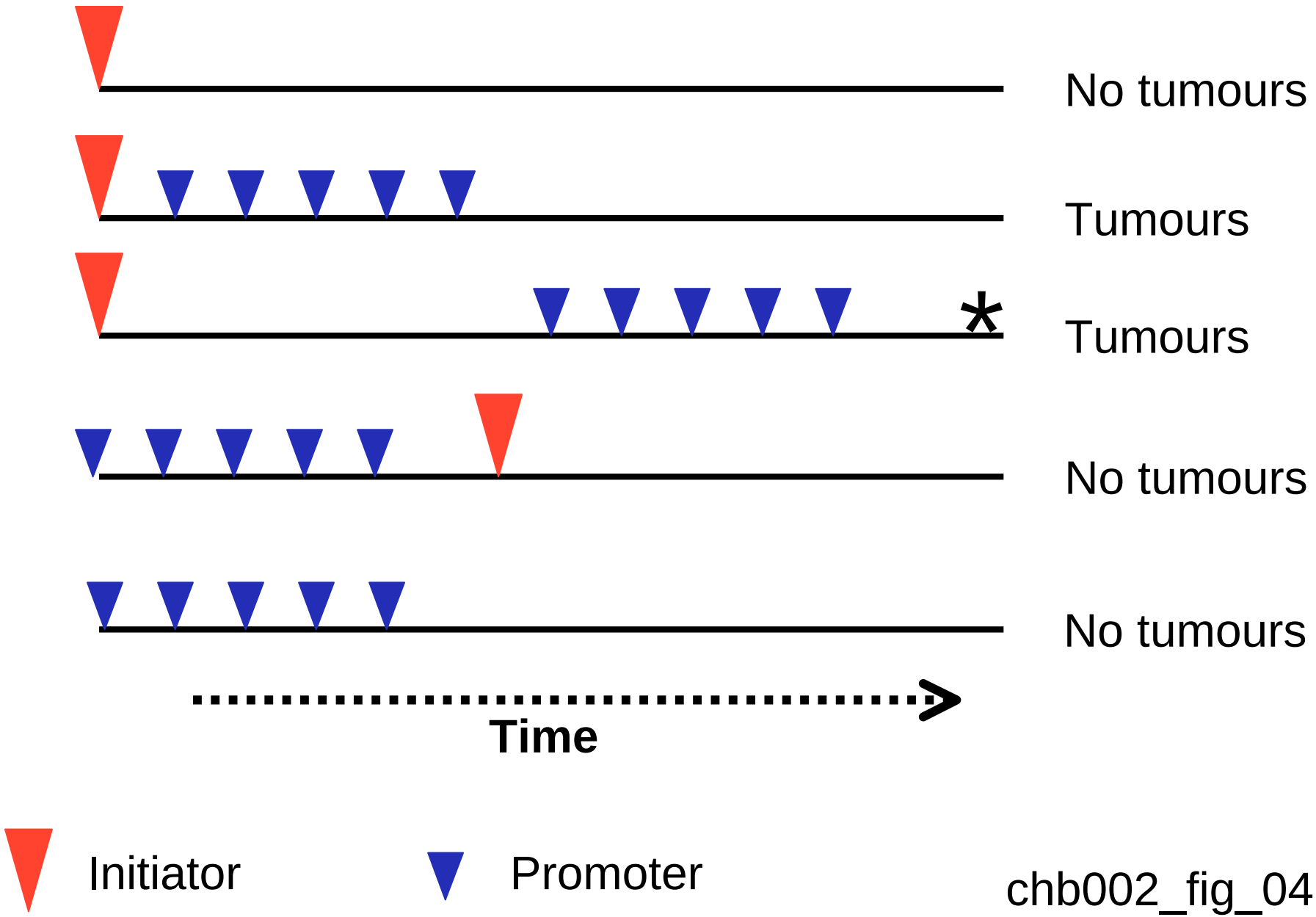


Journal of Pathology
J Pathol 2006; **209**: 287–297
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/path.2016

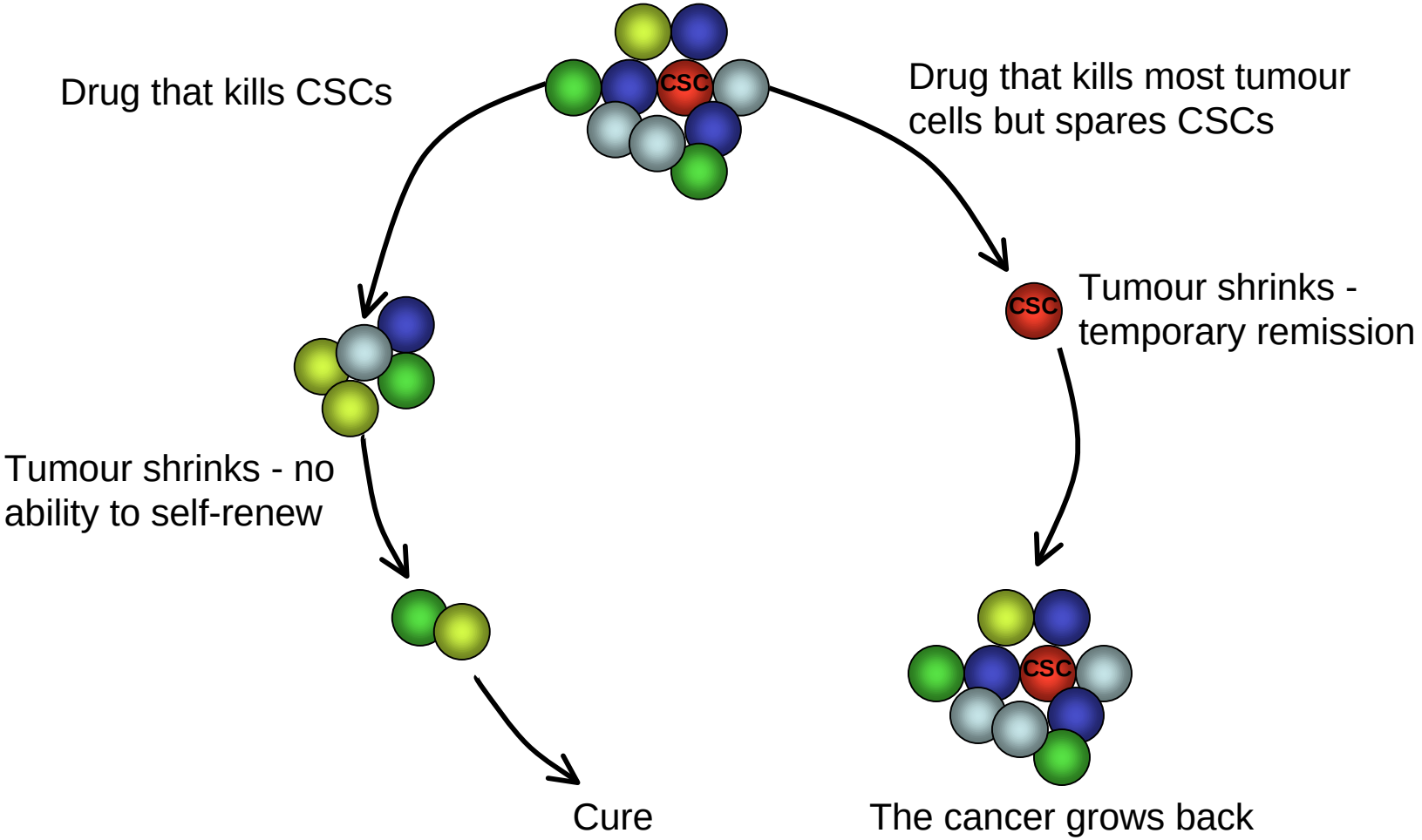
Review Article

Stem cells and cancer: an intimate relationship

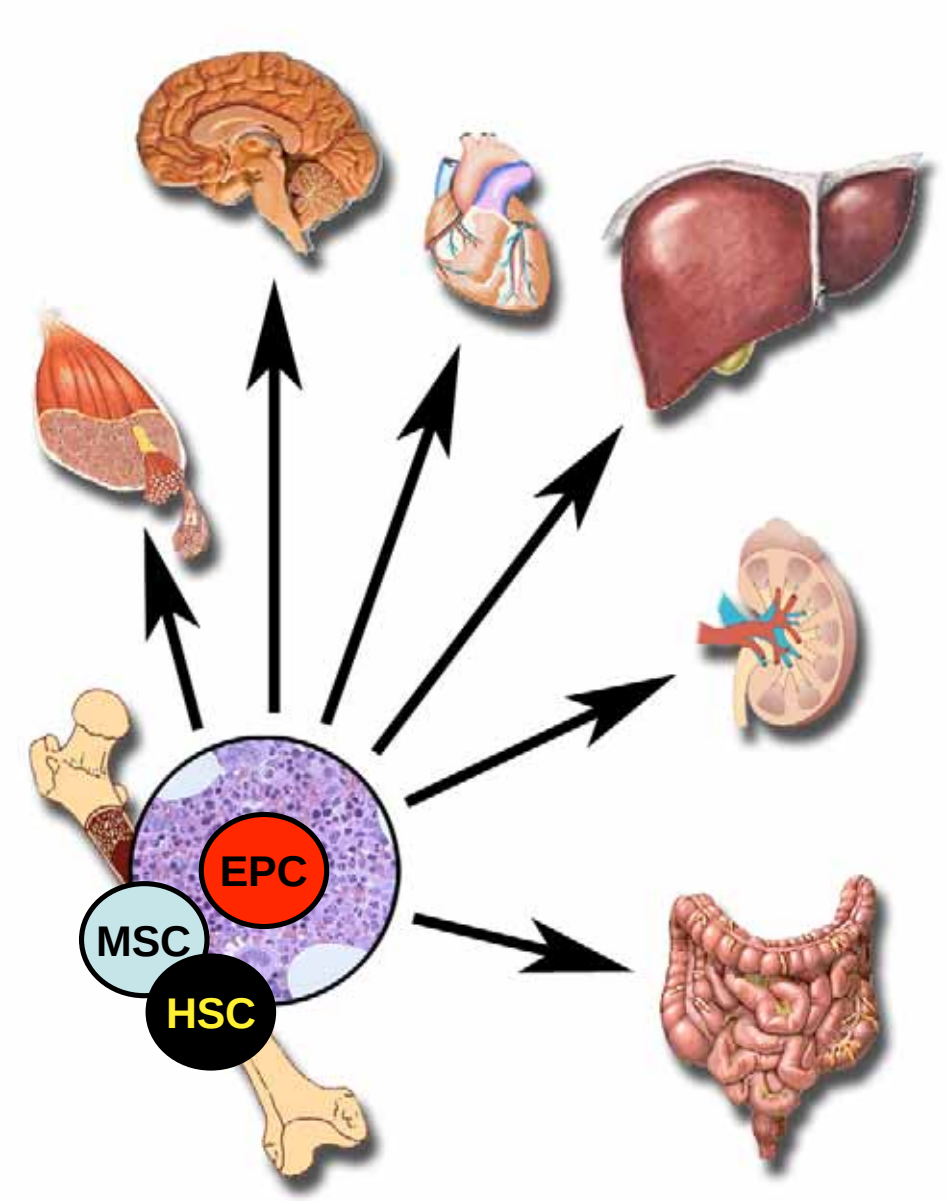
J Burkert,[1]* NA Wright[1,2] and MR Alison[1,2]
[1]*Histopathology Unit, Cancer Research UK, 44 Lincoln's Inn Fields, London WC2A 3PX, UK*
[2]*ICMS, Queen Mary's School of Medicine and Dentistry, London E1 2AT, UK*



chb002_fig_04


Drug that kills CSCs
CSC
Drug that kills most tumour cells but spares CSCs
CSC
Tumour shrinks - temporary remission
Tumour shrinks - no ability to self-renew
Cure
CSC
The cancer grows back

Bone Marrow hosts multipotent stem cells



Haematopoietic Stem Cells

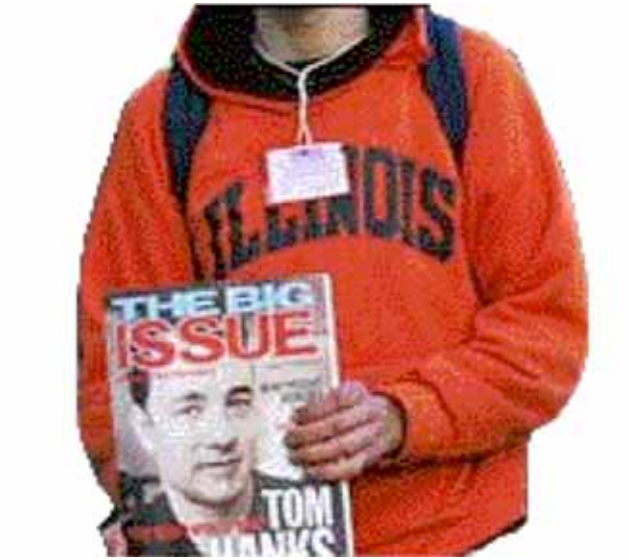
Mesenchymal Stem Cells

Endothelial Progenitor Cells

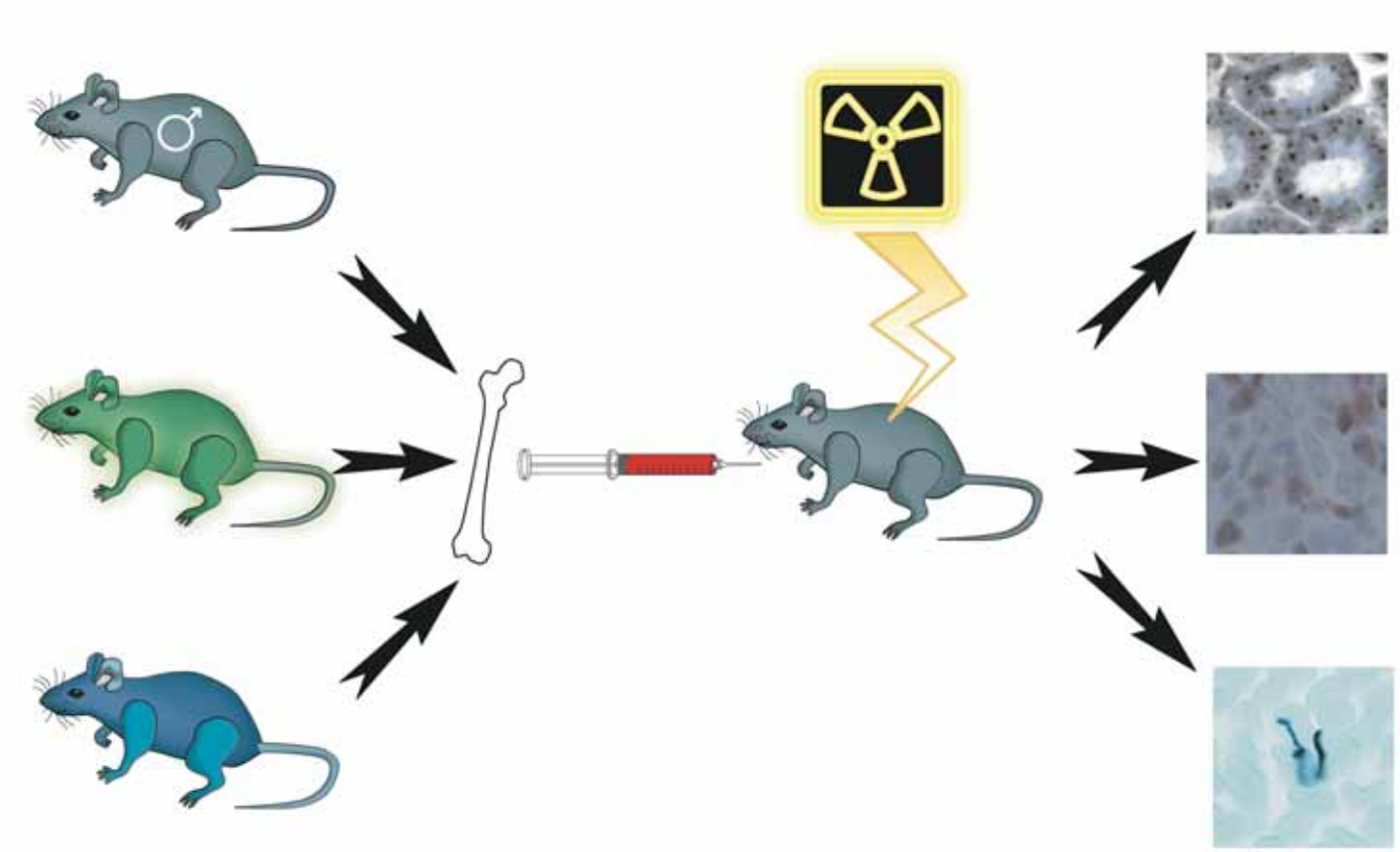
and what else?

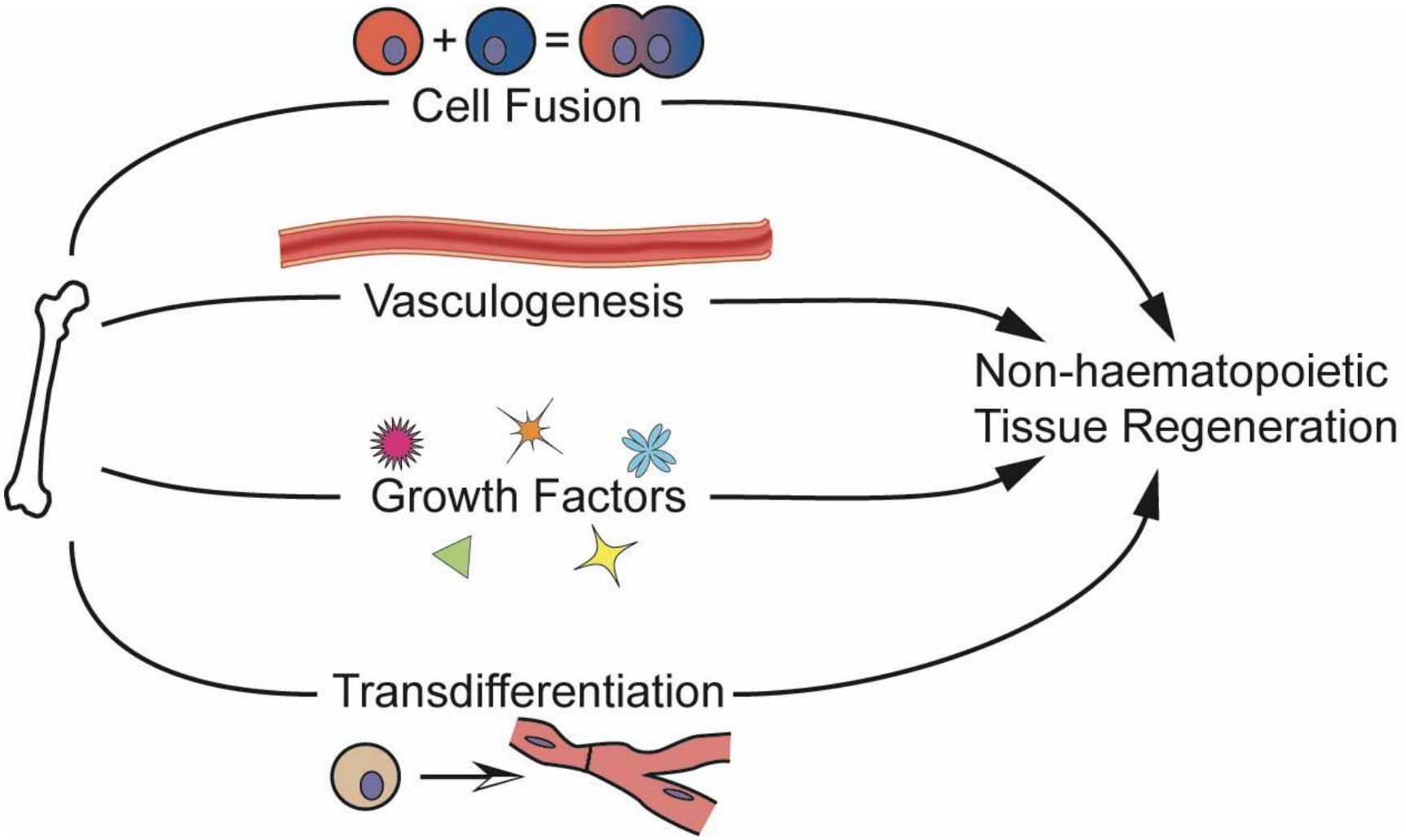
Stem Cell Plasticity - challenges traditional views of lineage commitment

Stem cell plasticity: the big issues



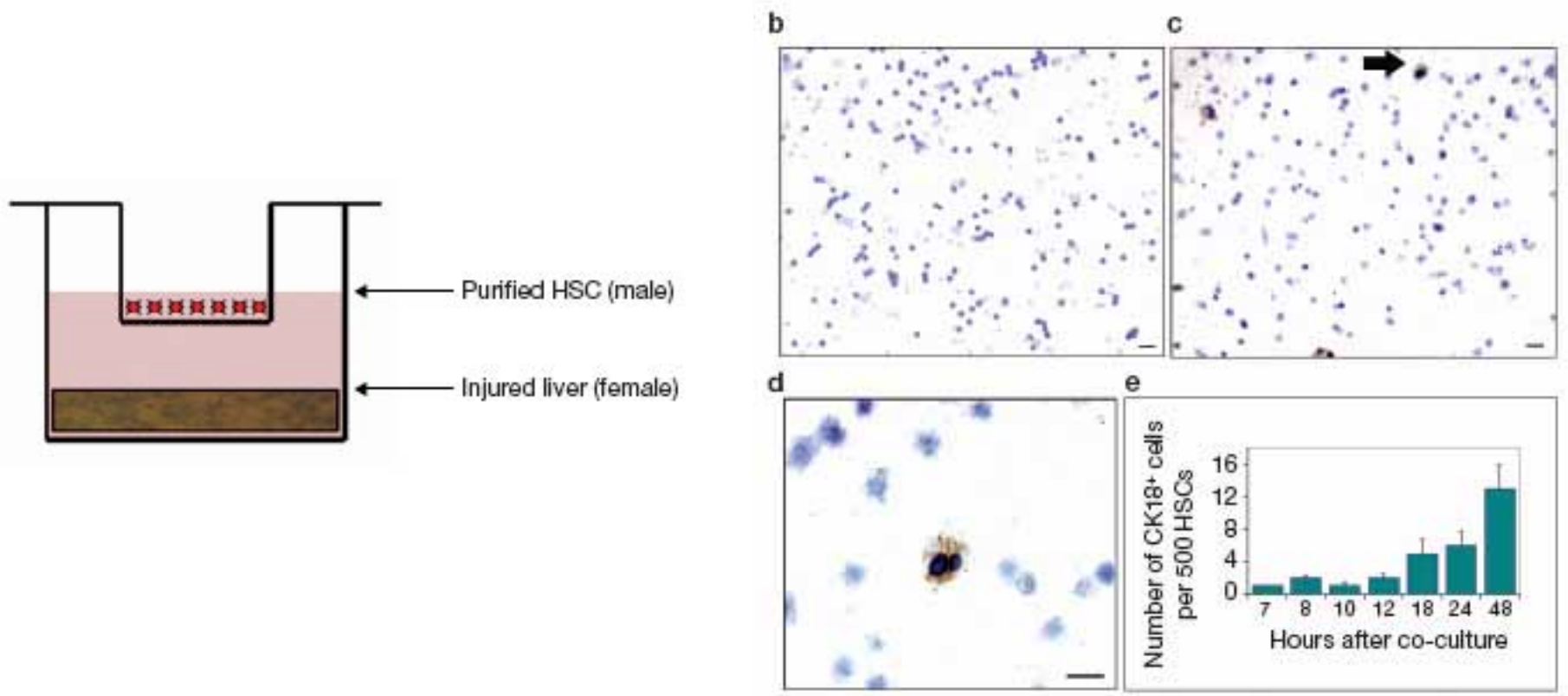
- Real
- Reproducible
- Fusion
- Function
- Clonogenic?




Cell Fusion
Vasculogenesis
Growth Factors
Transdifferentiation
Non-haematopoietic
Tissue Regeneration

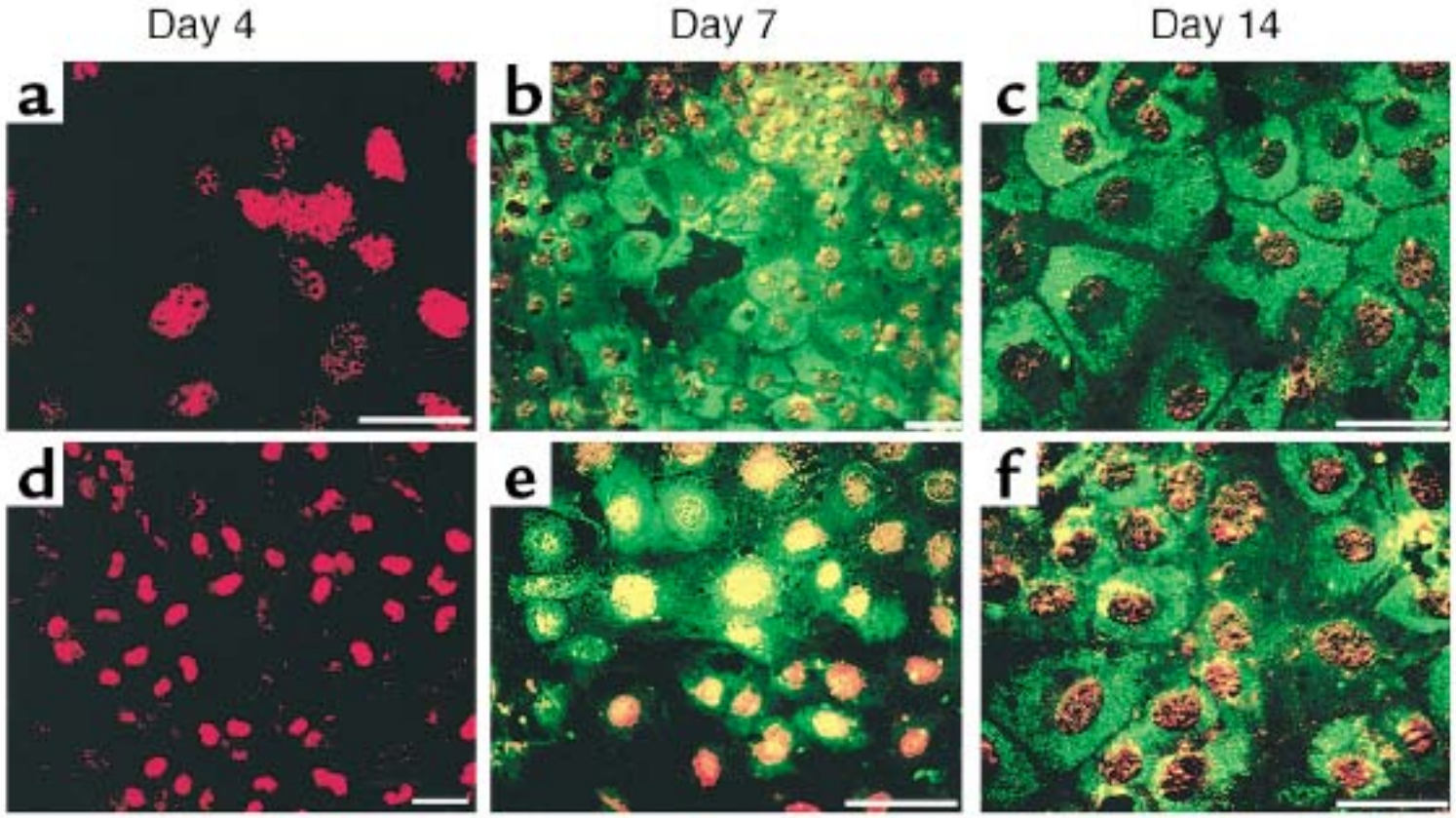
Hematopoietic stem cells convert into liver cells within days without fusion

Yoon-Young Jang[1], Michael I. Collector[1], Stephen B. Baylin[1], Anna Mae Diehl[2] and Saul J. Sharkis[1,3]



Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells

Robert E. Schwartz,[1] Morayma Reyes,[1] Lisa Koodie,[1] Yuehua Jiang,[1] Mark Blackstad,[1] Troy Lund,[1] Todd Lenvik,[1] Sandra Johnson,[1] Wei-Shou Hu,[2] and Catherine M. Verfaillie[1,3]



Matrigel + FGF4 + HGF

J. Clin. Invest. **109**:1291–1302 (2002).

NATURE |Vol 442|27 July 2006

THE TROUBLE WITH REPLICATION

The idea that readers should be able to replicate published scientific results is seen as the bedrock of modern science. But what if replication proves difficult or impossible? **Jim Giles** tracks the fate of one group of papers.

than replication. Her interpretation, though, has undergone something like replication; similar fossils that date from the same period have since been found and described in a way that conforms with her conclusions. In the case of the genome of *Dictyostelium discoideum*[2], an amoeba, few researchers would see the need to repeat the sequencing from scratch; in any case, the genome stored on the dictybase.org website can be updated should errors be identified.

Giant's signature

But for other papers from the 4 July issue, textbook status looks a long way off. One of those was authored by Sean Brittain and Terrance Rettig, both then based at the University of Notre Dame in Indiana. Their finding was an exciting one: they claimed, for the first time, to have seen H_3^+ ions in the disk of gas and dust surrounding a young star[7]. H_3^+ is seen in the atmospheres of Jupiter and Saturn, suggesting that the astronomers had spotted a gas giant in the act of formation.

Yet right from the start, other researchers wondered whether Brittain and Rettig really had seen H_3^+. The evidence was in the form of distinct frequencies of infrared radiation: H_3^+ emits at three particular frequencies, and Brittain and Rettig reported detecting emissions in only two of those three. Takeshi Oka of the University of Chicago in Illinois wrote a cautiously optimistic News and Views commentary on the finding in the same issue[8] but had his doubts about the result. "We used our earliest observation time to check," he says now. "We couldn't see it."

Over the next year, the two sets of authors exchanged their raw data in a bid to resolve their contradictory results. Such exchanges are never easy, given that the scientists are to some extent putting their reputations on the line. In this case, neither side seems to have completely

The test of time: most papers in this 2002 issue are looking good for their age — but who could tell which would stand up to testing?

Minnesota pitched in with a paper that seemed to offer a peaceful solution. Catherine Verfaillie and her colleagues described how they had isolated fully functioning stem cells from adult human bone marrow[10]. If the results were correct, all the benefits of stem cells could be realized by taking samples from the patient involved — no embryos, no cloning. To those with moral objections this sounded vastly preferable; to others it simply sounded easier. It looked like a win–win situation.

But four years later, the implications of the paper are still far from clear. "People found the paper amazing," says Stuart Orkin, a stem-cell biologist at Harvard University. "But there has been very little published literature since. There

Verfaillie counters that the procedure takes up to six weeks to master and that those who stayed for long enough have cracked it. Some, indeed, have published results[11].

Shy journals

Verfaillie adds that her own team has since ironed out problems with the serum it used and will soon publish a comprehensive methods paper describing the new protocol. But researchers who think they have derived pluripotent stem cells from human bone mar-