PÉCSI TUDOMÁNYEGYETEM
ÁLTALÁNOS ORVOSTUDOMÁNYI KAR
Immunológiai és Biotechnológiai Intézet

Biological therapies

Gene and stem cell therapies & Organ transplantation

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Lectures 21-22; 2019. 04. 11.

Outline

1. GENE THERAPY
2. STEM CELLS AND STEM CELL-BASED THERAPIES
3. ORGAN AND TISSUE TRANSPLANTATION

1. GENE THERAPY

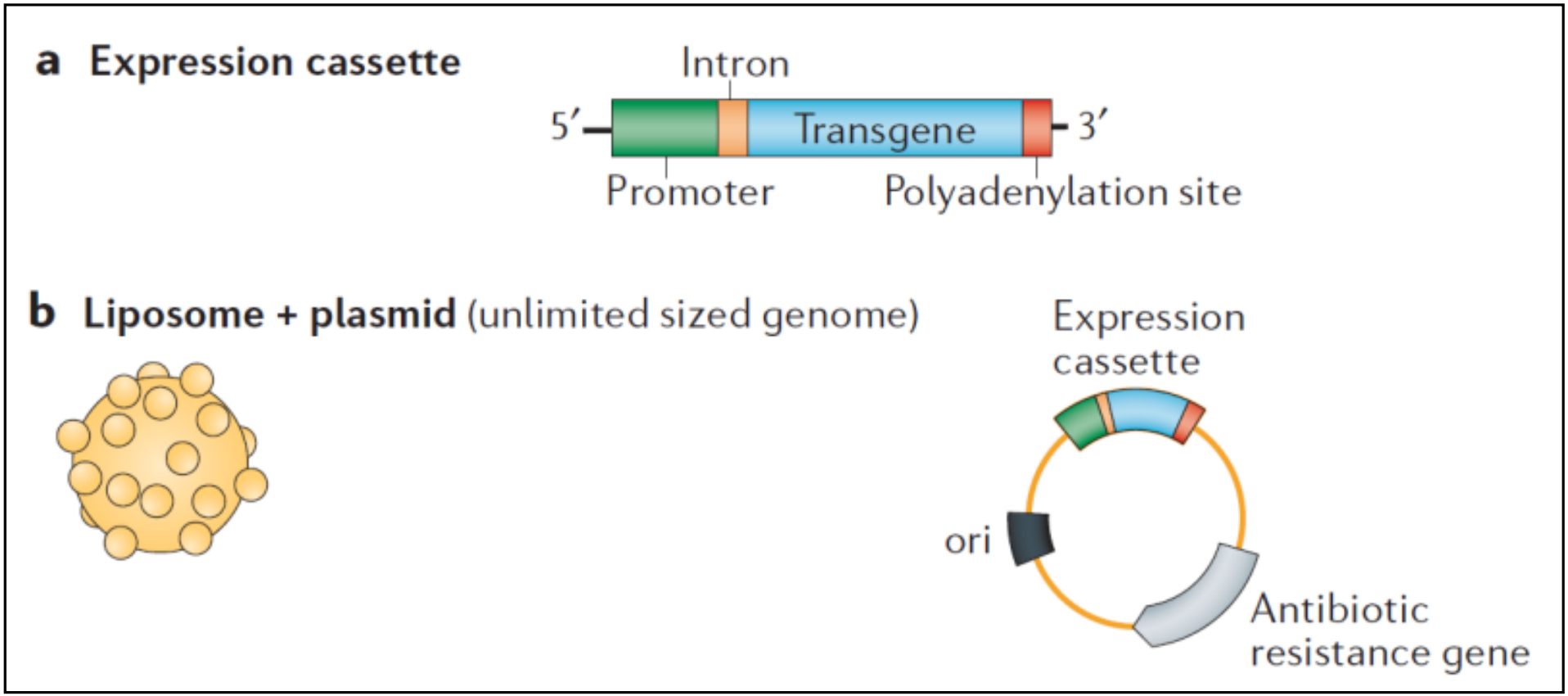
Genetic medicines: treatment strategies for hereditary disorders

The treatment of the more than **1,800 known monogenic hereditary disorders** will depend on the development of ‘genetic medicines’ — therapies that use the transfer of DNA and/or RNA to modify gene expression to correct or compensate for an abnormal phenotype.

Strategies include the **use of somatic stem cells, gene transfer, RNA modification and, in the future, embryonic stem cells.** Despite the efficacy of these technologies in treating experimental models of hereditary disorders, applying them successfully in the clinic is a great challenge, which will only be overcome by expending considerable intellectual and economic resources, and by solving societal concerns about modifications of the human genetic repertoire.

O’Connor & Crystal, Nature Rev Gen, 2006

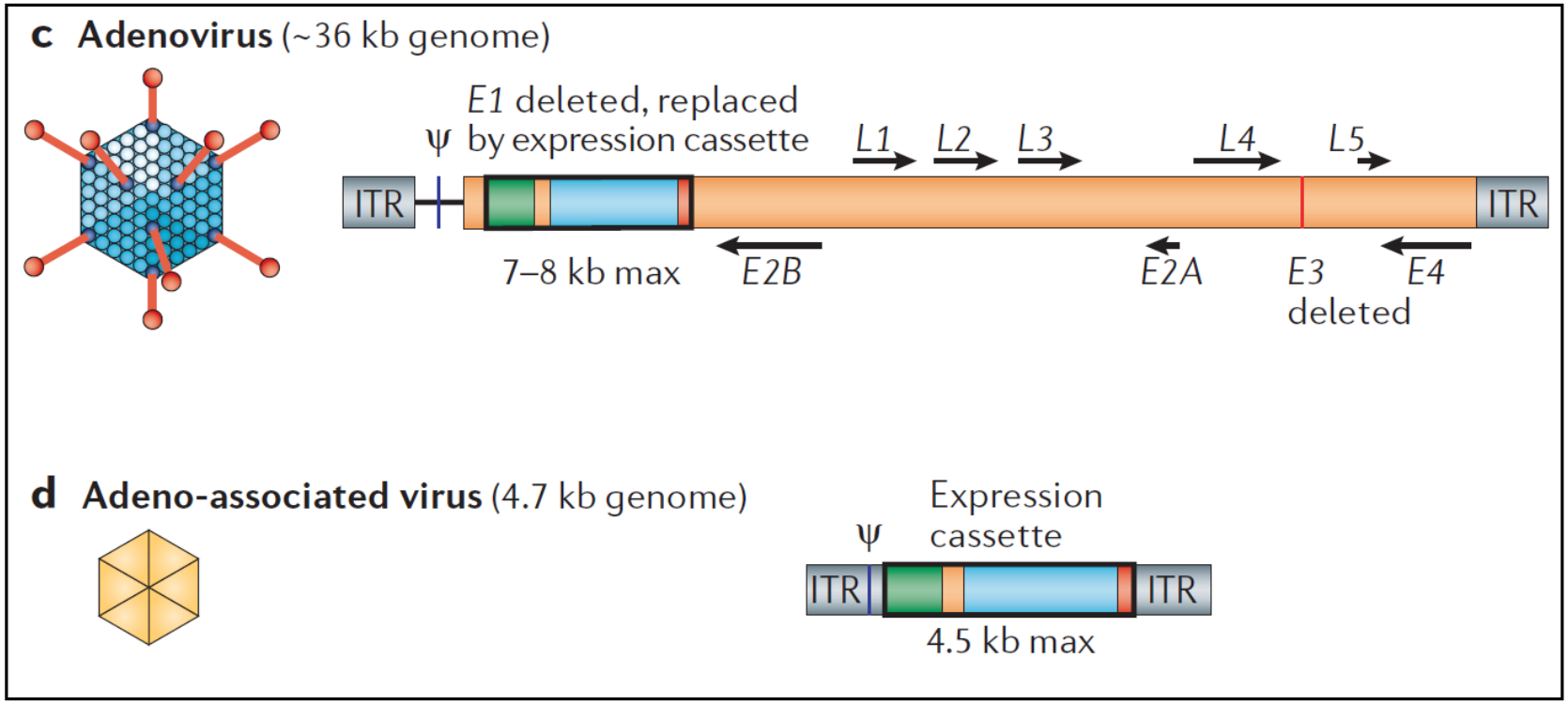
Gene-transfer vectors that are used to treat hereditary disorders (1)



There are five classes of gene-transfer vectors and a prototypic expression cassette that are used to treat hereditary disorders. Panel **a** shows a typical expression cassette. The therapeutic transgene is flanked at the 5′ end by the promoter and at the 3′ end by a polyadenylation site. Plasmid DNA has an unlimited size capacity and is delivered either naked or formulated with liposomes (**b**). The expression cassette contains a bacterial origin of DNA replication and an antibiotic resistance gene for production in bacteria.

O'Connor & Crystal, Nature Rev Gen, 2006

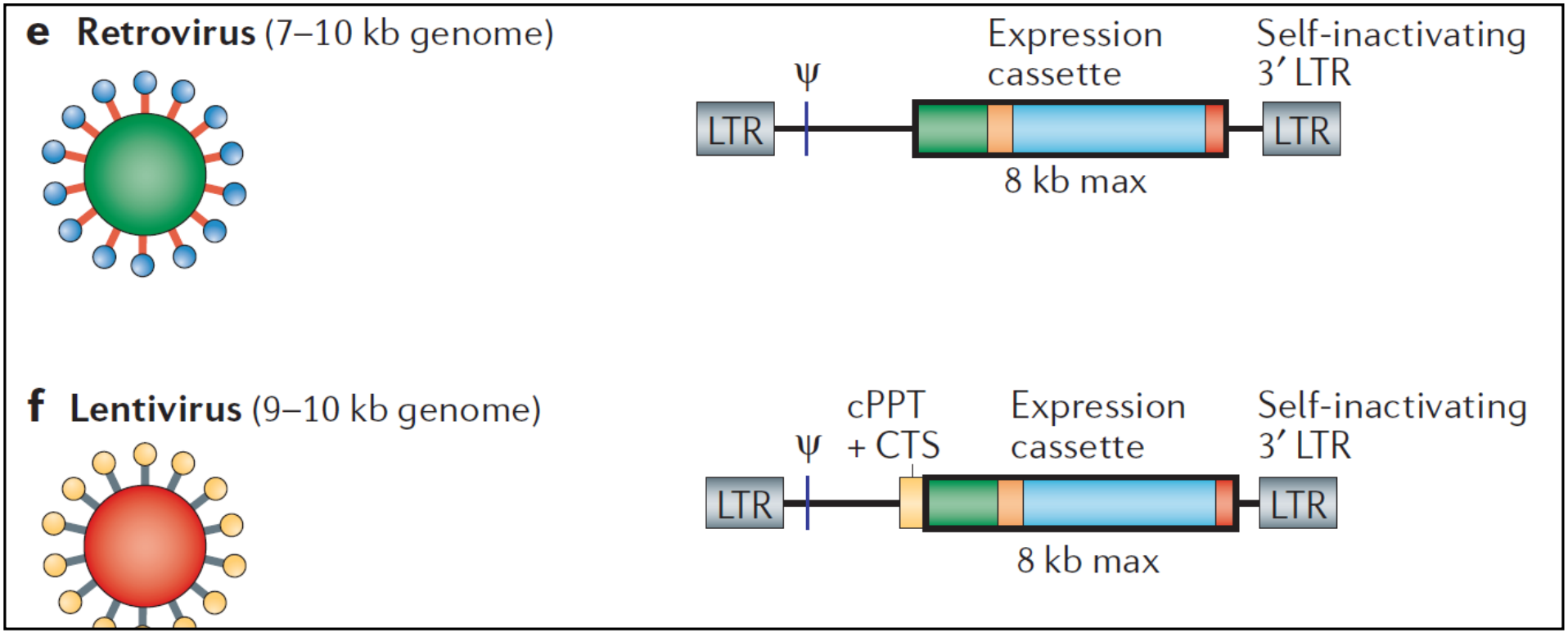
Gene-transfer vectors that are used to treat hereditary disorders (2)



Panel **c** shows a first generation adenovirus vector that is based on serotype 5. The 36-kb dsDNA genome of wild-type adenoviruses contains left- and right-inverted terminal repeats (ITR) that facilitate viral DNA replication, a packaging signal (Ψ), early (*E1–E4*) genes and late (*L1–L5*) genes. A typical adenovirus vector has the essential *E1* region deleted (to prevent replication) and lacks most *E3* genes (to increase the cargo space). Expression cassettes of 7–8 kb are inserted into the *E1* region. The 4.7-kb ssDNA genome of wild-type adeno-associated viruses (**d**) contains 5′ and 3′ ITRs and 2 genes, *rep* and *cap*. The 5′ ITR contains Ψ. Deletion of the viral *rep* and *cap* genes allows expression cassettes of up to 4.5 kb to be accommodated.

O'Connor & Crystal, Nature Rev Gen, 2006

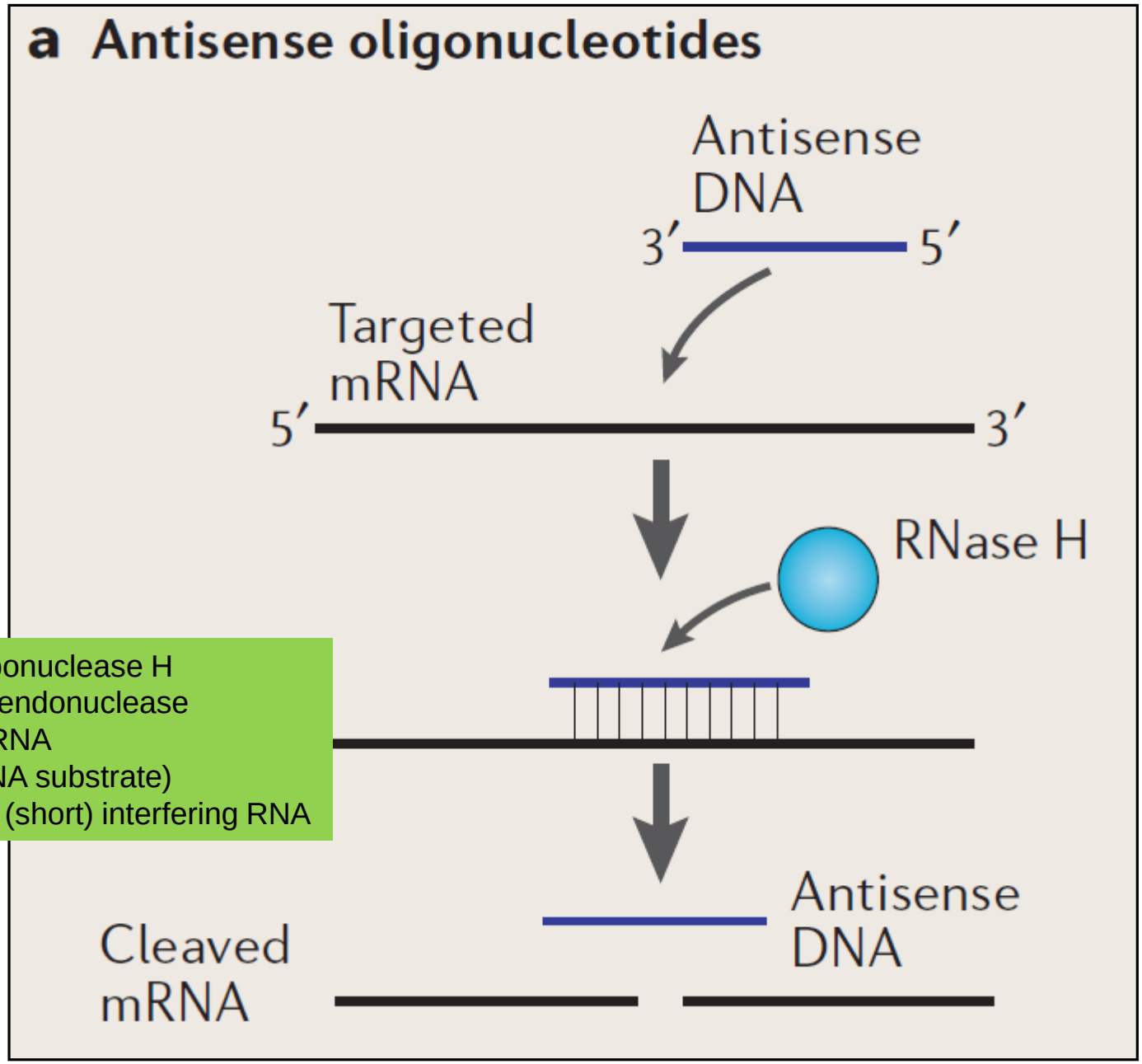
Gene-transfer vectors that are used to treat hereditary disorders (3)



CTS, Central termination sequence
cPPT, Central polypurine tract
LTR, Long terminal repeat
Ψ, Packaging element

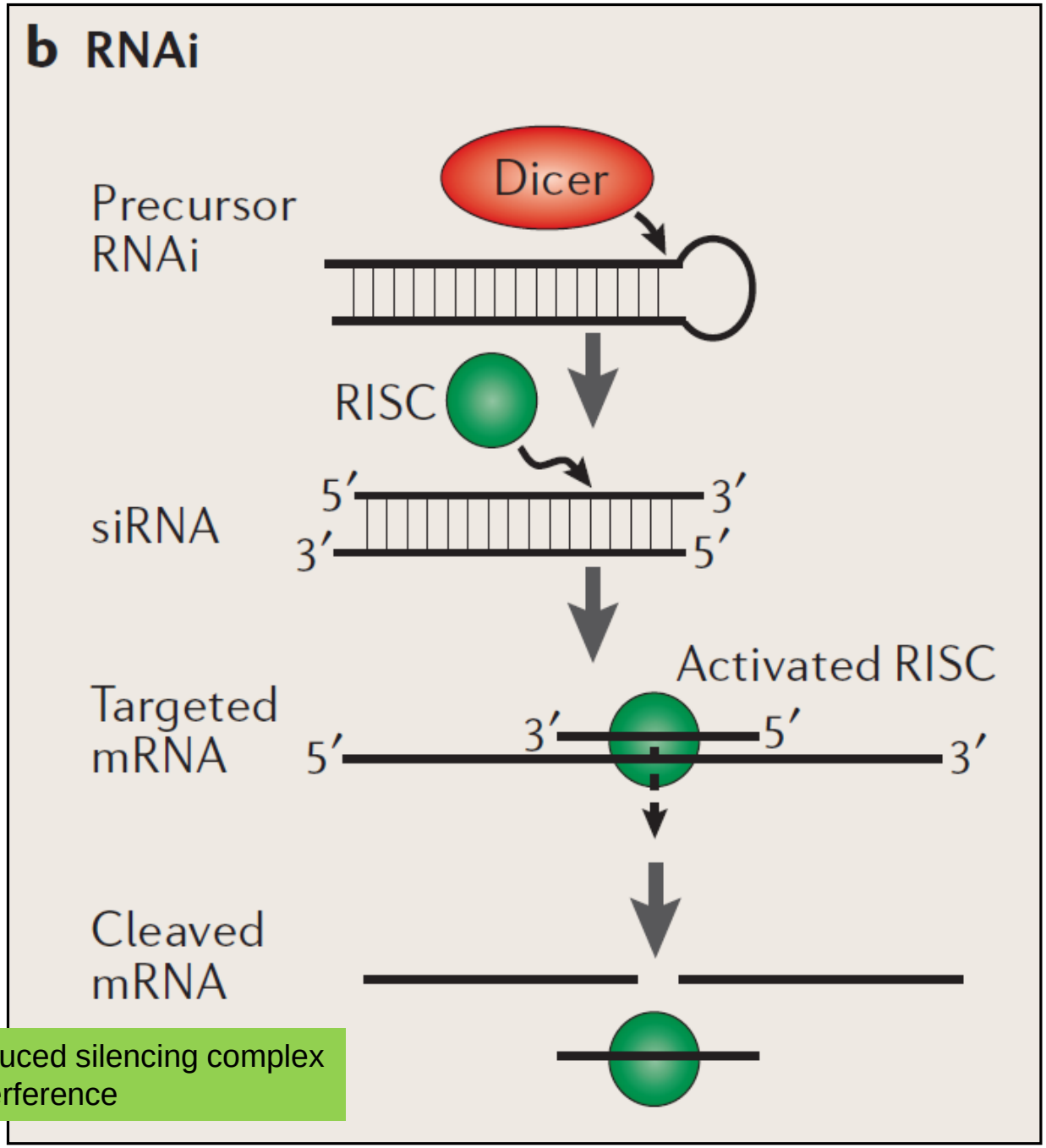
O'Connor & Crystal, Nature Rev Gen, 2006

RNA-modification strategies for genetic medicine



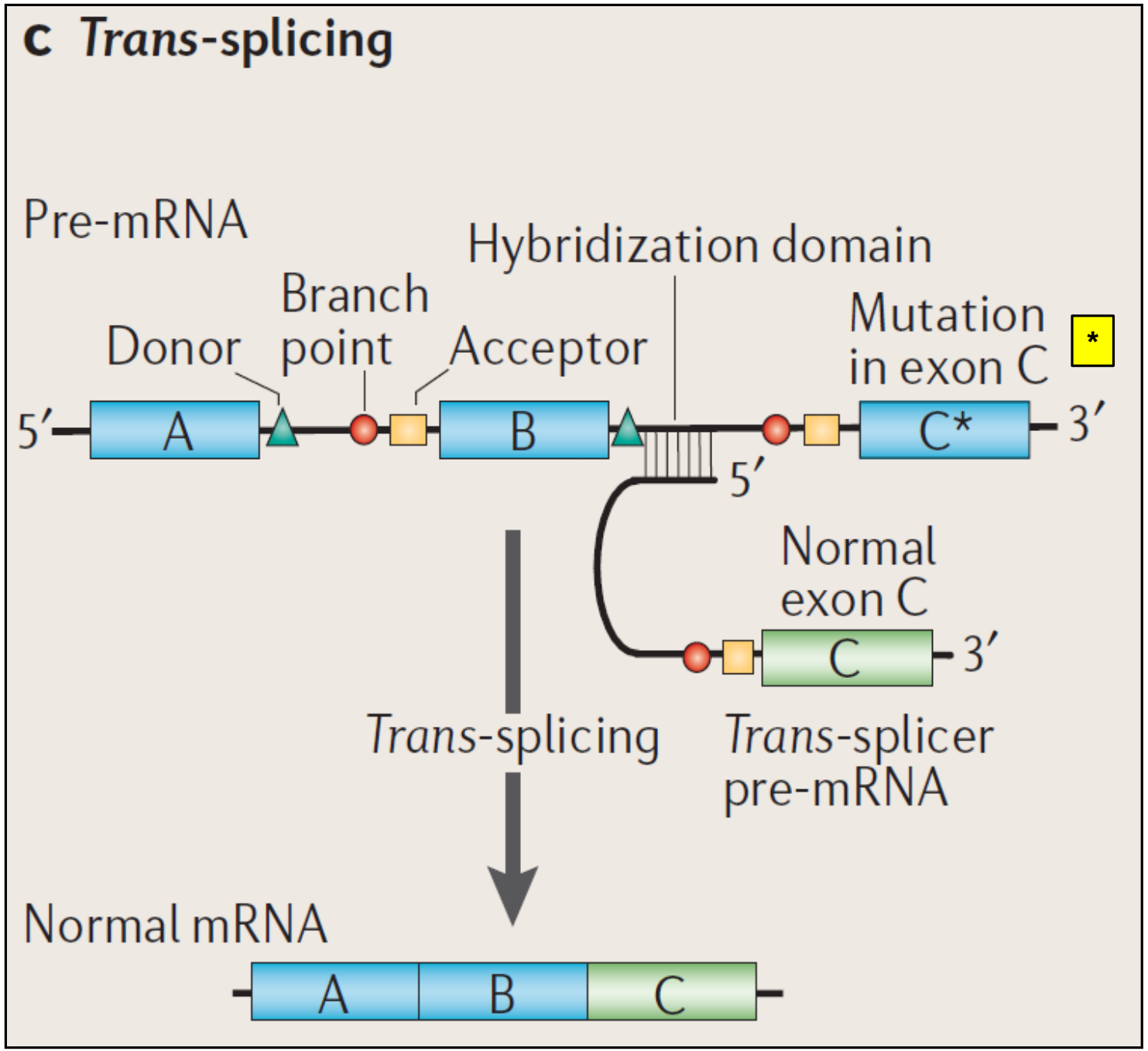
RNase H, Ribonuclease H
(non-specific endonuclease
that cleaves RNA
in an RNA/DNA substrate)
siRNA, Small (short) interfering RNA

O'Connor & Crystal, Nature Rev Gen, 2006

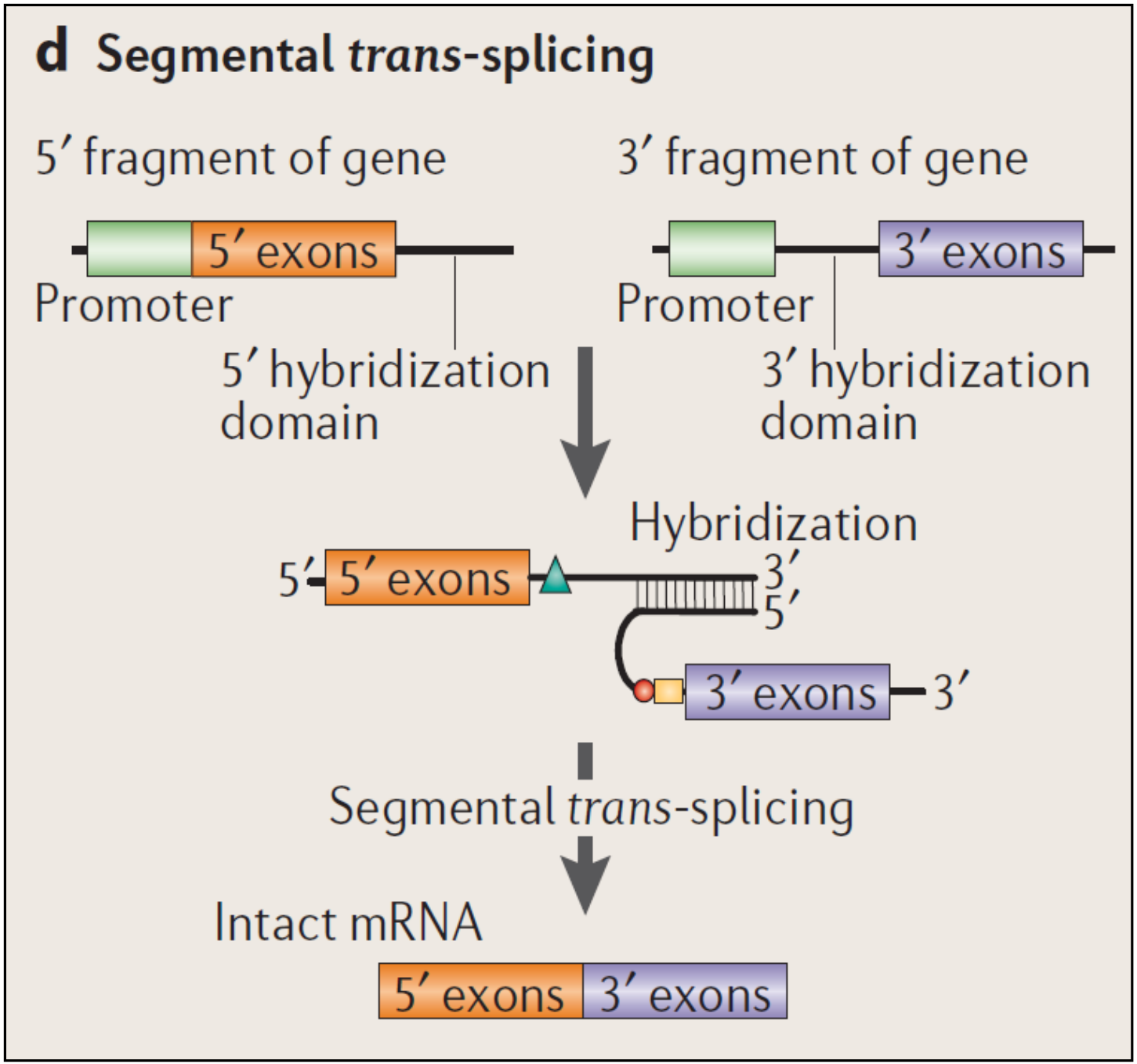


RISC, RNA-induced silencing complex
RNAi, RNA interference

O'Connor & Crystal, Nature Rev Gen, 2006

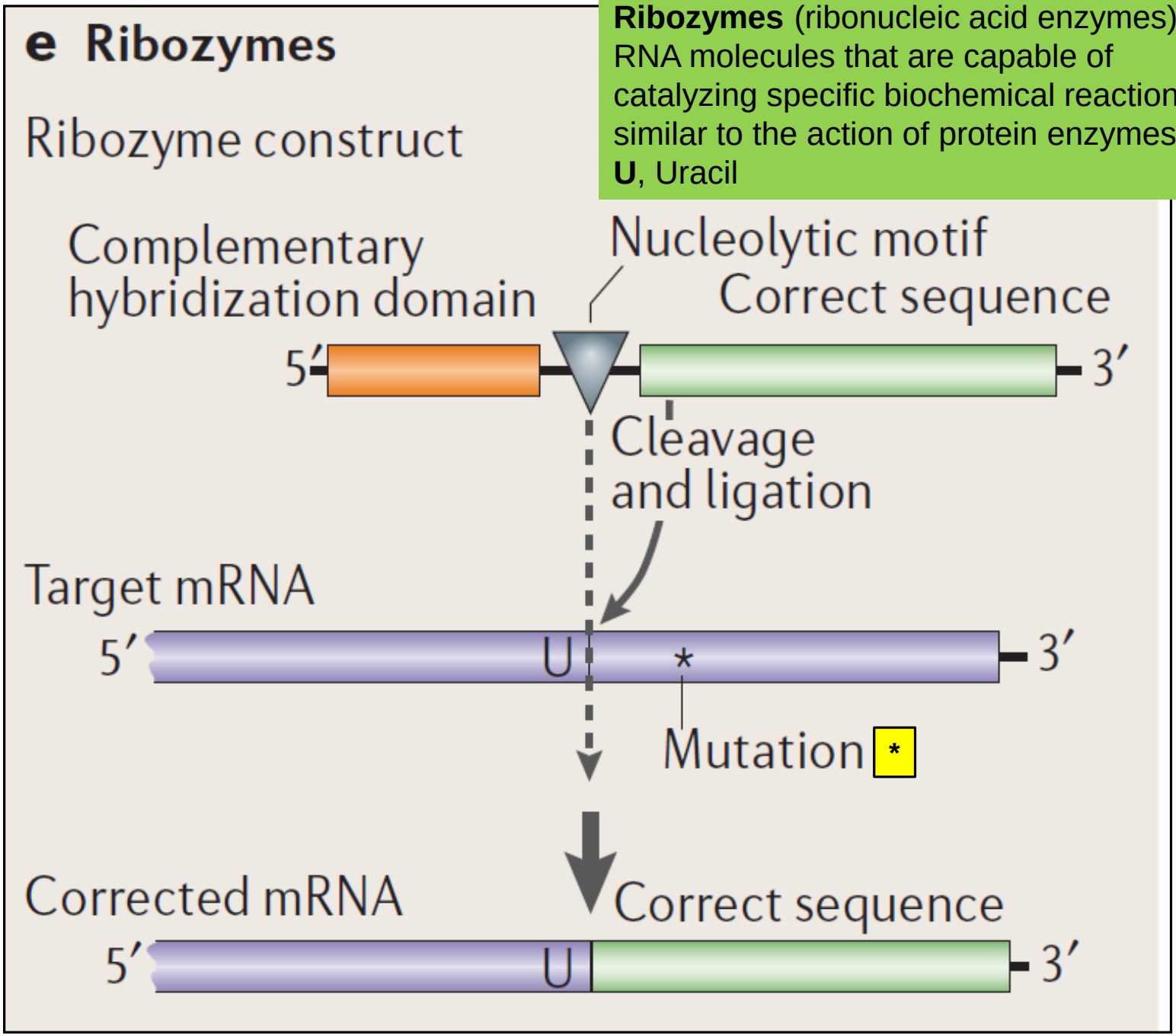


O'Connor & Crystal, Nature Rev Gen, 2006



O'Connor & Crystal, Nature Rev Gen, 2006

Ribozymes (ribonucleic acid enzymes) are RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of protein enzymes.
U, Uracil



O'Connor & Crystal, Nature Rev Gen, 2006

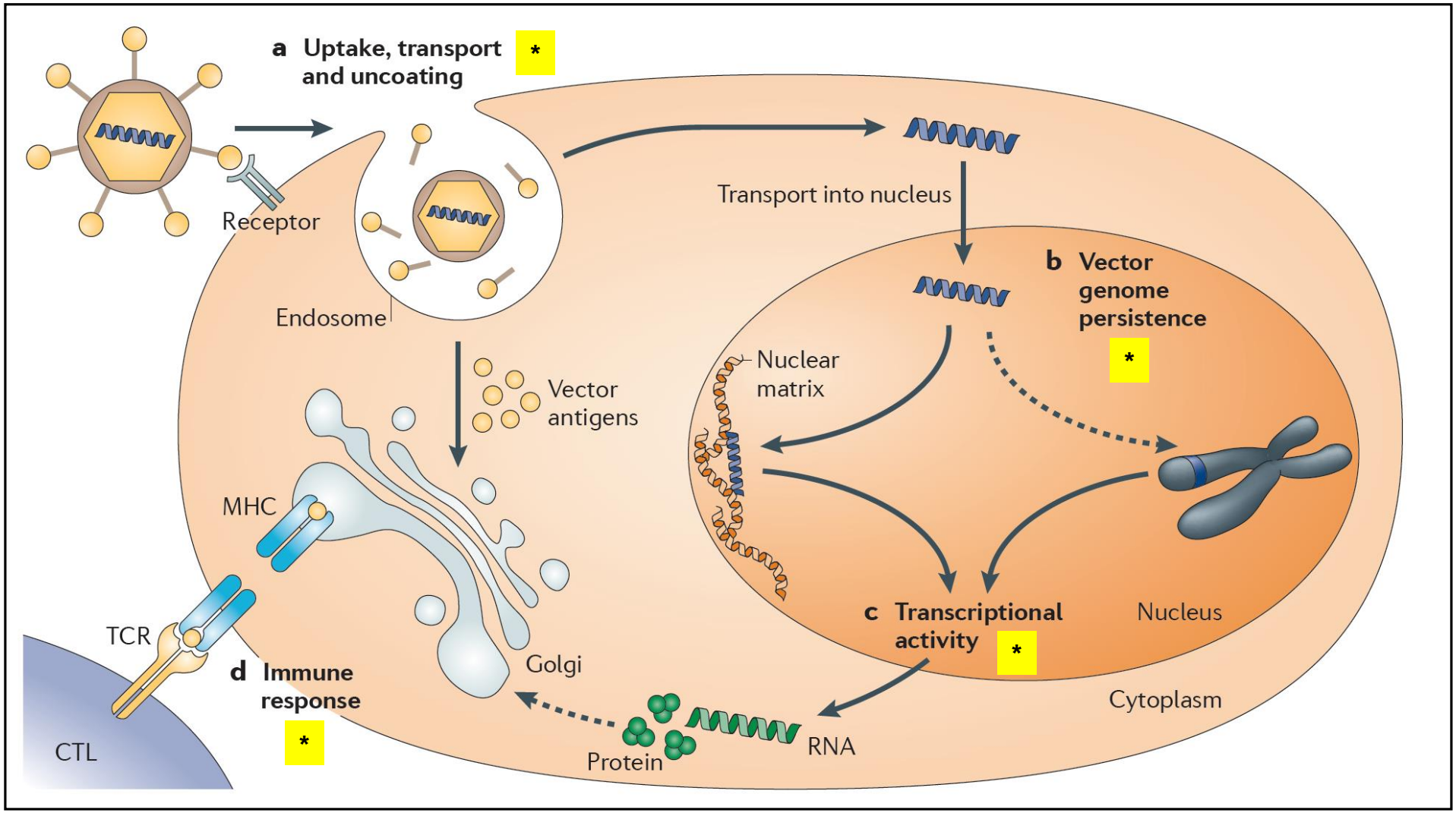
Main groups of viral vectors

Table 1 | **The main groups of viral vectors**

Vector	Genetic material	Packaging capacity	Tropism	Inflammatory potential	Vector genome forms	Main limitations	Main advantages
Enveloped 1							
Retrovirus	RNA	8 kb	Dividing cells only	Low	Integrated	Only transduces dividing cells; integration might induce oncogenesis in some applications	Persistent gene transfer in dividing cells
Lentivirus	RNA	8 kb	Broad	Low	Integrated	Integration might induce oncogenesis in some applications	Persistent gene transfer in most tissues
HSV-1	dsDNA	40 kb* 150 kb‡	Strong for neurons	High	Episomal	Inflammatory; transient transgene expression in cells other than neurons	Large packaging capacity; strong tropism for neurons
Non-enveloped 2							
AAV	ssDNA	<5 kb	Broad, with the possible exception of haematopoietic cells	Low	Episomal (>90%) Integrated (<10%)	Small packaging capacity	Non-inflammatory; non-pathogenic
Adenovirus	dsDNA	8 kb* 30 kb§	Broad	High	Episomal	Capsid mediates a potent inflammatory response	Extremely efficient transduction of most tissues

*Replication defective. ‡Amplicon. §Helper dependent. AAV, adeno-associated viral vector; dsDNA, double-stranded DNA; HSV-1, herpes simplex virus-1; ssDNA, single-stranded DNA.

Barriers to successful gene therapy



CTL, Cytotoxic T lymphocyte
MHC, Major histocompatibilty complex
TCR, T cell receptor

Kay MA, Nature Rev Gen, 2011

Figure 1 | **The four barriers to successful gene therapy.**

a | Uptake, transport and uncoating. Vectors bind to a cellular membrane and are internalized by various processes. Most uptake steps involve a ligand–receptor interaction. Once internalized, most vectors enter the endosome and undergo a complex set of reactions that can result in their full or partial degradation. Viruses have evolved effective mechanisms for escaping from the endosome; for example, adenoviruses lyse the endosome. Transport to the nucleus is also required for successful therapy.

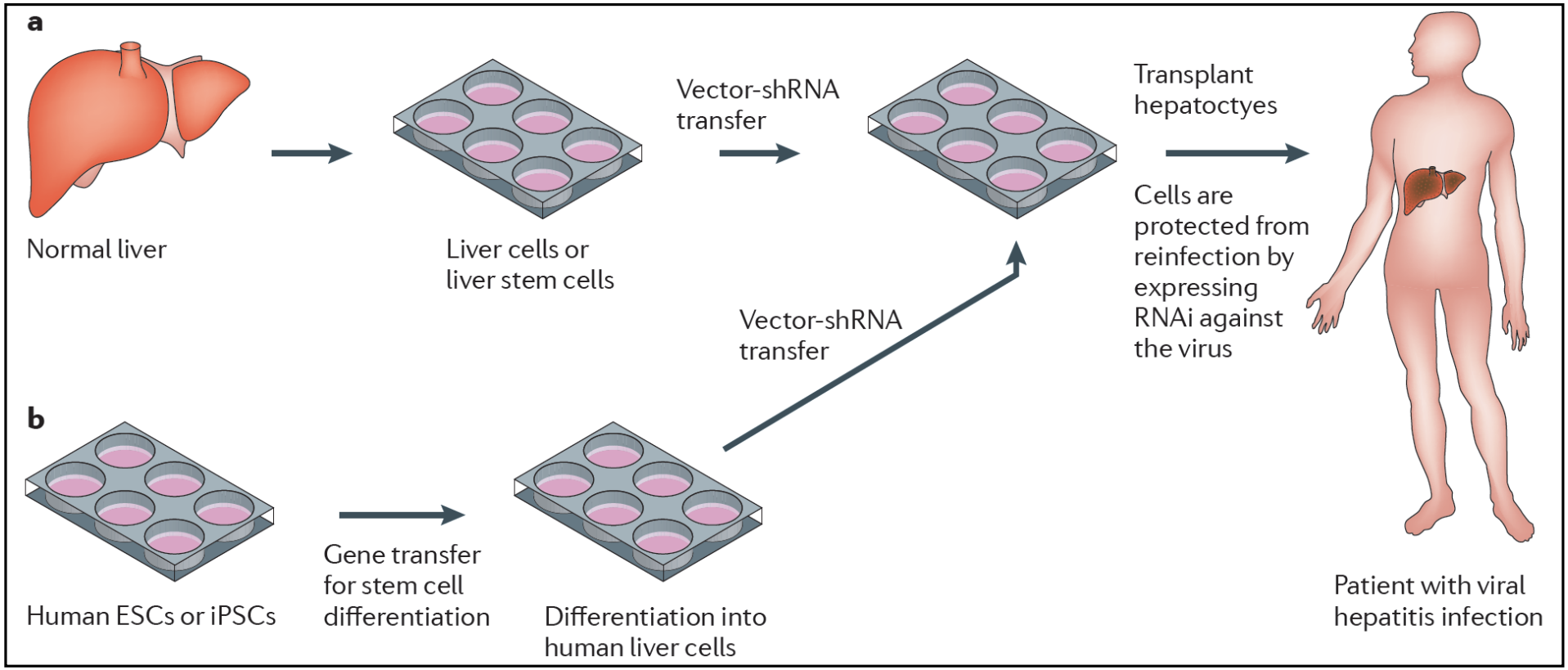
b | Vector genome persistence. Once the vector reaches the nucleus, it can be further processed. Depending on the vector, the DNA can exist as an episomal molecule (and associate with the nuclear matrix) or it can be integrated (by covalent attachment) into the host chromosome.

c | Transcriptional activity and transgene persistence are dependent on many factors, as described in the main text.

d | The immune response can limit the viability of the transduced cells and/or the expression of the transgene product. CTL, cytotoxic T cell lymphocyte; MHC, major histocompatibilty complex; TCR, T cell receptor.

Kay MA, Nature Rev Gen, 2011

Combining stem cells and gene therapy: an example application



ESCs, Embryonic stem cells
iPSCs, Induced pluripotent stem cells
shRNA, Short hairpin RNA

Kay MA, Nature Rev Gen, 2011

Figure 4 | **Combining stem cells and gene therapy: an example application.**

Patients with chronic liver disease from viral hepatitis (for example, HCV or HBV) infection who require a liver transplant might be amenable to hepatocellular transplantation of mature hepatocytes (**a**), or hepatocytes-derived from human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) (**b**). Not only might gene transfer be required to convert stem cells into hepatocytes (**b**) but, because the transplanted cells are likely to become reinfected by the hepatitis virus (as do whole liver organs after transplantation), gene transfer of a vector encoding RNAi (short hairpin RNAs (shRNAs)) directed against the virus would make the transplanted cells resistant or 'immune' to reinfection. The resistant cells can repopulate the liver over time and restore normal liver function.

Kay MA, Nature Rev Gen, 2011

Conditions for which human gene transfer trials have been approved (1)

Monogenic disorders 1

- Adrenoleukodystrophy
- α-1 antitrypsin deficiency
- Becker muscular dystrophy
- β-thalassaemia
- Canavan disease
- Chronic granulomatous disease
- Cystic fibrosis
- Duchenne muscular dystrophy
- Fabry disease
- Familial adenomatous polyposis
- Familial hypercholesterolaemia
- Fanconi anaemia
- Galactosialidosis
- Gaucher's disease
- Gyrate atrophy
- Haemophilia A and B
- Hurler syndrome
- Hunter syndrome
- Huntington's chorea
- Junctional epidermolysis bullosa
- Late infantile neuronal ceroid lipofuscinosis
- Leukocyte adherence deficiency
- Limb girdle muscular dystrophy
- Lipoprotein lipase deficiency
- Mucopolysaccharidosis type VII
- Ornithine transcarbamylase deficiency
- Pompe disease
- Purine nucleoside phosphorylase deficiency
- Recessive dystrophic epidermolysis bullosa
- Sickle cell disease
- Severe combined immunodeficiency
- Tay Sachs disease
- Wiskott–Aldrich syndrome

Ginn SL et al, J Gene Med, 2013

2

Cardiovascular disease

- Anaemia of end stage renal disease
- Angina pectoris (stable, unstable, refractory)
- Coronary artery stenosis
- Critical limb ischaemia
- Heart failure
- Intermittent claudication
- Myocardial ischaemia
- Peripheral vascular disease
- Pulmonary hypertension
- Venous ulcers

3

Infectious disease

- Adenovirus infection
- Cytomegalovirus infection
- Epstein–Barr virus
- Hepatitis B and C
- HIV/AIDS
- Influenza
- Japanese encephalitis
- Malaria
- Paediatric respiratory disease
- Respiratory syncytial virus
- Tetanus
- Tuberculosis

Conditions for which human gene transfer trials have been approved (2)

Ginn SL et al, J Gene Med, 2013

Cancer

Gynaecological – breast, ovary, cervix, vulva
Nervous system – glioblastoma, leptomeningeal carcinomatosis, glioma, astrocytoma, neuroblastoma, retinoblastoma
Gastrointestinal – colon, colorectal, liver metastases, post-hepatitis liver cancer, pancreas, gall bladder
Genitourinary – prostate, renal, bladder, anogenital neoplasia
Skin – melanoma (malignant/metastatic)
Head and neck – nasopharyngeal carcinoma, squamous cell carcinoma, oesophaegeal cancer
Lung – adenocarcinoma, small cell/nonsmall cell, mesothelioma
Haematological – leukaemia, lymphoma, multiple myeloma
Sarcoma
Germ cell
Li–Fraumeni syndrome
Thyroid

Neurological diseases

Alzheimer's disease
Amyotrophic lateral sclerosis
Carpal tunnel syndrome
Cubital tunnel syndrome
Diabetic neuropathy
Epilepsy
Multiple sclerosis
Myasthenia gravis
Parkinson's disease
Peripheral neuropathy
Pain

Ginn SL et al, J Gene Med, 2013

Conditions for which human gene transfer trials have been approved (3)

Ocular diseases
- Age-related macular degeneration
- Diabetic macular edema
- Glaucoma
- Retinitis pigmentosa
- Superficial corneal opacity
- Choroideraemia
- Leber congenital amaurosis

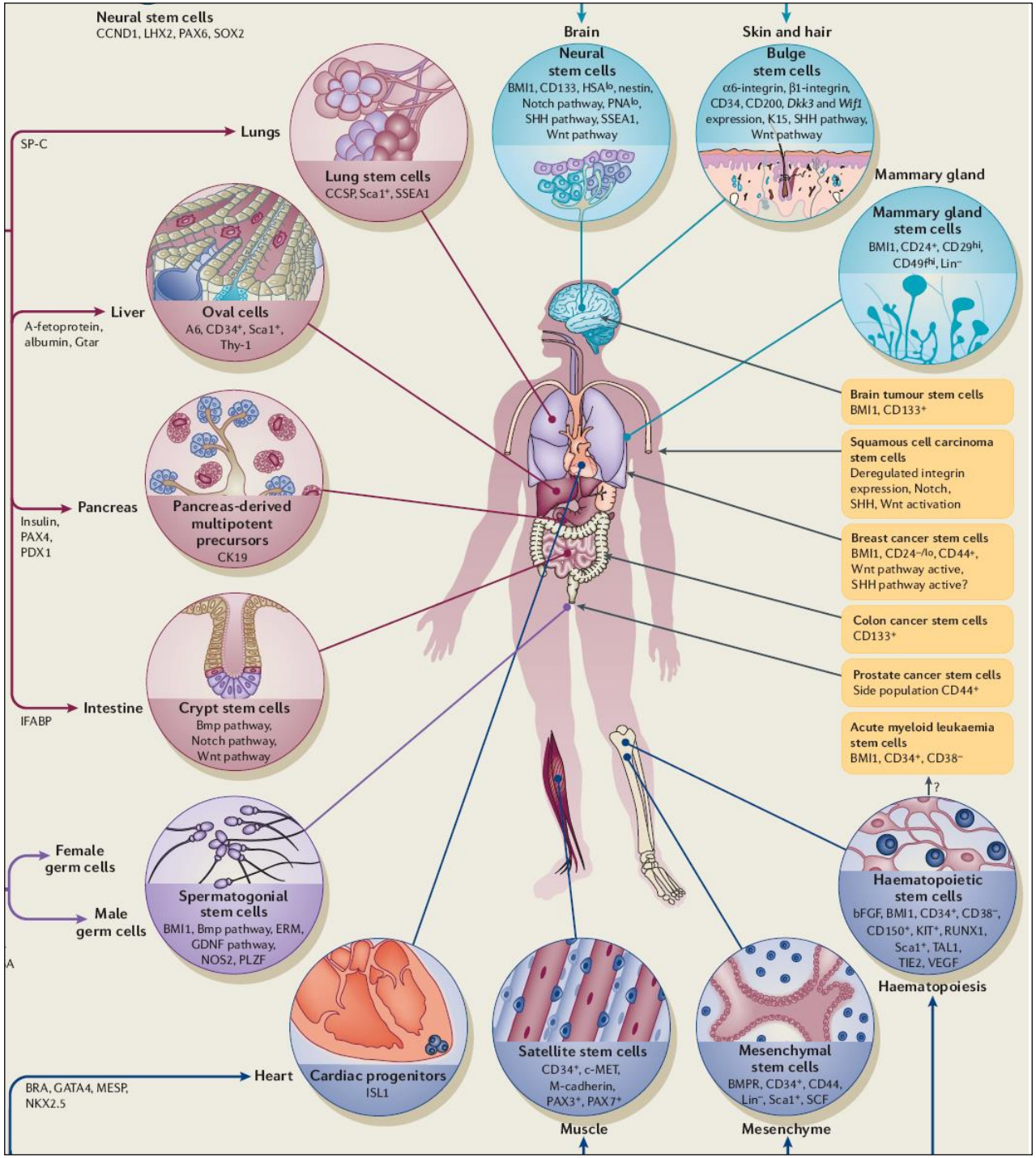
Inflammatory diseases
- Arthritis (rheumatoid, inflammatory, degenerative)
- Degenerative joint disease
- Ulcerative colitis
- Severe inflammatory disease of the rectum

Other diseases
- Chronic renal disease
- Erectile dysfunction
- Detrusor overactivity
- Parotid salivary hypofunction
- Oral mucositis
- Fractures
- Type I diabetes
- Diabetic ulcer/foot ulcer
- Graft versus host disease/transplant patients

Conditions for which human gene transfer trials have been approved (4)

Ginn SL et al, J Gene Med, 2013

2. STEM CELLS AND STEM CELL-BASED THERAPIES



Stem cells in various organs and tissues

Watt & Eggan, Nature Rev Cancer, 2006

Glossary of stem cell-related terms

Adult stem cells
Allogeneic transplantation
Autologous transplantation
Blastocyst
Bone marrow stromal cell
Cell line
Cell type
Cloning
Cytoplasm
Differentiation
Ectoderm
Embryo
Embryoid bodies
Embryonic germline cells
Embryonic stem cell
Endoderm
Fetus
Germ layers
Hematopoietic stem cells
Hematopoietic cell transplantation
Heterologous
Histocompatible
Homologous
Homologous recombination
Human embryonic stem cell
Inner cell mass
In vitro fertilization
Mesemchymal stem cell
Mesoderm
Morphology
Multipotent stem cells
Neural stem cell
Nuclear transfer
Nucleus
Oligopotent progenitor cells
Parthenogenesis
Plasticity
Phenotype
Pluripotent stem cells
Post-implantation embryo
Pre-implantation embryos
Progenitor cell
Regenerative medicine
Reproductive cloning
Somatic cells
Somatic cell nuclear transfer
Stem cells
Therapeutic cloning
Totipotent stem cells
Transdifferentiation
Transplantation biology
Trophoblast
Umbilical cord stem cells
Unipotent stem cells
Zygote

ISSCR, International Society for Stem Cell Research

www.isscr.org/home/resources/learn-about-stem-cells/stem-cell-glossary#inner

Germline stem cells: Origin and destiny

Germline stem cells are key to genome transmission to future generations.

Over recent years, there have been numerous insights into the regulatory mechanisms that govern both germ cell specification and the maintenance of the germline in adults.

Complex regulatory interactions with both the niche and the environment modulate germline stem cell function.

Lehmann R, Cell Stem Cell, 2012

Mouse spermatogonial niche

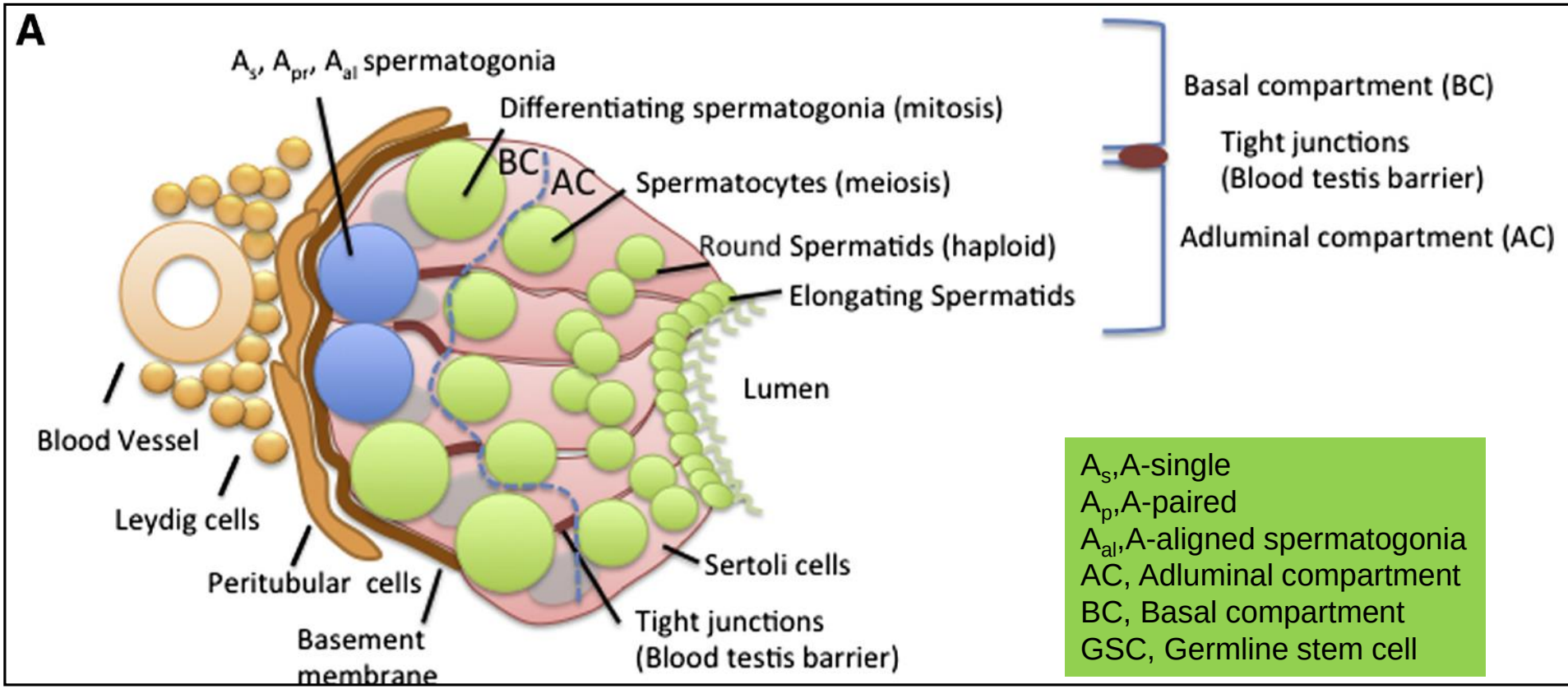


Figure 4. (**A**) Drawing of **GSC compartment** as a quadrant of cross sections of a seminiferous tubule. The entire **spermiogenesis process,** starting from **self-renewing spermatogonia** to release of elongated sperm into the lumen of the seminiferous tubule, occurs within the environment of large, somatic Sertoli cells. Tight junctions separate the basal compartment, which contains early spermiogenic stages, from the adluminal compartment filled with later stages.

Lehmann R, Cell Stem Cell, 2012

Hematopoietic stem cells

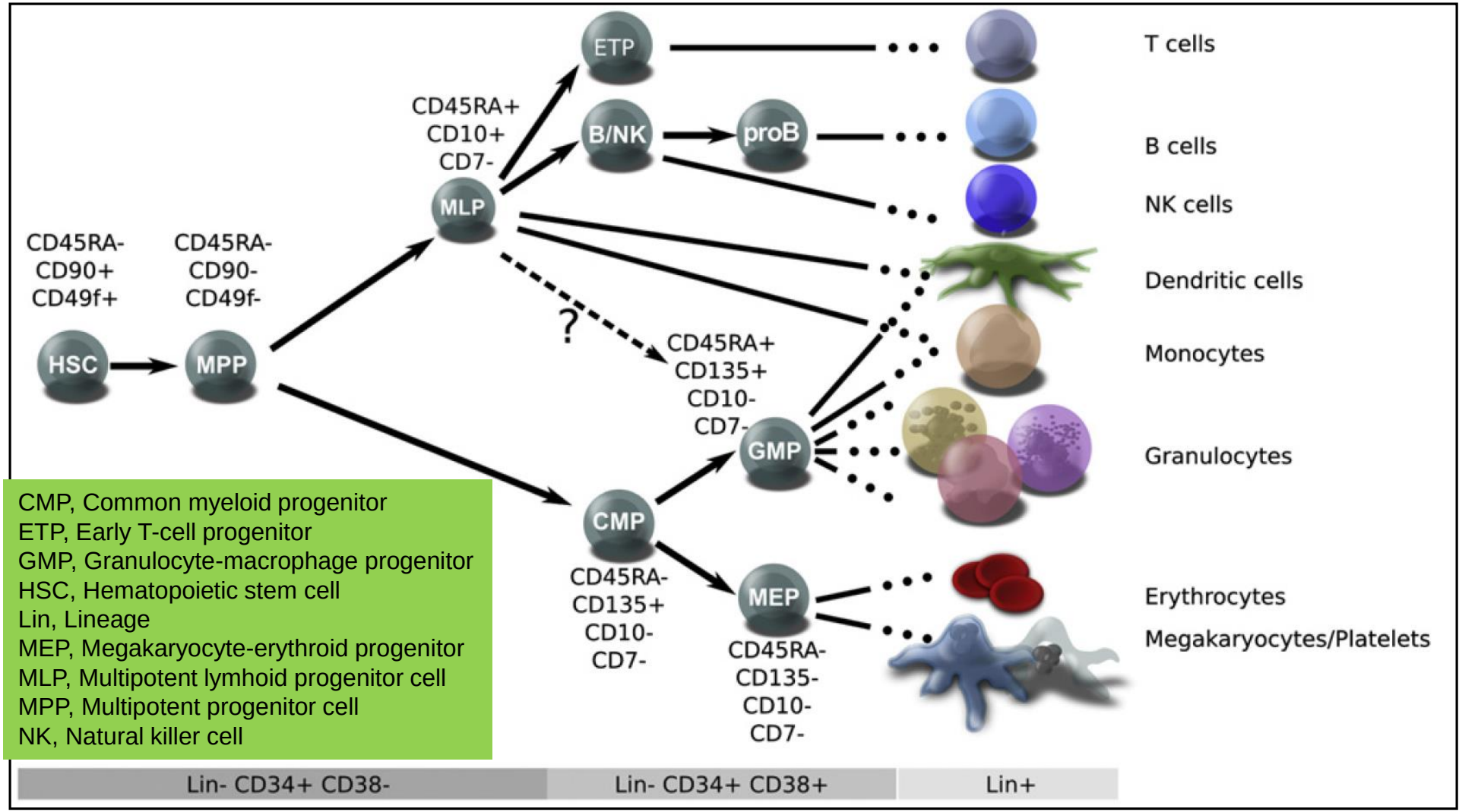
Despite its complexity, blood is probably the best understood developmental system, largely due to seminal experimentation in the mouse.

Clinically, hematopoietic stem cell (HSC) transplantation represents the most widely deployed regenerative therapy, but human HSCs have only been characterized relatively recently.

The discovery that immune-deficient mice could be engrafted with human cells provided a powerful approach for studying HSCs.

Doulatov S et al, Cell Stem Cell, 2012

Current model of lineage determination in the adult human hematopoietic hierarchy

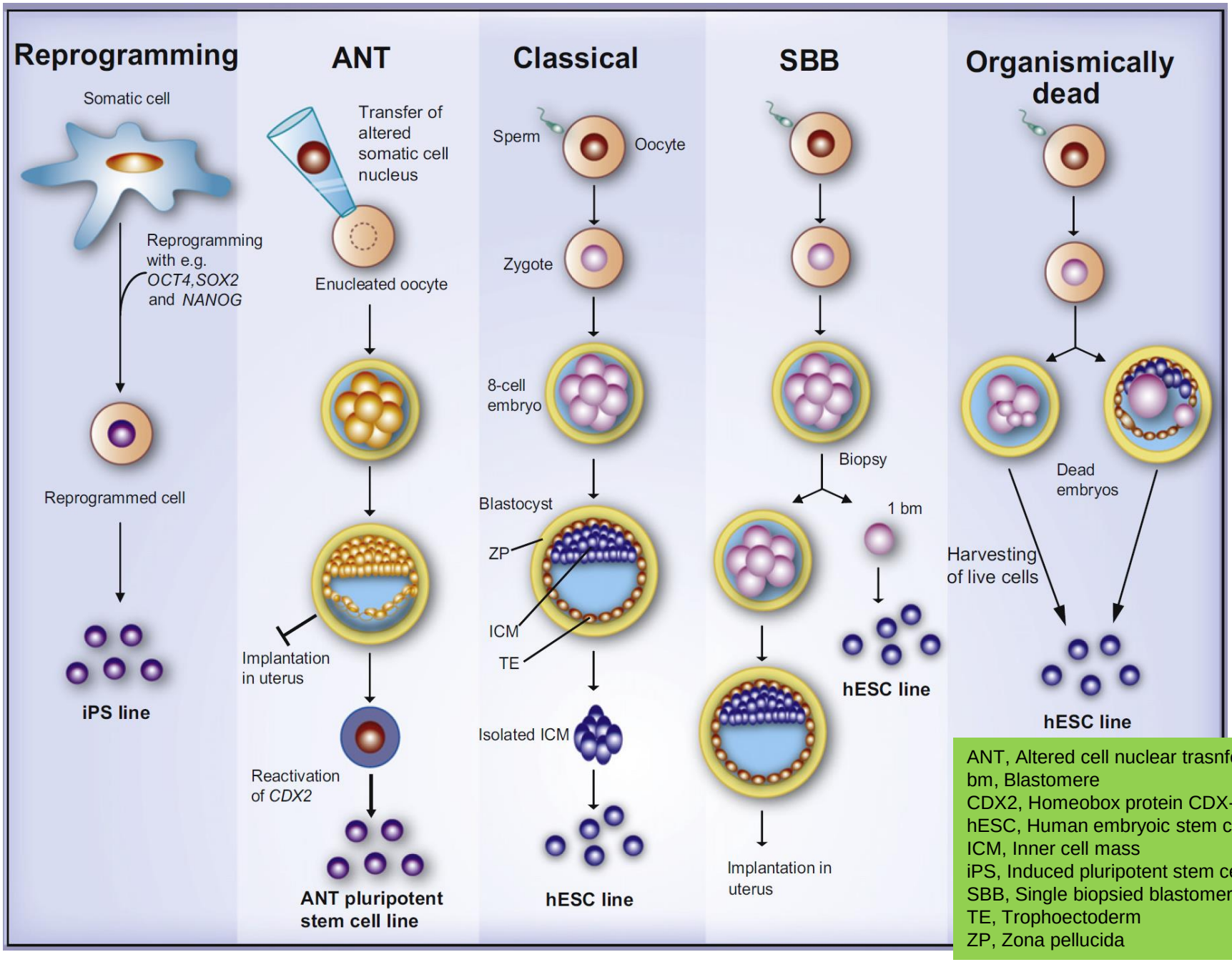


CMP, Common myeloid progenitor
ETP, Early T-cell progenitor
GMP, Granulocyte-macrophage progenitor
HSC, Hematopoietic stem cell
Lin, Lineage
MEP, Megakaryocyte-erythroid progenitor
MLP, Multipotent lymhoid progenitor cell
MPP, Multipotent progenitor cell
NK, Natural killer cell

Doulatov S et al, Cell Stem Cell, 2012

Human embryonic stem cells

Human ESCs: Classical and alternative sources



ANT, Altered cell nuclear trasnfer
bm, Blastomere
CDX2, Homeobox protein CDX-2
hESC, Human embryoic stem cell
ICM, Inner cell mass
iPS, Induced pluripotent stem cell
SBB, Single biopsied blastomere
TE, Trophoectoderm
ZP, Zona pellucida

Gavrilov S et al, in Atala A et al Principles of Regenerative Medicine, 2nd ed, Elsevier, 2011

Stem cells from amniotic fluid

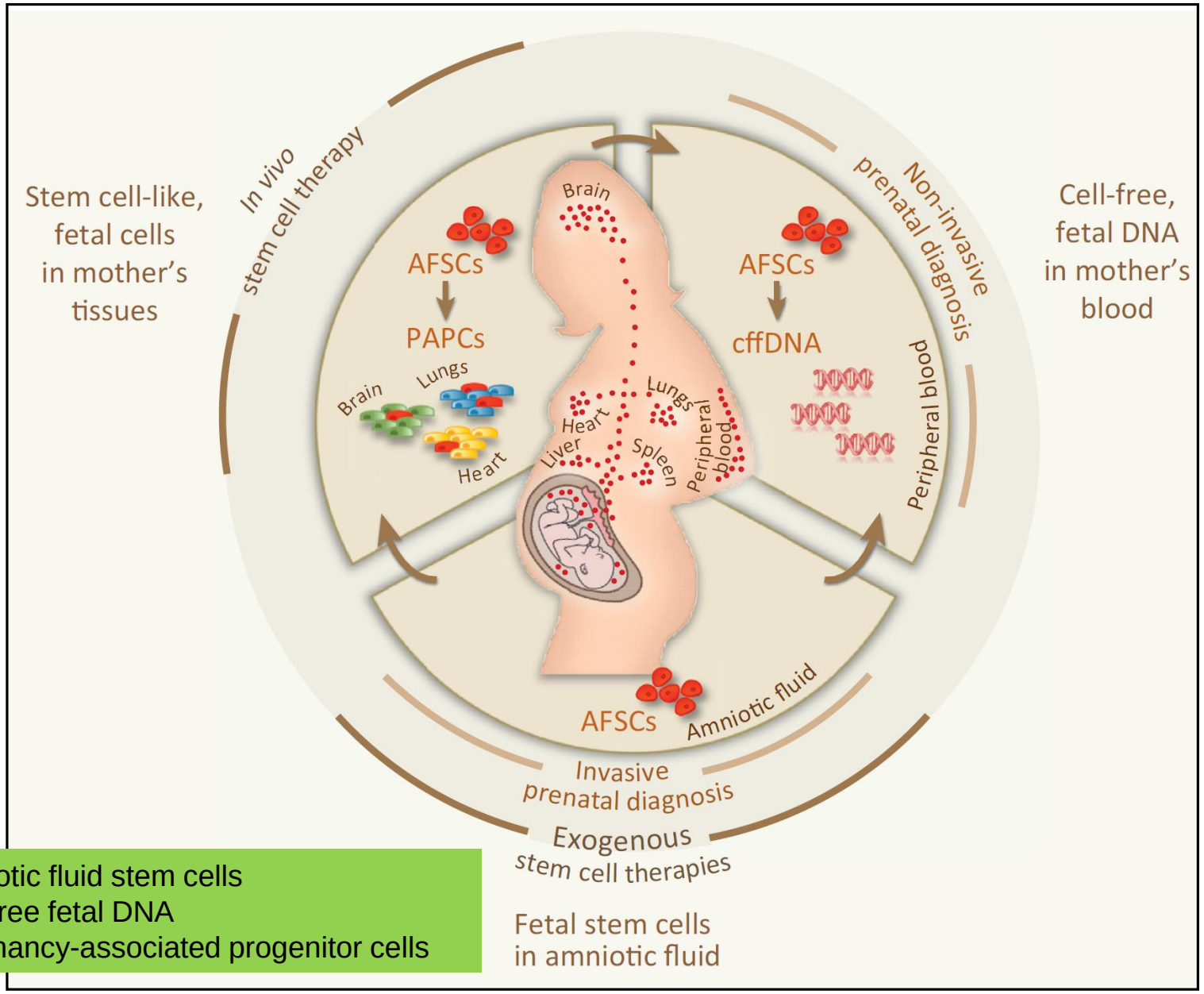
Fetal cells (and cell-free, fetal DNA used for non-invasive prenatal diagnosis) are known to exist in the circulation of pregnant women.

These cells exhibit stem cell properties when they differentiate at the site of injured maternal tissue, but the origin of these fetal, natural, and probably reparative cells is unknown.

During pregnancy, mobilized pluripotent fetal stem cells of yet unidentified *in vivo* significance float in the amniotic fluid, and we argue that circulating fetal cells and the pluripotent amniotic fluid cells might share a common origin.

Rosner & Hengstschläger, Trends Mol Med, 2013

Amniotic fluid stem cells as the source for fetal cell microchimerism



AFSCs, Amniotic fluid stem cells
cffDNA, Cell-free fetal DNA
PAPCs, Pregnancy-associated progenitor cells

Rosner & Hengstschläger, Trends Mol Med, 2013

Figure 1
Amniotic fluid stem cells as the source for fetal cell microchimerism.

An illustration of the suggested relationship between amniotic fluid stem cells and fetal microchimeric cells and DNA in the mother's tissue and blood. Amniotic fluid, routinely collected during invasive prenatal diagnosis, contains pluripotent, non-tumorigenic AFSCs, which might be the missing link to the unknown origin of PAPCs and cffDNA. In this model, mobilized and viable AFSCs (or probably cells differentiated from AFSCs) that reach the maternal circulation could be, or give rise to, what we currently recognize as PAPCs, directly engrafting to healthy, injured, or diseased maternal tissues or resting in stem cell niches, and potentially available over the course of a lifetime. Similarly, cffDNA found in the mother's peripheral blood and used for noninvasive prenatal diagnosis might be the direct consequence of the apoptotic disposal of excess AFSCs in the amniotic fluid, or of defective AFSCs floating in the maternal circulation or engrafted into tissues. In summary, this model suggests AFSCs to be the cell type of origin of both PAPCs and cffDNA, and favors a new, dual role for AFSCs in stem cell-based regenerative therapies, including their usage in classic, exogenous transplantation therapy and their potential involvement in tissue regeneration and repair in vivo.

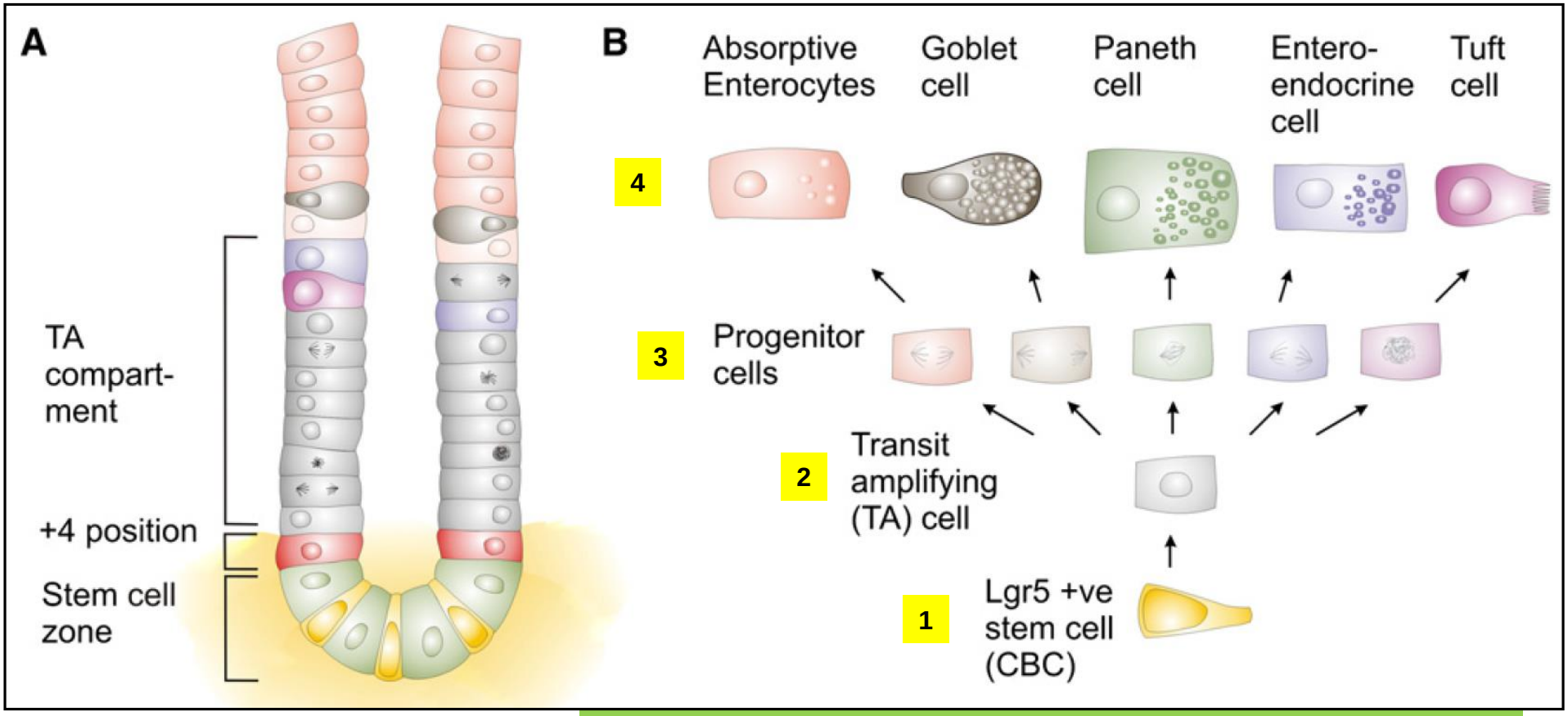
Abbreviations: cffDNA, cell-free, fetal DNA; PAPCs, pregnancy-associated progenitor cells; AFSCs, amniotic fluid stem cells.

Tissue-resident adult stem cell populations of rapidly self-renewing organs: Intestine, stomach, skin

- The epithelial lining of the intestine, stomach, and skin is continuously exposed to environmental assault, imposing a requirement for regular self-renewal.
- Resident adult stem cell populations drive this renewal, and much effort has been invested in revealing their identity.
- Reliable adult stem cell biomarkers would accelerate our understanding of stem cell roles in tissue homeostasis and cancer.
- Membrane-expressed markers would also facilitate isolation of these adult stem cell populations for exploitation of their regenerative potential.

Barker N et al, Cell Stem Cell, 2010

Adult stem cell-driven epithelial renewal in the small intestine (1)

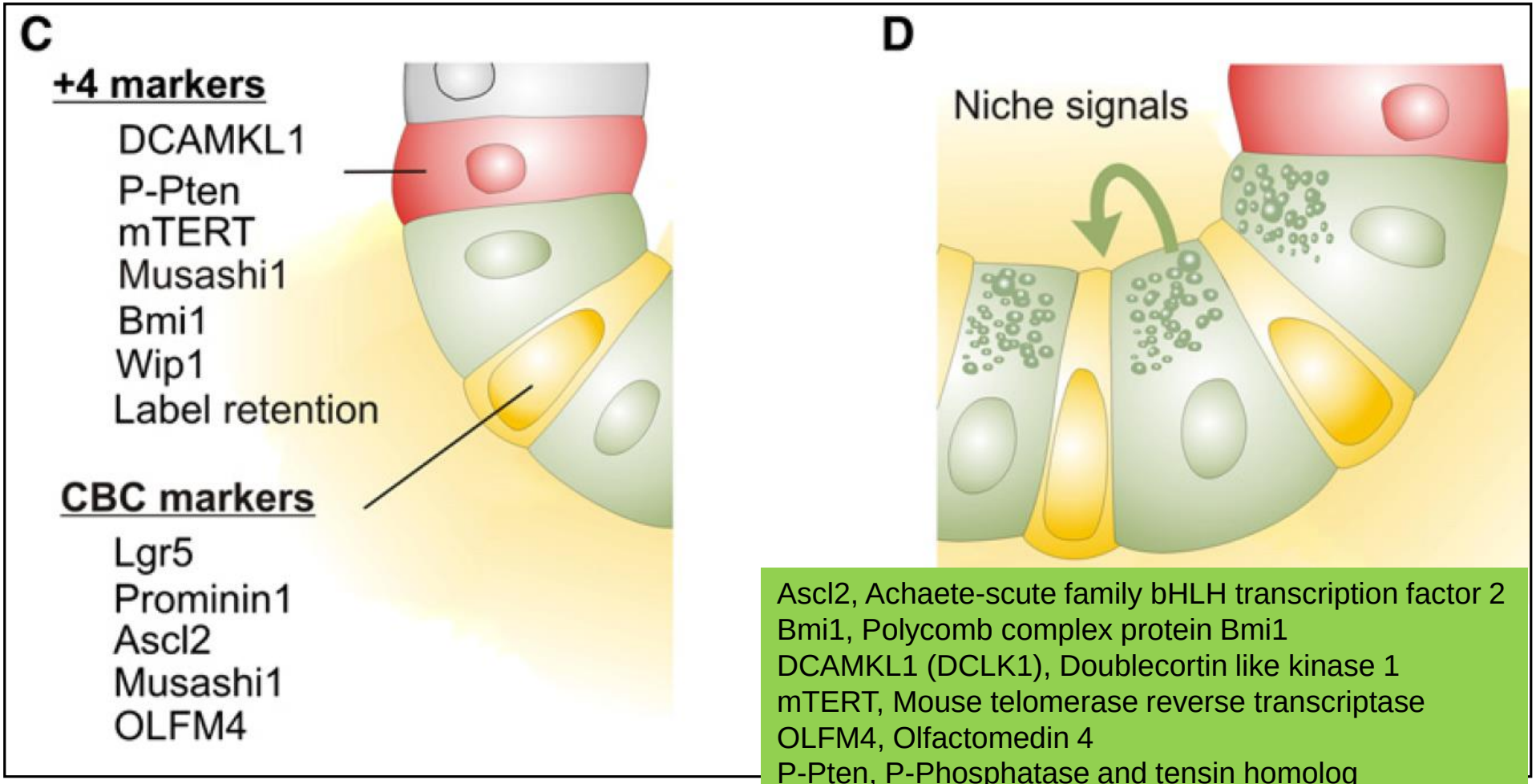


CBC, Crypt base columnar cell
Lgr5, Leucine-rich repeat-containing G-protein coupled receptor 5
TA, Transit amplifying

Figure 1
(**A**) The general architecture of a crypt.
(**B**) Flowchart depicting generation of functional epithelial cells from LGR5+ intestinal stem cells.

Barker N et al, Cell Stem Cell, 2010

Adult stem cell-driven epithelial renewal in the small intestine (2)



Ascl2, Achaete-scute family bHLH transcription factor 2
Bmi1, Polycomb complex protein Bmi1
DCAMKL1 (DCLK1), Doublecortin like kinase 1
mTERT, Mouse telomerase reverse transcriptase
OLFM4, Olfactomedin 4
P-Pten, P-Phosphatase and tensin homolog
Wip1, Wild-type p53-induced phosphatase 1

Figure 1
(C) Summary of the published +4 and CBC stem cell markers.
(D) Paneth Cells are a crucial niche component for the LGR5+ stem cells at the crypt base.

Barker N et al, Cell Stem Cell, 2010

Adult stem cell-driven epithelial renewal in the pyloric stomach (1)

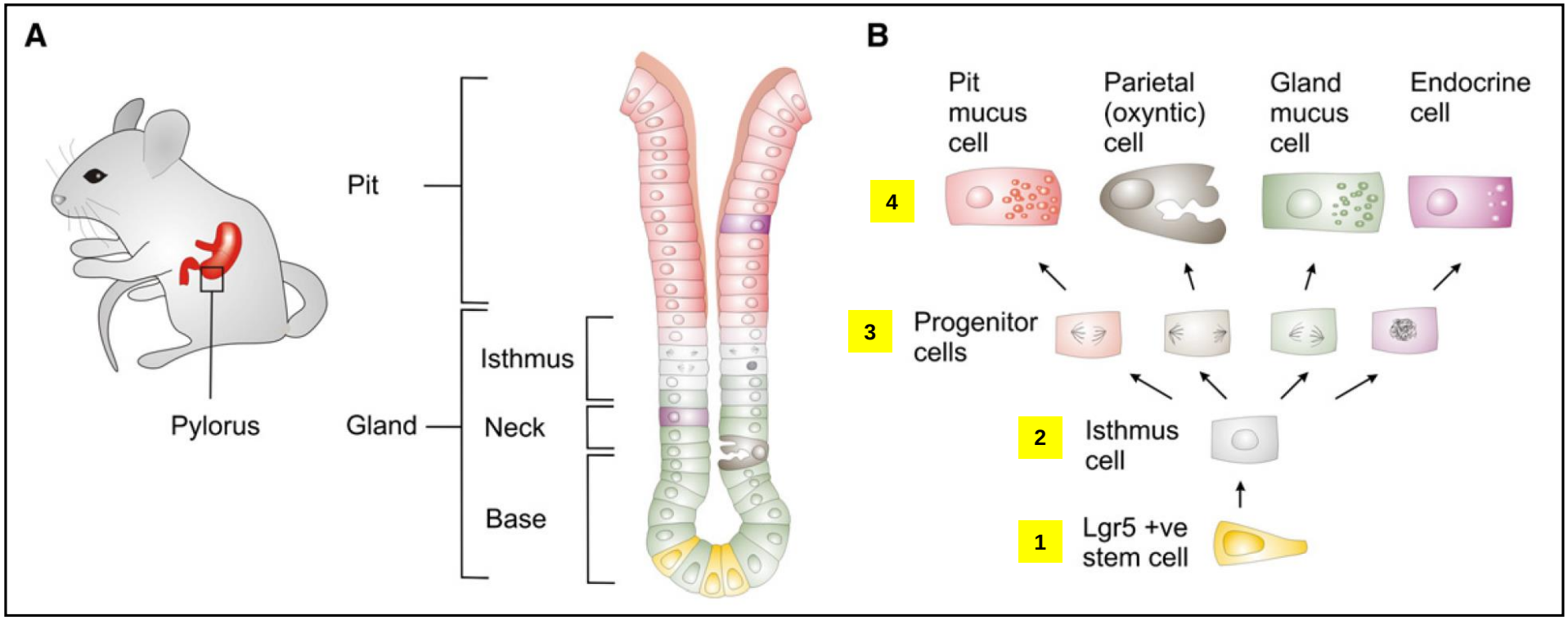
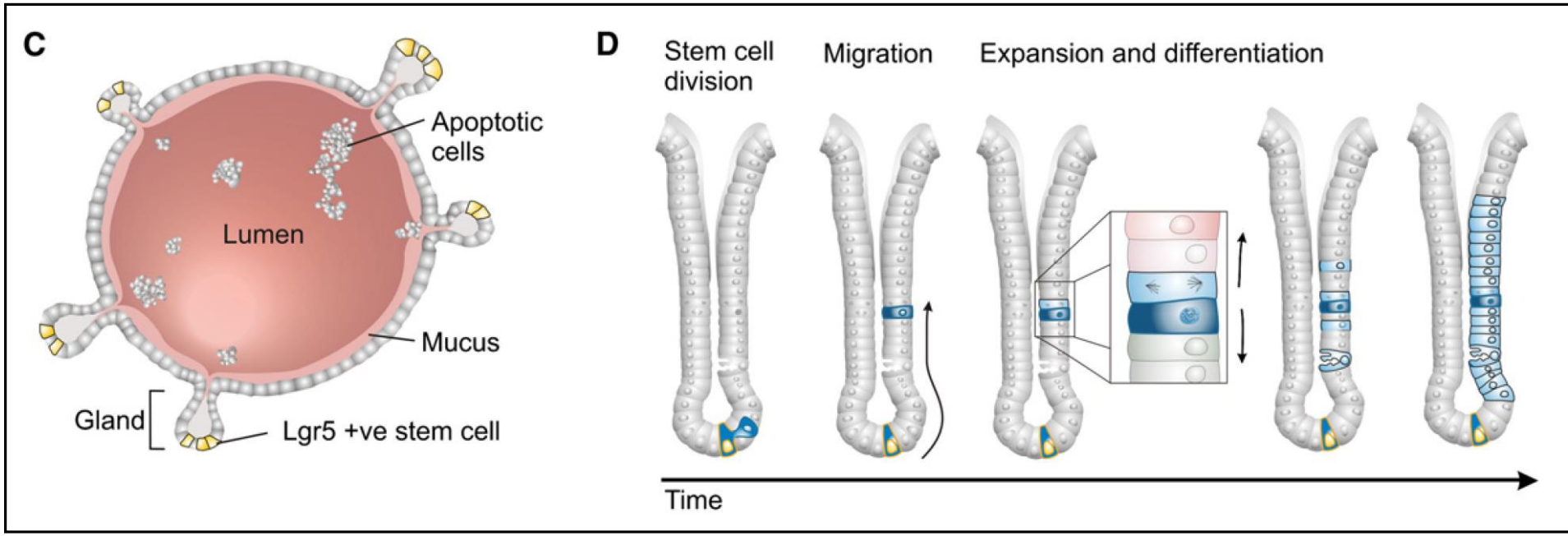


Figure 2
(**A**) The location and general architecture of pyloric gastric units.
(**B**) Flowchart depicting generation of functional epithelial cells from LGR5+ pyloric stem cells.

Barker N et al, Cell Stem Cell, 2010

Adult stem cell-driven epithelial renewal in the pyloric stomach (2)



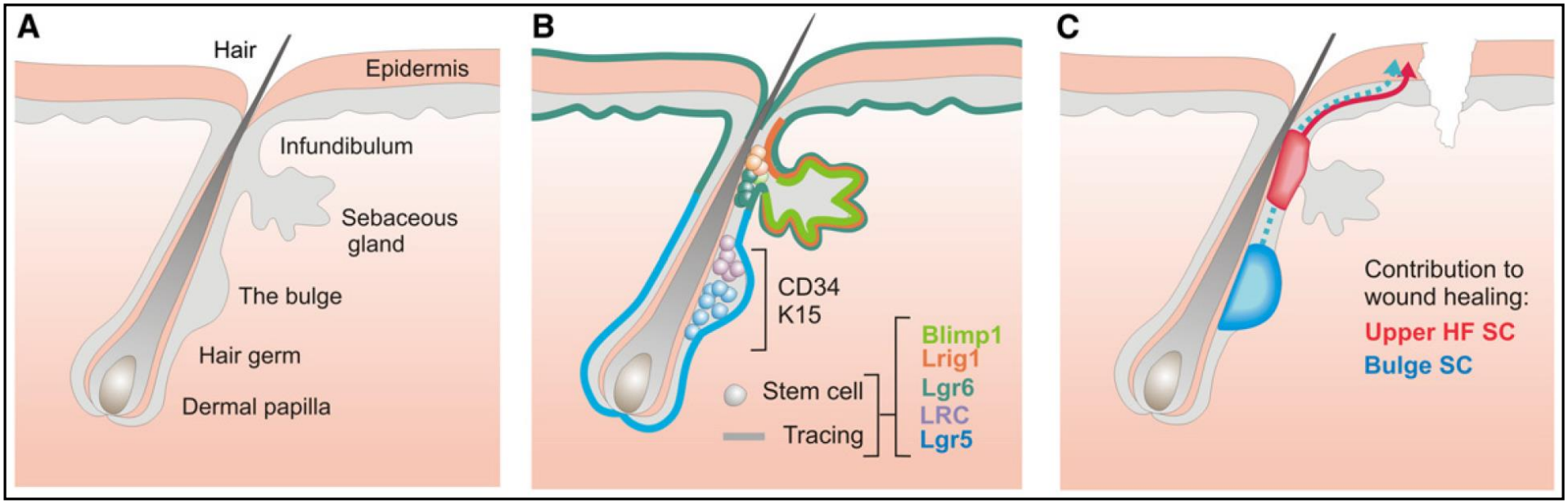
Barker N et al, Cell Stem Cell, 2010

Figure 2

(**C**) Cartoon of a self-renewing gastric organoid grown from a single LGR5+ pyloric stem cell. Multiple glands harboring LGR5+ stem cells at their base are interconnected by mature pit epithelium. Apoptotic cells are shed into the central lumen as the organoids epithelium constantly renews.

(**D**) A model for LGR5+ stem cell-driven epithelial renewal: LGR5+ stem cell progeny rapidly migrate to the isthmus, where they undergo rapid clonal expansion to generate committed progenitors, which subsequently terminally differentiate toward the functional cell lineages as they migrate bidirectionally toward the pit or the gland.

Adult stem cell-driven epithelial renewal in the skin under physiological conditions and following injury



Blimp1, B-Lymphocyte-Induced Maturation Protein 1
CD34, CD34 molecule, highly glycosylated, and phosphorylated by protein kinase C
K15, Keratin 15
Lgr5, Leucine-rich repeat-containing G-protein coupled receptor 5
LRC, Leukocyte receptor complex
Lrig1, Leucine-rich repeats and immunoglobulin-like domains protein 1
Upper HF SC, Upper hair follicle stem cell
SC, Stem cell

Figure 3
(**A**) The general architecture of a hair follicle in adult skin.
(**B**) Summary of the various adult stem cell compartments and their contribution to skin homeostasis under physiological conditions.
(**C**) The contribution of the adult stem cell populations to wound healing.

Stem cells in the face: Tooth regeneration and beyond

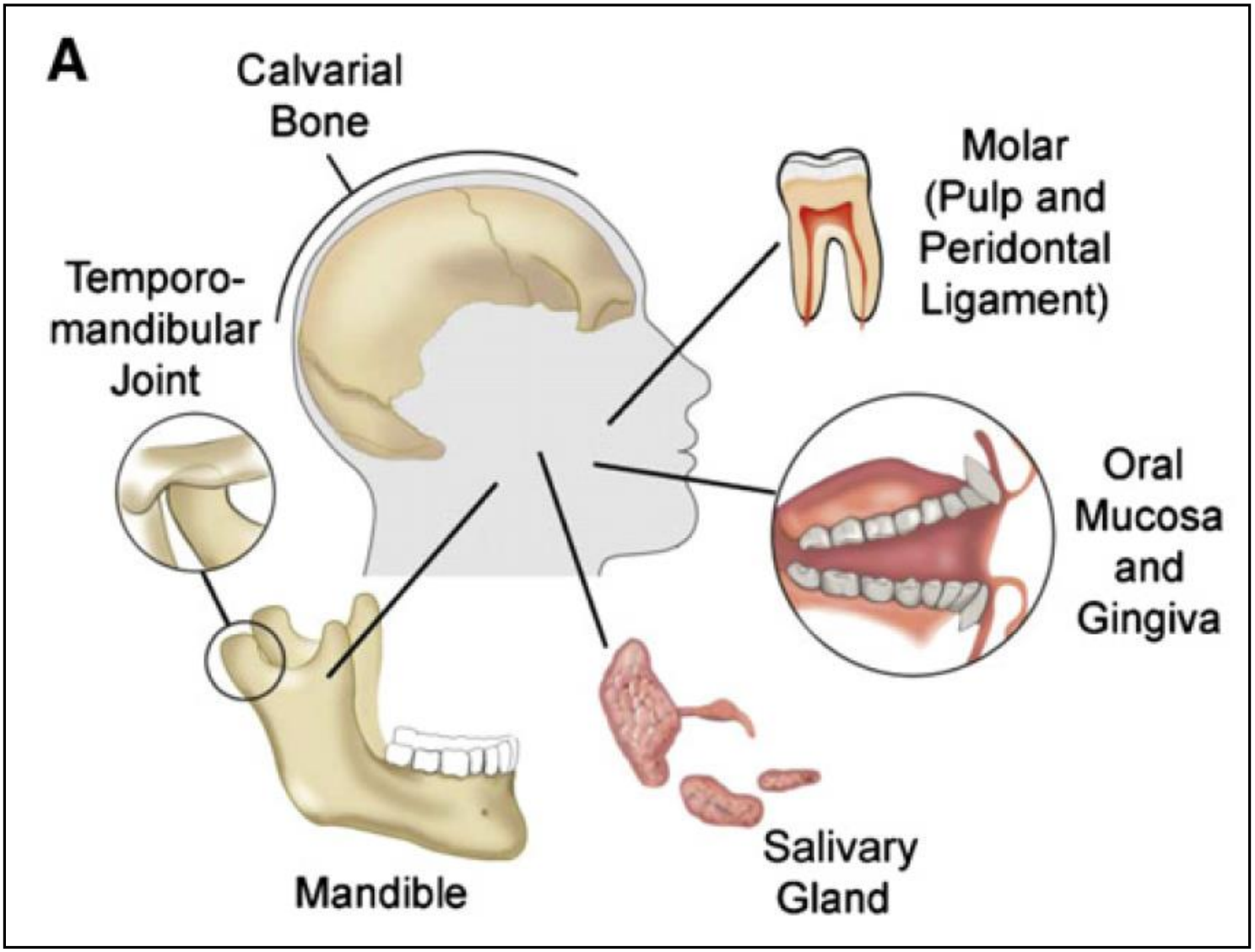
The face distinguishes one person from another.

Postnatal orofacial tissues harbor rare cells that exhibit stem cell properties.

Despite unmet clinical needs for reconstruction of tissues lost in congenital anomalies, infections, trauma, or tumor resection, how orofacial stem/progenitor cells contribute to tissue development, pathogenesis, and regeneration is largely obscure.

Mao & Prockop, Cell Stem Cell, 2012

Human orofacial tissues from which stem/progenitor cells have been studied



Mao & Prockop, Cell Stem Cell, 2012

Figure 1. (**A**) Epithelial stem cells reside in the developing tooth germ, oral epithelium, and salivary gland. Connective tissue stem/progenitor cells (of mesenchyme/ mesoderm origin) have been isolated from calvarial bone, tooth pulp, dental papilla, the periodontal ligament, and marrow of alveolar bone.

Adipose stem cells

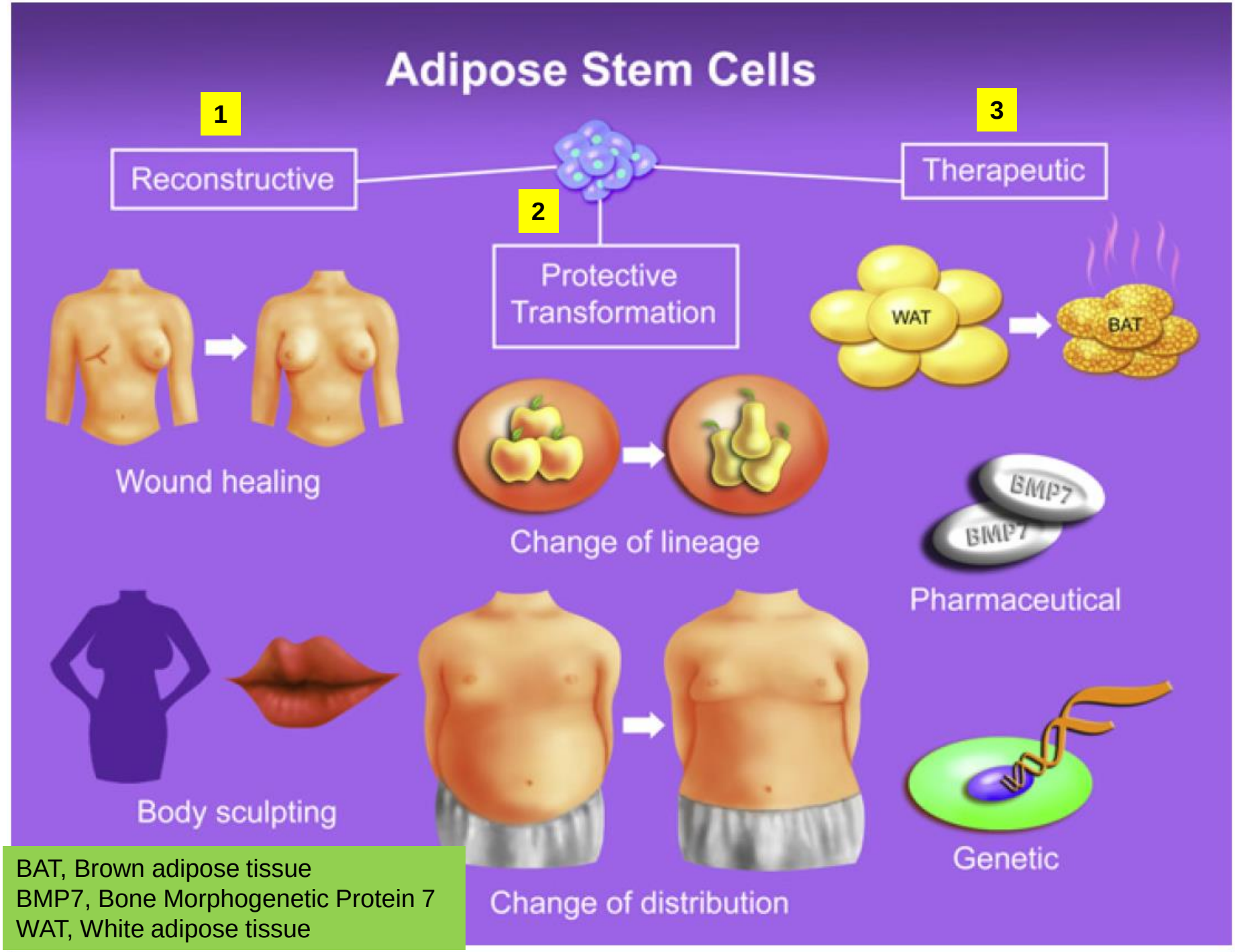
"eat ye that which is good and let your soul delight itself in fatness."

Isaiah 55:2, ~750 BCE

"Our people are killing themselves with a fork."

Rev. G. Roland, 21st century

Zeve D et al, Cell Stem Cell, 2009



BAT, Brown adipose tissue
BMP7, Bone Morphogenetic Protein 7
WAT, White adipose tissue

Zeve D et al, Cell Stem Cell, 2009

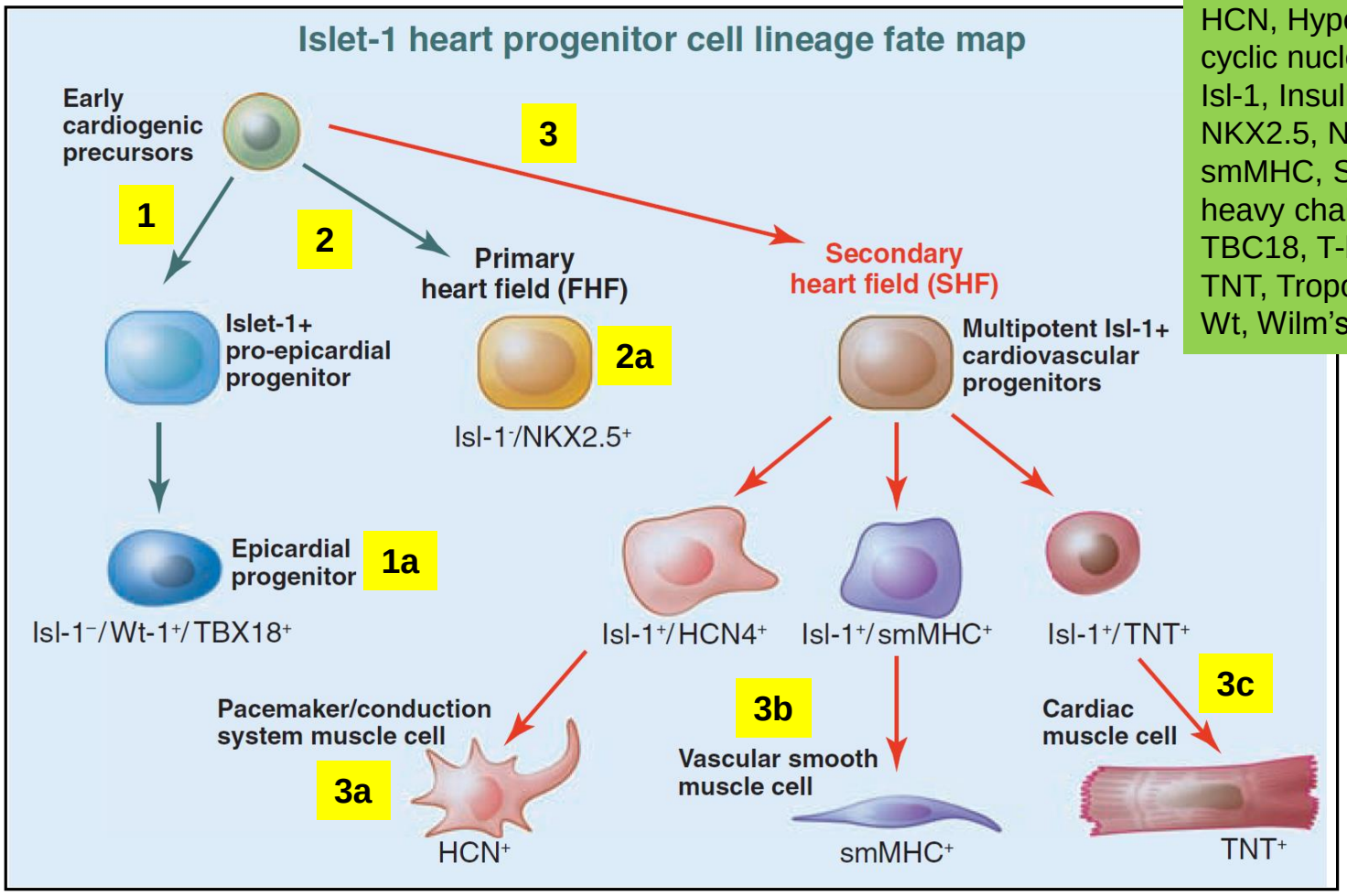
Figure 3. Adipose stem cells have clinical promise

Adipose stem cells have a variety of clinical applications: reconstructive applications, protective transformations, and therapeutic applications. Reconstructive applications broadly encompass surgical, genetic or traumatic defects as well as purely cosmetic purposes. Protective transformations denote induction of a heightened subcutaneous, metabolically protective, adipose signature (pear fat), preferentially over a visceral one (apple fat); this latter type is strongly associated with disease risk. These protections, which do not reduce total fat mass, either change the phenotype of extant depots or change where the fat is located, diverting fat storage to subcutaneous depots. Therapeutic refers to molding the white, energy storing, adipose lineage to a brown-like, energy burning, adipocyte fate, thereby reducing obesity and metabolic dysfunction. These approaches exploit the proclivity of adipose lineage cells to form adipocytes, a feature that paradoxically may be exploited to cure obesity and diabetes.

Zeve D et al, Cell Stem Cell, 2009

Cardiogenesis and the complex biology of regenerative cardiovascular medicine

Multipotent heart progenitors in the Isl1 lineage

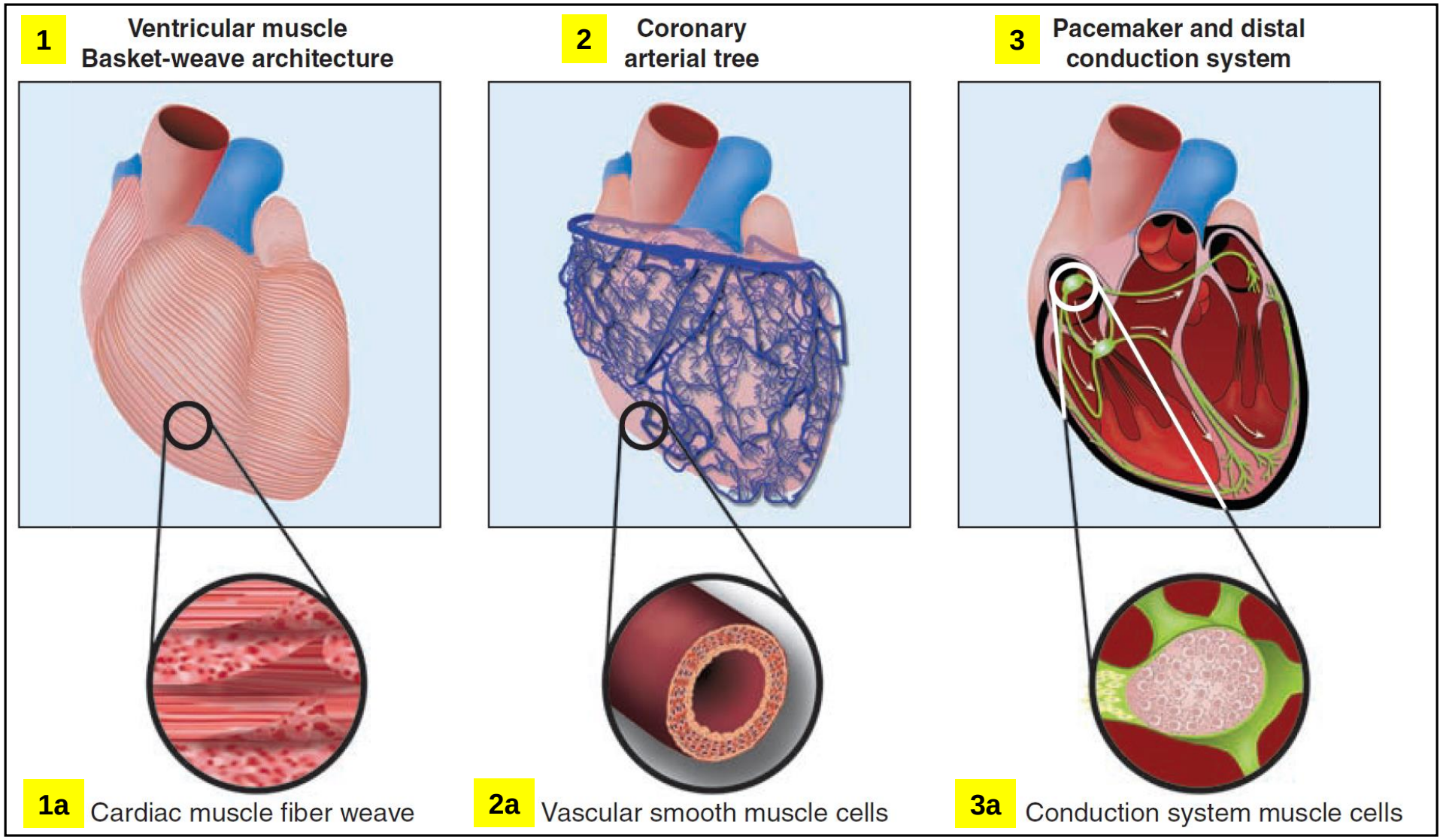


HCN, Hyperpolarization-activated cyclic nucleotide–gated channel
Isl-1, Insulin Gene Enhancer Protein
NKX2.5, NK2 homeobox 5
smMHC, Smooth muscle myosin heavy chain
TBC18, T-box transcription factor 18
TNT, Troponin T
Wt, Wilm's tumor

Chien KR et al, Science, 2008

Fig. 2. Multipotent heart progenitors in the Isl1 lineage. Early mesoderm-derived cardiac precursors give rise to progenitors in the first and second heart fields (FHF and SHF, respectively). The LIM–homeodomain transcription factor Isl1 marks a multipotent cardiovascular progenitor that can give rise to myocardium, conduction system, smooth muscle cells, and endothelial lineages. The developmental potential of FHF progenitors is not well established because of an absence of specific molecular markers for that field and an inability to isolate purified progenitor populations of that lineage. Recent work has also identified a third multipotent set of epicardial progenitors that appears to arise from a very early Isl1 precursors and to express the transcription factors Tbx18 or Wt1. TNT indicates troponin T; MHC, myosin heavy chain.

3D structure of ventricular muscle basket weave, coronary arterial tree, and pacemaker and conduction system

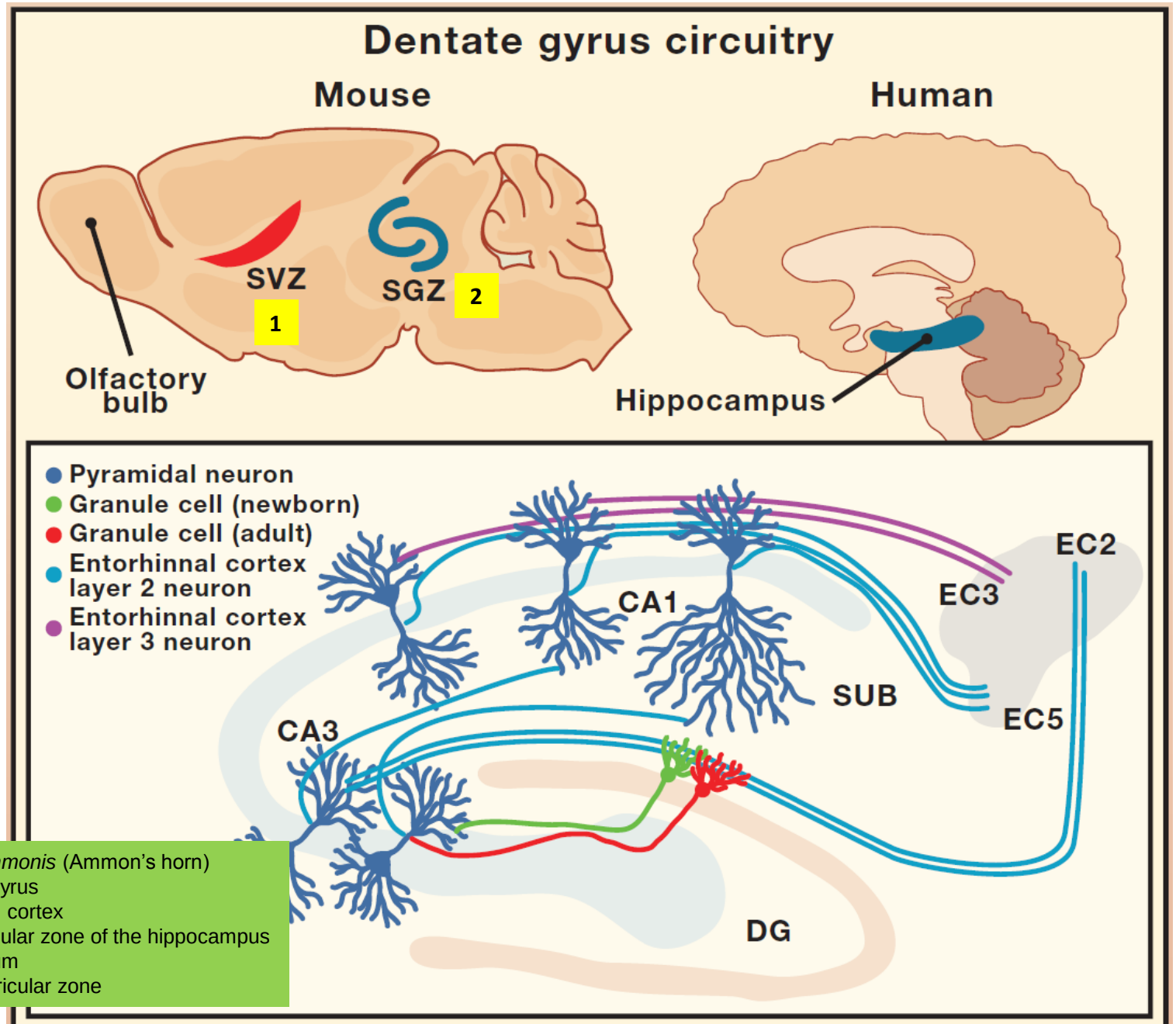


Chien KR et al, Science, 2008

Adult neurogenesis in the mammalian brain

Mechanisms and functional implications of adult neurogenesis

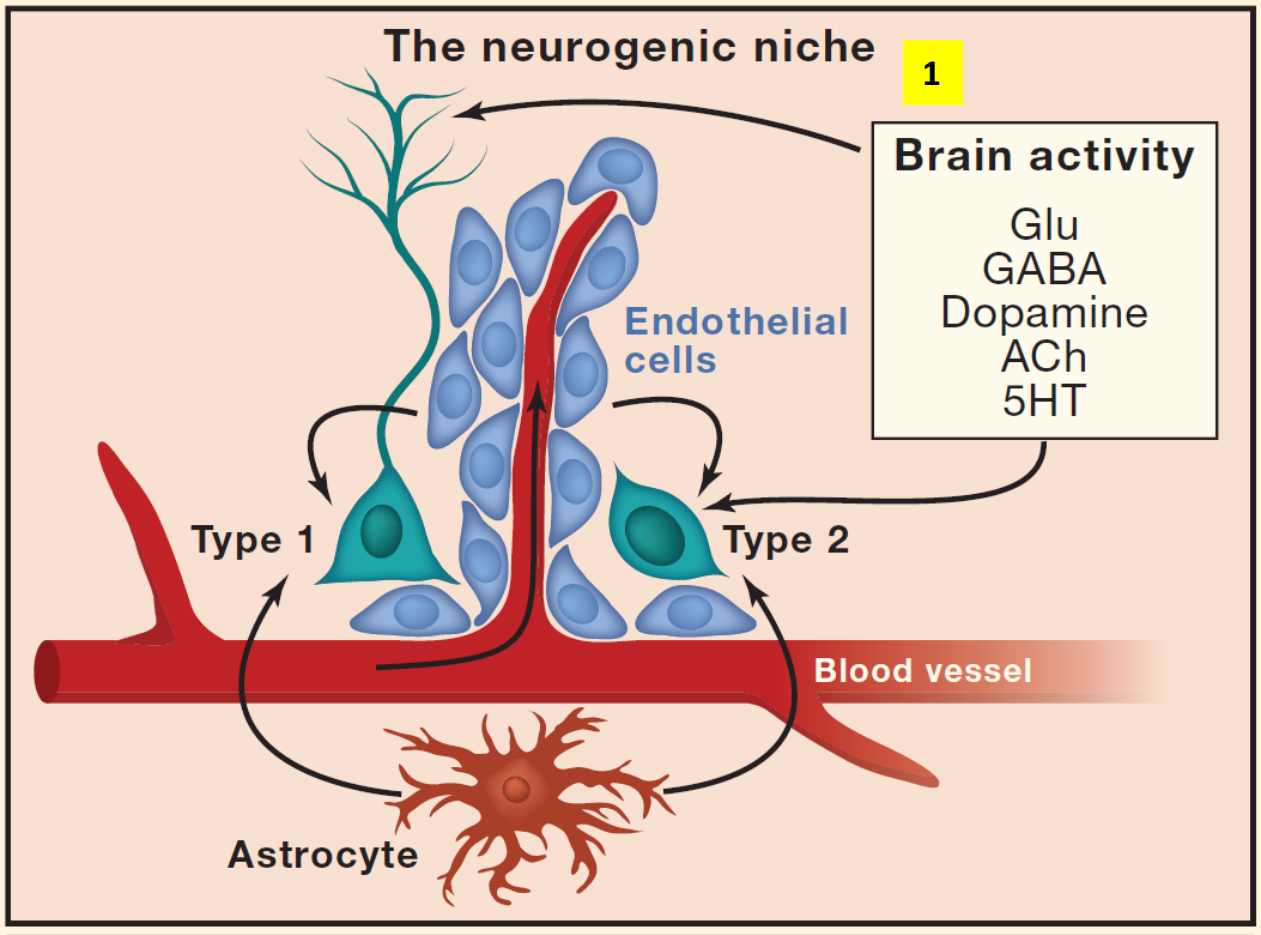
Adult hippocampal neurogenesis



CA, *Cornu Ammonis* (Ammon's horn)
DG, Dentate gyrus
EC, Entorhinal cortex
SGZ, Subgranular zone of the hippocampus
SUB, Subiculum
SVZ, Subventricular zone

Vadodaria & Gage, Cell, 2014

The neurogenic niche



2 Niche-specific contributors

Vasculature, astrocytes, microglia, granule cells, local interneurons

3 Local secreted factors

Wnt, IGF, VEGF, BDNF, IL4, IFNγ, TGFβ, IL-6, IL1-β, IGFBP-6, TNFα

4 Endocrine regulators

Thyroid, estrogen, endorphins, leptin, testosterone, corticosterone

ACh, Acetylcholine
BDNF, Brain-derived neurotrophic factor
GABA, Gamma-Aminobutyric acid
Glu, Glutamic acid
IFNγ, Interferon γ
IGF, Insulin-like growth factor
IGFBP-6, Insulin-like growth factor binding protein 6
IL1β, Interleukin 1β
IL4, Interleukin 4
IL6, Interleukin 6
TNFα, Tumor necrosis factor α
VEGF, Vascular endothelial growth factor
Wnt, Wingless-type MMTV integration site family protein
5HT, Serotonin (5-Hydroxytryptamine)

Vadodaria & Gage, Cell, 2014

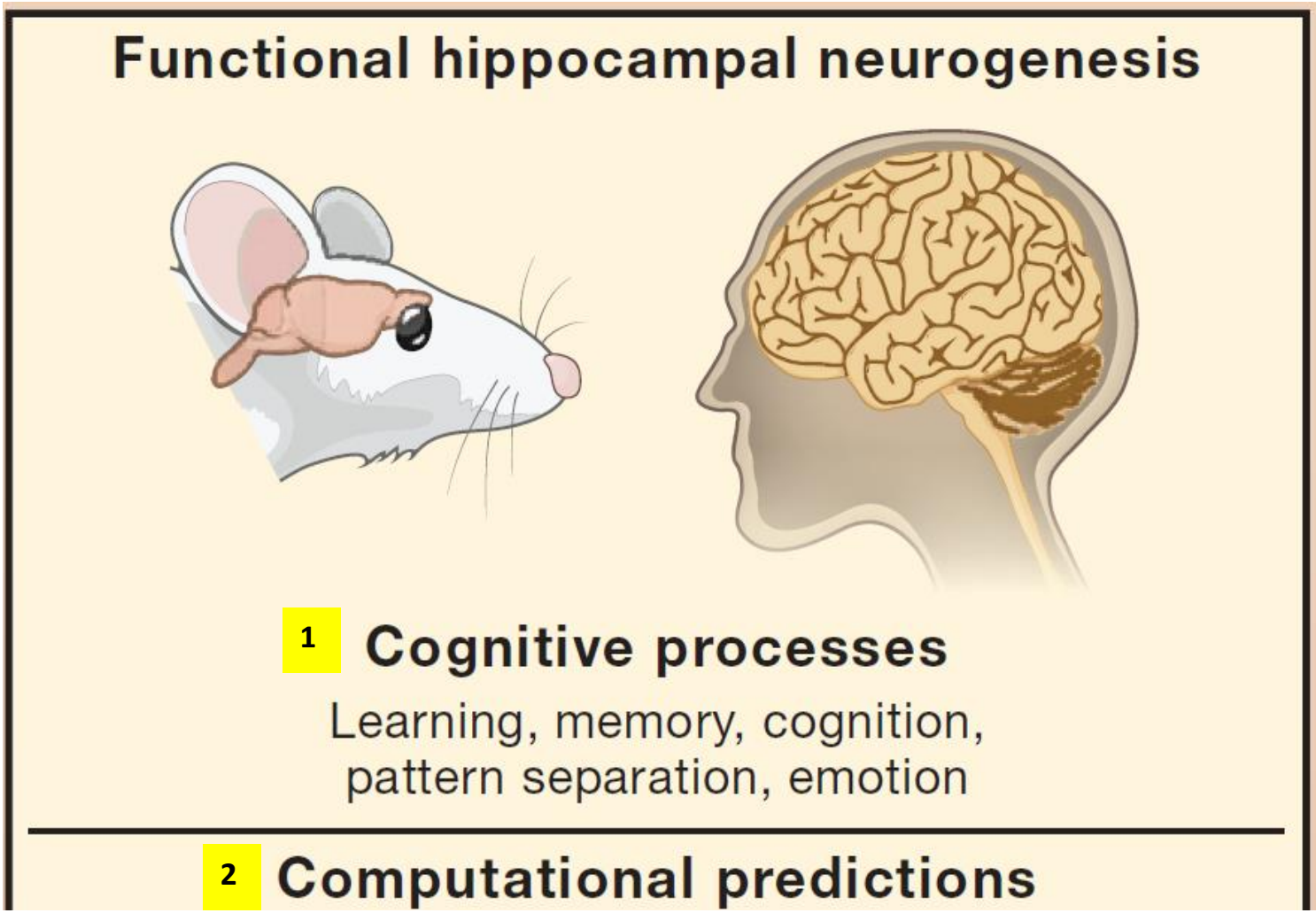
Regulators of hippocampal neurogenesis

Vadodaria & Gage, Cell, 2014

Regulators/stage 1	Proliferation 2	Differentiation/ survival/maturation 3
Behavioral/ environment	Running, learning, calorie restriction, seizure, ischemia Aging, stress, inflammation	Learning, enriched environment, activity Stress
Neurotransmitters	Serotonin, dopamine, acetylcholine, norepinephrine GABA	GABA, dopamine, glutamate, acetylcholine Norepinephrine
Growth factors/ morphogens	IGF-1, FGF-2, Shh, Wnt3, VEGF Notch	BDNF-TrkB, NT3, Wnt3, FGF2
Molecular mediators (Tx factors; epigenetic regulators)	GADD45b, miR-137, DISC1 FMRP	MBD1, MECP2, FMRP, DISC1, Cdk5, Klf9, Akt-mTOR HDACs **Epigenome modification**

BDNF, Brain-derived neurotrophic factor; Cdk5, Cyclin-dependent kinase 5; DISC1, Disrupted in schizophrenia 1; FGF-2, Fibroblast growth factor; FMRP, Familial mental retardation protein; GABA, Gamma-Aminobutyric acid; GADD45b, Growth arrest and DNA-damage-inducible 45 beta; HDAC, Histone deacetylase; IGF-1, Insulin-like growth factor 1; Klf9, Krueppel-like factor 9; MBD1, Methyl-CpG-binding domain protein 1; MECP2, Methyl-CpG binding protein 2; miR-137, microRNA-137; mTOR, Mammalian target of rapamycin; NT3, Neurotrophin 3; Shh, Sonic hedgehog; VEGF, Vascular endothelial growth factor

Functional hippocampal neurogenesis

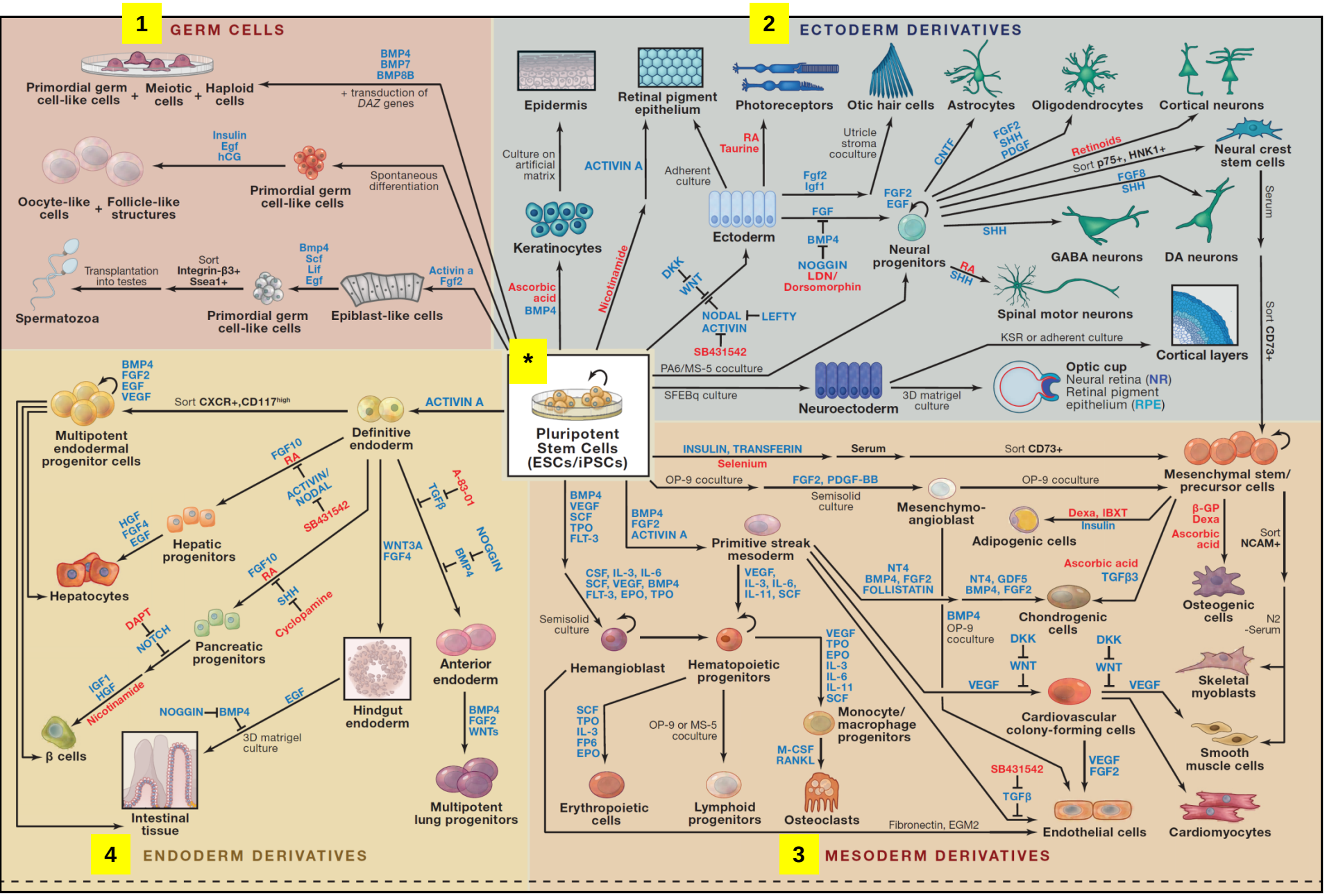


3 Regeneration

Vadodaria & Gage, Cell, 2014
(modified by Najbauer J, 2017)

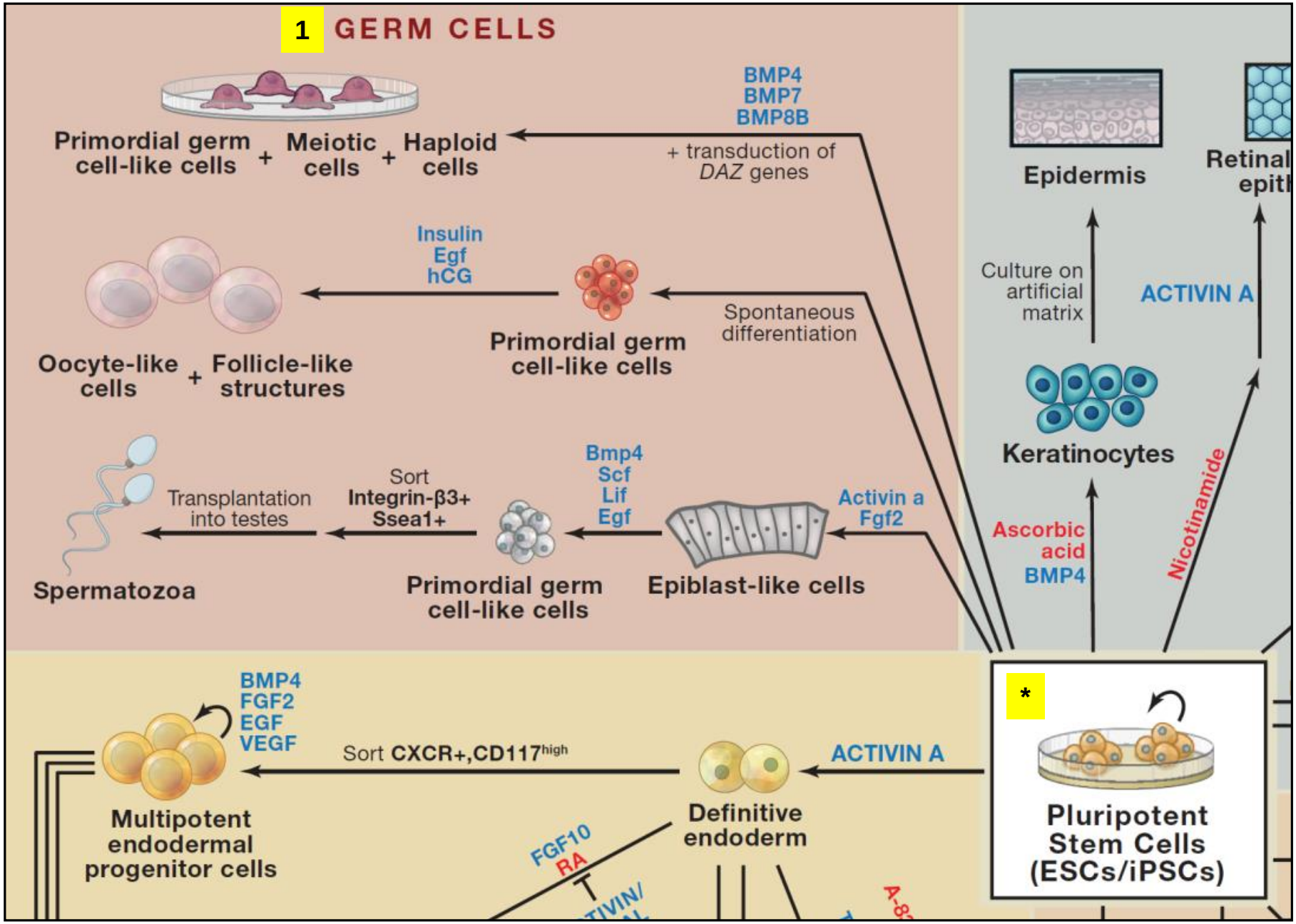
Directed differentiation of pluripotent stem cells

Directed differentiation of pluripotent stem cells



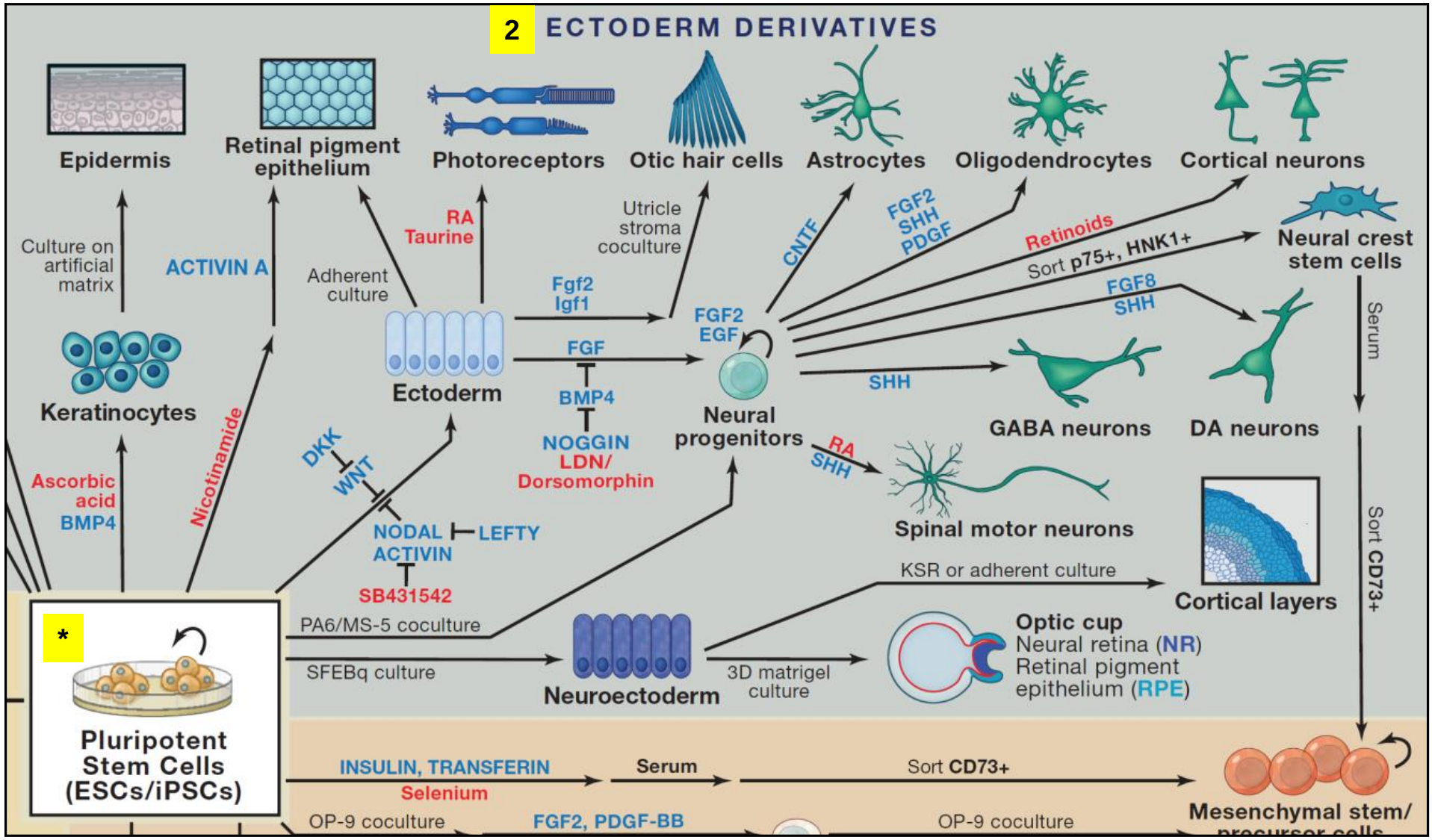
William LA et al, Cell, 2012

Directed Differentiation of Pluripotent Stem Cells – Germ Cells



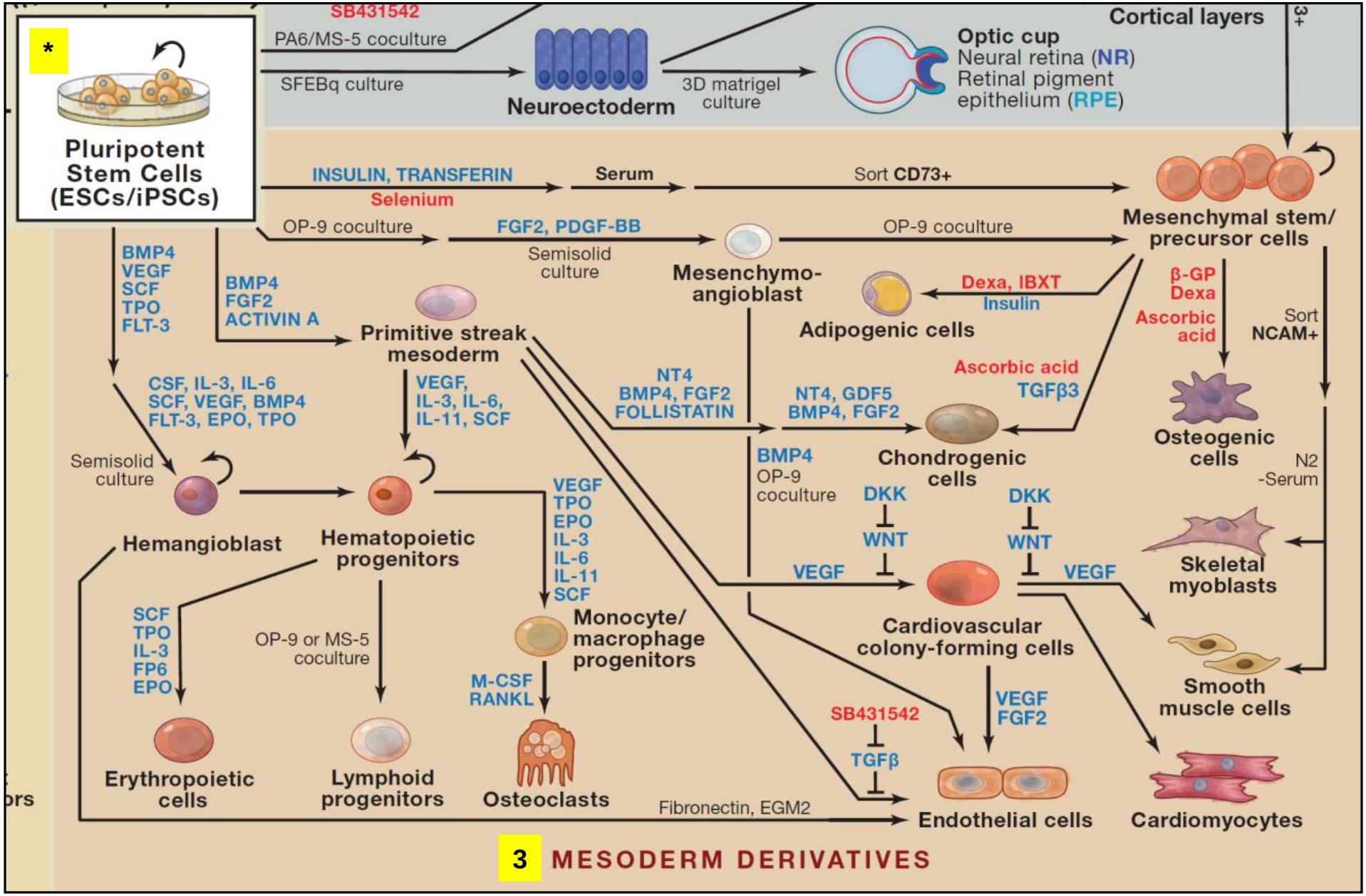
William LA et al, Cell, 2012

Directed Differentiation of Pluripotent Stem Cells – Ectoderm Derivatives



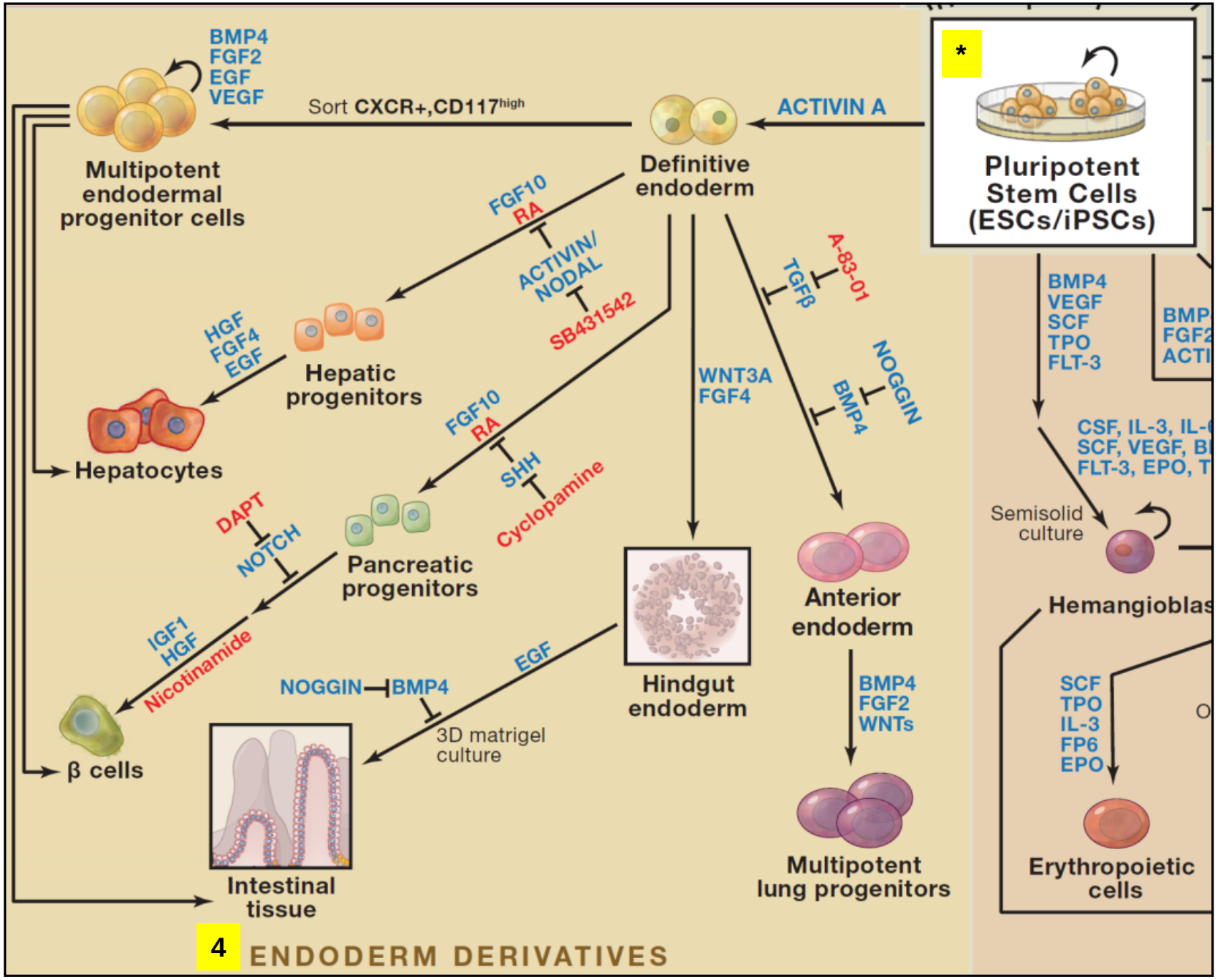
William LA et al, Cell, 2012

Pluripotent Stem Cells – Mesoderm Derivatives



William LA et al, Cell, 2012

Directed Differentiation of Pluripotent Stem Cells – Endoderm Derivatives



William LA et al, Cell, 2012

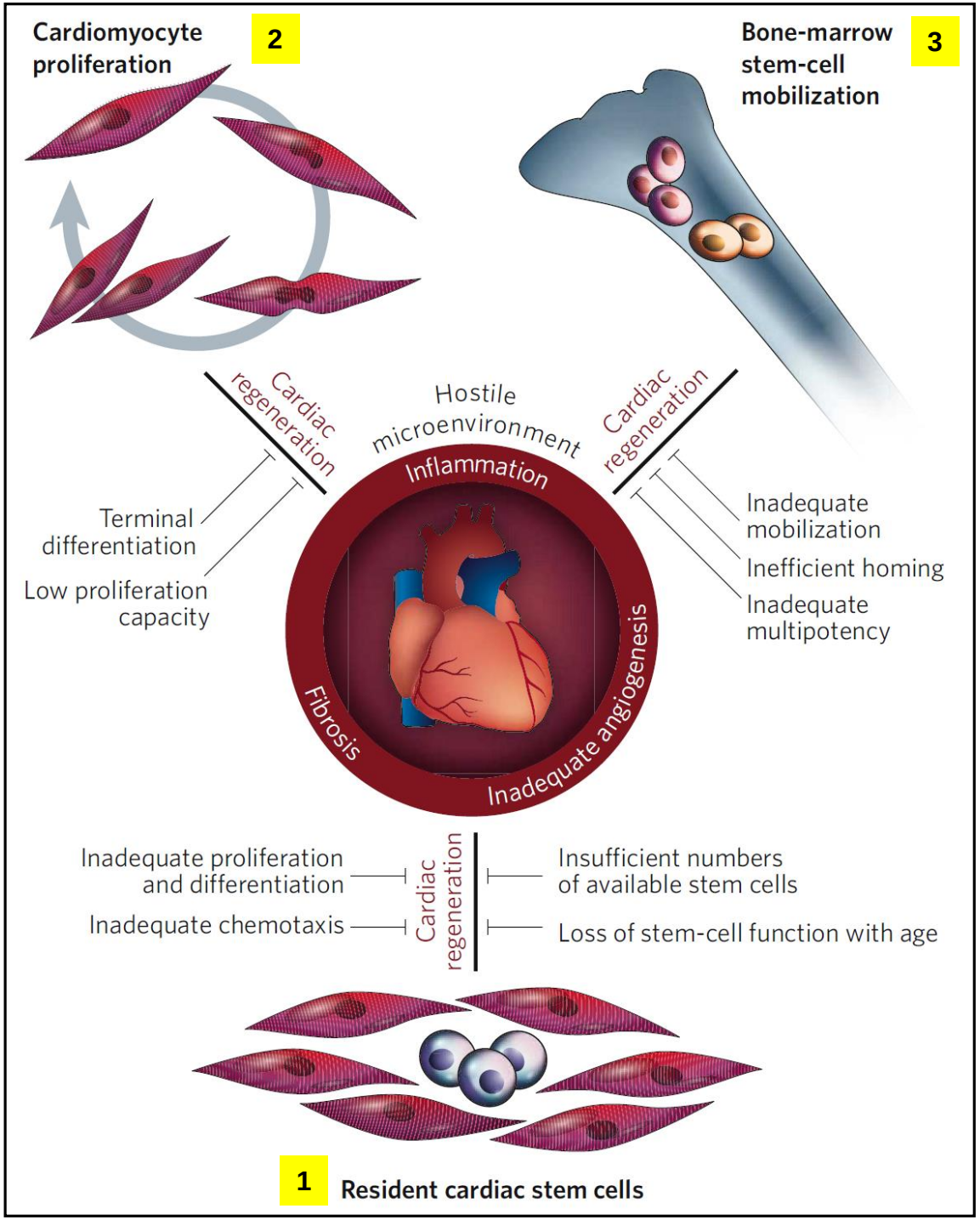
Abbreviations

β-GP, β-glycerol phosphate; **BMP**, bone morphogenetic protein; **CNTF**, ciliary neurotrophic factor; **CSF**, colony-stimulating factor; **Dexa**, dexamethasone; **DKK**, Dickkopf; **EGF**, epidermal growth factor; **EPO**, erythropoietin; **FGF**, fibroblast growth factor; **FLT**, fms-like tyrosine kinase ligand; **FP6**, IL-6 + IL-6 soluble receptor; **GDF**, growth differentiation factor; **hCG**, human chorionic gonadotropin; **HGF**, hepatocyte growth factor; **IBXT**, isobutylxanthine; **IGF**, insulin-like growth factor; **IL**, interleukin; **KSR**, knockout serum replacement; **Lif**, leukemia inhibitory factor; **M-CSF**, macrophage colony-stimulating factor; **NT4**, neurotrophin 4; **PDGF**, platelet-derived growth factor; **RA**, retinoic acid; **RANKL**, receptor activator of nuclear factor kappa b ligand; **SCF**, stem cell factor; **SFEBq**, Serum-free of embryoid body-like aggregates; **SHH**, Sonic hedgehog; **TGF-β**, transforming growth factor β; **TPO**, thrombopoietin; **VEGF**, vascular endothelial growth factor.

William LA et al, Cell, 2012

Examples of stem cell therapies

Stem-cell therapy for cardiac disease



Mechanisms of, and potential barriers to, endogenous cardiac regeneration

Segers & Lee, Nature, 2008

Cell types and mechanisms proposed for cardiac therapy

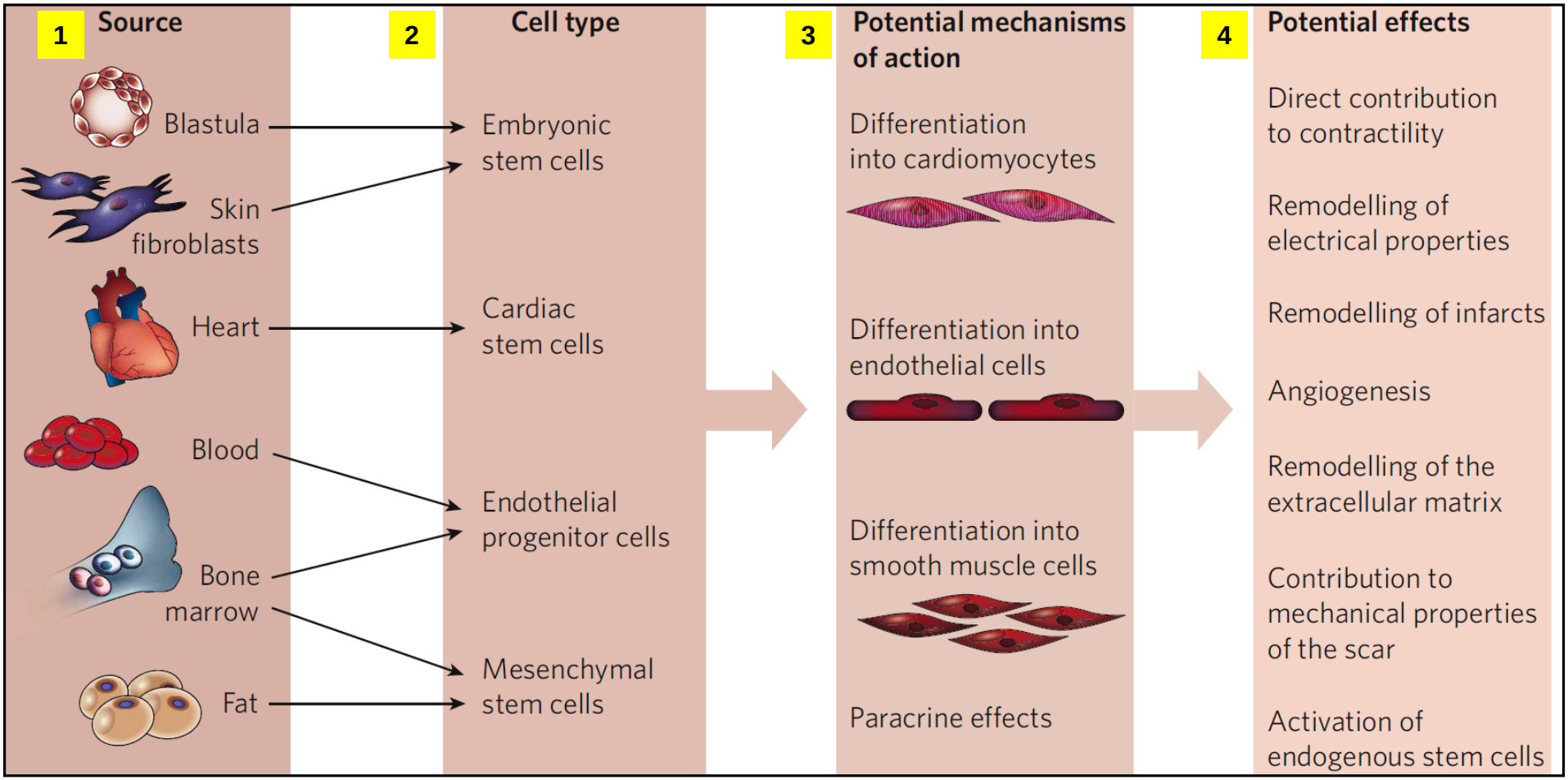
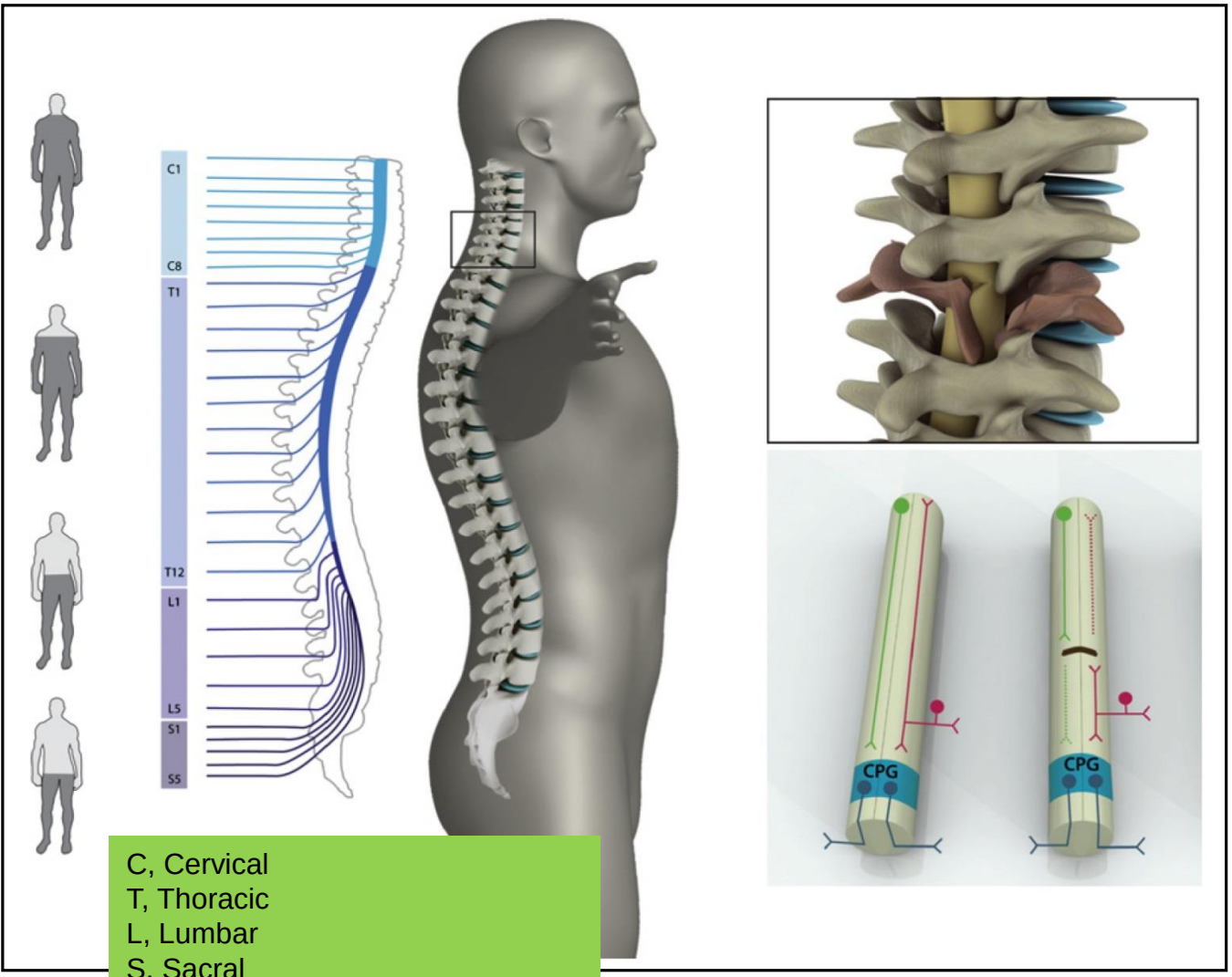


Figure 2. Many cell types and mechanisms have been proposed for cardiac therapy. Stem cells and progenitor cells can be isolated from either autologous or allogeneic sources. Different types of stem cell and progenitor cell have been shown to improve cardiac function through various mechanisms, including the formation of new myocytes, endothelial cells and vascular smooth muscle cells, as well as through paracrine effects.

Segers & Lee, Nature, 2008

Stem cells for spinal cord repair

Spinal cord injury results in disruption of motor control and sensory input below the level of the lesion



C, Cervical
T, Thoracic
L, Lumbar
S, Sacral
CPG, Central pattern generator

Barnabé-Heider & Frisén, Cell Stem Cell, 2008

Figure 1. Spinal cord injury is most often caused by trauma, which results in dislocation of vertebrae and damage to the spinal cord. Motor control and sensory input is lost below the level of the lesion (shaded area in figures to the left) due to the severance of long ascending sensory (red) and descending motor (green) axons. The local circuitry, including central pattern generators (CPG) responsible for coordinated locomotor function, remains largely intact in uninjured segments.

Mechanisms by which transplanted stem cell-derived cells may facilitate regeneration

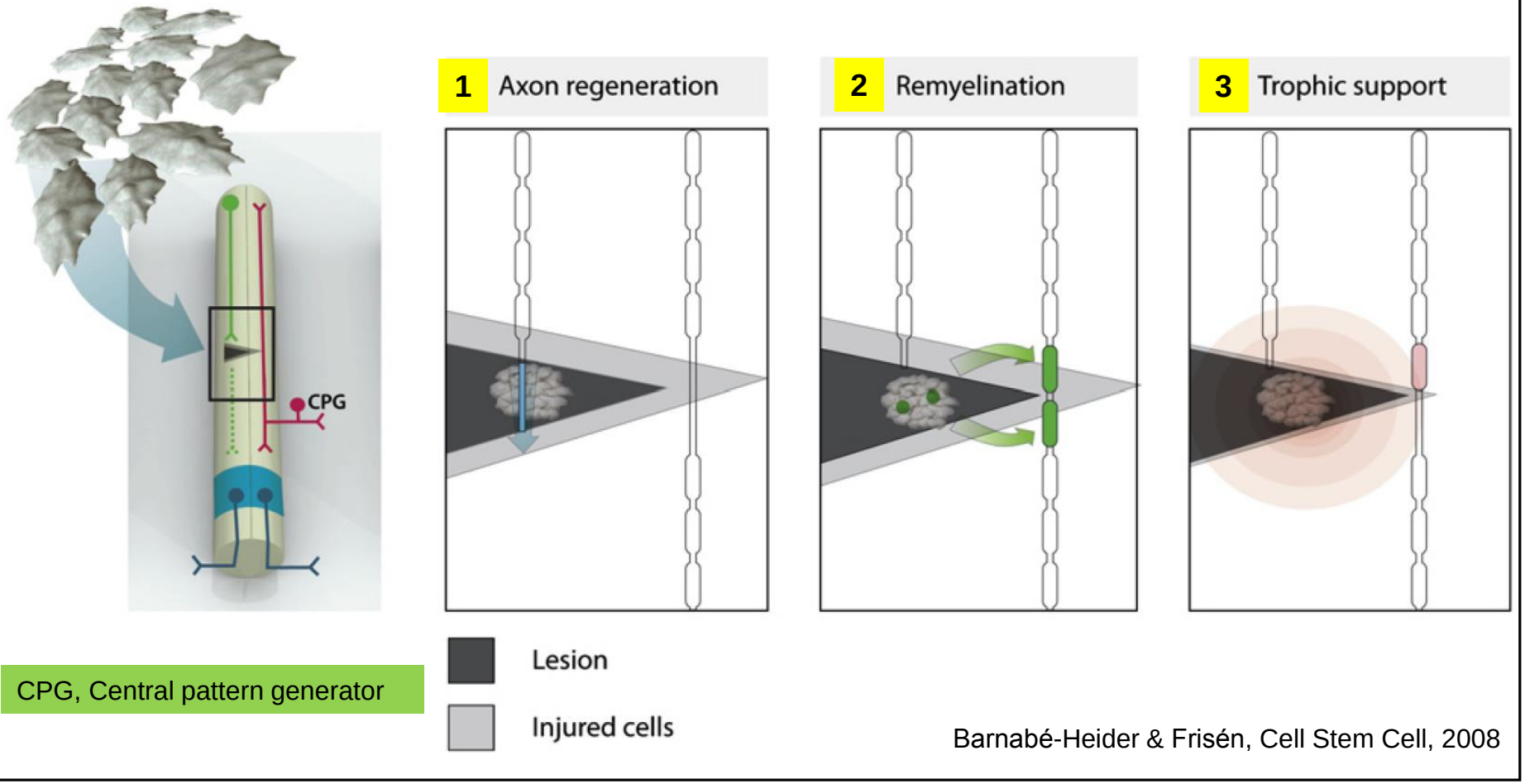


Figure 2. It is often difficult to establish the mechanism by which transplanted cells may facilitate functional recovery. Likely mechanisms include creating a permissive substrate for axonal growth, providing cells that remyelinate spared but demyelinated axons, and supplying trophic support reducing the damage and rescuing, for example, neurons and oligodendrocytes. Transplanted cells may in addition (not depicted) enhance axonal plasticity and replace lost neurons to reconstruct local circuitry.

Stem cell therapy against gliomas

Malignant gliomas are one of the most lethal cancers, and despite extensive research very little progress has been made in improving prognosis.

Multimodality treatment combining surgery, radiation, and chemotherapy is the current gold standard, but effective treatment remains difficult due to the invasive nature and high recurrence of gliomas.

Stem cell-based therapy using neural, mesenchymal, or hematopoietic stem cells may be an alternative approach because it is tumor selective and allows targeted therapy that spares healthy brain tissue.

Stem cells can be used to establish a long-term antitumor response by stimulating the immune system and delivering prodrug metabolizing genes, or oncolytic viruses.

Bovenberg et al, Trends Mol Med, 2013

Stem cell-based delivery of different therapeutics to gliomas

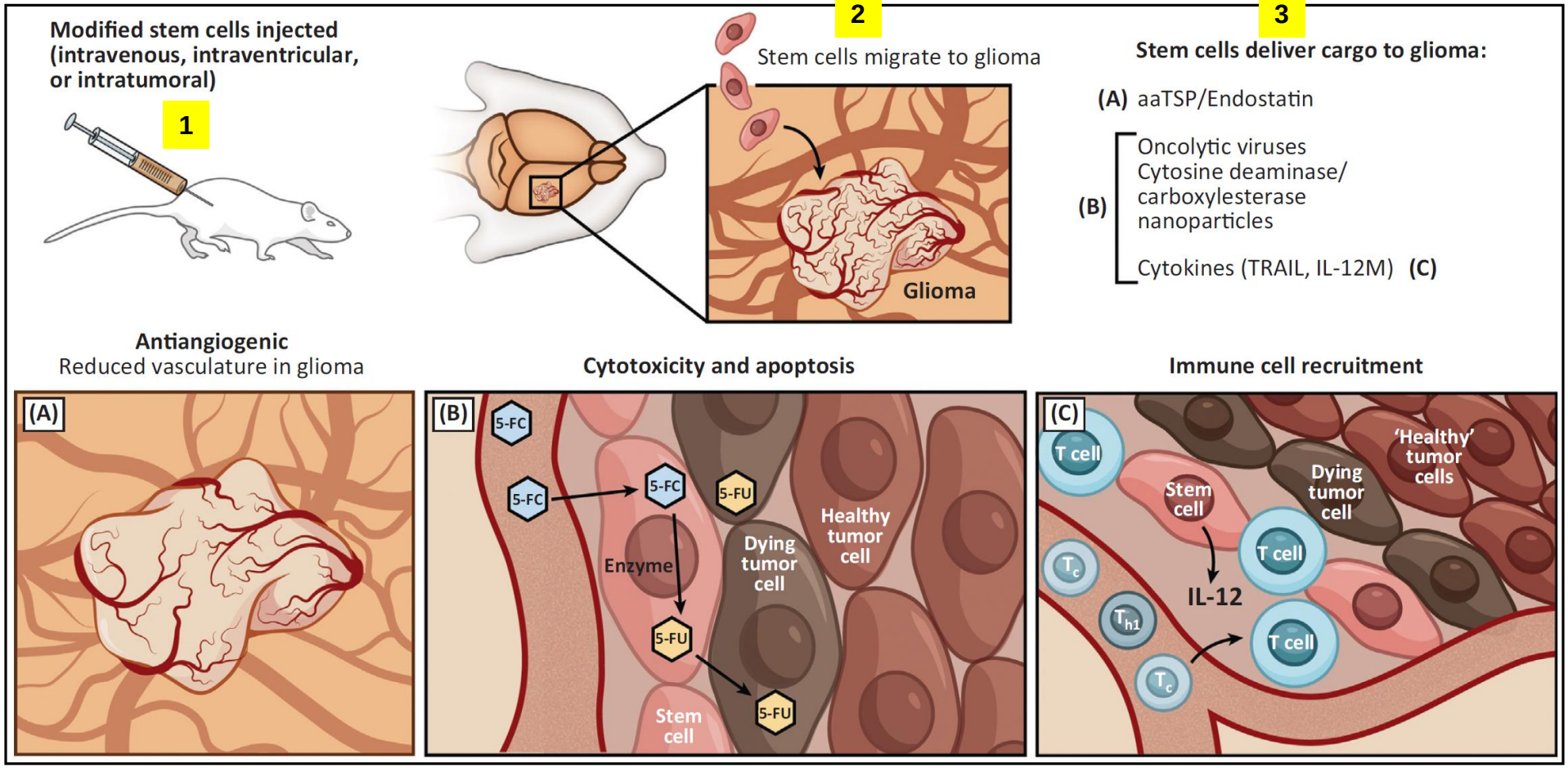
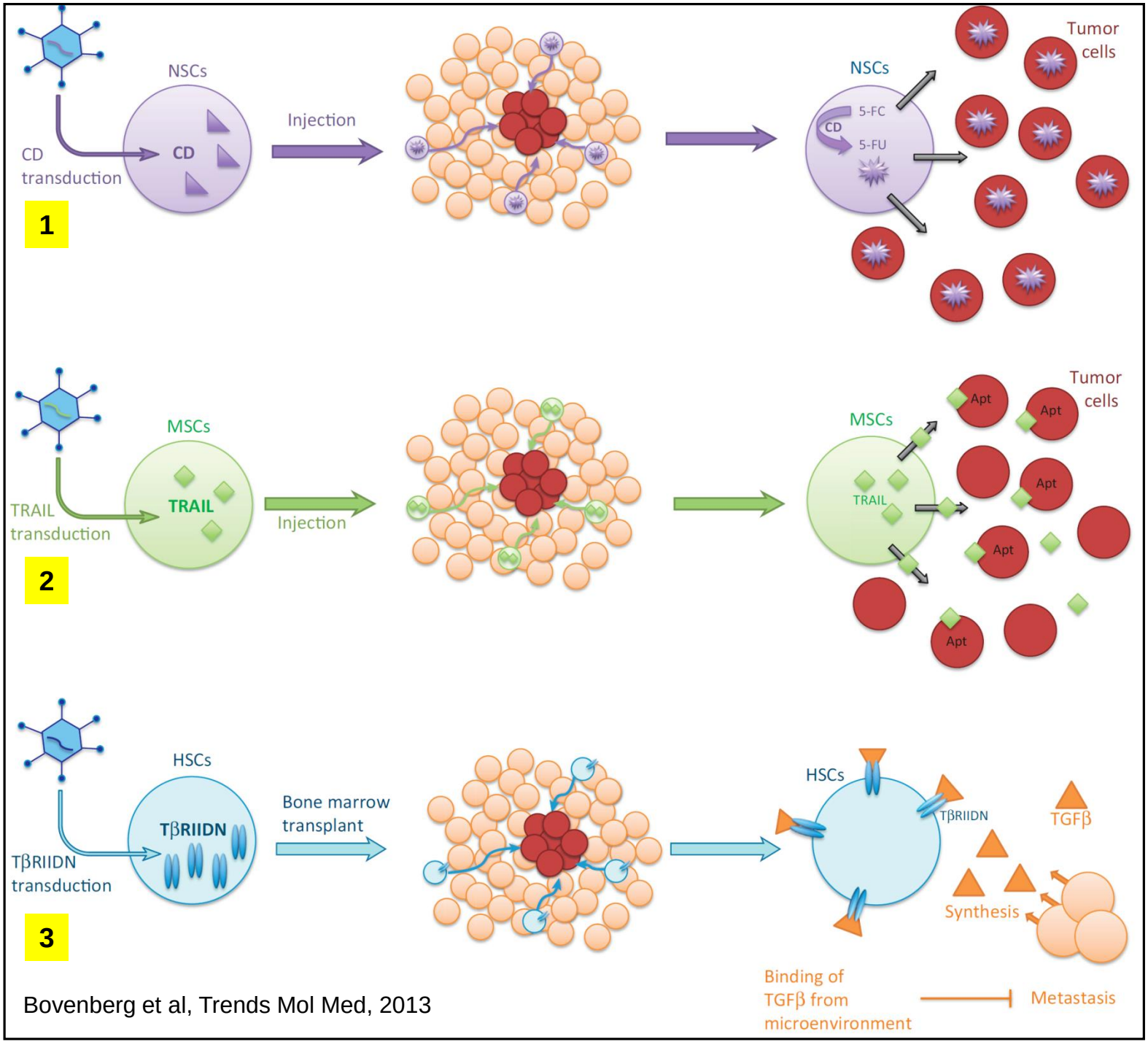


Figure 1. Overview of stem cell-based delivery of different therapeutics to gliomas. Several forms of therapy can be delivered by modified stem cells, including antiangiogenic factors such as aaTSP or endostatin (A), oncolytic viruses or enzymes capable of processing prodrugs such as 5-FC to cytotoxic compounds (B), and immune regulatory factors such as interleukin (IL)-12 that can recruit antitumor immune cells (C). *Abbreviations:* aaTSP, antiangiogenic protein thrombospondin; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Th1, T helper 1 cell; Tc, cytotoxic T cell.

Bovenberg et al, Trends Mol Med, 2013



Bovenberg et al, Trends Mol Med, 2013

Figure 2. Examples of stem cell-based therapies against gliomas. Many variations on stem cell therapy are possible, and three are depicted here using mesenchymal, neuronal, and hematopoietic stem cells (MSCs, NSCs, and HSCs, respectively). *Abbreviations:* TGFβ, transforming growth factor β; Apt, apoptosis; CD, cytosine deaminase; TRAIL, tumor necrosis factor apoptosis-inducing ligand; TβRIIDN, dominant negative mutant of transforming growth factor β receptor II.

Bovenberg et al, Trends Mol Med, 2013

Stem cell therapies against malignant gliomas (1)

Stem cell function A	Approach B	Transgene/modification strategy C	Application D
Cargo delivery	Cytokine 1	Expression of sTRAIL-luciferase fusion; NSC	Visualization of TRAIL-mediated therapy
		sTRAIL plus bortezomib; NSC	Glioma sensitization to TRAIL
		sTRAIL plus mTOR inhibitor; NSC	Glioma sensitization to TRAIL
		sTRAIL; MSC	Proof-of-principle MSC-mediated TRAIL therapy
		IL-12 expression; MSC	Immunotherapy
	Enzyme/prodrug activation 2	aaTSP-1 expression; NSC	Antiangiogenesis therapy
		rCE expression; NSC	SN-38-mediated therapy
		rCE expression; MSC	SN-38-mediated therapy
		Endostatin and/or carboxylesterase 2; MSC	Antiangiogenesis therapy
		CD expression; NSC	5-FC therapy
		CD expression; MSC	5-FC therapy
		HSV-TK and VPA; MSC	Enhanced efficacy of HSV-TK-mediated therapy
	Oncolytic virus 3	CRAD-S-pk7 expression; NSC	Proof-of-principle
			Enhanced carrier system
	Nanoparticles 4	NPs loaded with coumarin-6; MSC	Proof-of-principle NP-mediated delivery system
		NPs loaded with Fc-diOH; MSC	NP delivery
	5	NPs carrying silicia nanorattle dox; MSC	NP delivery
	NSC delivery to glioma	Coating with sECM; NSC	Improved NSC delivery

aaTSP, Antiangiogenic protein thrombospondin; CRAD-S-pk7, Conditionally replicative adenovirus; Fc-diOH, Ferrociphenol (anti-cancer drug); HSV-TK, Herpes simplex virus thymidine kinase; MSC, Mesenchymal stem cell; NPs, Nanoparticles; NSC, Neural stem cell; rCE, Rabbit carboxylesterase; sECM, Synthetic extracellular matrix; SN-38, Antineoplastic drug (topoisomerase I inhibitor); sTRAIL, Soluble tumor necrosis factor-related apoptosis-inducing ligand; VPA, Valproic acid; 5-FC, 5-Fluorocytosine

Stem cell therapies against malignant gliomas (2)

Stem cell function A	Approach B	Transgene/modification strategy C	Application D
Enhancement of the stem cell model	Routes of administration 1	Intraventricular injections	Improved delivery mode
		Intratumoral vs extratumoral injections; MSC	Proof-of-principle; improved delivery mode
	Factors regulating tropism 2	CXCR4 overexpression; MSC	Enhanced glioma tropism
		IL-8 and/or CXCR1 overexpression; MSC	Enhanced glioma tropism
		Overexpression of various cytokines; MSC	Enhanced glioma tropism
	Improved cellular vehicles 3	iPSCs generated from embryonic fibroblasts; ESC	Proof-of-principle
		NSC differentiation; ESC	Proof-of-principle
		EPC; hNIS and FePro expression; HSC	Proof-of-principle; imaging
		TβRIIDN expression; HSC	Proof-of-principle; immunotherapy

Abbreviations: MSC, mesenchymal stem cell; NSC, neural stem cell; ESC, embryonic stem cell; HSC, hematopoietic stem cell; NP, nanoparticle; sTRAIL, secretable tumor necrosis factor apoptosis-inducing ligand; IL-12, interleukin-12; Fc-diOH, ferrociphenol; dox, doxorubicin; HSV-TK, herpes simplex virus thymidine kinase; VPA, valproic acid; CD, cytosine deaminase; rCE, rabbit carboxylesterase; IL-8, interleukin-8; aaTSP-1, antiangiogenic protein thrombospondin; sECM, synthetic extracellular matrix; iPSCs, induced pluripotent stem cells; FePro, ferumoxides–protamine sulfate; TβRIIDN, transforming growth factor β receptor II; 5-FC, 5-fluorocytosine.

hNIS, human sodium iodide symporter

Bovenberg et al, Trends Mol Med, 2013

3. ORGAN AND TISSUE TRANSPLANTATION

Types of graft

■ ***Autograft*** — tissue grafted back on to the original donor.

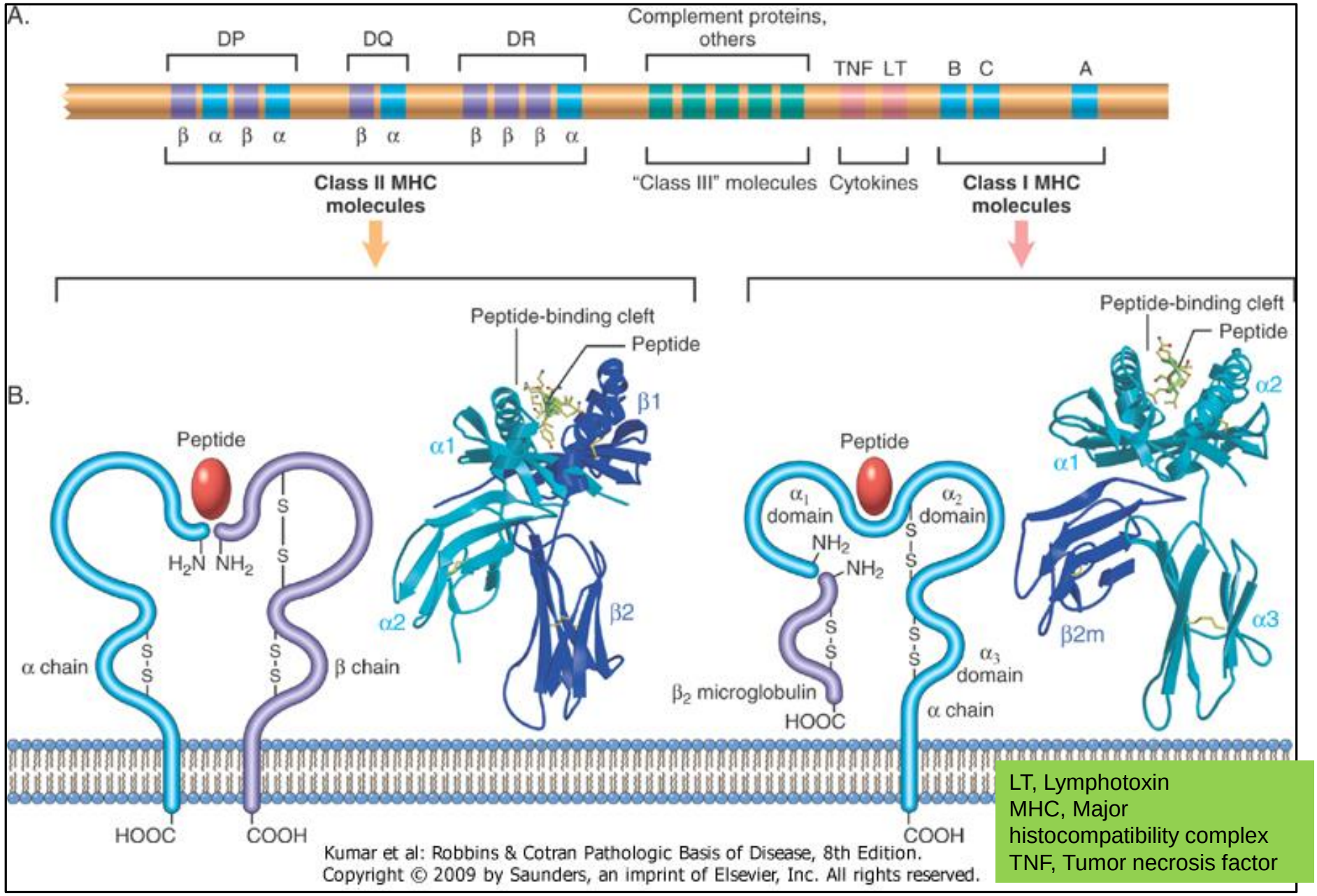
■ ***Isograft*** — graft between **syngeneic** individuals (i.e. of identical genetic constitution) such as identical twins or mice of the same pure inbred strain.

■ ***Allograft*** — graft between **allogeneic** individuals (i.e. members of the same species but different genetic constitution), e.g. human to human and one mouse strain to another.

■ ***Xenograft*** — graft between **xenogeneic** individuals (i.e. of different species), e.g. pig to human.

Roitt's Essential Immunology, 12th ed, 2011

The human leukocyte antigen (HLA) complex and the structure of HLA molecules



LT, Lymphotoxin
MHC, Major histocompatibility complex
TNF, Tumor necrosis factor

Skin graft rejection is the result of a T cell-mediated anti-graft response

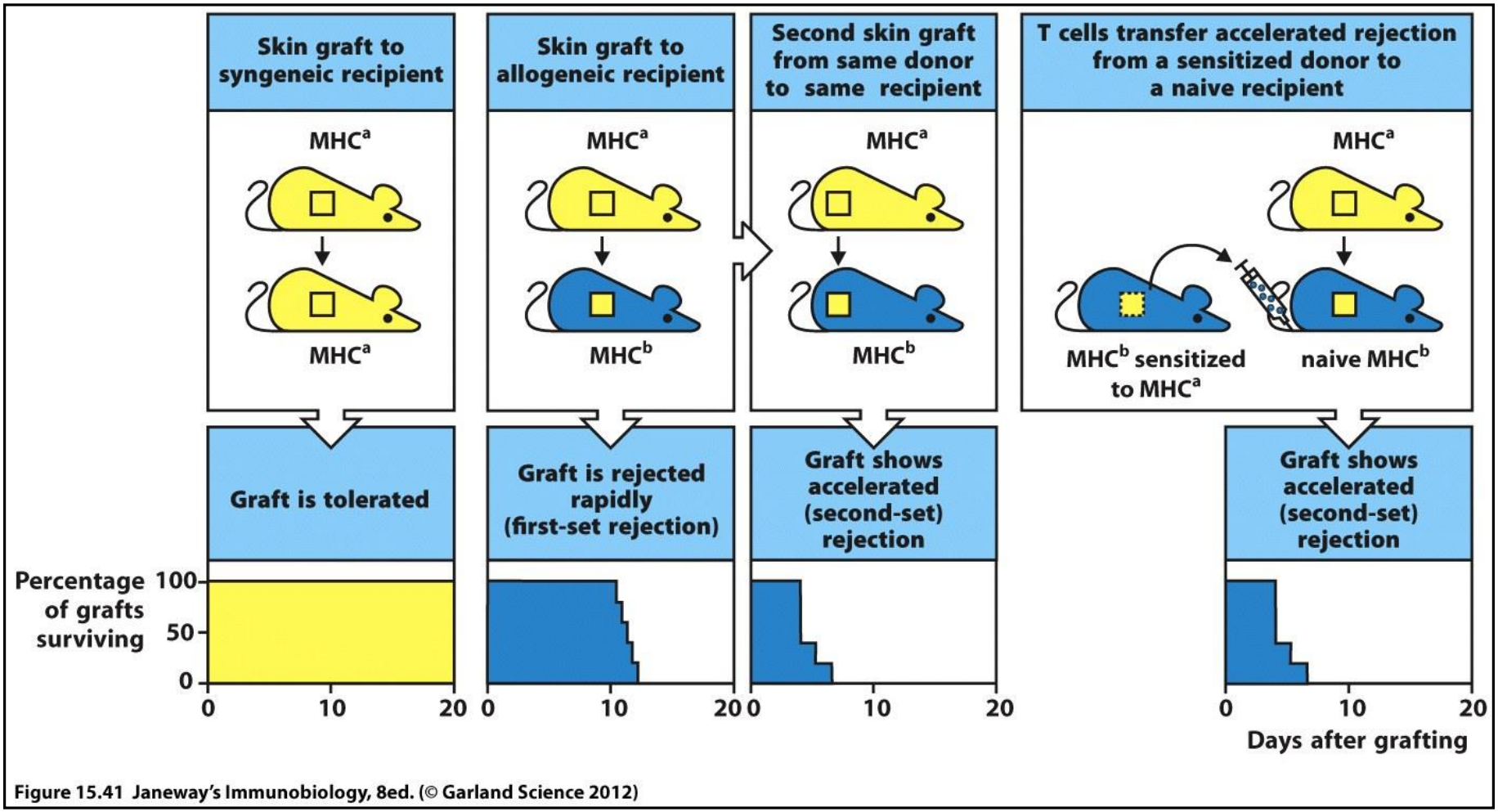


Fig. 15.41 Grafts that are syngeneic are permanently accepted (**first panels**), but grafts differing at the MHC are rejected about 10-13 days after grafting (first-set rejection, **second panels**). When a mouse is grafted for a second time with skin from the same donor, it rejects the second graft faster (**third panels**). This is called a second-set rejection, and the accelerated response is MHC-specific; skin from a second donor of the same MHC type is rejected equally fast, whereas skin from an MHC-different donor is rejected in a first-set pattern (not shown). Naive mice that are given T cells from a sensitized donor behave as if they had already been grafted (**final panels**).

Even complete matching at the MHC does not ensure graft survival

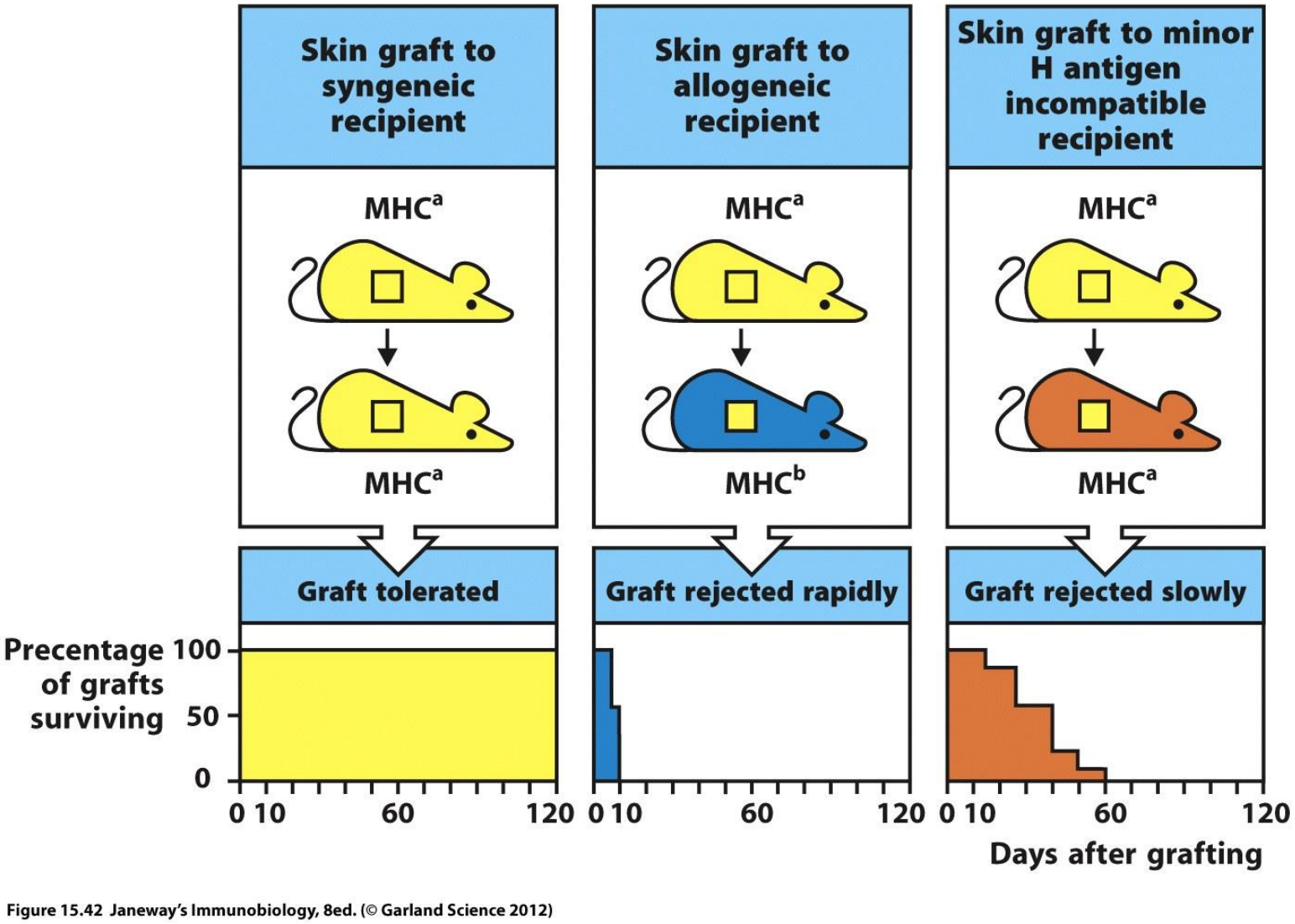


Figure 15.42 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Fig. 15.42 Although syngeneic grafts are not rejected (left panels), MHC-identical grafts from donors that differ at other loci (minor H antigen loci) are rejected (right panels), albeit more slowly than MHC-disparate grafts (center panels).

Alloantigens in grafted organs are recognized in two different ways

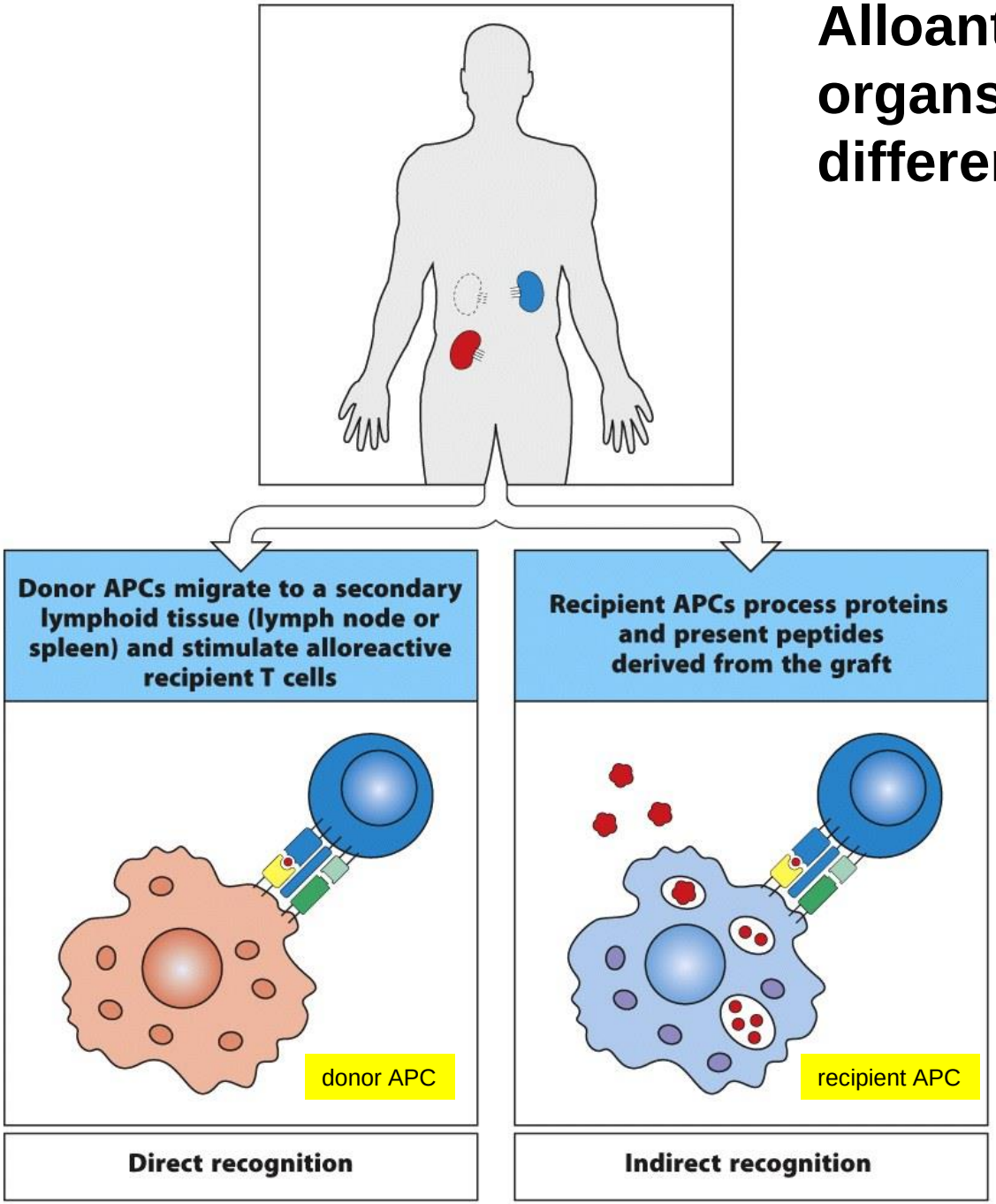
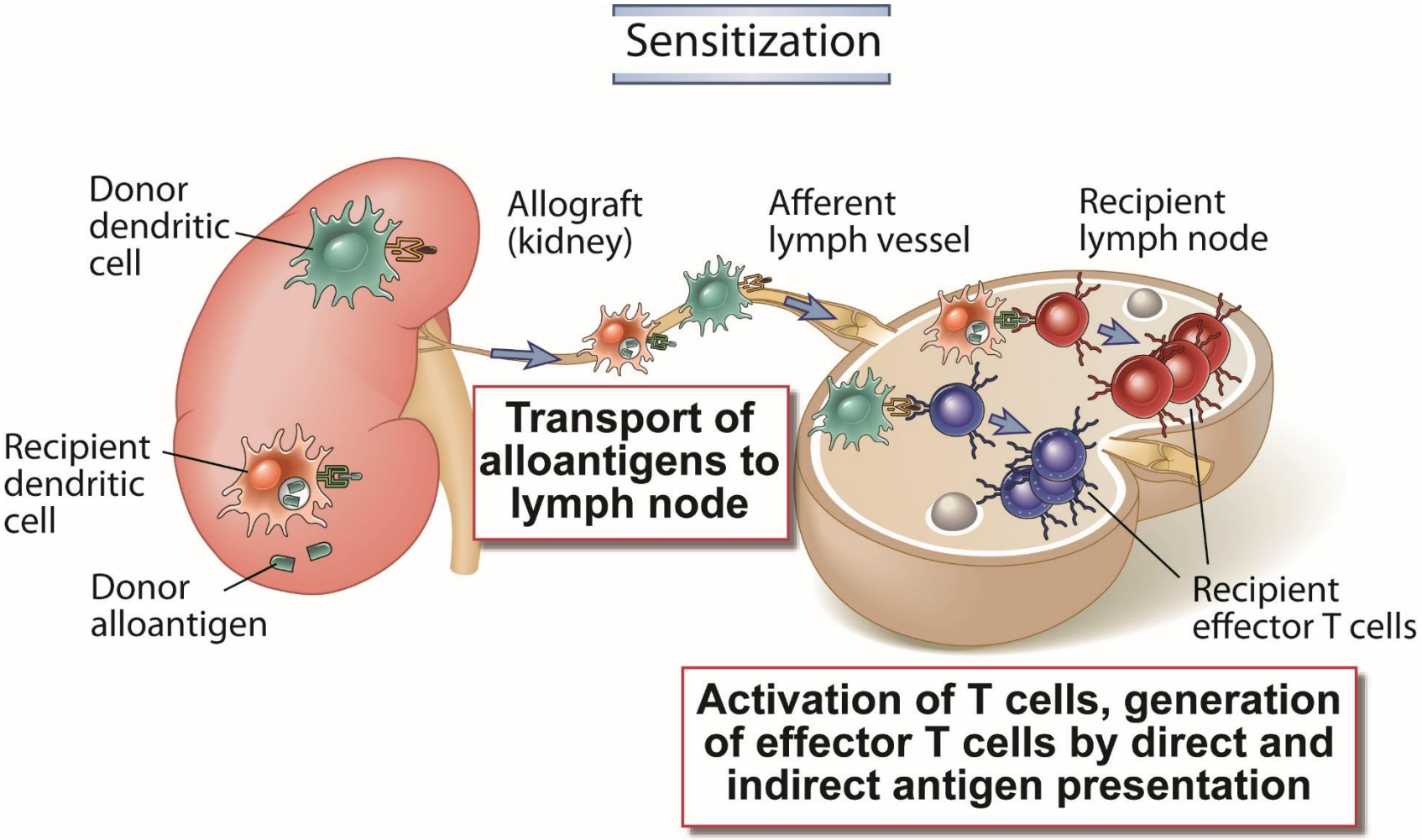


Figure 15.45 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Fig. 15.45 Direct recognition of a grafted organ (red in upper panel) is by T cells whose receptors have specificity for the allogeneic MHC class I or class II molecule in combination with peptide. These alloreactive T cells are stimulated by donor antigen-presenting cells (APCs), which express both the allogeneic MHC molecule and co-stimulatory activity (lower left panel). Indirect recognition of the graft (lower right panel) involves T cells whose receptors are specific for allogeneic peptides derived from the grafted organ. Proteins from the graft (red) are taken up and processed by the recipient's antigen-presenting cells and are therefore presented by self (recipient) MHC class I or class II molecules.

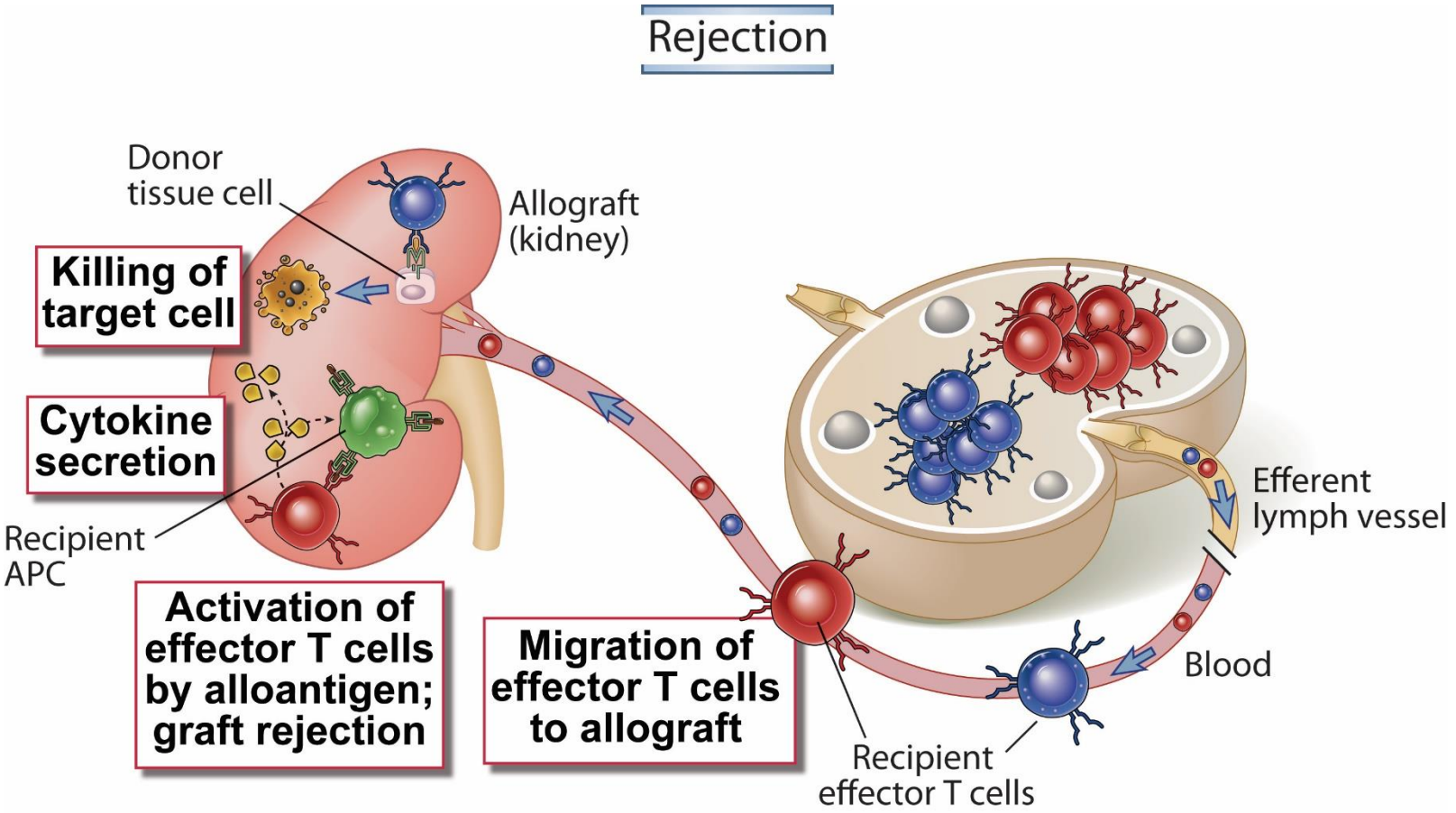
Activation of Alloreactive T cells (1)



A, In the case of direct allorecognition, donor dendritic cells in the allograft migrate to secondary lymphoid tissues, where they present allogeneic MHC molecules to host T cells.

Fig. 16-6

Activation of alloreactive T cells (2)



B, In the case of indirect allorecognition, recipient dendritic cells that have entered the allograft transport donor MHC proteins to secondary lymphoid tissues and present peptides derived from these MHC proteins to alloreactive host T cells. In both cases, the T cells become activated and differentiate into effector cells. The alloreactive effector T cells migrate into the allograft, become reactivated by alloantigen, and mediate damage. Lymphatic drainage of grafted organs is not well described, and therefore the location of the relevant lymph nodes is uncertain.

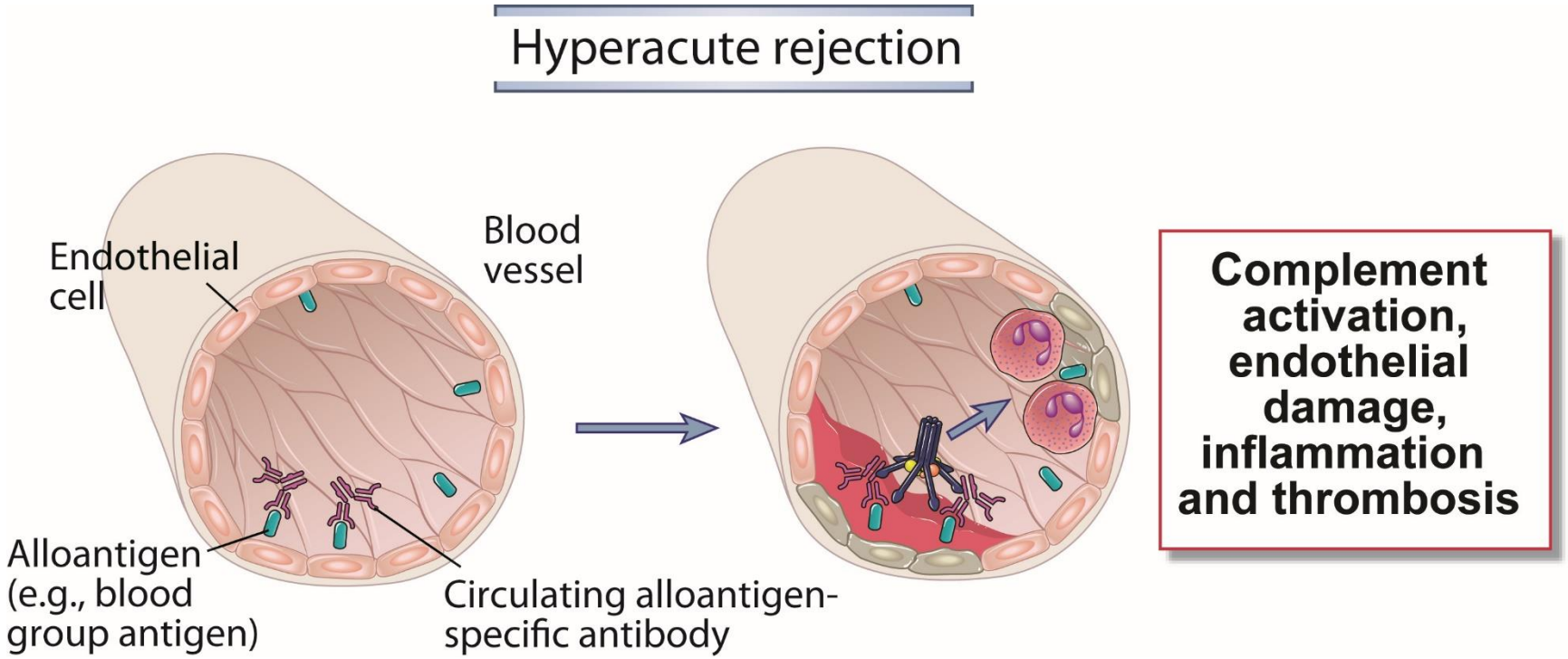
Fig. 16-6

Tissues commonly transplanted in clinical medicine

Tissue transplanted	No. of grafts in USA (2009)*	5-year graft survival
Kidney	17,683	81.4%#
Liver	6,320	68.3%
Heart	2,241	74.0%
Pancreas and pancreas/ kidney	1,233	53.4%†
Lung	1,690	50.6%
Intestine	180	~ 48.4%
Cornea	~40,000	~ 70%
HSC transplants	15,000‡	40%/60%‡

Figure 15.47 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

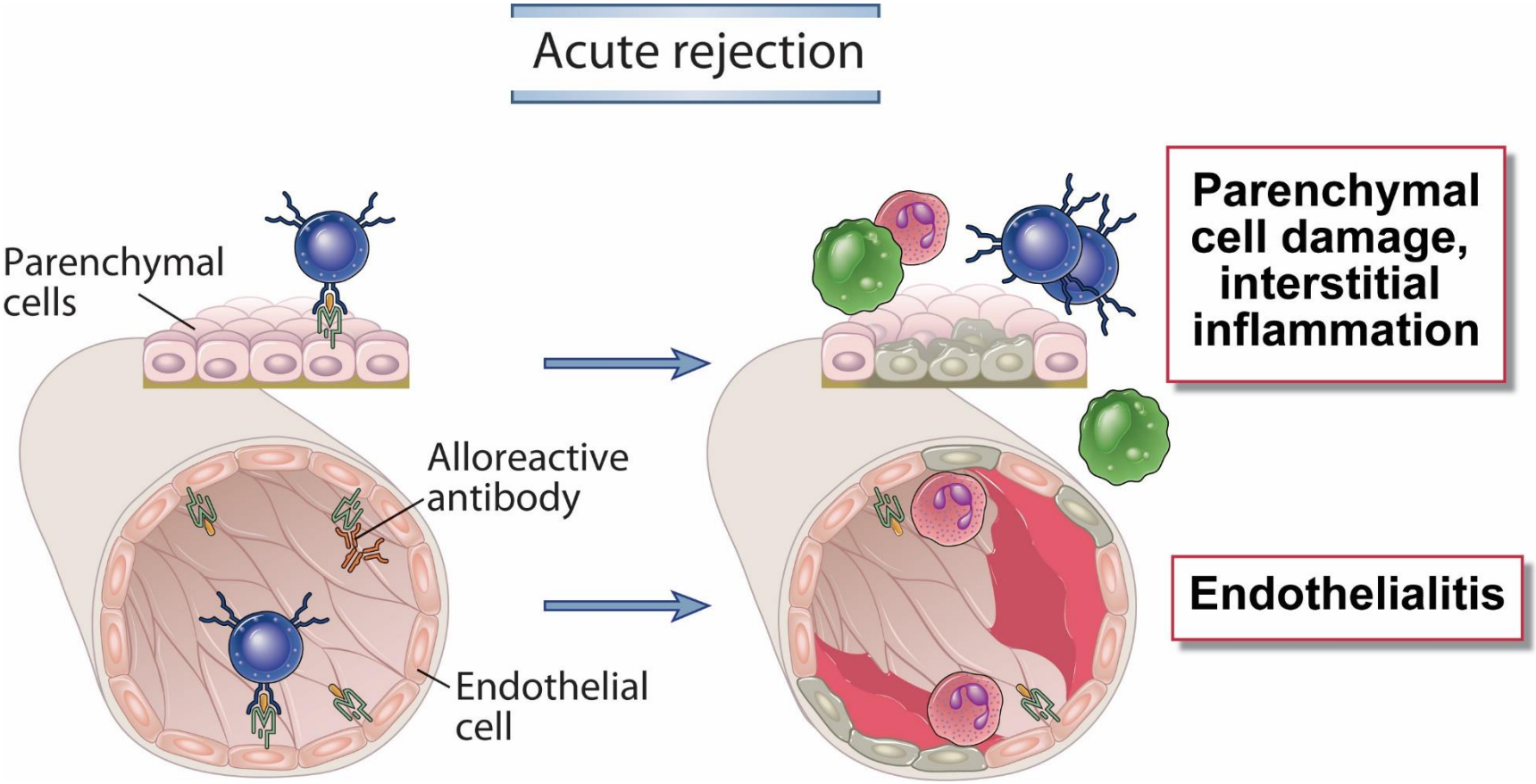
Immune mechanisms of graft rejection (1)



A, In hyperacute rejection, preformed antibodies reactive with vascular endothelium activate complement and trigger rapid intravascular thrombosis and necrosis of the vessel wall.

Fig. 16-8A

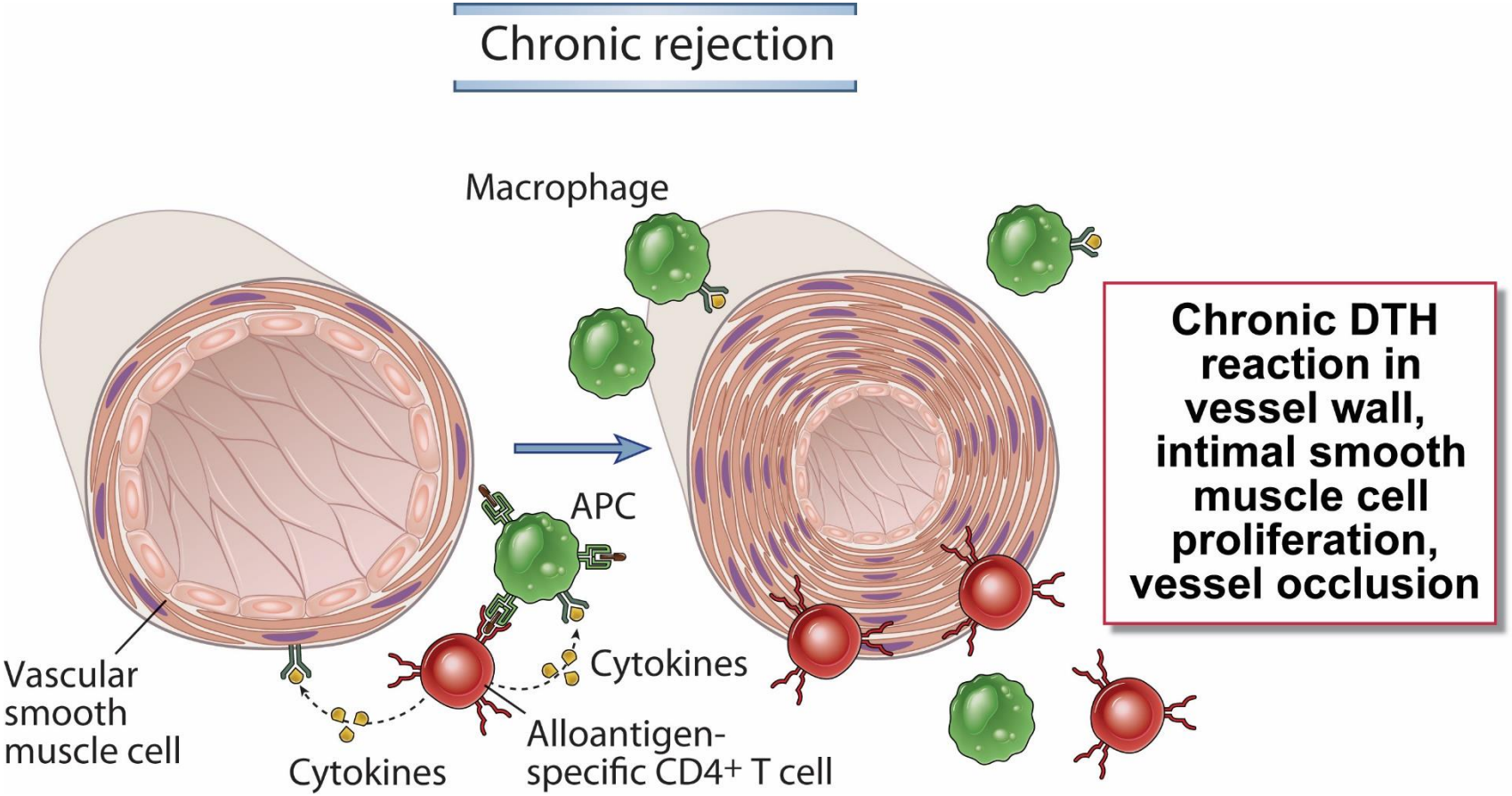
Immune mechanisms of graft rejection (2)



B, In acute rejection, CD8+ T lymphocytes reactive with alloantigens on endothelial cells and parenchymal cells mediate damage to these cell types. Alloreactive antibodies formed after engraftment may also contribute to vascular injury.

Fig. 16-8B

Immune mechanisms of graft rejection (3)



C, In chronic rejection with graft arteriosclerosis, injury to the vessel wall leads to intimal smooth muscle cell proliferation and luminal occlusion. This lesion may be caused by a chronic DTH reaction to alloantigens in the vessel wall.

Fig. 16-8C

Mechanisms of immunosuppressive drugs

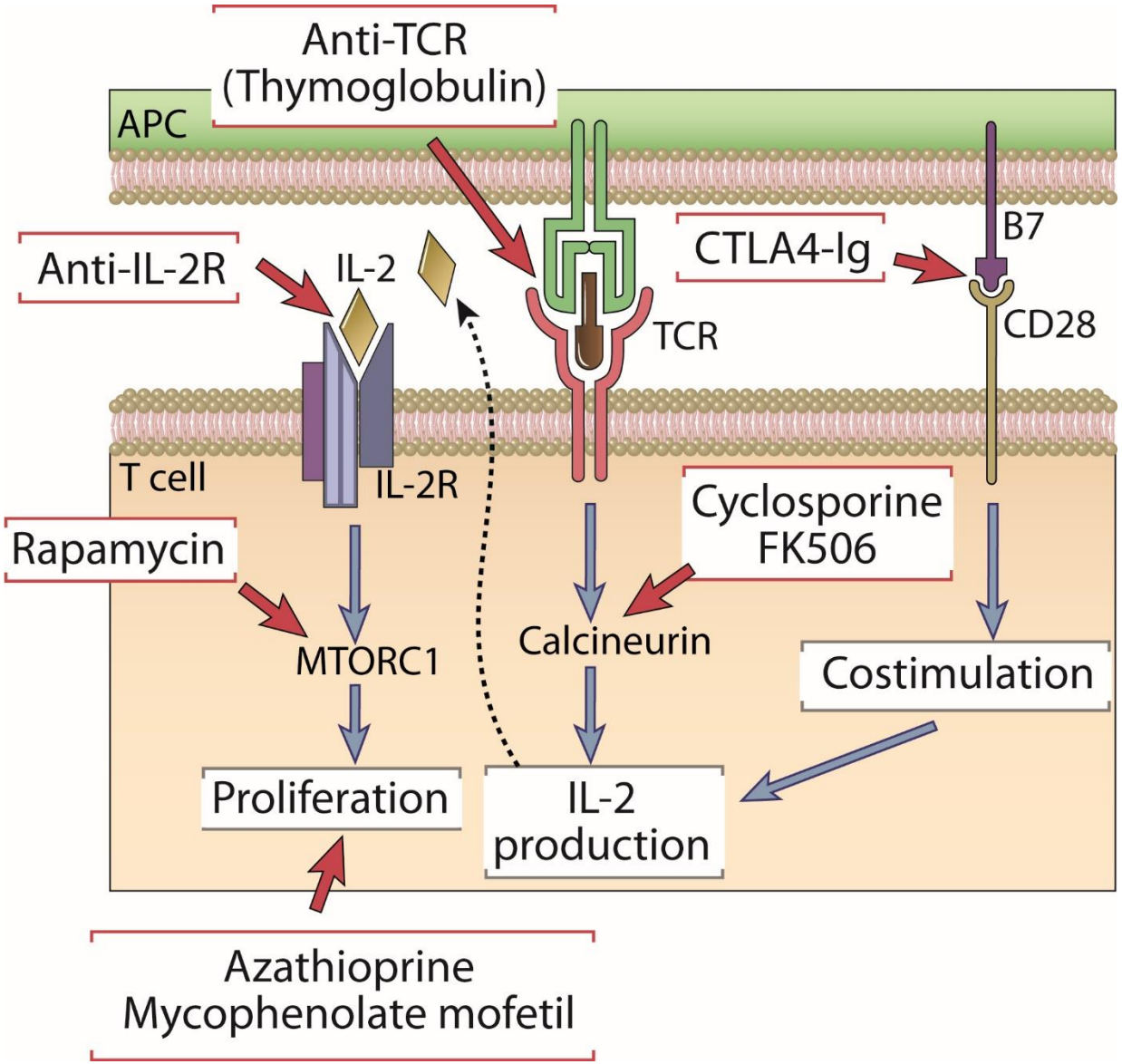
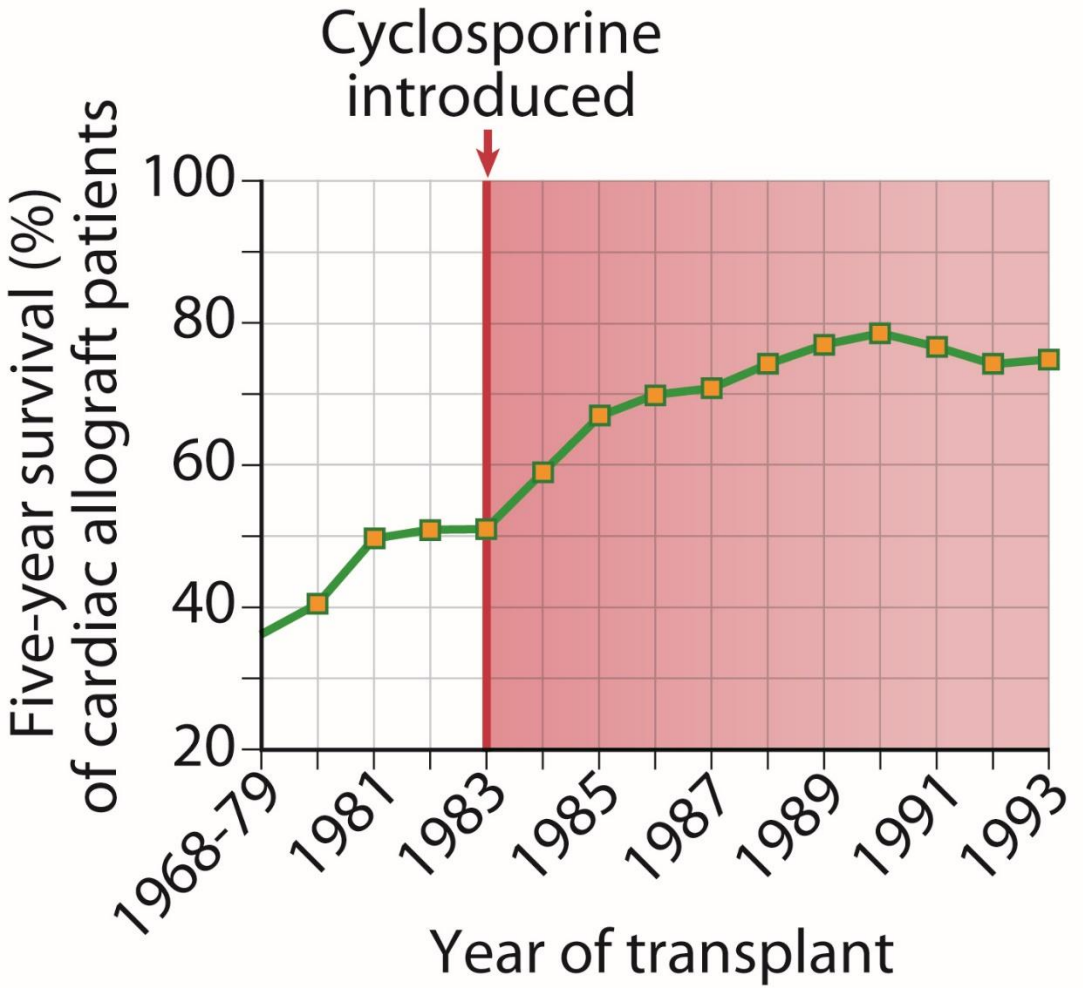


Fig. 16-10

Influence of cyclosporine on graft survival



Five-year survival rates for patients receiving cardiac allografts increased significantly beginning when cyclosporine was introduced in 1983.

Fig. 16-11

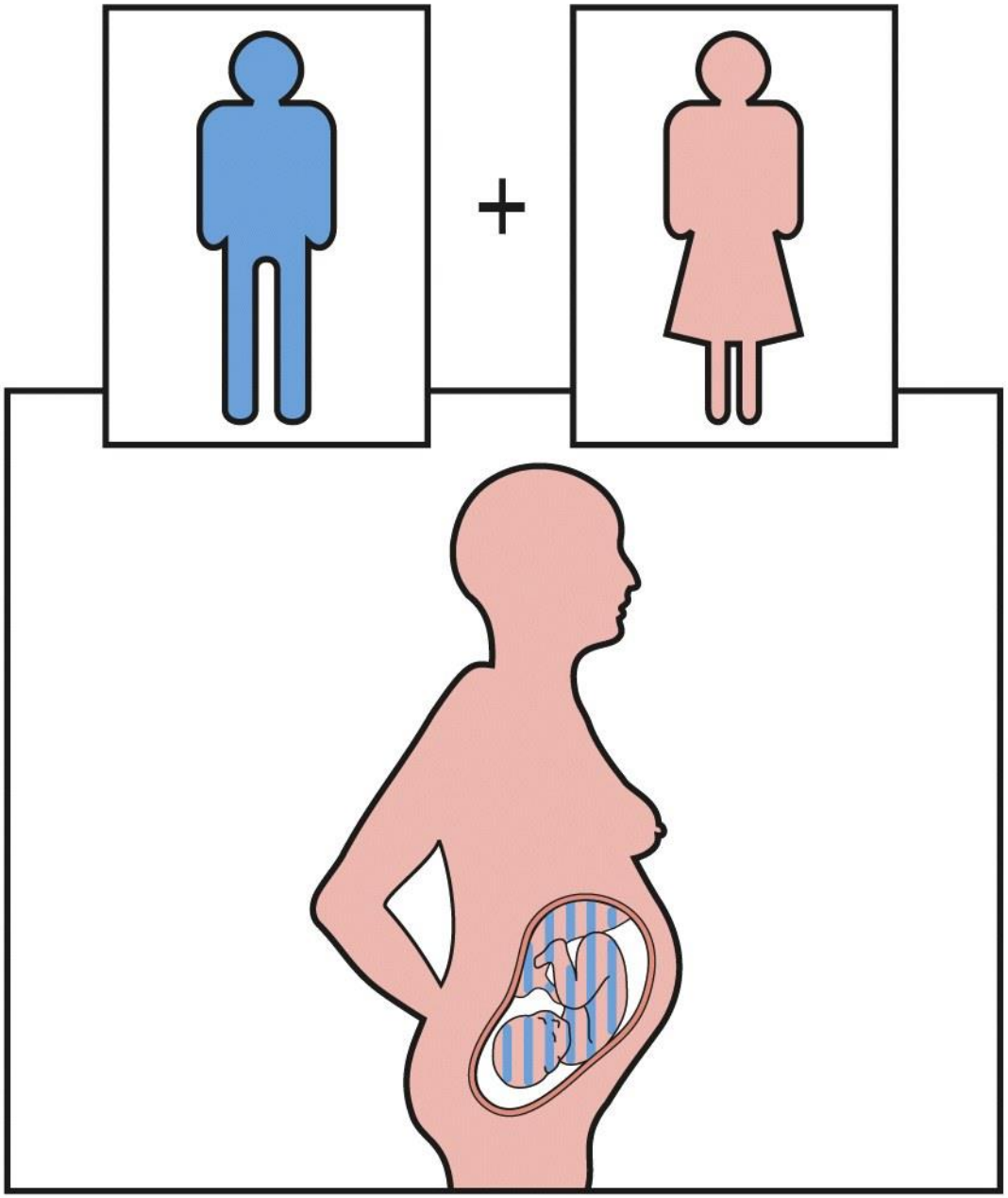


Figure 15.50 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

The fetus is an allograft that is not rejected

Fig. 15.50 Although the fetus carries MHC molecules derived from the father, and other foreign antigens, it is not rejected. Even when the mother bears several children to the same father, no sign of immunological rejection is seen.

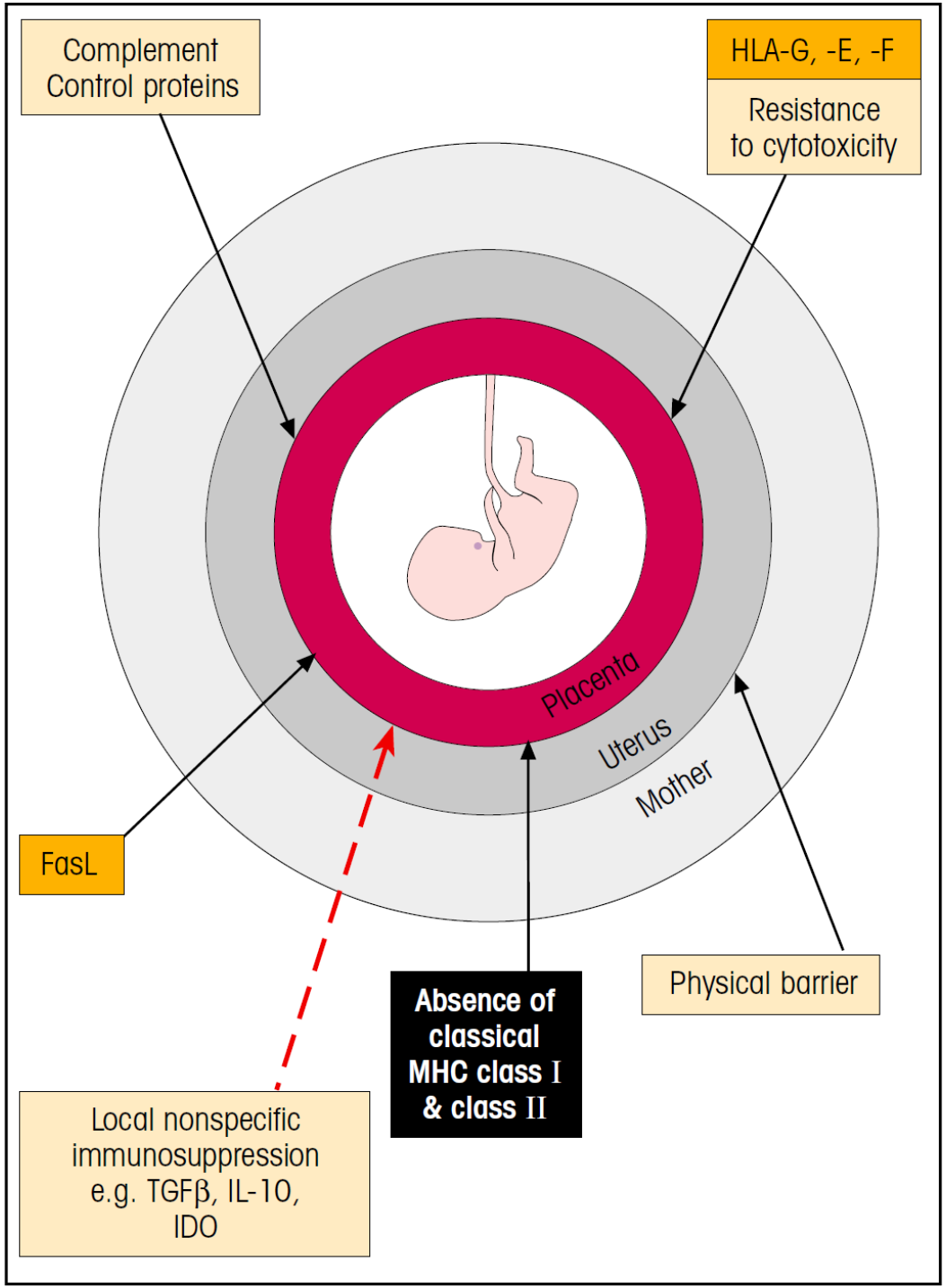


Figure 16.20. Mechanisms postulated to account for the survival of the fetus as an allograft in the mother.

FasL, Fas ligand
HLA, Human leucocyte antigen
IDO, Indoleamine 2,3 – dioxygenase
IL-10, Interleukin-10
MHC, Major histocompatibility complex
TGFβ, Transforming growth factor β

Roitt's Essential Immunology