Gene Therapy
基因療法

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Mar. 23, 2011

參考資料

科學人
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2.基因療法再顯聲威2008

3. Gene Therapy in Wikipedia
http://en.wikipedia.org/wiki/Gene_therapy

基因是什麼?

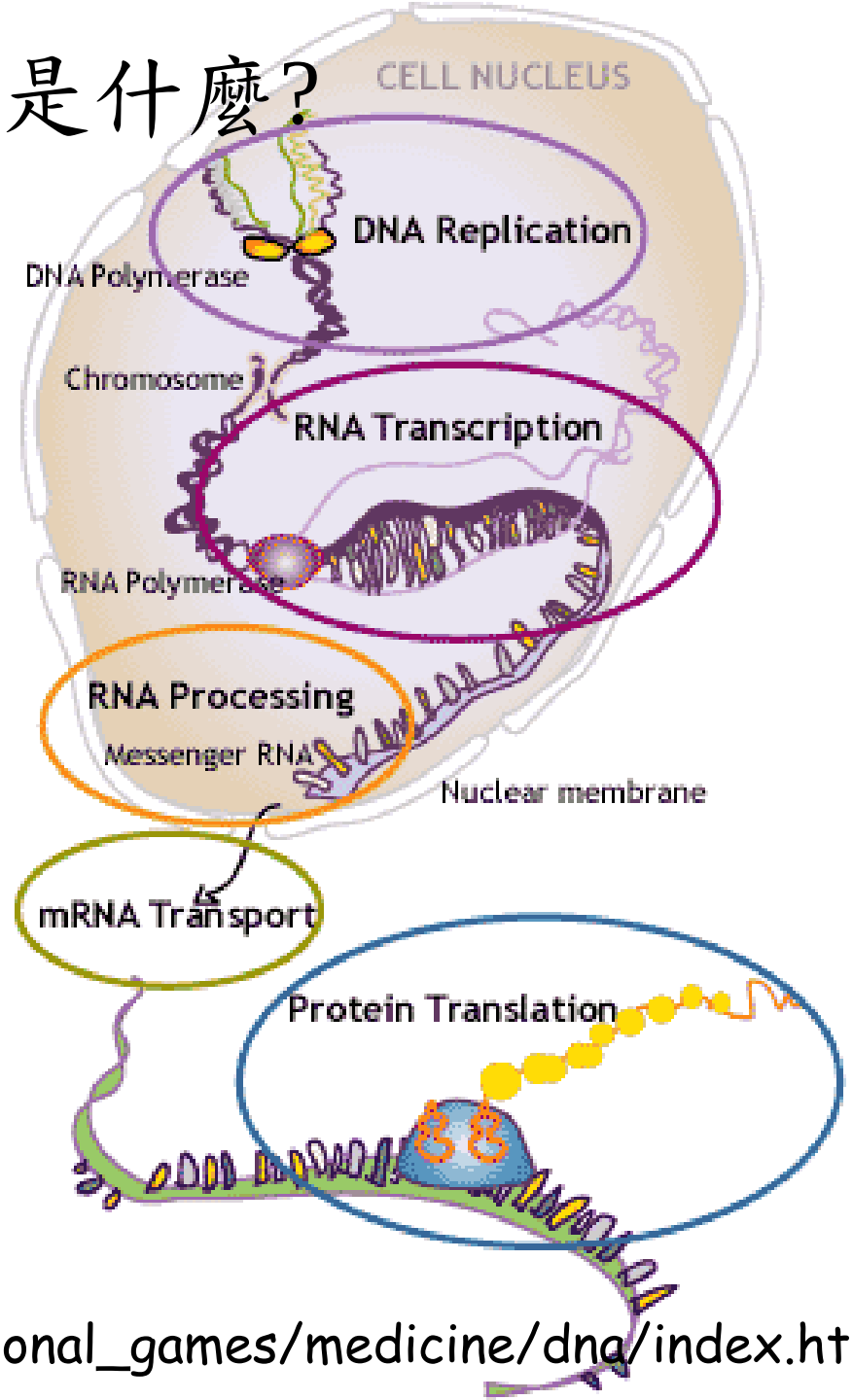
DNA

↓

RNA

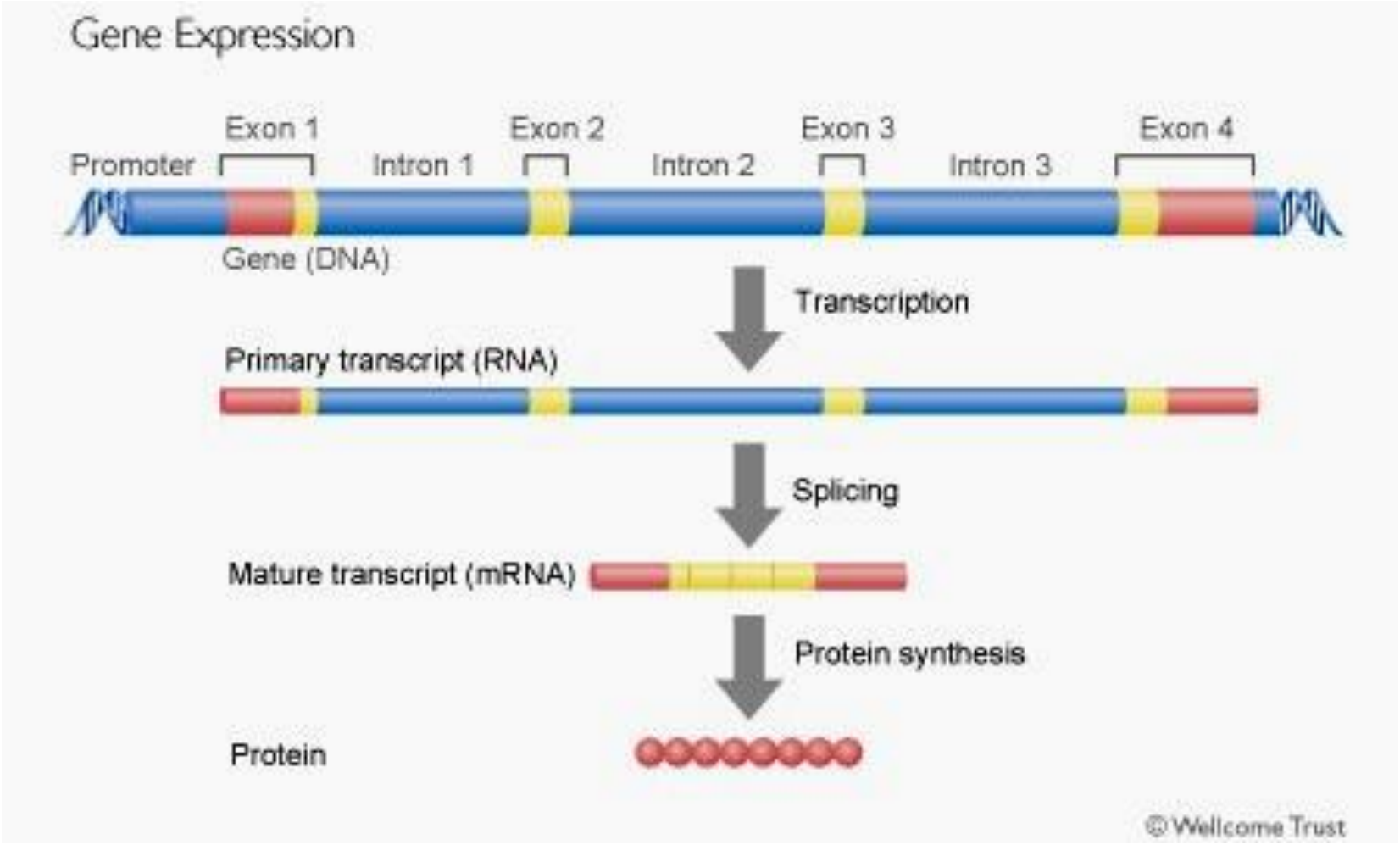
↓

蛋白質



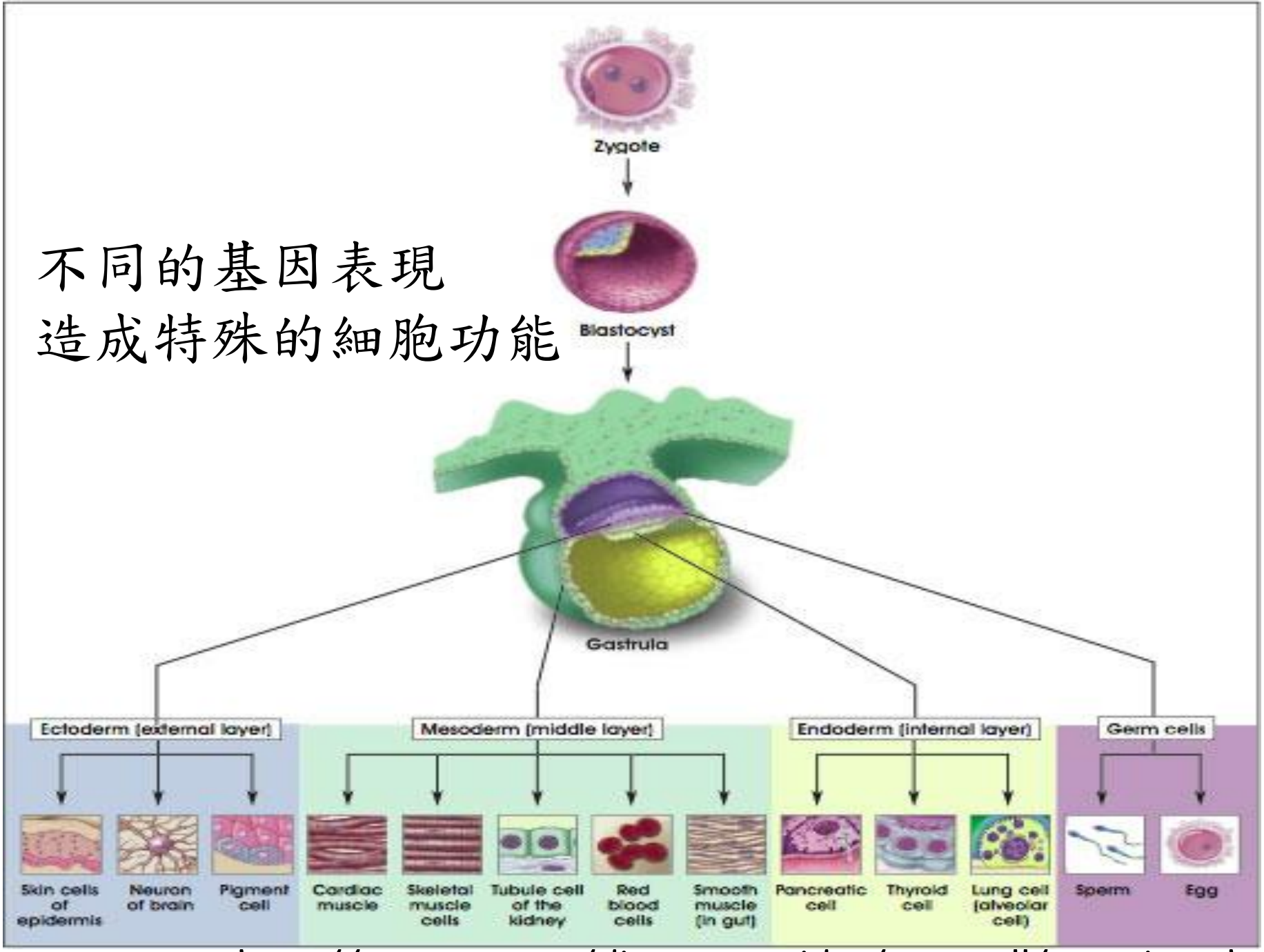
http://nobelprize.org/educational_games/medicine/dna/index.html

基因是什麼?



genome.wellcome.ac.uk/doc_WTD020757.html

不同的基因表現
造成特殊的細胞功能



http://www.csa.com/discoveryguides/stemcell/overview.php

Gene Therapy

基因 療法

基因
突變/缺失 → 細胞與組織
功能異常 → 疾病

確定
缺失基因 ← 致病機制 ← 疾病診斷

什麼是基因療法?

什麼疾病適合用基因療法?

1. 這疾病是因單一(或多重)基因異常所造成
2. 有嚴重症狀且沒有適當治療方法
3. 缺失基因(機制)是已知的
4. 正常的基因可以矯正缺失的功能
5. 有適當的載體用以傳遞正常的基因

疾病	缺陷基因	標的細胞
嚴重性免疫不全症 (SCID)	adenosine deaminase, γ_c cytokine receptor	骨髓細胞， T 淋巴球
高式症 (Gaucher disease)	glucocerebrosidase	巨噬細胞
血紅素病變 (sickle cell anemia or α -and β -thalassemias)	α 或 β -globin	紅血球細胞
血友病 (hemophilia A and B)	Factors Ⅷ or Ⅸ	肝細胞
囊性纖維化症 (cystic fibrosis)	CFTR	肺表皮細胞
杜氏持續性肌肉萎縮症 (Duchenne muscular dystrophy)	dystrophin	肌肉細胞
巴金森症 (Parkinson disease)	dopamine	神經細胞
肺氣腫 (emphysema)	alpha-1 antitrypsin	肺表皮細胞

基因療法的步驟

1. 確定缺失基因
2. 準備正常基因表現系統
3. 將基因表現系統與載體結合
4. 將在載體送入標的細胞
5. 基因正常表現
6. 修復缺失功能

基因療法的載體

病毒載體

反轉錄病毒

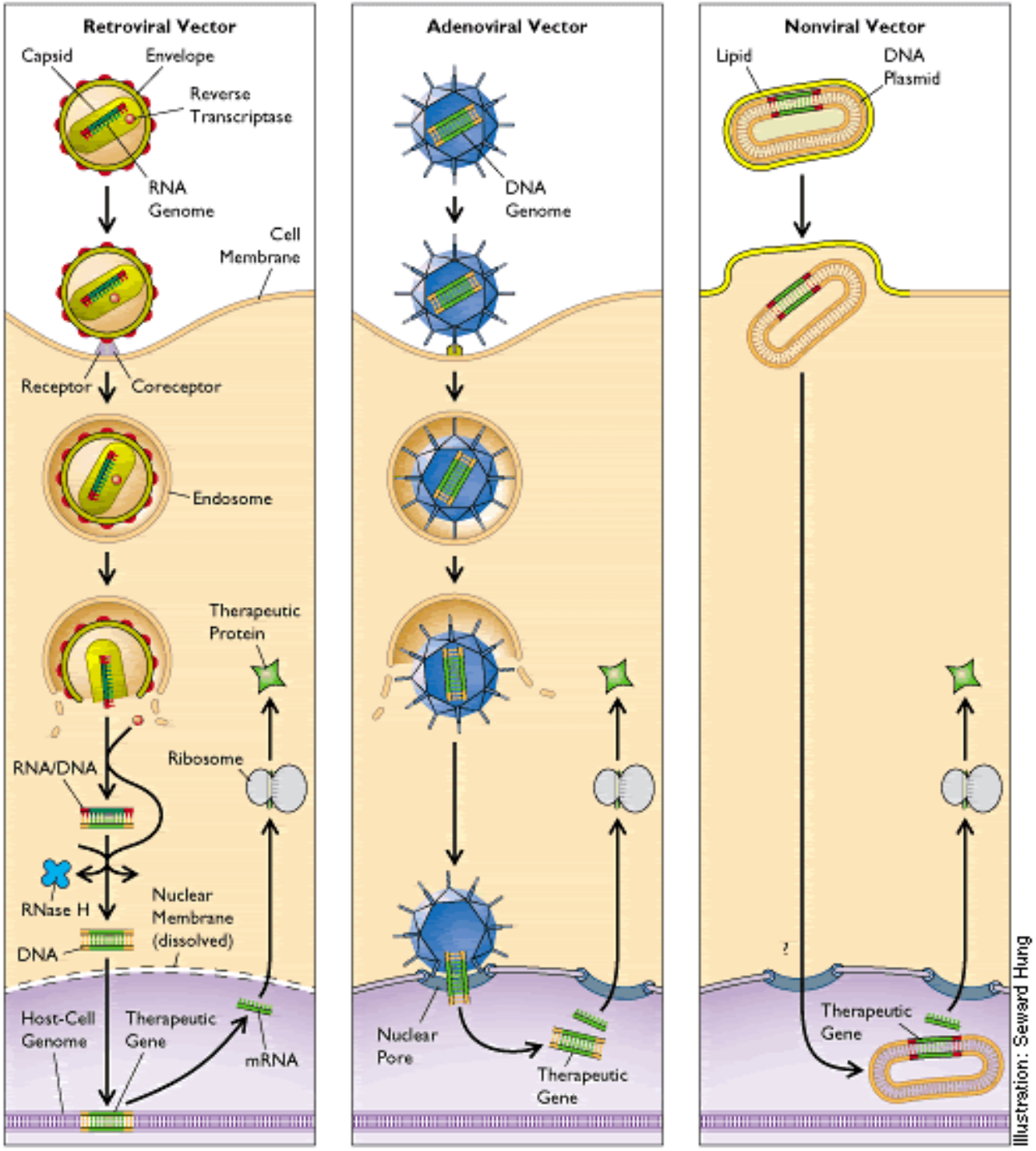
腺病毒

腺病毒附屬病毒

皰疹病毒

非病毒載體

脂質體


Retroviral Vector
Capsid
Envelope
Reverse Transcriptase
RNA Genome
Cell Membrane
Receptor
Coreceptor
Endosome
Therapeutic Protein
RNA/DNA
Ribosome
RNase H
Nuclear Membrane (dissolved)
DNA
Host-Cell Genome
Therapeutic Gene
mRNA
Adenoviral Vector
DNA Genome
Nuclear Pore
Therapeutic Gene
Nonviral Vector
Lipid
DNA Plasmid
Therapeutic Gene
Illustration: Seward Hung

載體優缺點

反轉錄病毒	腺病毒	腺病毒附屬病毒	脂質體
準備方便	準備方便	準備方便	準備方便
效果持久	效果短暫	效果持久	效果短暫
產量低	產量高	產量低	產量高
致癌危險	無致癌危險	無致癌危險	無致癌危險
基因表現不穩	表現量高	表現不穩	表現不穩
	免疫反應	承載能力低	可調控專一性

直接傳送的基因療法

(多用於疫苗發展)

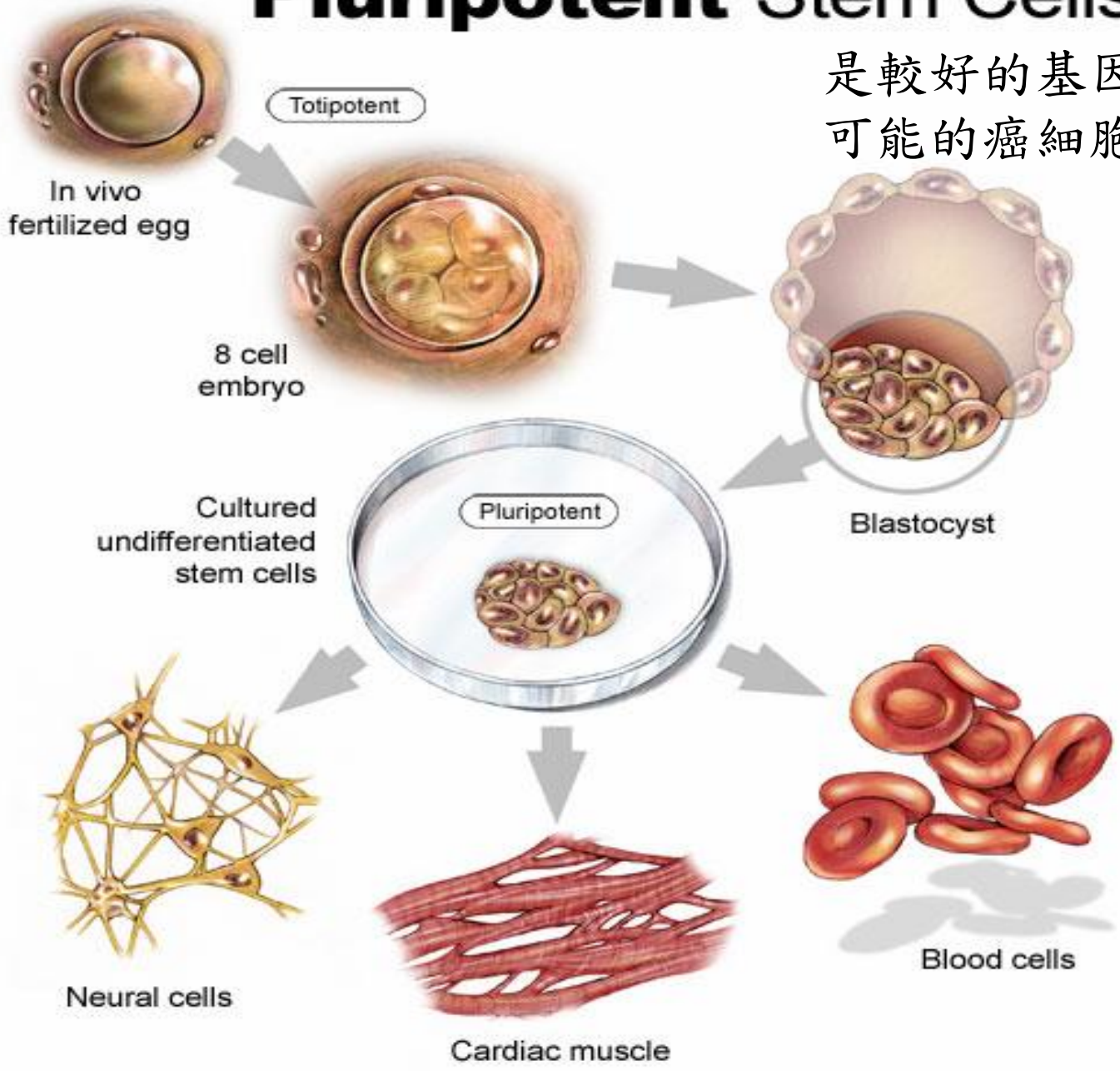
電孔洞法

基因槍法

直接注射

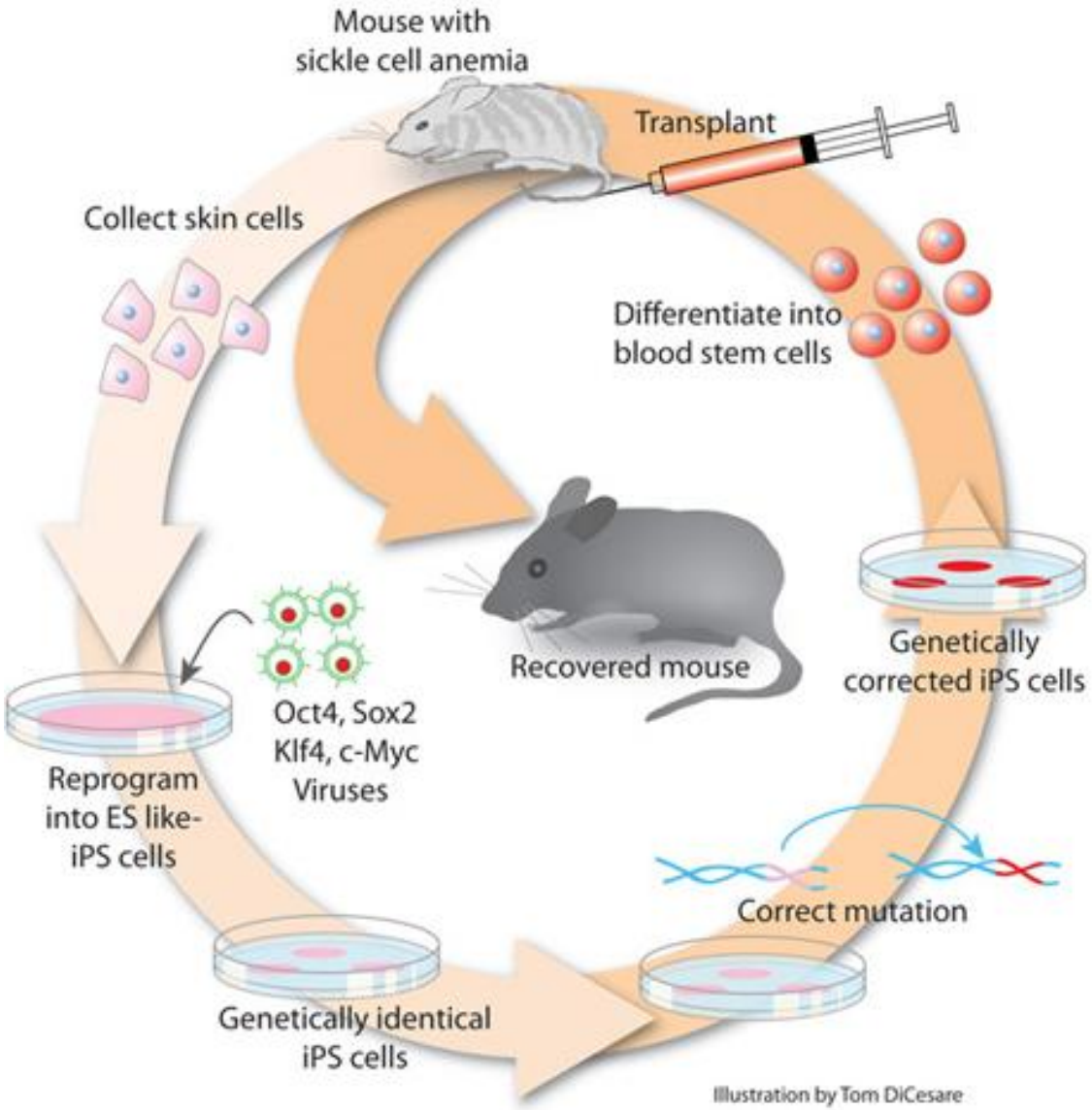
Pluripotent Stem Cells

是較好的基因治療工具或是可能的癌細胞?



http://www.csa.com/discoveryguides/stemcell/overview.php

Induced pluripotent stem cells (誘導多功能幹細胞)
In gene therapy



基因療法適用疾病

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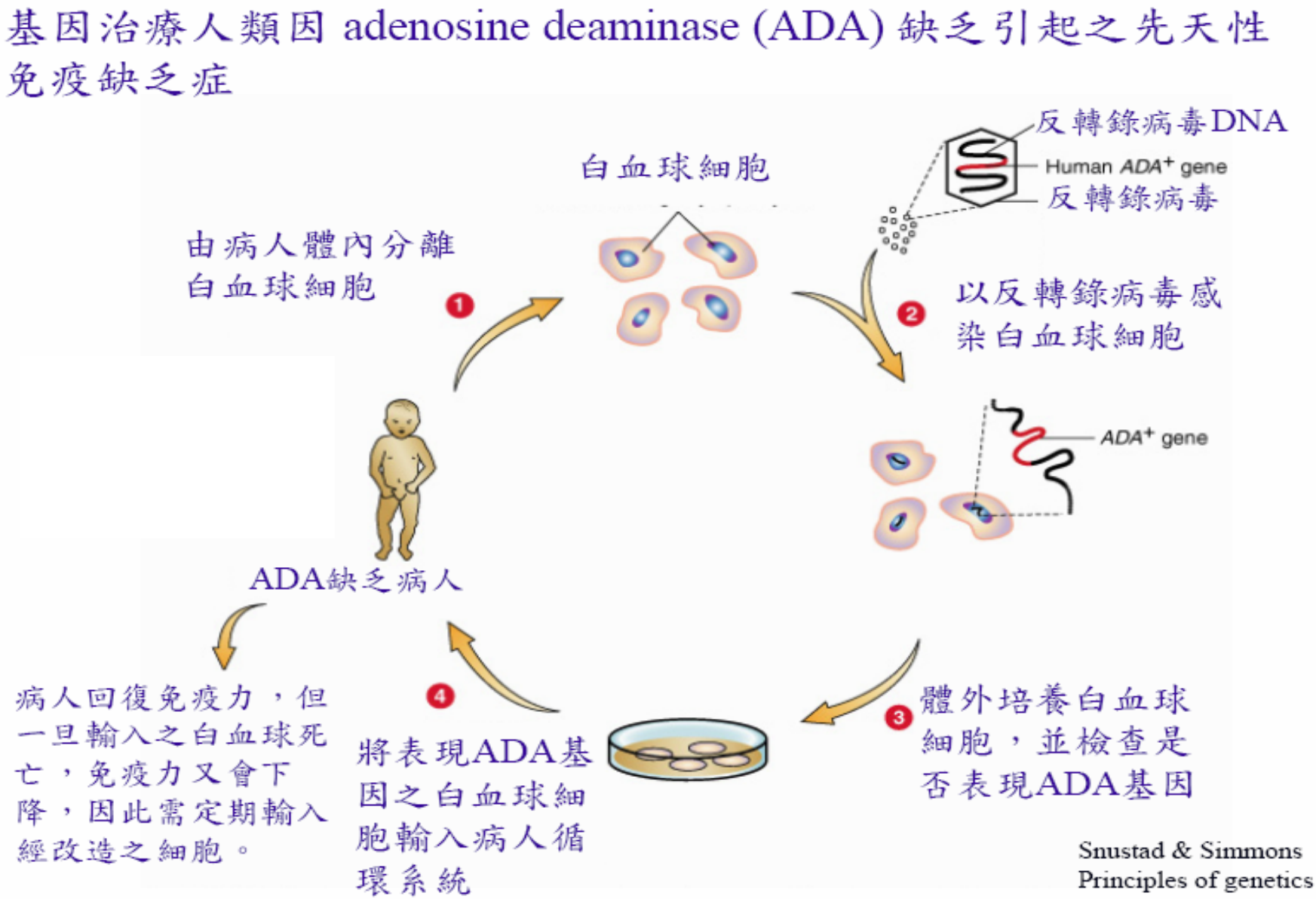
Severe combined Immunodeficiency
嚴重合併免疫不全症

25%的病患是缺乏adenosine deaminase (ADA)，無法代謝Purines， 這對免疫T與B細胞毒性很強。病人先天性免疫不全。

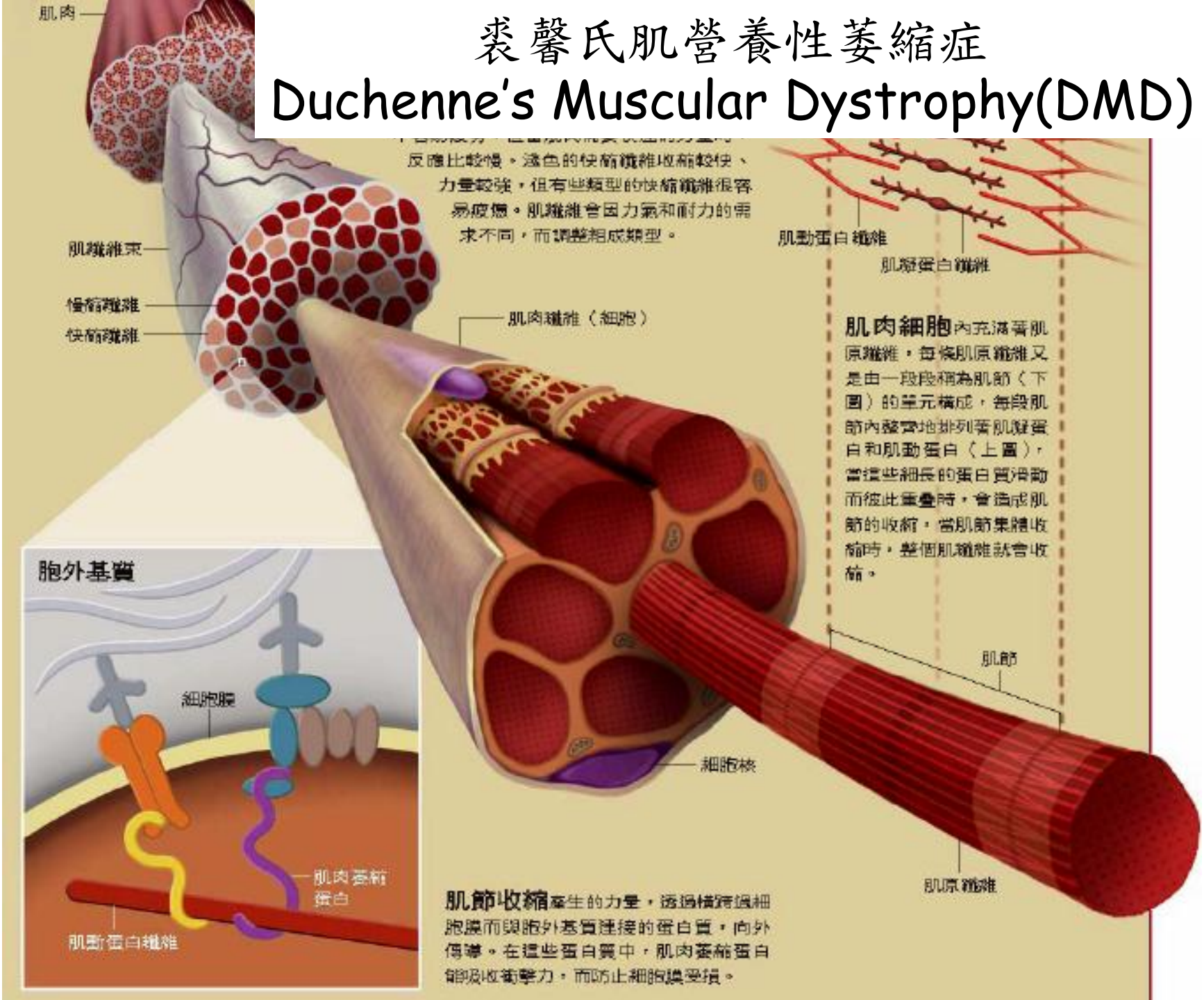
其他治療方法

1.隔離傳染病原
2.外給ADA
3.免疫相容的骨髓移植

W. French Anderson, M.D., R. Michael Blaese, M.D., and Kenneth Culver, M.D.performed the first approved gene therapy on four years old Ashanthi DeSilva, at National Institutes of Health, USA (Sep 14, 1990)



裘馨氏肌營養性萎縮症
Duchenne's Muscular Dystrophy(DMD)



裘馨氏肌營養性萎縮症
Duchenne's Muscular Dystrophy, DMD

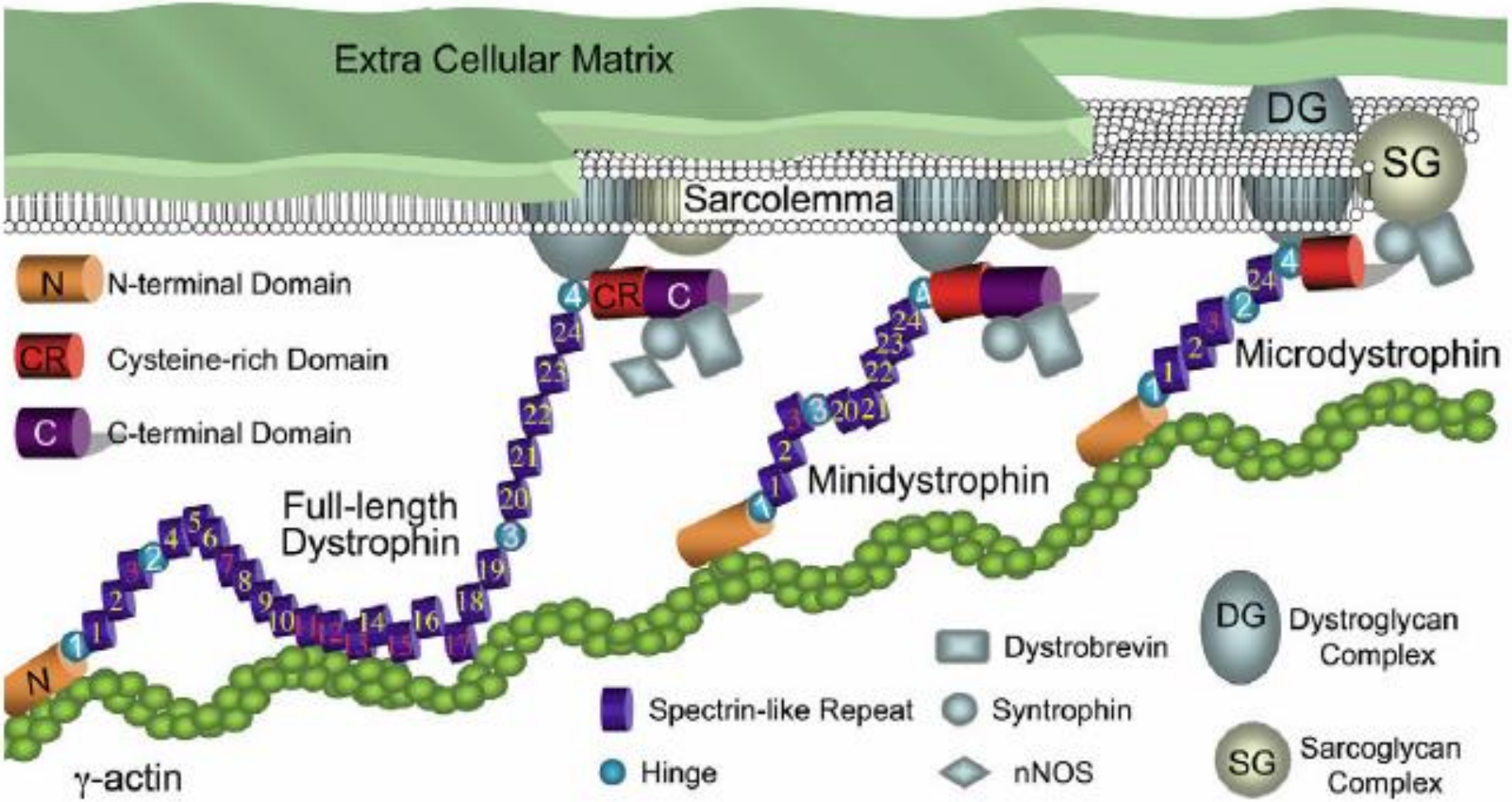
- 肌肉細胞缺乏dystrophin蛋白質，此蛋白質可保護肌肉細胞在一般運動時不致因施力而造成損傷。

- 一般人肌肉損傷可自行修復，但DMD患者肌肉耗損高於修護速度；老化肌肉則修復速度慢於耗損。導致肌纖維死亡。

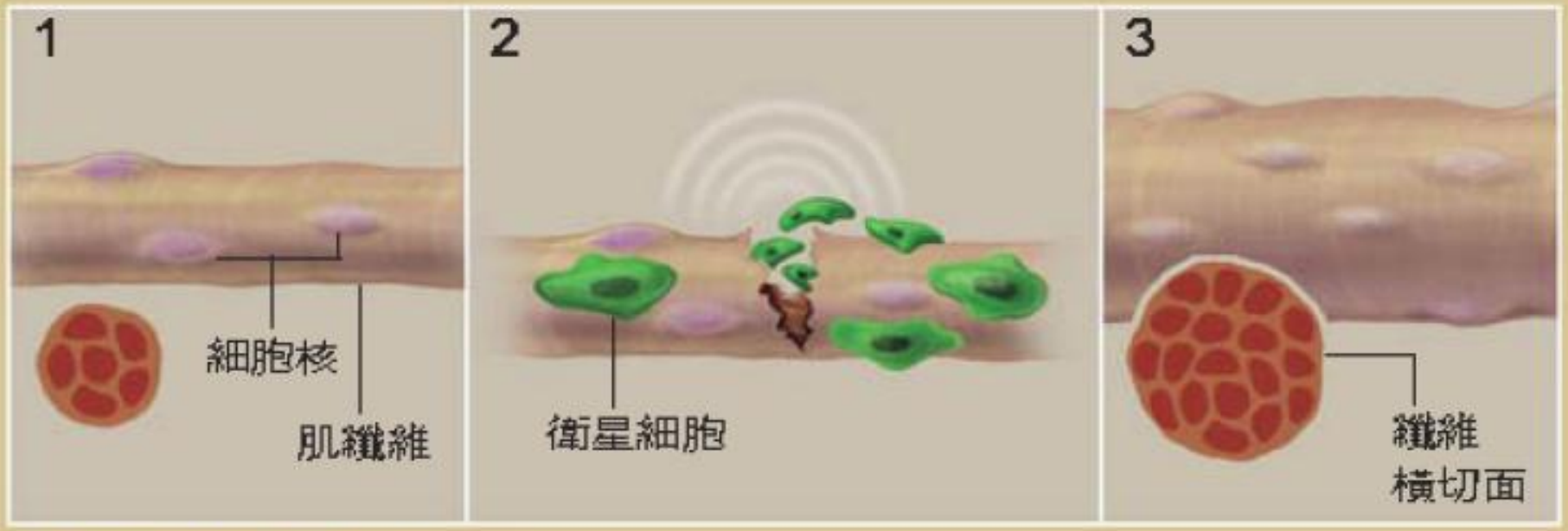
- 肌肉生長和修復受化學信號的影響，化學訊號又受基因活性控制。因老化或疾病耗損的肌肉，可透過添加合成的基因，以強化或阻斷這些訊號。

- dystrophin基因很大，只能用修剪過的micro-dystrophin

-- 引述自科學人

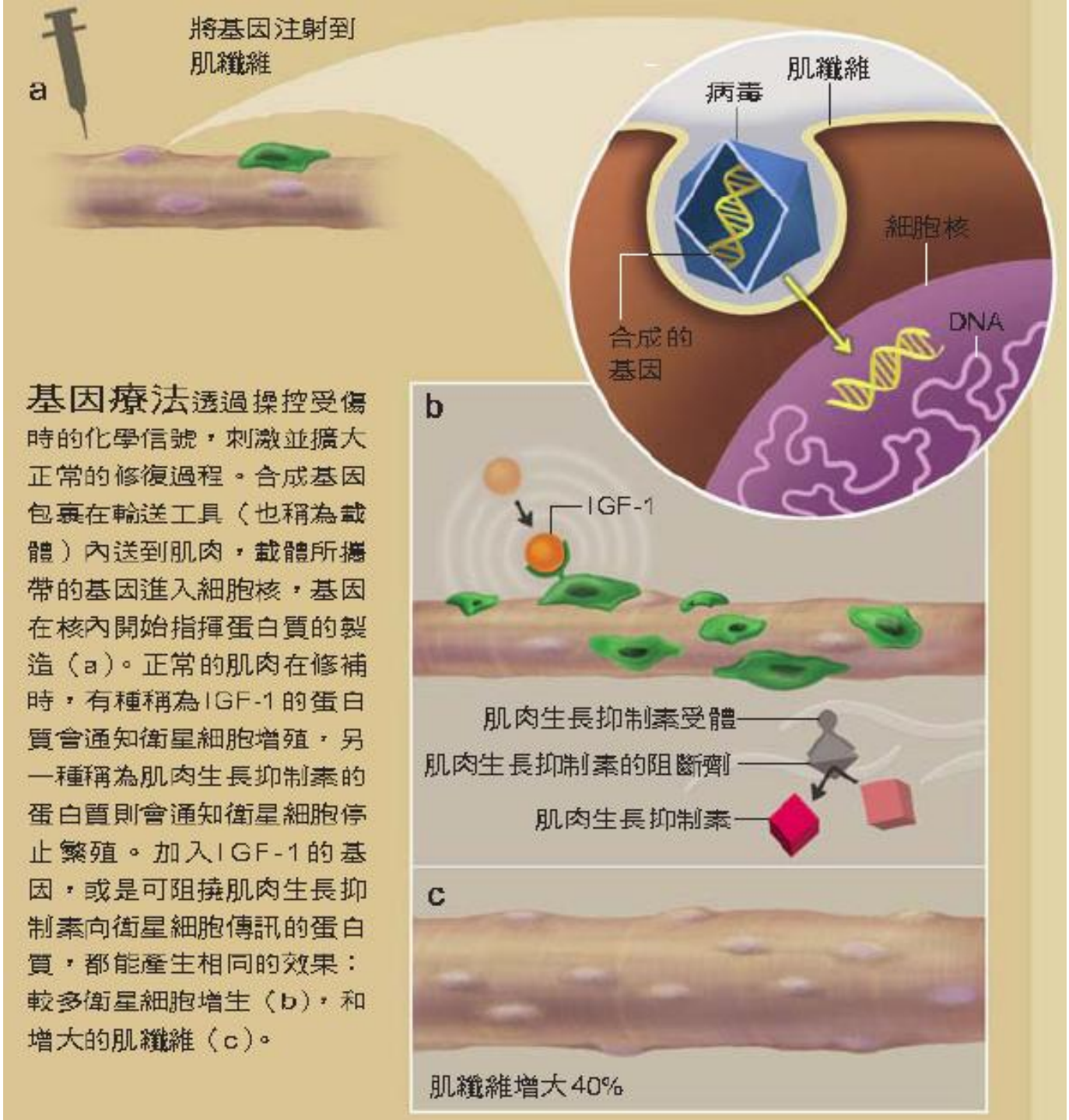


Human Molecular Genetics, 2006, Vol. 15, Review Issue No. 2
doi:10.1093/hmg/ddl180



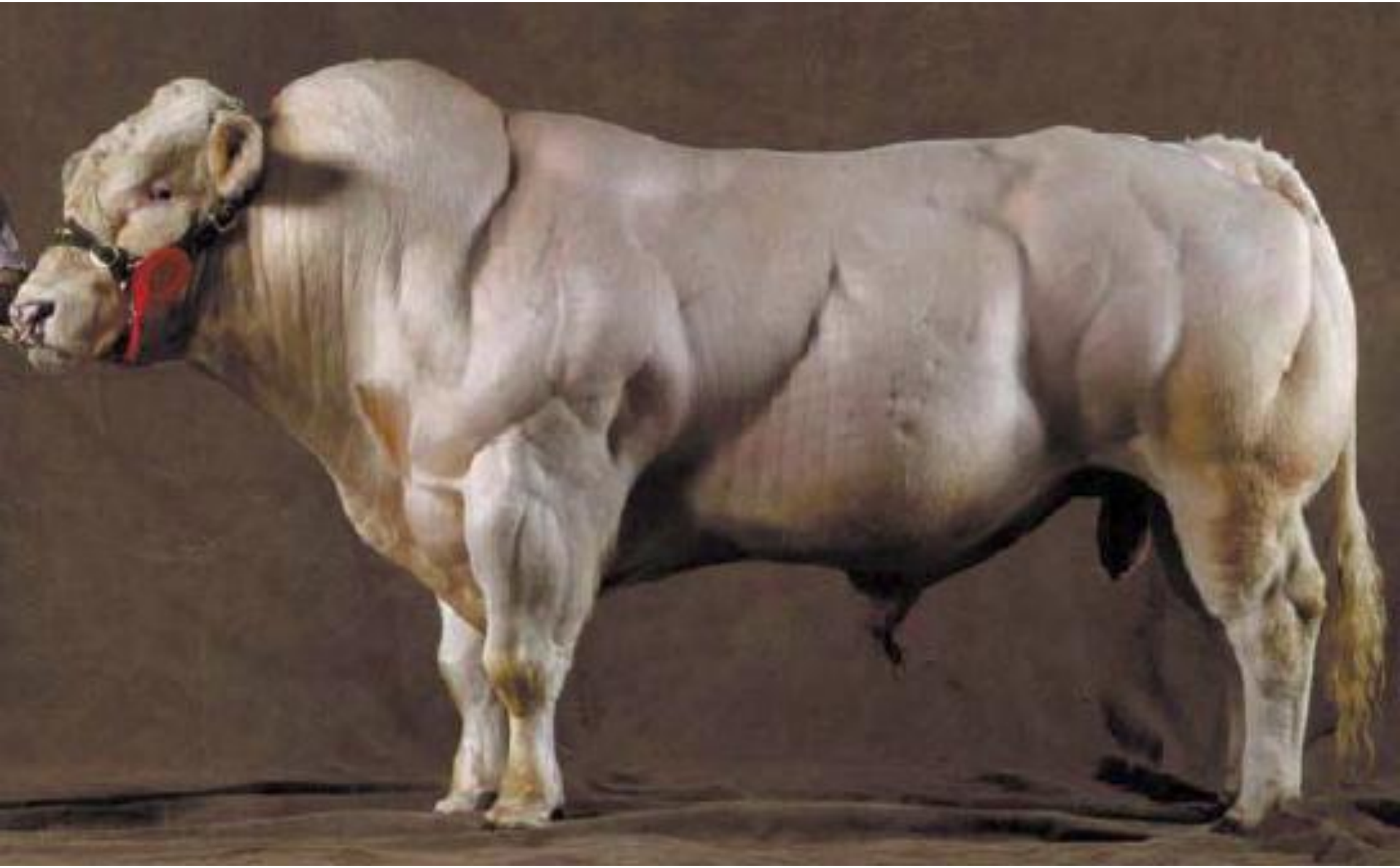
在正常的肌肉中，肌纖維上的多個細胞核（1）會驅動新蛋白質的製造。當肌肉需要修補時，傷口釋出的化學信號，會引來衛星細胞。衛星細胞會先分裂，再與肌纖維融合，讓本身的細胞核加入生產蛋白質的行列（2）。新加入的細胞核和新的肌原纖維，讓修復後的肌纖維比受傷前更粗壯（3）。

IGF1 & myostatin

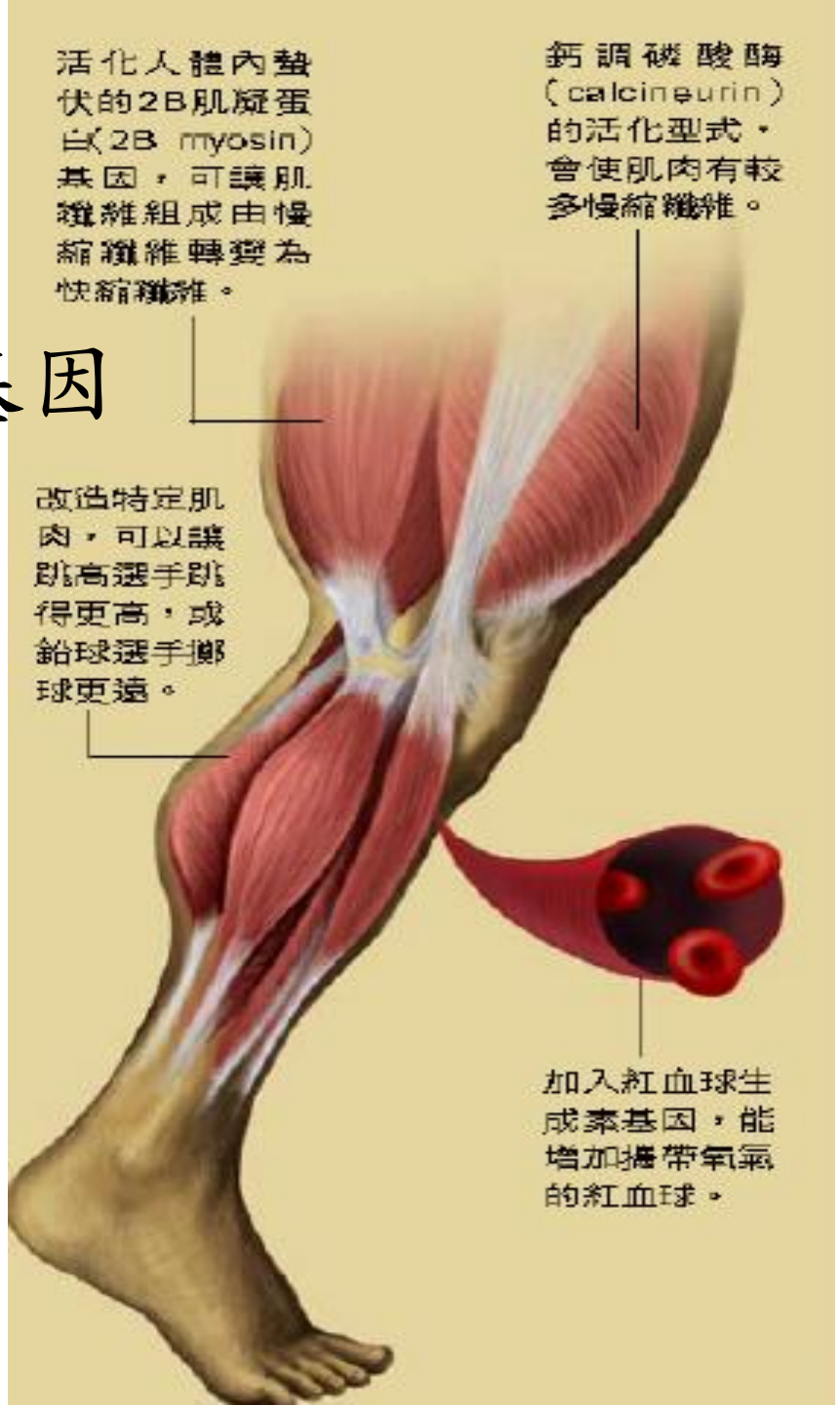


基因療法透過操控受傷時的化學信號，刺激並擴大正常的修復過程。合成基因包裹在輸送工具（也稱為載體）內送到肌肉，載體所攜帶的基因進入細胞核，基因在核內開始指揮蛋白質的製造（a）。正常的肌肉在修補時，有種稱為IGF-1的蛋白質會通知衛星細胞增殖，另一種稱為肌肉生長抑制素的蛋白質則會通知衛星細胞停止繁殖。加入IGF-1的基因，或是可阻撓肌肉生長抑制素向衛星細胞傳訊的蛋白質，都能產生相同的效果：較多衛星細胞增生（b），和增大的肌纖維（c）。

肌肉生長抑制素突變的比利時藍牛



其他影響肌功能之基因


活化人體內潛伏的2B肌凝蛋白(2B myosin)基因，可讓肌纖維組成由慢縮纖維轉變為快縮纖維。
鈣調磷酸酶(calcineurin)的活化型式，會使肌肉有較多慢縮纖維。
改造特定肌肉，可以讓跳高選手跳得更高，或鉛球選手擲球更遠。
加入紅血球生成素基因，能增加攜帶氧氣的紅血球。

癌症基因療法

1. 將抑癌基因(抑制細胞生長或促進細胞凋亡的基因)引入癌細胞，藉此阻止腫瘤生長。
2. 將自殺基因引入癌細胞。此類基因產物會將無毒藥物轉化為毒性物質，藉此殺死腫瘤細胞。
3. 引入抗血管新生分子，藉阻斷血管生成來抑制腫瘤生長。
4.引入腫瘤抗原基因，藉促進免疫功能來除去腫瘤細胞。

(引入之基因不見得是缺失基因)

其他疾病治療與預防

心血管疾病

類風濕性關節炎

神經退化疾病

慢性傳染病

疫苗給予

基因療法的危險性

危險的治療過程

危險的外緣基因

危險的載體

一九九九年十月美國賓州大學威爾森（James W. Wilson）醫師以腺病毒質體，進行鳥胺酸胺甲醯轉移（Ornithine transcarbamylase; OTC）基因缺陷之治療（此會造成肝臟的疾病），該研究的前十七個受試者都沒事，但第十八個病患—來自於美國亞歷桑那州的青少年Jesse Gelsinger的卻因嚴重的副作用造成全身器官壞死而死亡；此並引起了廣泛的爭議，促使對於人體進行基因治療所應顧及的倫理、法律和政策上的重新反省，美國國會並因此舉行聽證會；諸此種種，皆為此新興科技蒙上了一層陰影。OTC為先天性基因缺陷，病因為肝臟OTC酵素缺陷，進而使得血中氮氨囤積量過高造成毒性。該疾病治療方式為以含有OTC基因腺病毒基因載體注射入肝動脈，而Jesse Gelsinger接受此療法時，發生急性中毒反應，最後因肝臟及全身器官壞死而過逝。

Another Child Developed Cancer: SCID Trials Suspended

ABC Tue, 14 Jan 2003

ABC News reports that the FDA suspended 30 gene therapy trials after a second child developed leukemia. So far, two boys who had undergone the experimental gene therapy treatment for immunodeficiency, "bubble boy disease" -- or SCID--developed leukemia. "In gene therapy, a vector -- usually but not always a virus -- is used to carry a healthy gene into the cells of patients." The FDA has been reviewing 150 gene therapy trials that used a retrovirus as a vector. Since October, about 50 of those trials had closed down because all the patients enrolled for gene therapy had died of their diseases.

Published by AAAS

Questions Remain on Cause of Death in Arthritis Trial

The patient, Jolee Mohr of Springfield, Illinois, had suffered from rheumatoid arthritis since she was 21. Through her rheumatologist, she enrolled in a phase I/II safety trial of a new treatment for arthritis sponsored by Targeted Genetics Corp. in Seattle, Washington. The study involved injecting into joints AAV that carried a gene coding for a protein that inhibits a proinflammatory cytokine called tumor necrosis factor α (TNF-α). Mohr received an initial injection in her right knee on 26 February and a second on 2 July.

After the second injection, she developed flulike symptoms. Ten days later, she was admitted to the hospital and was later transported to the University of Chicago Hospital. She died there after massive organ failure on 24 July. The Food and Drug Administration immediately put the trial on hold (*Science*, 3 August, p. 580).

以外源基因創造運動健將 可能嗎? 可行嗎?

基因改造不是基因療法

正當性、合理性是否存在

預期利益是否超越安全考量

評量因素會隨時代改變

'Marathon' mouse keeps on running

Mice can go further with the modified gene

A "marathon" mouse which can run twice as far as a normal rodent has been bred by a US-South Korean team of scientists

BBC, Tuesday, 24 August, 2004

Open access, freely available online PLoS BIOLOGY

Regulation of Muscle Fiber Type and Running Endurance by PPARδ

Yong-Xu Wang[1], Chun-Li Zhang[1], Ruth T. Yu[1], Helen K. Cho[1], Michael C. Nelson[1,2], Corinne R. Bayuga-Ocampo[1], Jungyeob Ham[3], Heonjoong Kang[3], Ronald M. Evans[1,2*]

1 Gene Expression Laboratory, Salk Institute, La Jolla, California, United States of America, 2 Howard Hughes Medical Institute, La Jolla, California, United States of America, 3 Marine Biotechnology Laboratory, School of Earth and Environmental Sciences, Seoul National University, Seoul, Korea

Endurance exercise training can promote an adaptive muscle fiber transformation and an increase of mitochondrial biogenesis by triggering scripted changes in gene expression. However, no transcription factor has yet been identified that can direct this process. We describe the engineering of a mouse capable of continuous running of up to twice the distance of a wild-type littermate. This was achieved by targeted expression of an activated form of peroxisome proliferator-activated receptor δ (PPARδ) in skeletal muscle, which induces a switch to form increased numbers of type I muscle fibers. Treatment of wild-type mice with PPARδ agonist elicits a similar type I fiber gene expression profile in muscle. Moreover, these genetically generated fibers confer resistance to obesity with improved metabolic profiles, even in the absence of exercise. These results demonstrate that complex physiologic properties such as fatigue, endurance, and running capacity can be molecularly analyzed and manipulated.

Citation: Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, et al. (2004) Regulation of muscle fiber type and running endurance by PPARδ. PLoS Biol 2(10): e294.

“**Almost by definition, athletes and elite runners would have an interest in this because it might make their exercise more Efficient**”

Ronald Evans, Salk Institute

基改威力鼠 連跑6小時不累

【聯合報／編譯王先棠／報導】2007.11.03 03:07 am

老鼠也能跑馬拉松。美國學者透過基因工程成功繁殖出能連續跑六個小時不用休息的「威力鼠」（mighty mouse）。學者表示，這種威力鼠的相關研究，可以促進新藥研發，甚至藉此強化運動員的體能。

Supplemental Material can be found at:
http://www.jbc.org/cgi/content/full/M706127200/DC1

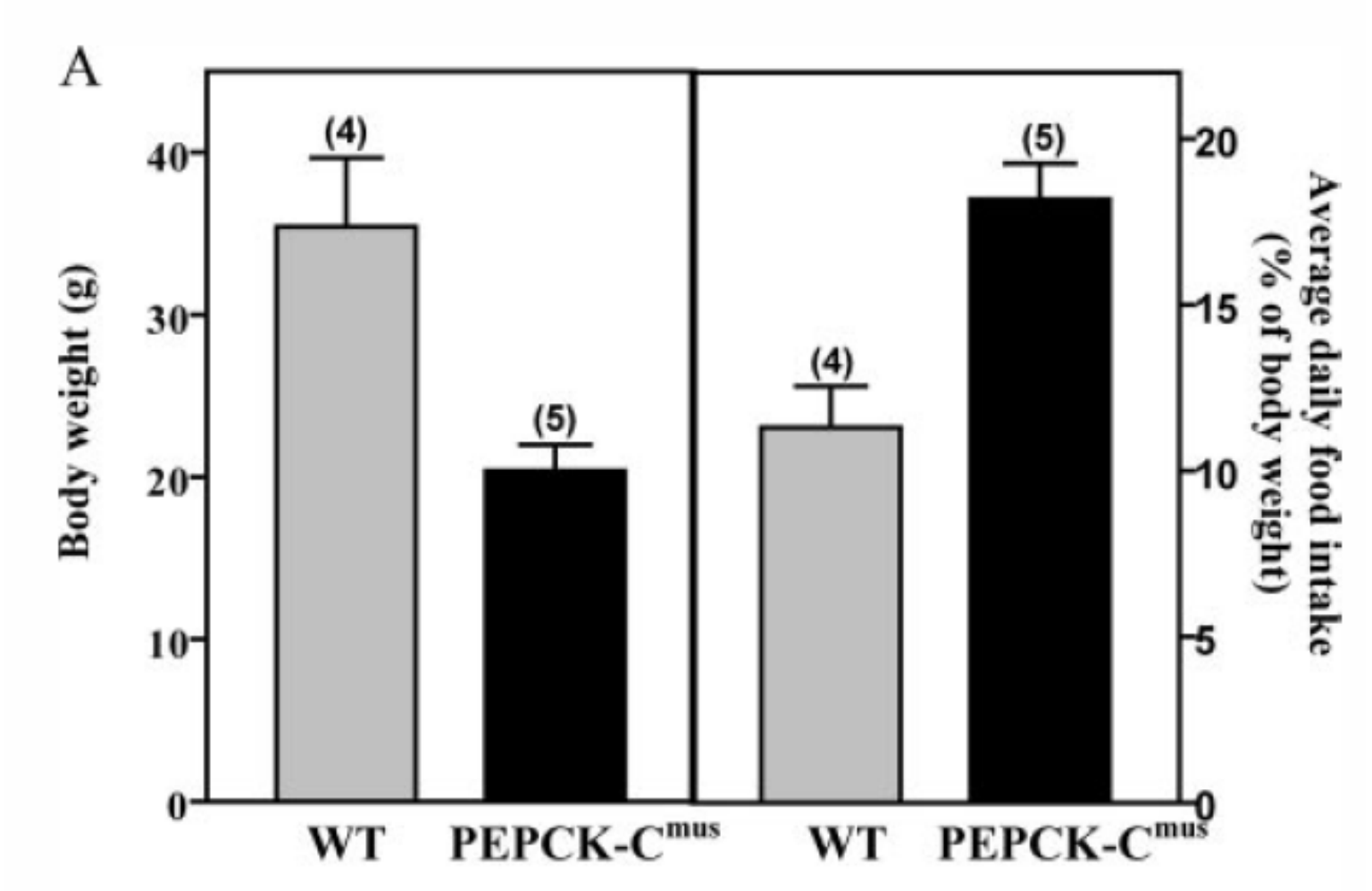
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 282, NO. 45, pp. 32844–32855, November 9, 2007

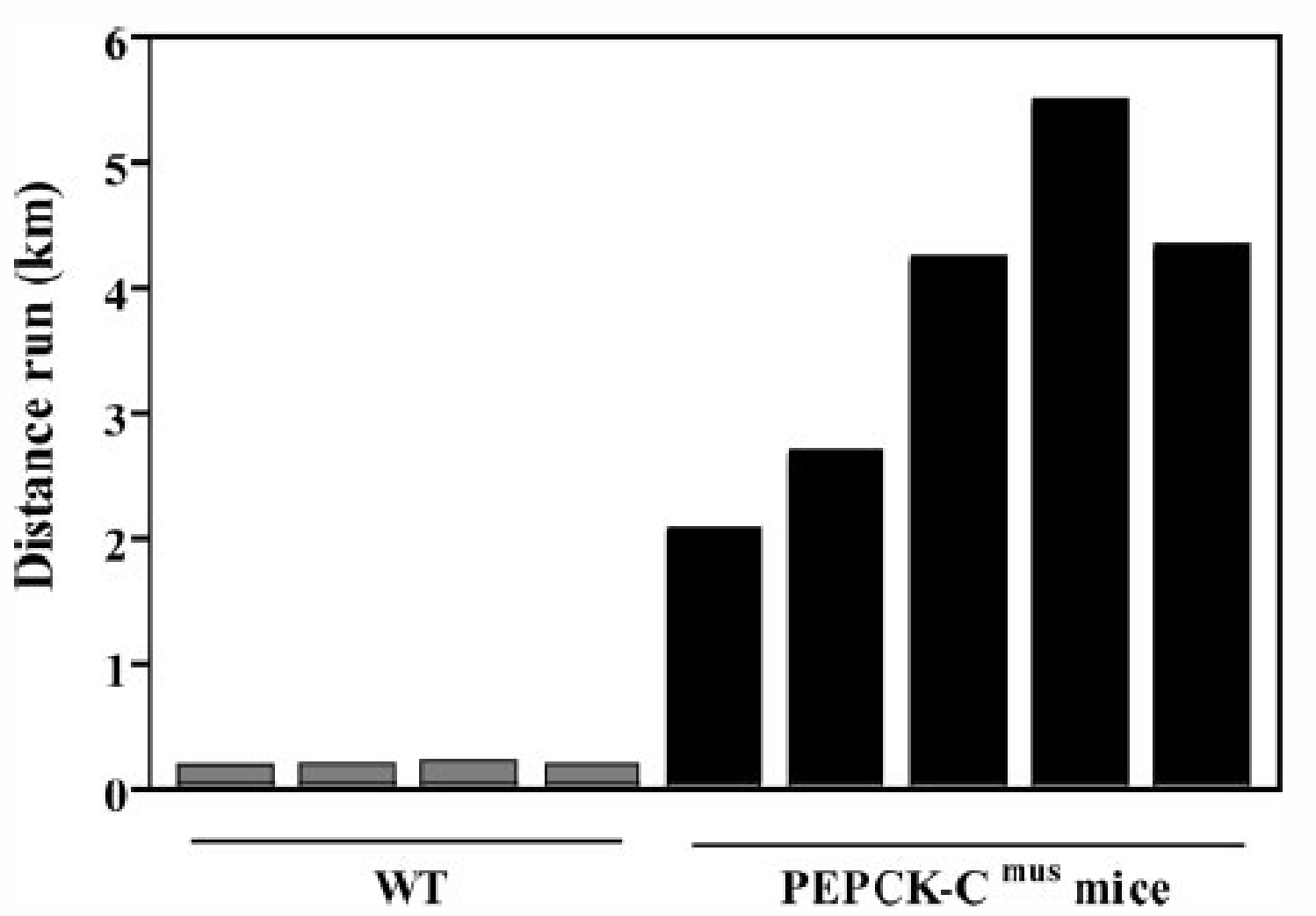
Overexpression of the Cytosolic Form of Phosphoenolpyruvate Carboxykinase (GTP) in Skeletal Muscle Repatterns Energy Metabolism in the Mouse*[S]◆

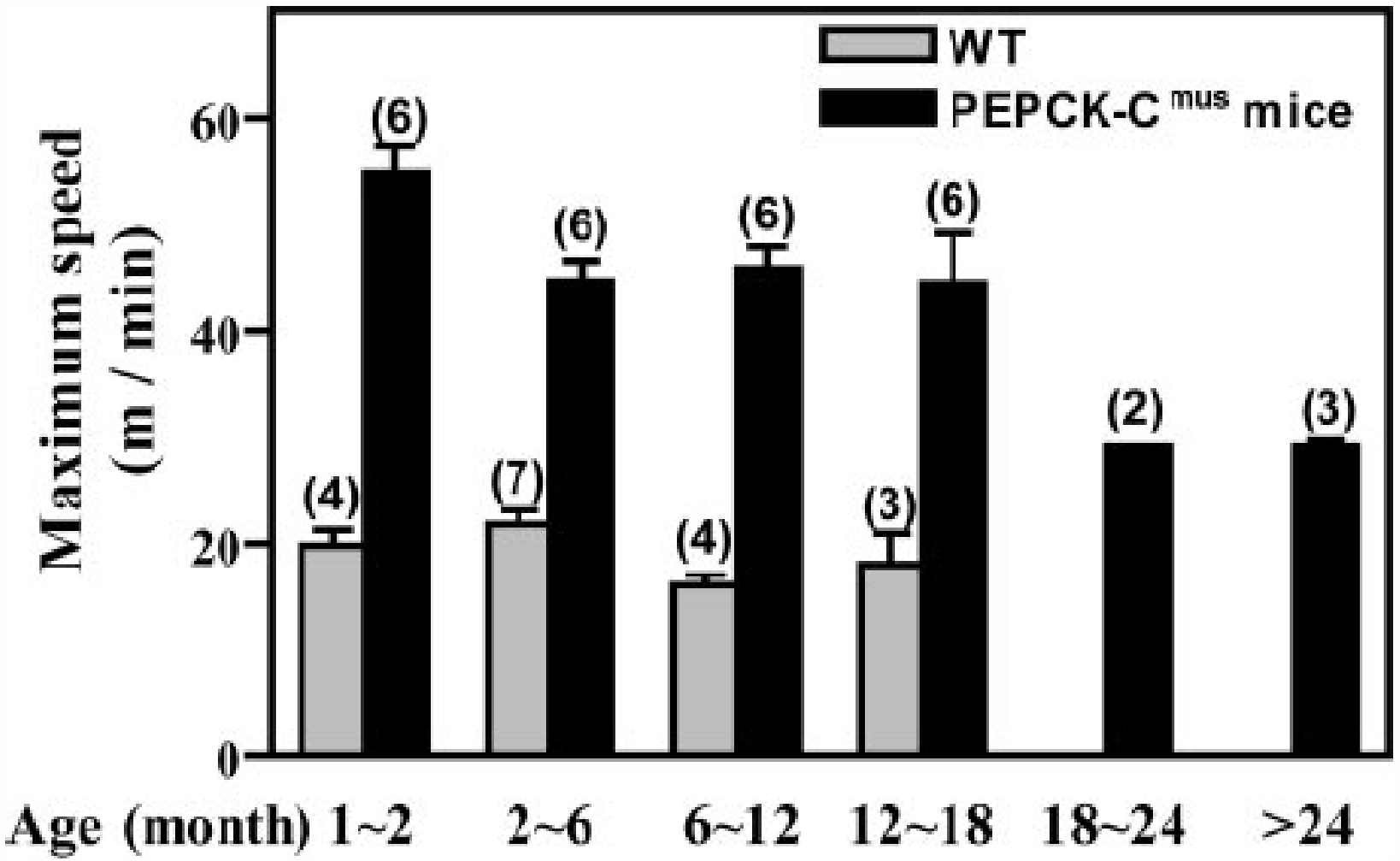
Received for publication, July 25, 2007, and in revised form, August 21, 2007 Published, JBC Papers in Press, August 23, 2007, DOI 10.1074/jbc.M706127200

Parvin Hakimi‡, Jianqi Yang‡, Gemma Casadesus§, Duna Massillon¶, Fatima Tolentino-Silva‖, Colleen K. Nye‡, Marco E. Cabrera‖**, David R. Hagen‡, Christopher B. Utter‡, Yacoub Baghdy‡, David H. Johnson‖, David L. Wilson‖, John P. Kirwan‡‡, Satish C. Kalhan‡‡, and Richard W. Hanson‡1**

*From the Departments of ‡Biochemistry, ¶Nutrition, **Pediatrics, §Neuroscience, and ‖Biomedical Engineering, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106-4935 and the ‡‡Department of Gastroenterology/Hepatology and Pathobiology, Cleveland Clinic Foundation, Cleveland, Ohio 44195*


A
Body weight (g)
Average daily food intake
(% of body weight)
40
30
20
10
0
20
15
10
5
0
(4)
(5)
(4)
(5)
WT
PEPCK-C^mus
WT
PEPCK-C^mus


Distance run (km)
6
5
4
3
2
1
0
WT
PEPCK-C mus mice


WT
PEPCK-C mus mice
Maximum speed (m / min)
0
20
40
60
(4)
(6)
(7)
(6)
(4)
(6)
(3)
(6)
(2)
(3)
Age (month) 1~2
2~6
6~12
12~18
18~24
>24

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基因改造不是基因療法

正當性、合理性是否存在

預期利益是否超越安全考量

評量因素會隨時代改變