Nucleus and Nucleic Acids
細胞核與核酸

認知單元: 細胞核與核酸

大綱

- 真核細胞 vs 原核細胞
- 細胞核: 核膜, 運輸, 超微結構
- 核酸: DNA 和基因
- 基因表達: 轉錄, 調節性 RNA, mRNA剪接
- 轉譯: 蛋白質合成
- RNA 定序, 利用RNA的基因療法, RNA 藥物
- 生物科技與生活

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生物醫學科學研究所
INSTITUTE OF BIOMEDICAL SCIENCES

認知單元: 細胞核與核酸

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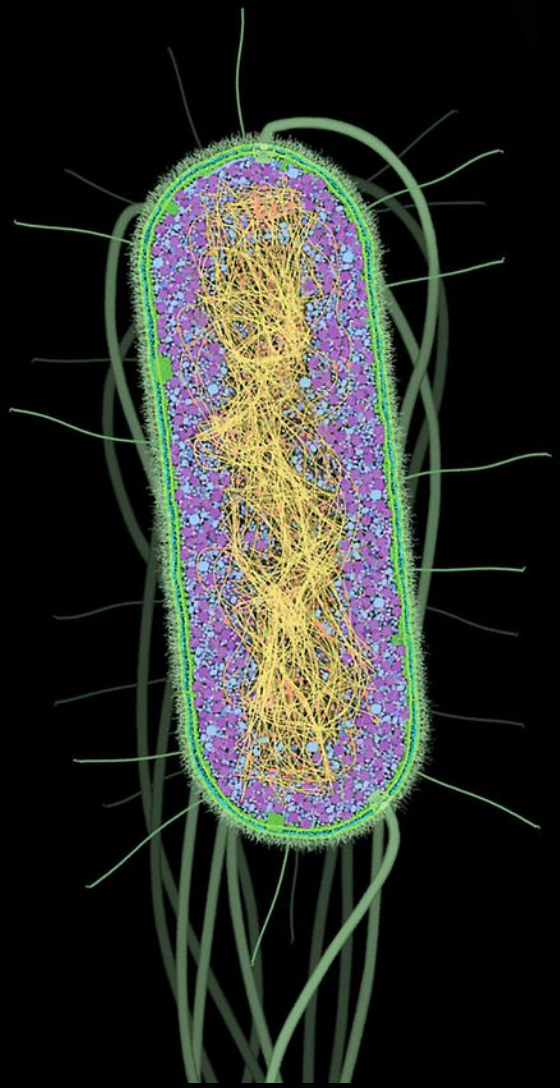
癌症組/表觀遺傳學實驗室



原核生物 (細菌)

真核生物細胞:
出現有膜包圍的胞器
包括細胞核

From fine art america

細胞膜 vs 核膜

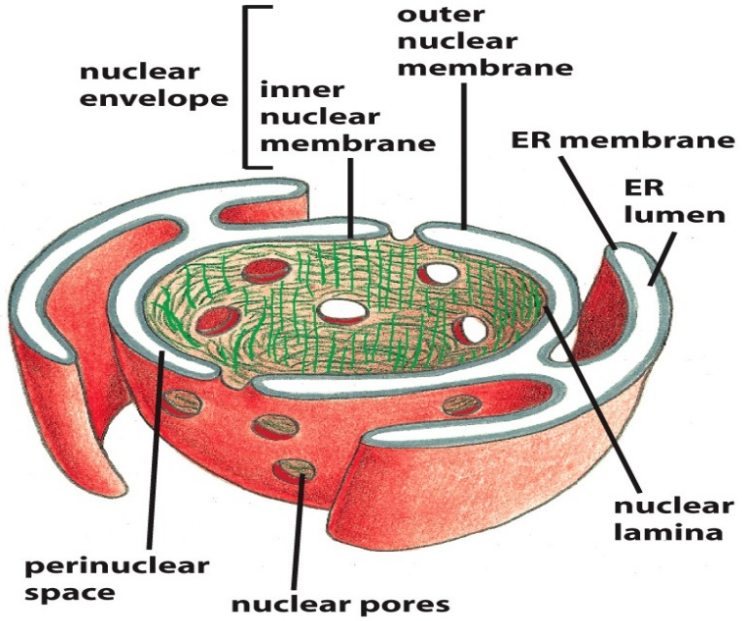


Figure 12-8 Molecular Biology of the Cell 5/e (© Garland Science 2008)

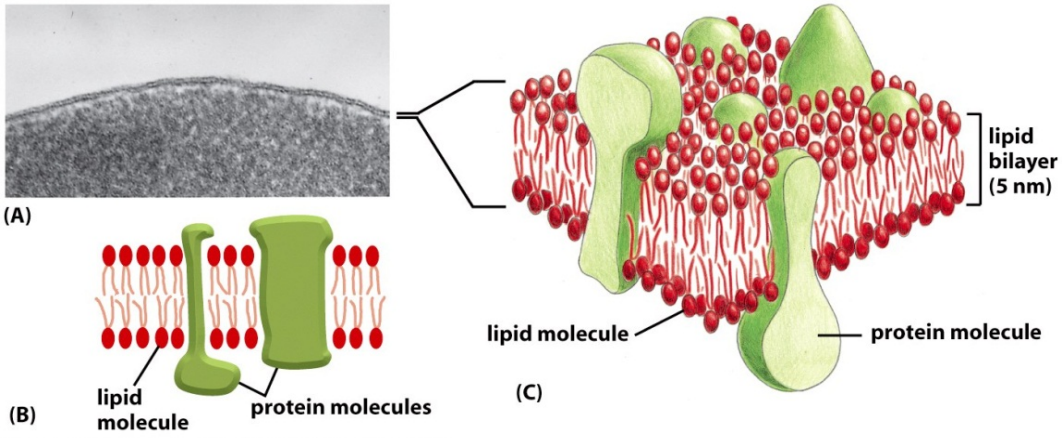
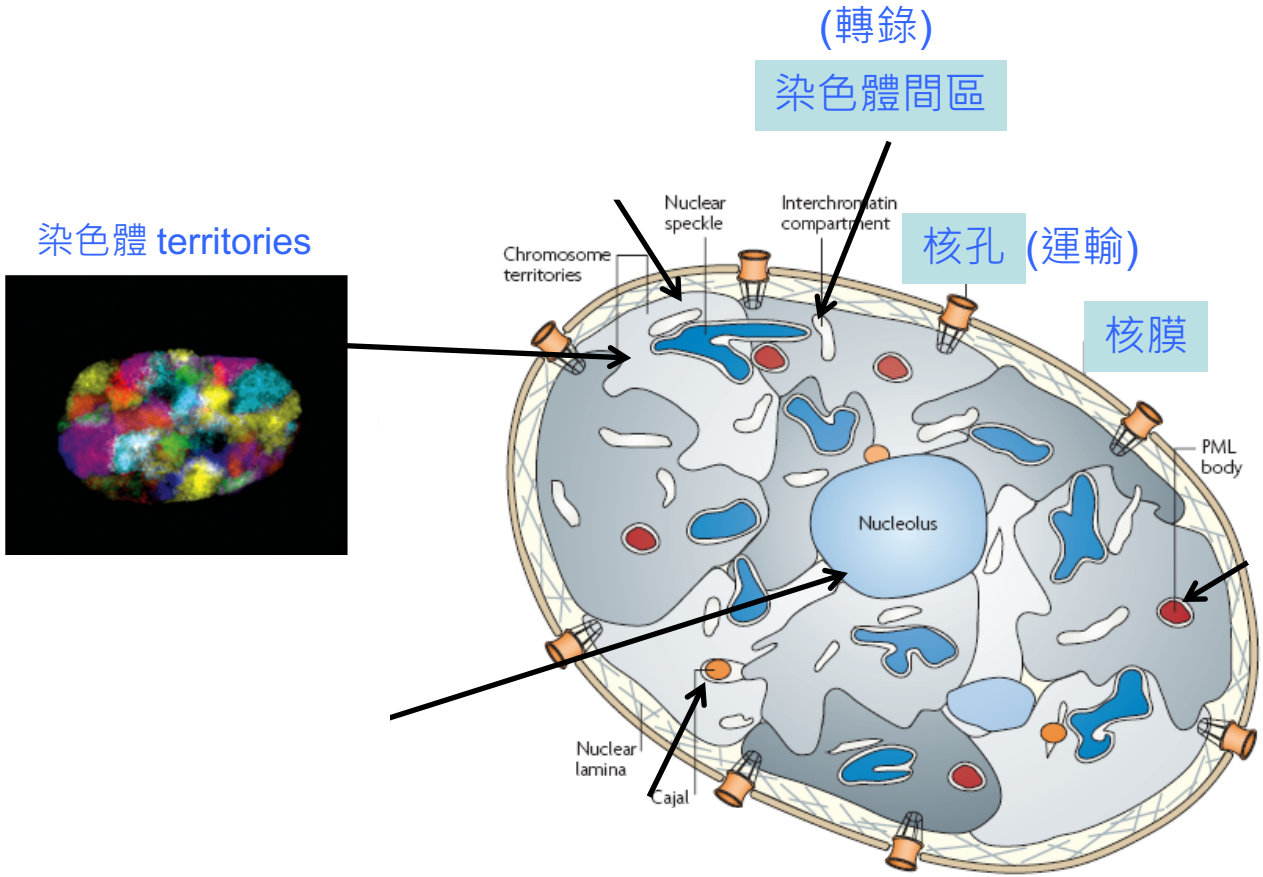


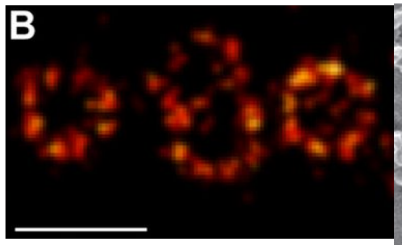
Figure 10-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- ❖細胞核膜分隔了染色體 (複製和表達) 和核醣體 (產生蛋白質) 存在的位置
- ❖細胞膜和核膜的最大不同處
  - 細胞膜沒有 ”(大分子) 通道”
  - 核膜為雙層膜，有蛋白質組成的通道 (即 nuclear pore complex; 核孔) 讓蛋白質及 RNA 等大型分子進出

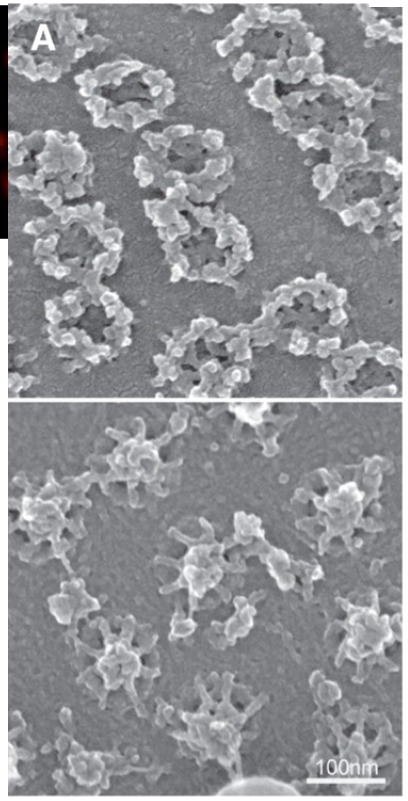
真核細胞的細胞核及內部的超微結構

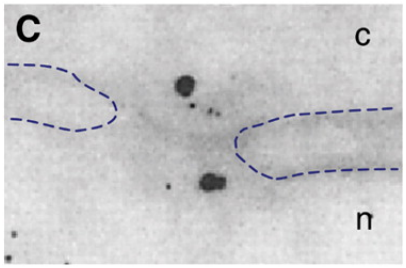


細胞核運輸: 大分子如何通過核膜



Ultra-resolution microscopy GP210



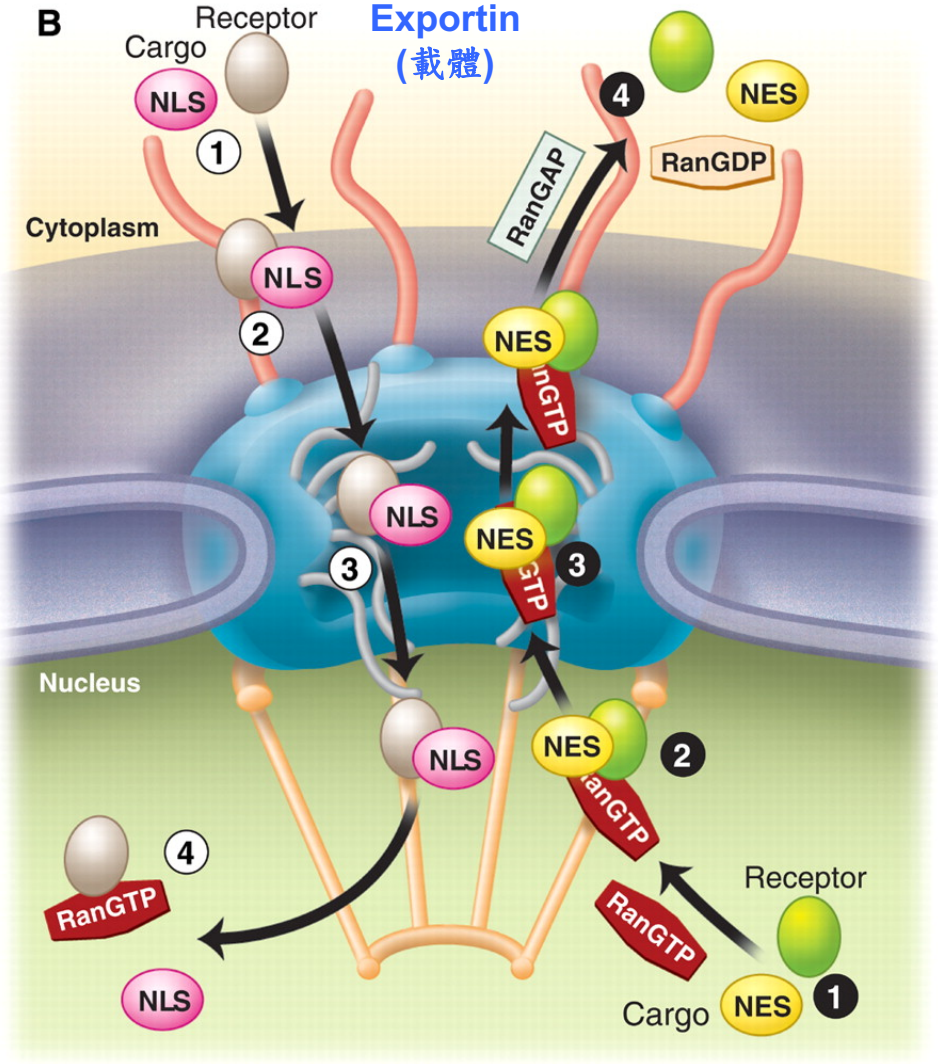


Electron microscopy

蛋白分子
(貨物 cargo)

Importin
or
Exportin
(載體)

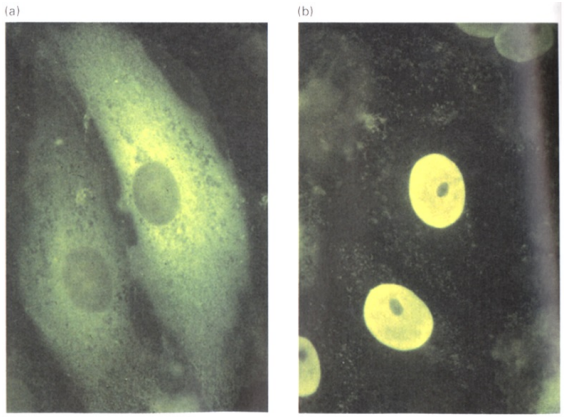
Passport:
NLS: Nuclear localization signal
NES: Nuclear export signal

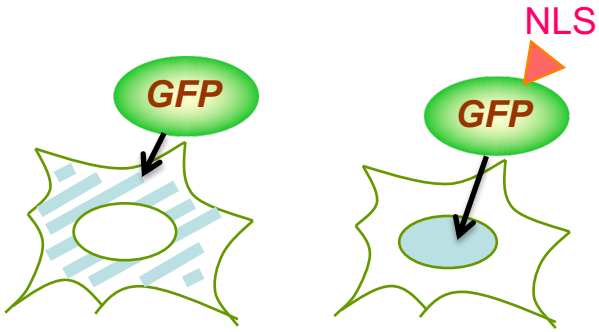


細胞核運輸的研究方法

蛋白分子進入細胞核

The nuclear localization signal (NLS) (進核訊號 like a “ticket”; 車票) allows protein transport into the nucleus.

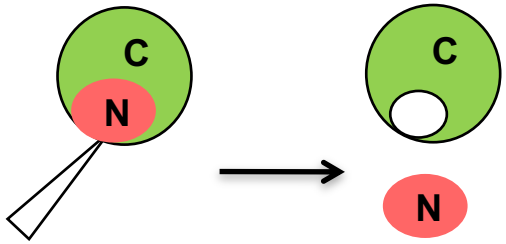




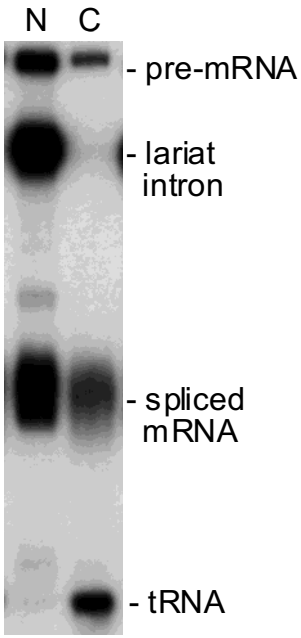
NLS: SV40病毒的 large T-antigen 的一段胺基酸序列 (PKKKRKV)

RNA運出細胞核

Xenopus oocyte
角蛙卵母細胞



將RNA顯微注射至卵母細胞的核中

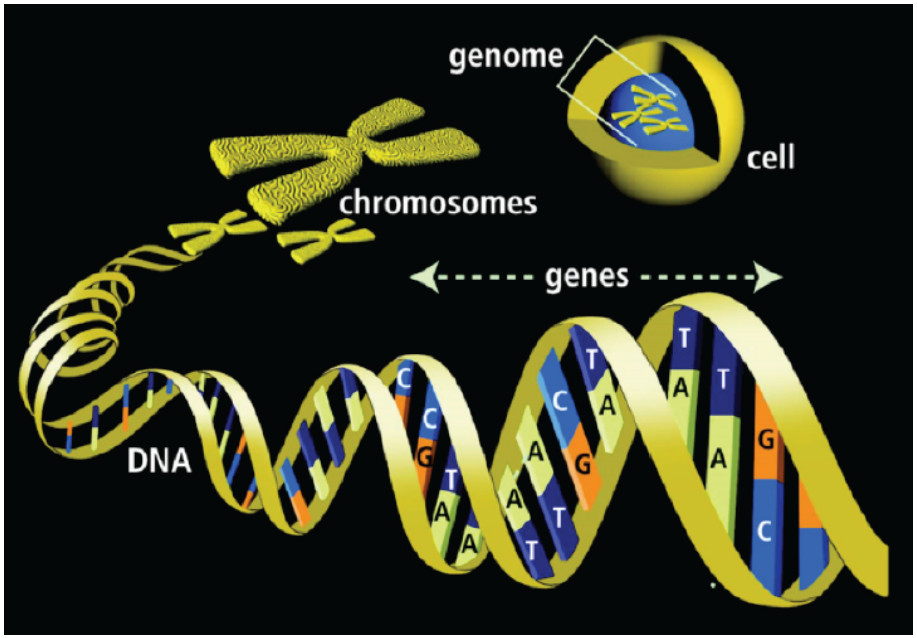


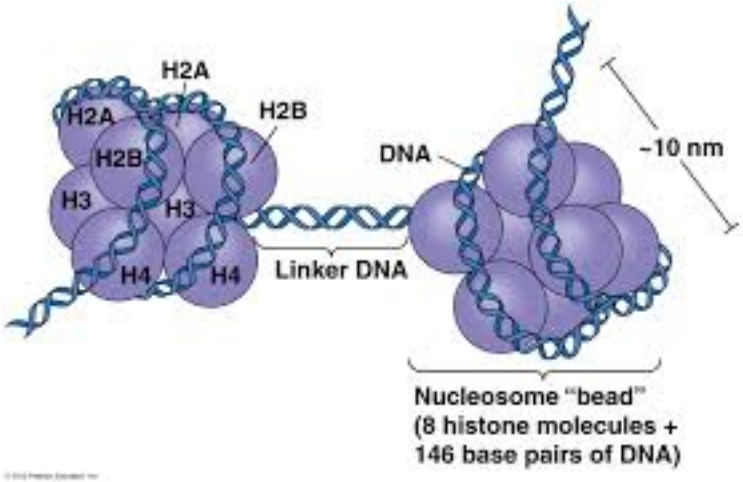
染色體與基因

Chromosome and Gene

Human genome
23 pairs of chromosomes
3.0×10^9 base pairs: 3,000 megabase (Mb)
~ 21000 genes

Species	*Escherichia coli*	*Gallus gallus*	*Homo sapiens*	*Daphnia pulex*	*Oryza sativa*
Number of Genes	~4,200	~17,000	~21,000	~31,000	~38,000
Common Name	 Bacteria	 Chicken	 Human	 Water flea	 Rice

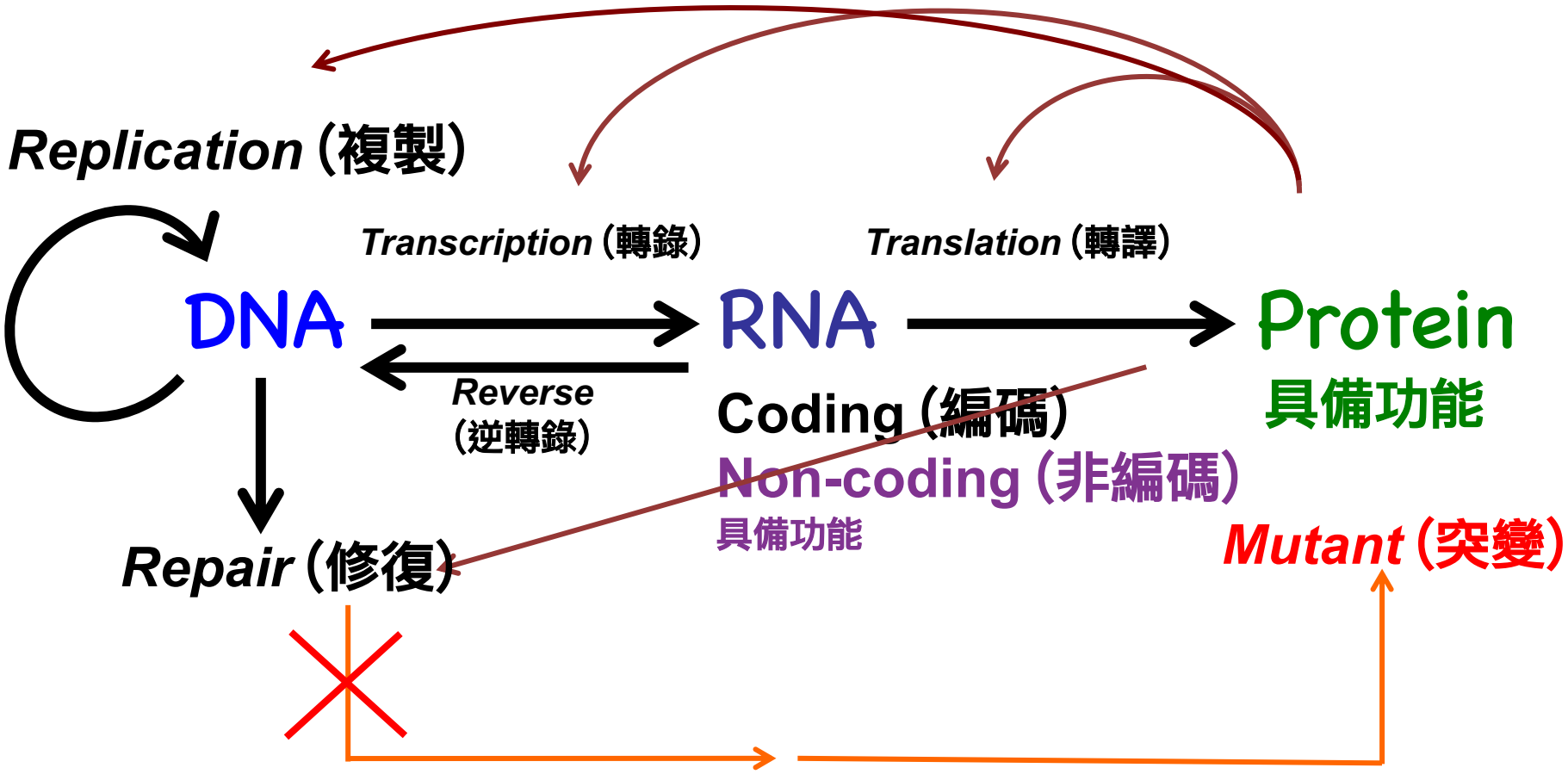




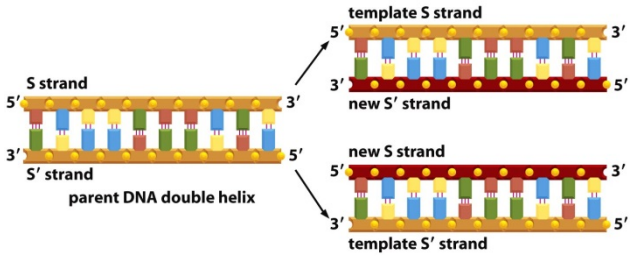
真核生物的DNA和蛋白質 (histones)形成染色質/絲(chromatin)

核酸: DNA和基因

分子生物學的中心法則

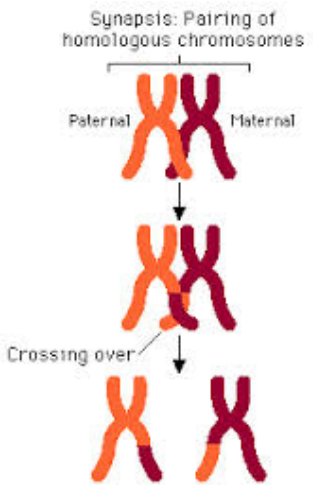

Replication (複製)
Transcription (轉錄)
Translation (轉譯)
DNA
RNA
Protein
Reverse
(逆轉錄)
Coding (編碼)
具備功能
Non-coding (非編碼)
具備功能
Repair (修復)
Mutant (突變)

DNA的複製, 重組, 修復和修飾



複製
維持每個細胞內的遺傳物質維持相同
DNA聚合酶 (DNA polymerases)

重組
減數分裂時非姐妹染色單體 (母方和父方)上的基因重新組合



DNA 複製 (replication)
重組 (recombination)
修復 (repair)
修飾 (modification)

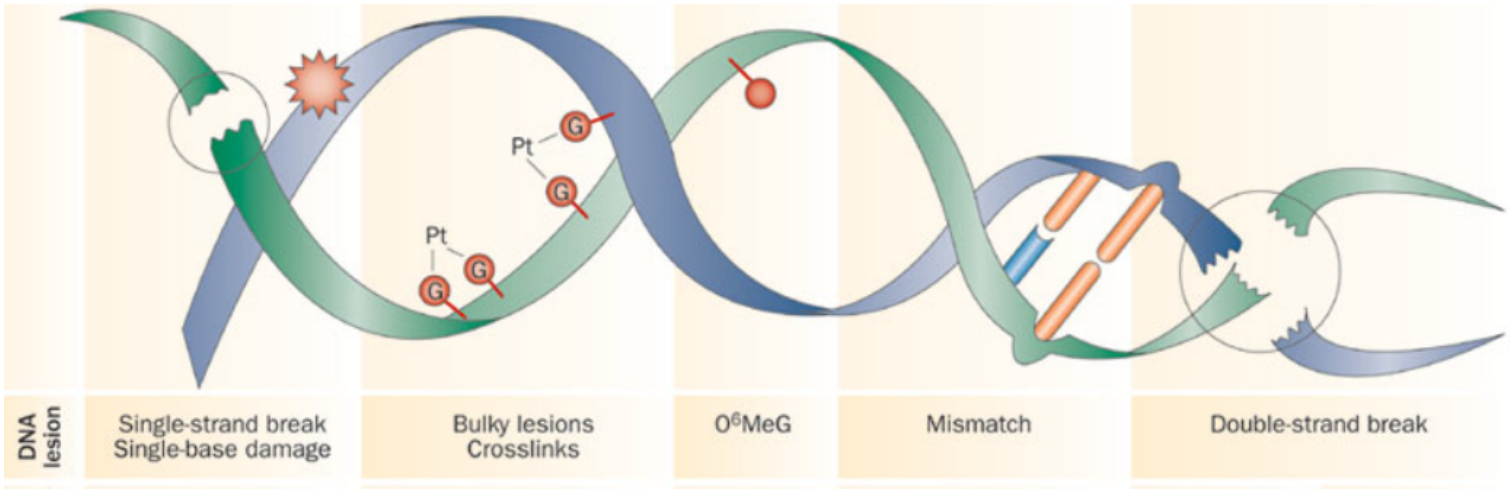
修復
修復複製過程有可能發生的錯誤
人類基因組: 3×10^9 base pairs
複製之錯誤機率 10^{-9}-10^{-11}
(the mutation rate: 1 per cell division.)
避免環境因素 (如紫外線和放射線)造成的DNA損傷和突變

修飾
基因體印記 (genomic imprinting):
生物體會特定的對其父系或母系染色體上的某些基因進行甲基化印記(imprinting)，造成該基因的關閉

表觀遺傳學 epigenetics

DNA 損傷與疾病

DNA的複製或重組錯誤
DNA的修復功能喪失
游離輻射, 化學藥物, 紫外線
細胞內的活性氧物質造成鹼基的氧化作用



單股斷裂　雙嚙啶鍵結　鹼基甲基化或氧化　錯誤配對　雙股斷裂

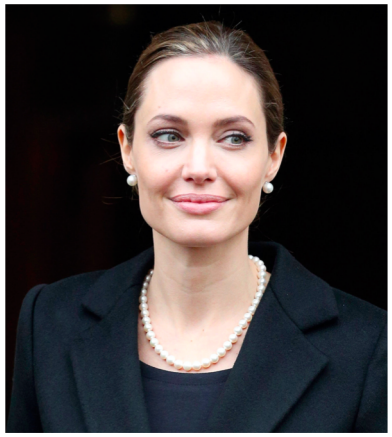
免疫疾病　**癌症**　老化　神經疾病

Nobel Prize in Chemistry 2015

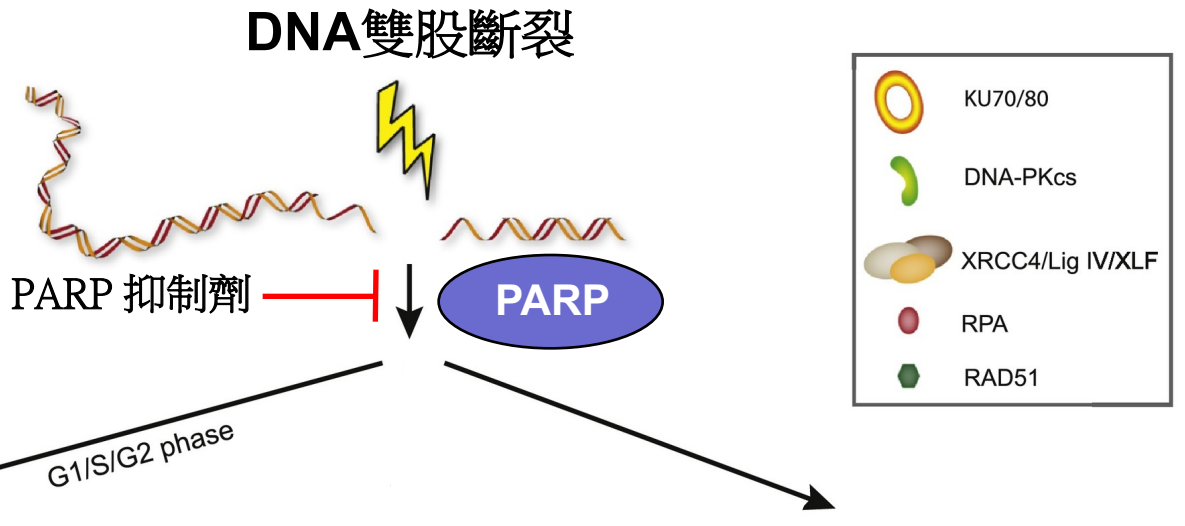
Prize in Chemistry for 2015 to
Tomas Lindahl (UK)
Paul Modrich (Duke University)
Aziz Sancar (Uni North Carolina)
"for mechanistic studies of DNA repair"

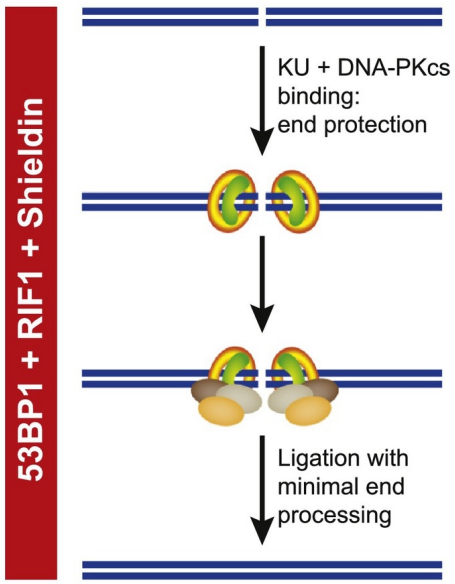
The cells' toolbox for DNA repair: how cells repair damaged DNA and safeguard the genetic information

DNA雙股斷裂之修復



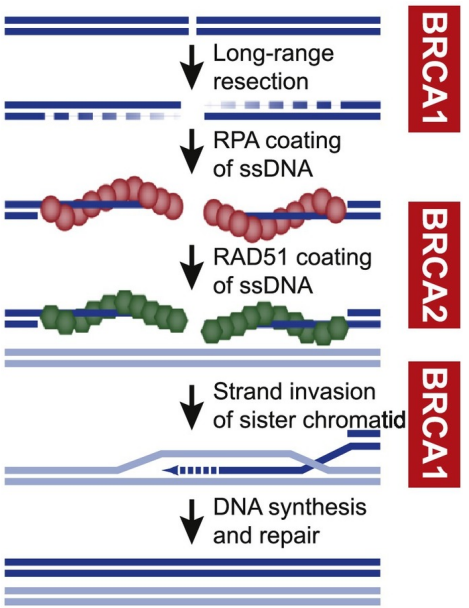
Angelina Jolie





DNA黏合酶

BRCA1/2 基因缺陷搭配 PARP 抑制劑可引發癌細胞合成致死 (Synthetic Lethality)

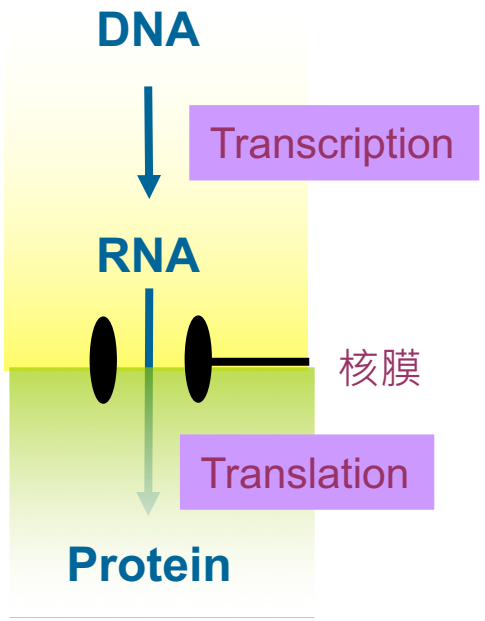


Non-homologous end-joining 非同源性末端接合

Homologous recombination 同源性重組

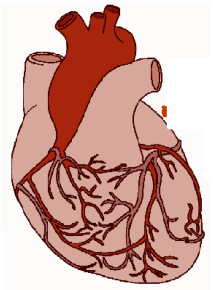
基因表達 (gene expression)

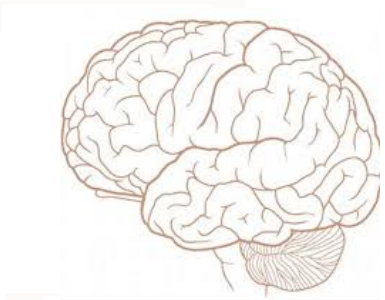
DNA轉錄成RNA再轉譯成蛋白質

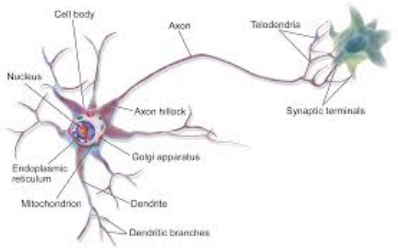


❖ 基因表達的調控: 不同種類的細胞 (肌肉細胞、神經細胞、肝臟細胞, *etc*) 或同一種類的細胞在不同時期 (生長期、分化期、衰老期, *etc*) 及不同環境下 (營養、毒物、病毒侵襲, *etc*) 有不同的基因表達

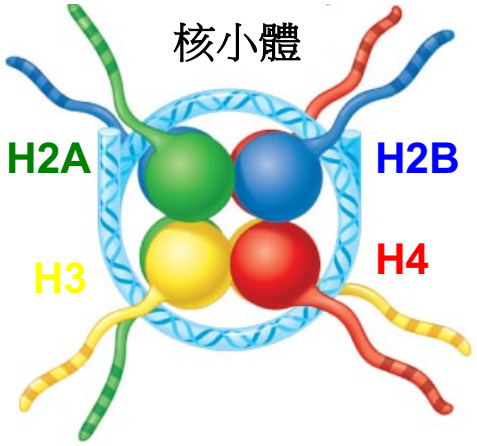
•細胞分化 (differentiation):
藉由不同基因表達,具有相同基因的細胞能執行不同的功能也會有不一樣的命運







遺傳訊息也存在於染色質中



Methylation
(甲基化)
Acetylation
(乙醯化)
Phosphorylation
(磷酸化)
Ubiquitination
(泛素化)
.....

Chromosome
(染色體)

Nucleus
(細胞核)

Heterochromatin
(異染色質)

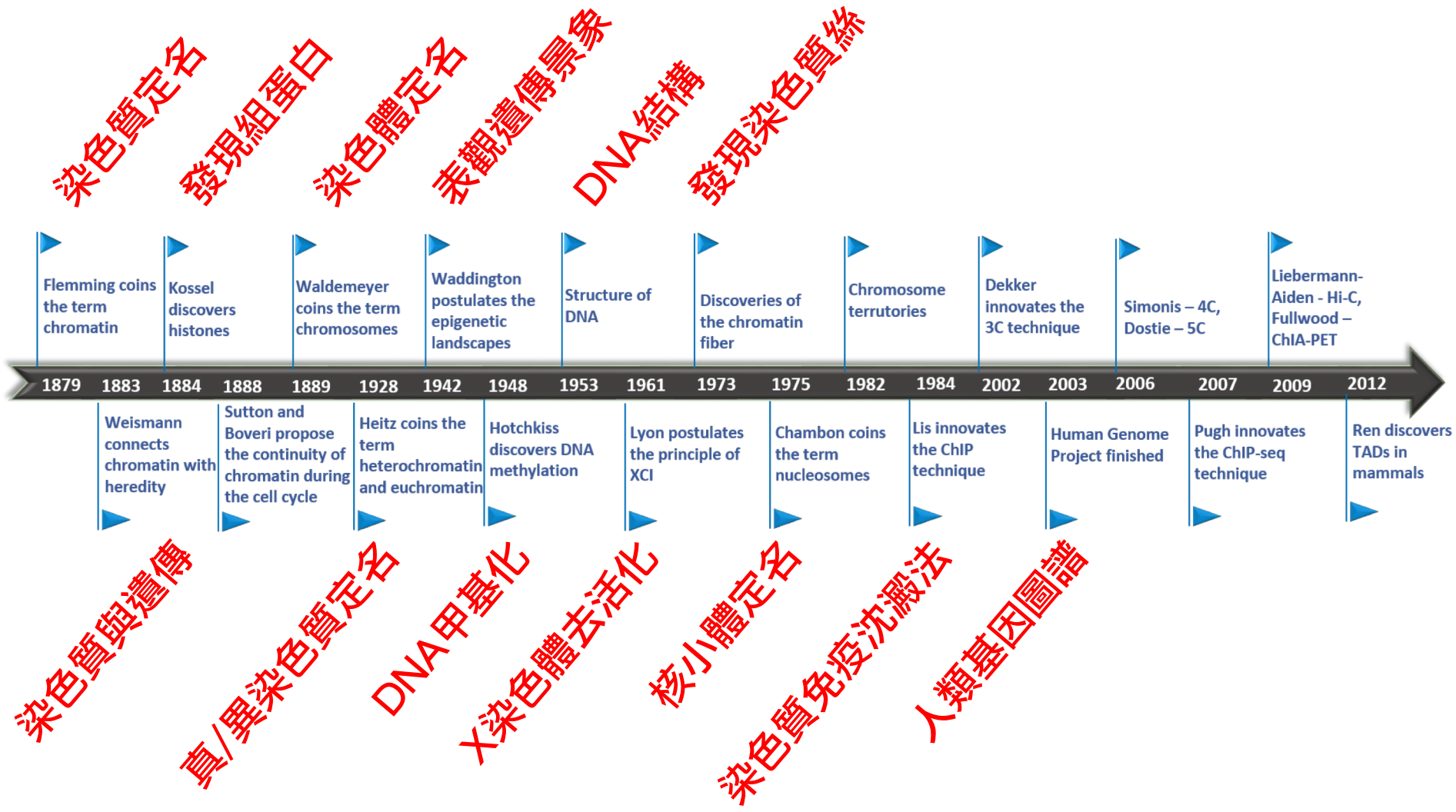
Euchromatin
(真染色質)

Nucleosome
(核小體)

Methylation
(甲基化)

DNA

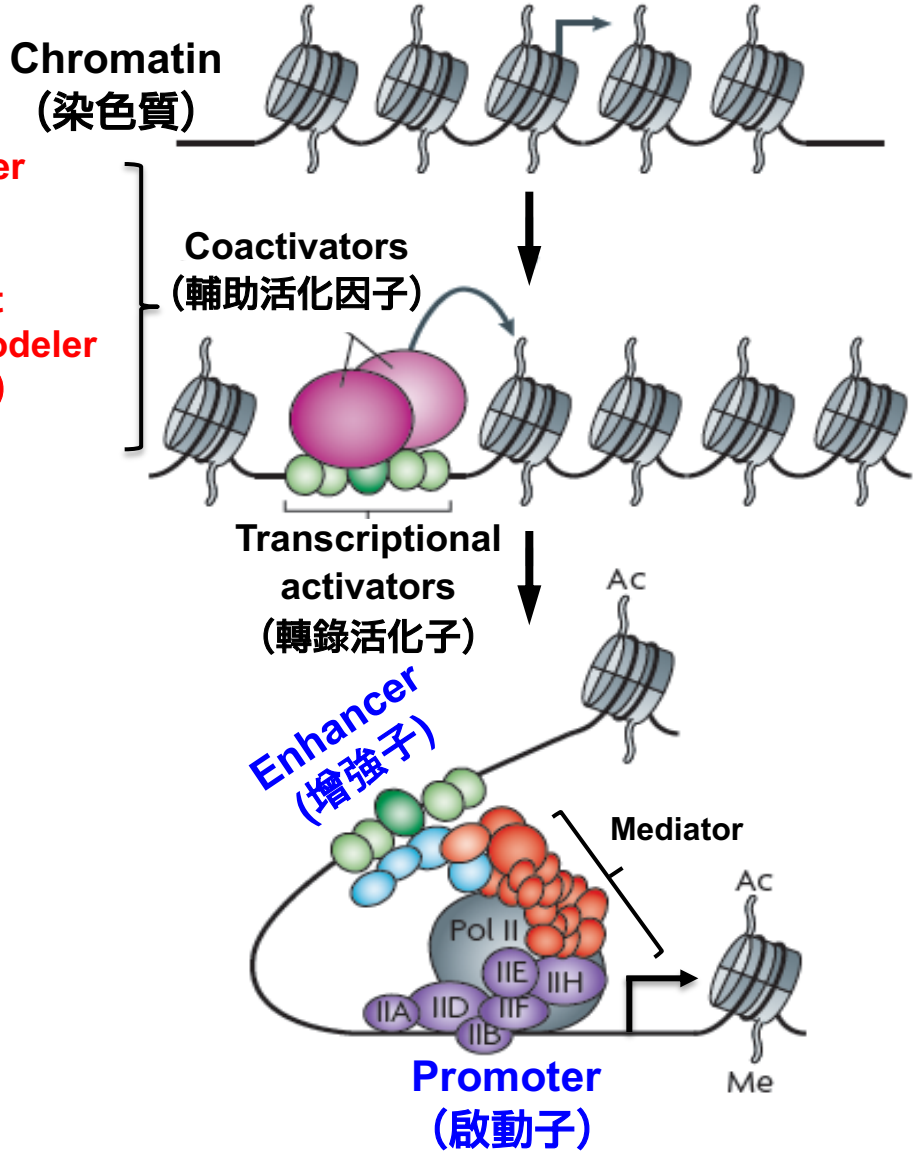
表觀遺傳學研究年表



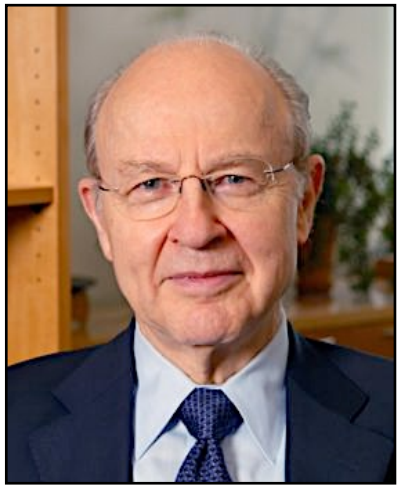
基因轉錄的啟動與作用機制

David Allis
(The Rockefeller University)

- **Histone modifier**
  (組蛋白修飾酶)
- **ATP-dependent chromatin remodeler**
  (染色質重塑蛋白)
- **Mediator**
  (仲介體)



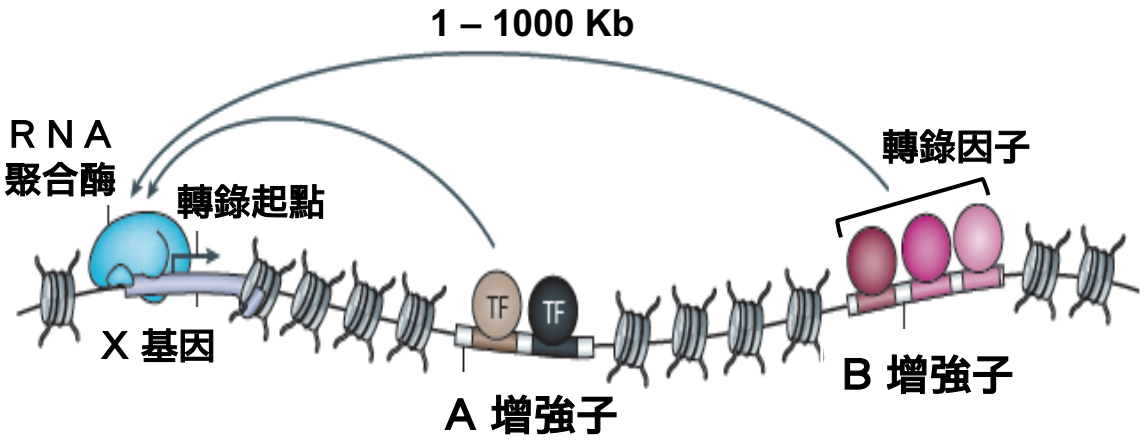
Robert (Bob) Tjian
(UC-Berkeley/HHMI)

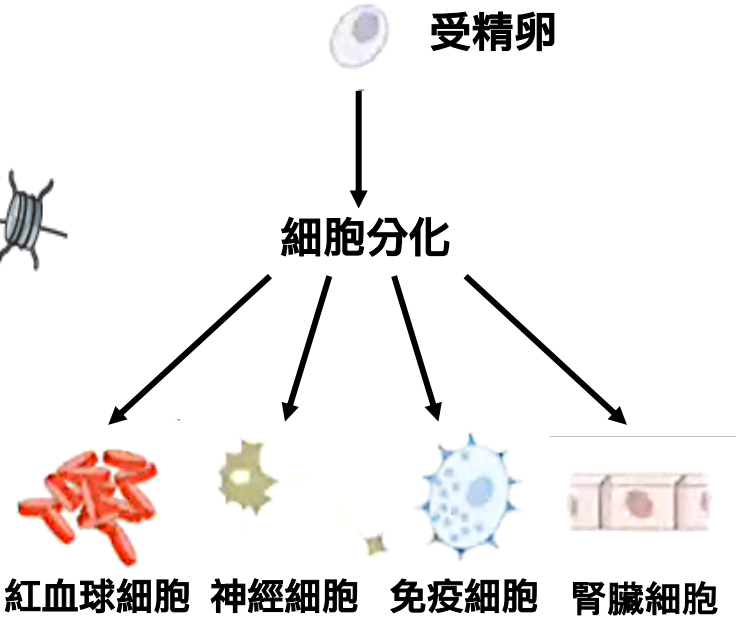


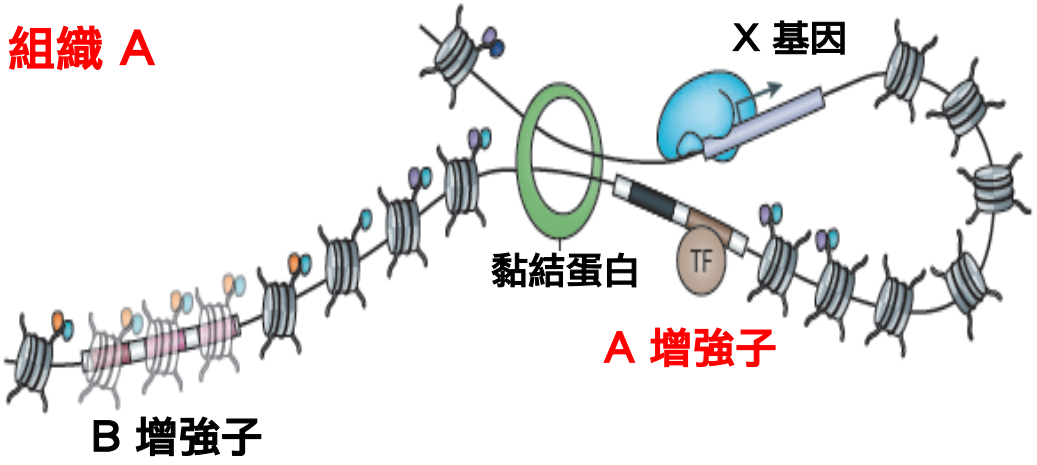
Robert (Bob) Roeder
(The Rockefeller University)

Adapted from Malik and Roeder (2010) ***Nat. Rev. Genet.***

啟動子決定獨特的基因表現型態

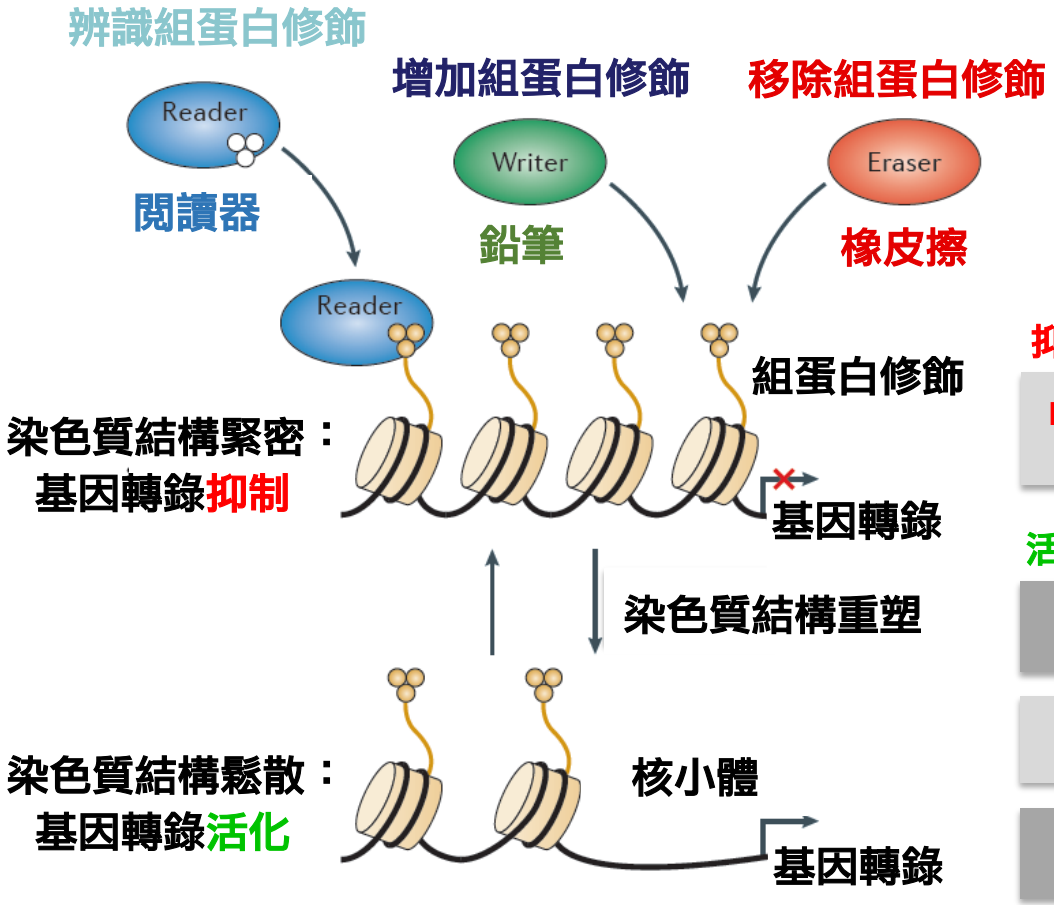


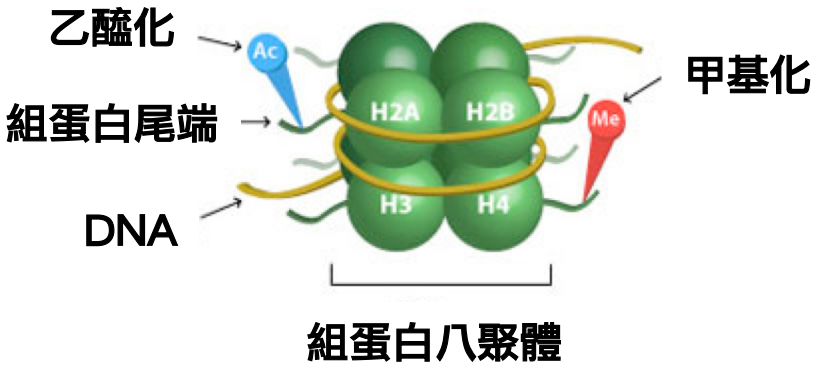




Adapted from Shlyueva/Stampfel/Stark (2014) ***Nat. Rev. Genet.***

組蛋白修飾與染色質結構有關



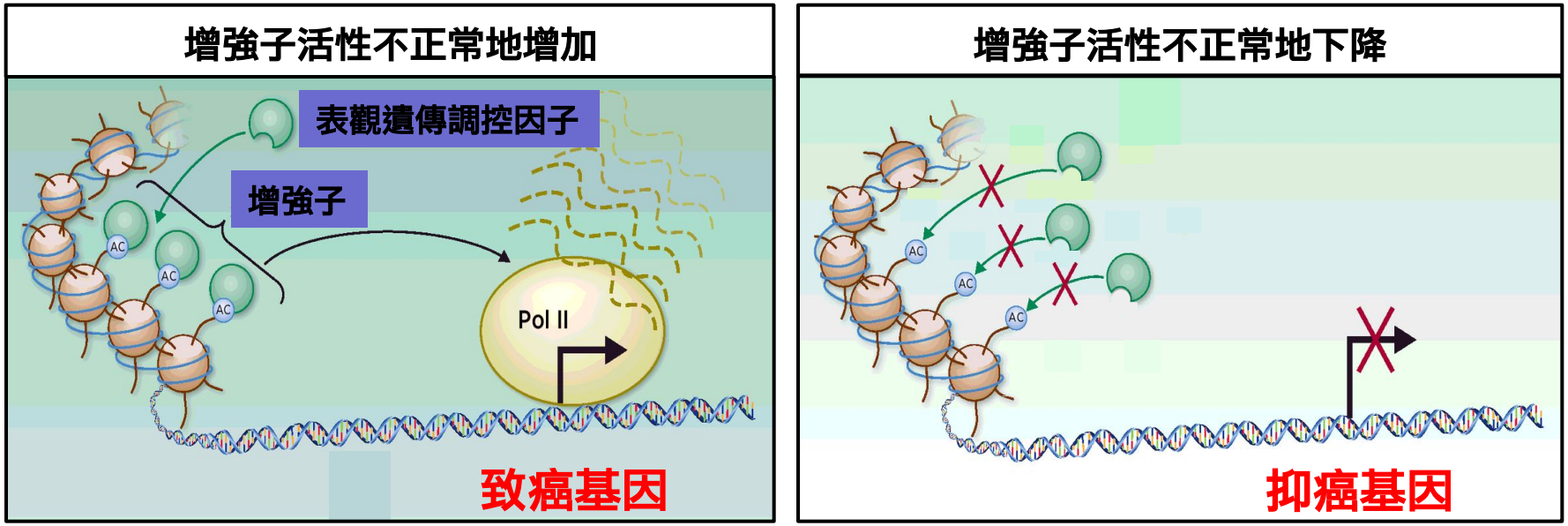


抑制型修飾	鉛筆	閱讀器	橡皮擦
H3K27me3	PRC2 (EZH2)	PRC1	UTX/Jmjd3

活化型修飾			
H3K27ac	p300/CBP	Bromodomain	HDACs
H3K4me3	SET1A/1B	PHD finger	PHF8
H3K4me1	MLL3/4	PHD finger	LSD1

增強子失調將導致不正常基因表現

增強子的活性一旦失去控制，可能導致腫瘤細胞的產生



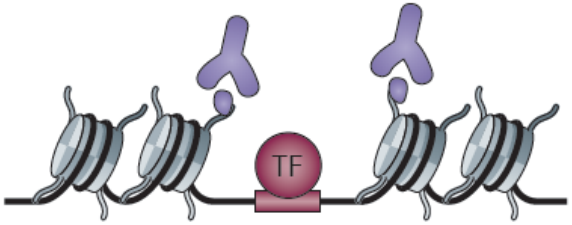
Modified from Evan/Evans (2017) *Clin. Cancer Res.*

調控增強子為基因療法開了一扇窗

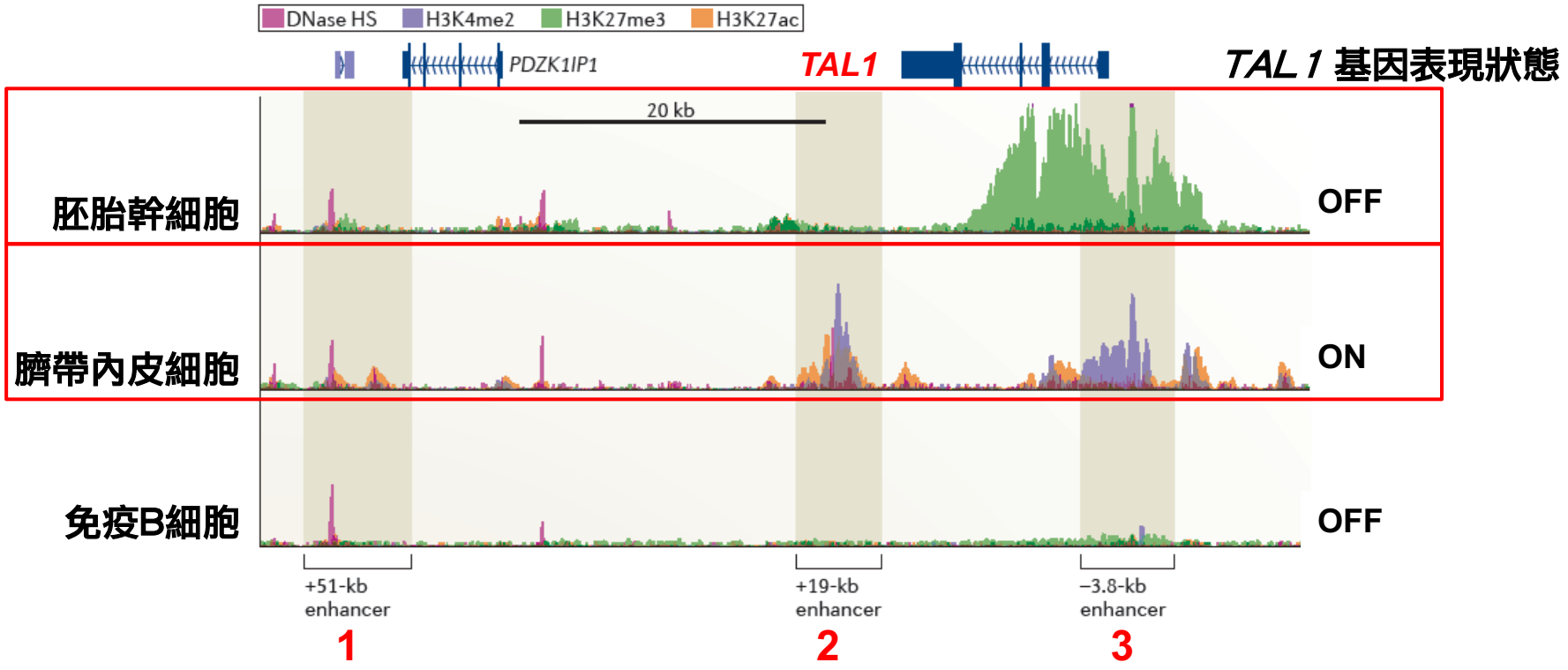
Modified from Willyard (2017) *Nature Med.*

染色質修飾型態可判定增強子活性

染色質免疫沈澱法

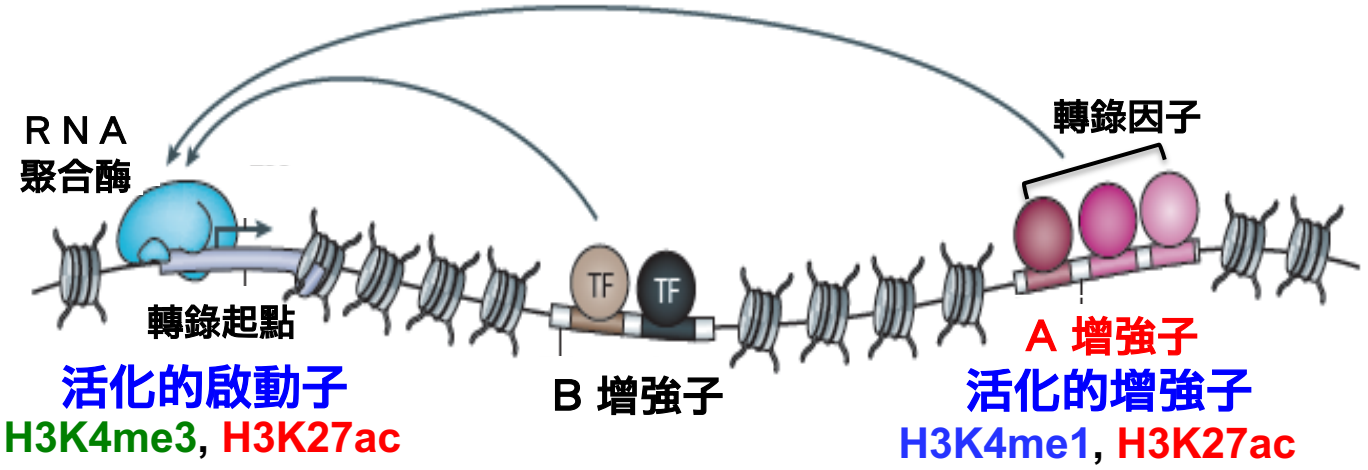


染色質修飾景象

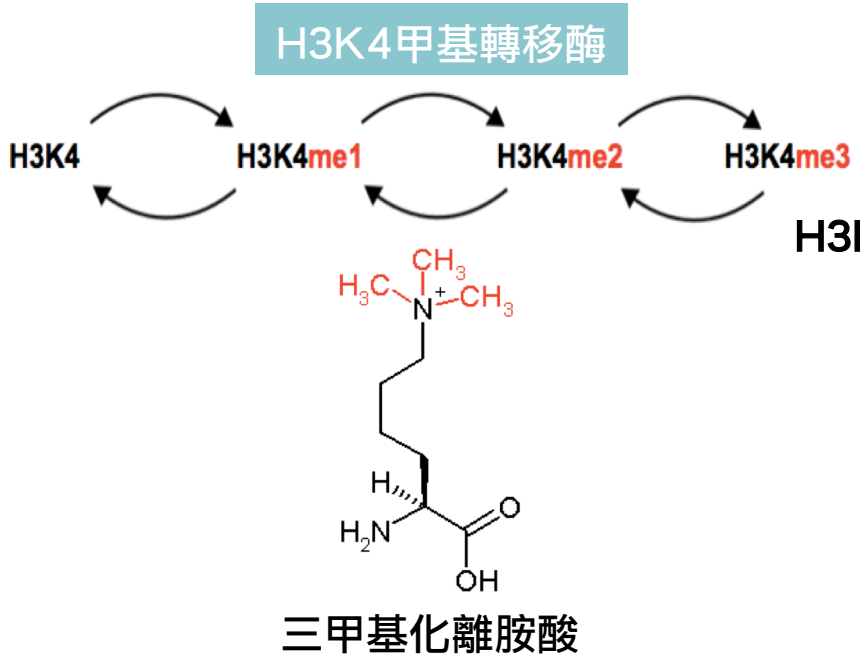


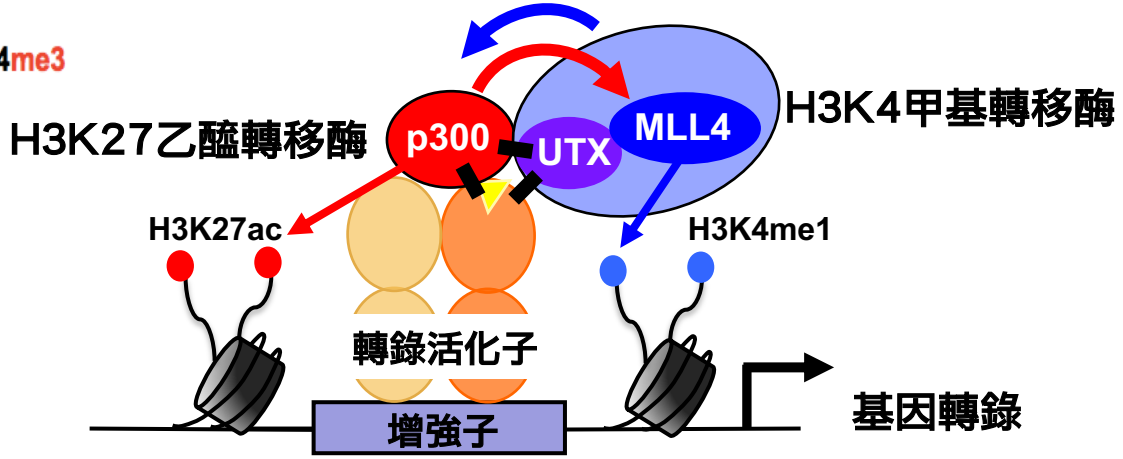
Adapted from Heinz/Glass (2015) *Nat. Rev. Mol. Cell Biol.*

H3K4me1與H3K27ac賦予增強子活性

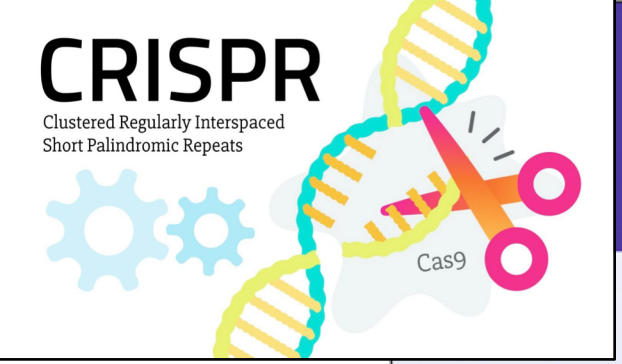


Adapted from Shlyueva/Stampfel/Stark (2014) *Nat. Rev. Genet.*





Wang/Roeder (2017) ***Molecular Cell***, 67:308–321.



How CRISPR works

1. Cas9蛋白與引導RNA形成複合體

Cas9

2. 此複合體可攻擊與引導RNA互補之基因體DNA

Guide RNA

3. 此複合體可接續切斷雙股DNA

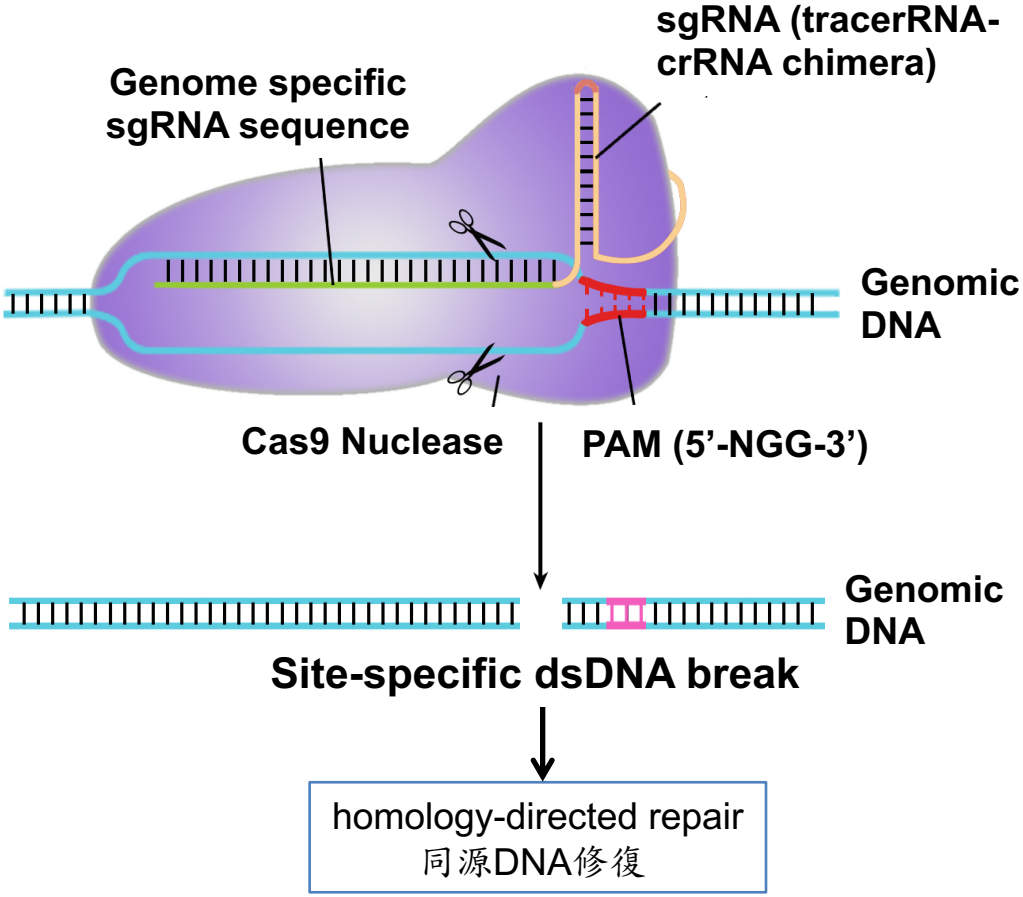
Programmed DNA

4. 經設計之DNA序列可以插入並取代斷裂的DNA序列

Credit: *MRS Bulletin*

利用RNA引導基因組編輯之基因療法

源起於細菌切斷外來 (噬菌體) DNA 的一種免疫策略，設計出具有基因專一性的 RNA去引導 DNA 剪切酶 Cas9 找到並切斷目標基因，然後修復其突變。



CRISPR: 群聚且有規律間隔的短回文重複序列 (clustered regularly interspaced short palindromic repeats)

The gRNA (引導RNA) guides the Cas9 nuclease (DNA 剪切酶) to the target sequence (標的基因). There, the Cas9 generates a double strand break, which then stimulate error-prone nonhomologous end joining or homology-directed repair. (斷裂再修復)

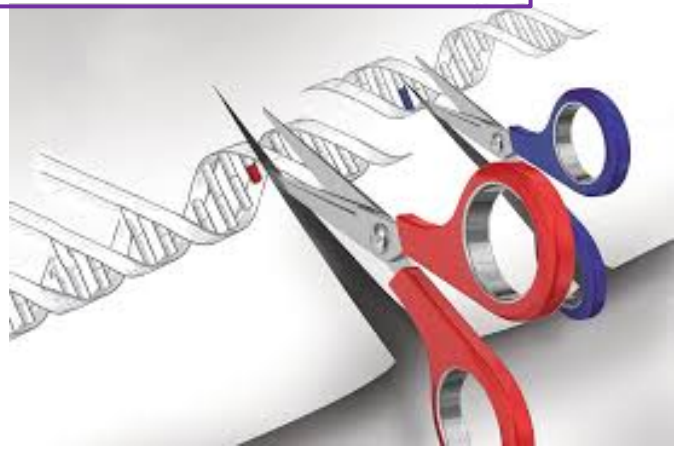
唐獎 2016：Genome editing

Jennifer Doudna

Emmanuelle Charpentier

Feng Zheng (張峰)

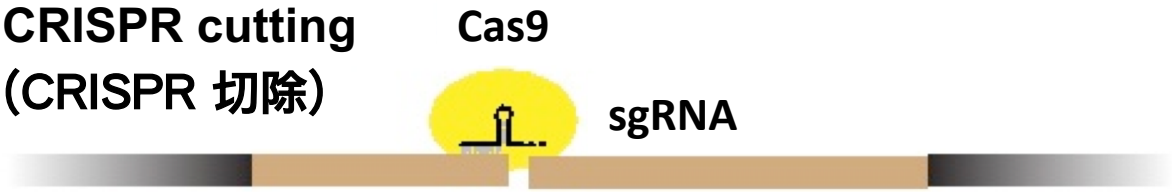
諾貝爾獎 2020



利用RNA的基因療法

以CRISPR為基礎之基因轉錄療法

A. CRISPR cutting
(CRISPR 切除)

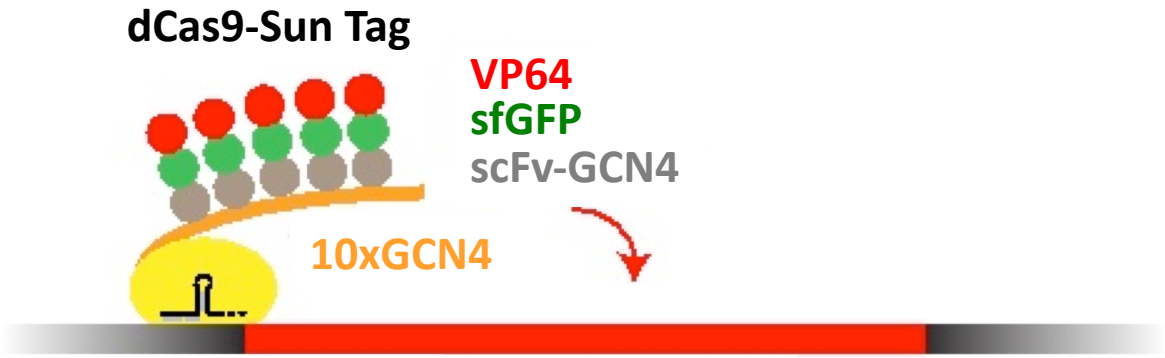


基因編輯 / 基因去活化

B. CRISPRi (interference)(CRISPR 基因轉錄抑制)

啟動子/轉錄起點 基因轉錄抑制

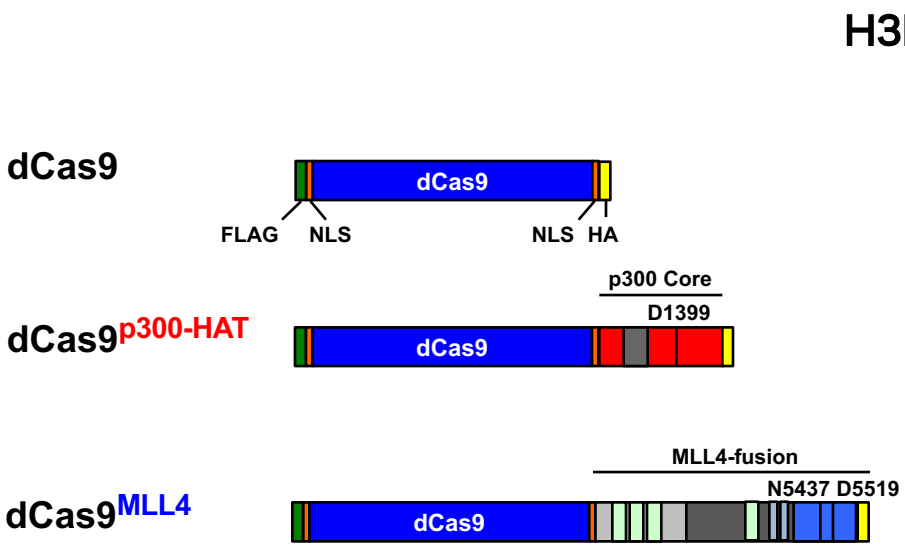
C. CRISPRa (activation) (CRISPR 基因轉錄活化)

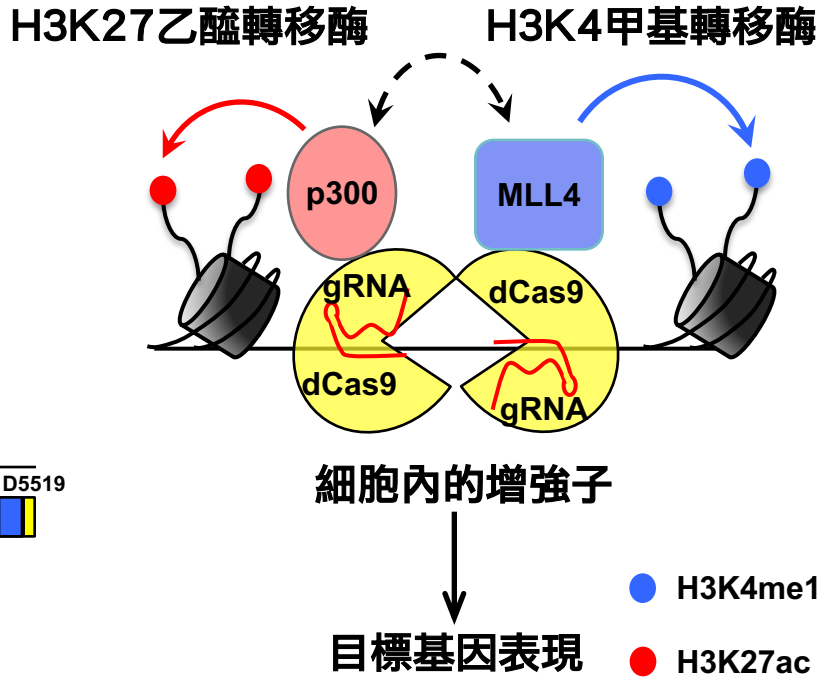


啟動子/轉錄起點 基因轉錄活化

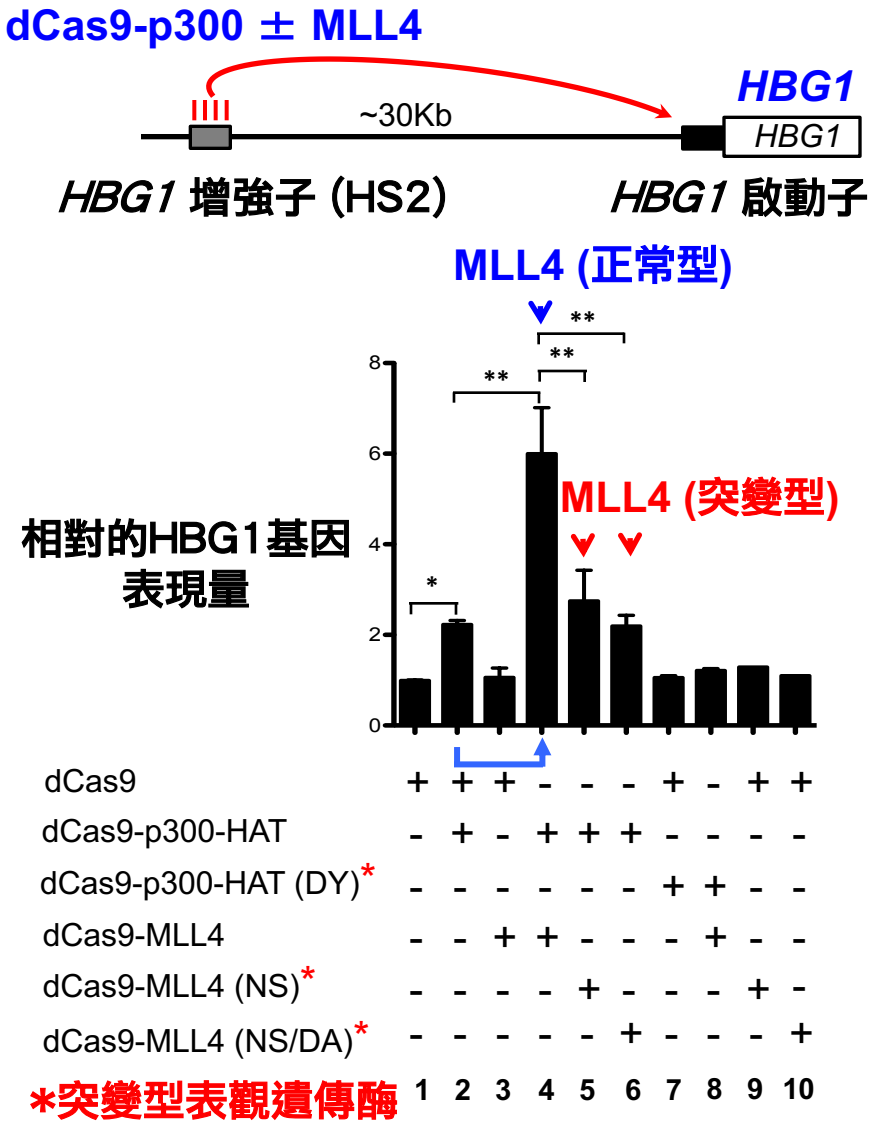
Adapted from Dr. Martin Kampmann, NCI, Office of Cancer Genomics

CRISPR-dCas9染色質修飾編輯技術





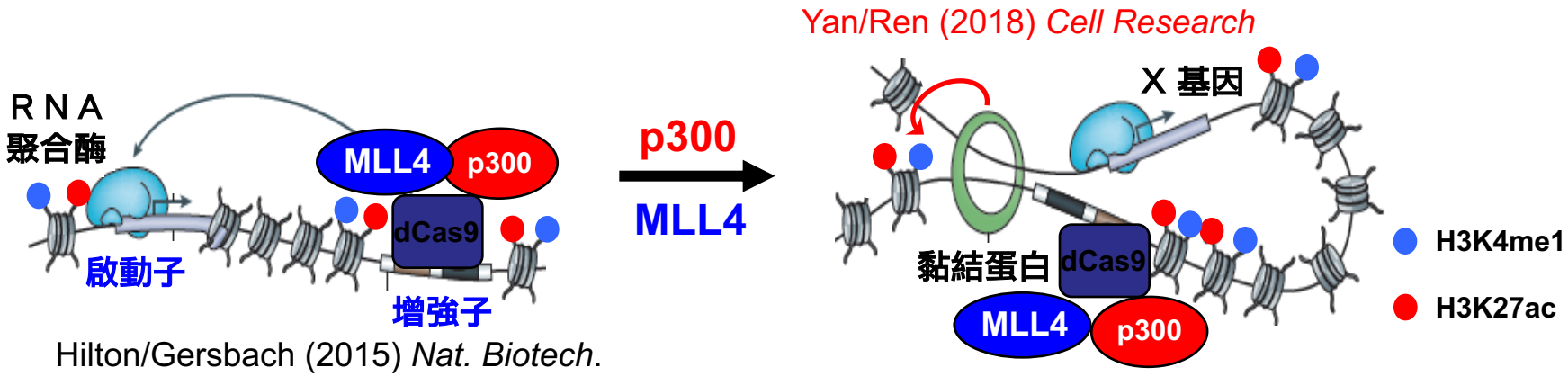
增強子染色質修飾誘發目標基因表現



Wang/Roeder (2017) *Molecular Cell*

CRISPR-dCas9編輯技術應用與展望

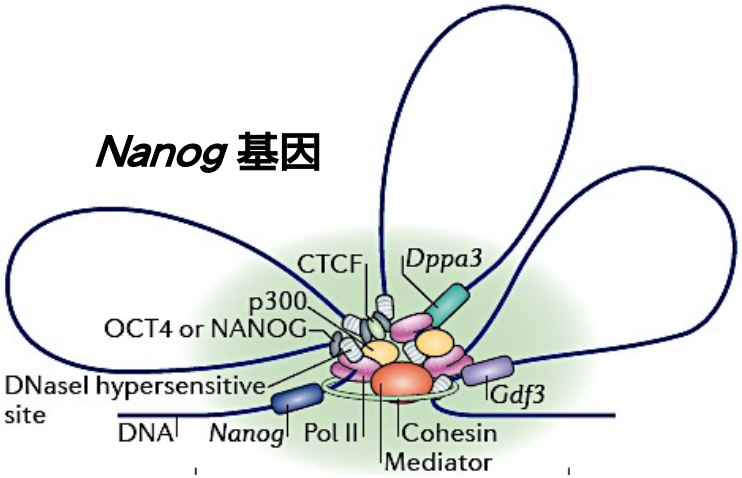
- 增強子獲得特定的染色質修飾 (H3K4me1, H3K27ac) 可能提供結合位點, 使得黏結蛋白可以被吸引過來而誘發增強子與啟動子形成環形結構.

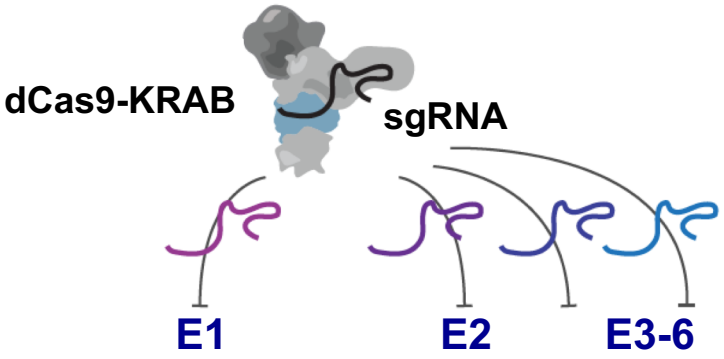


Hilton/Gersbach (2015) *Nat. Biotech.*

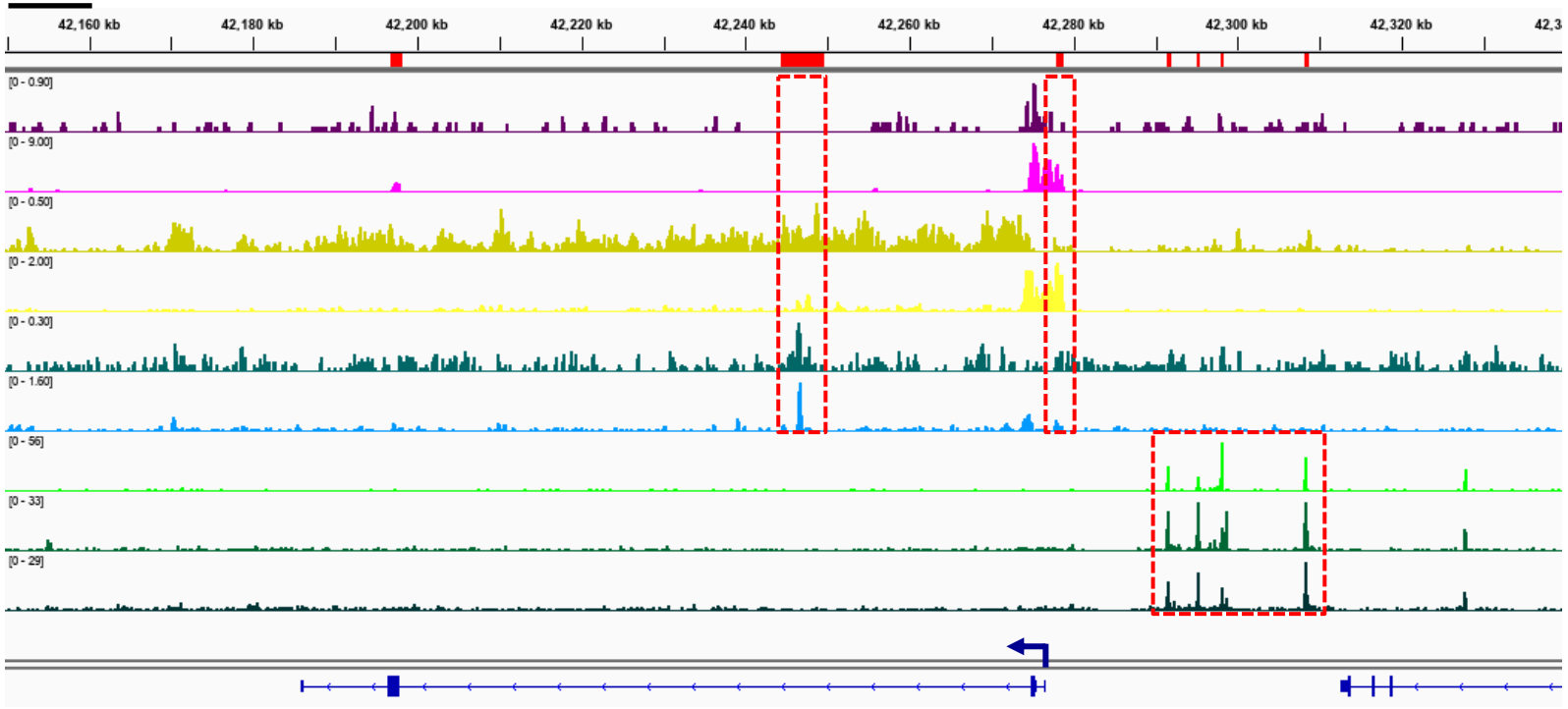
CRISPR-dCas9染色質修飾編輯技術:

CRISPRi 基因轉錄抑制檢測增強子

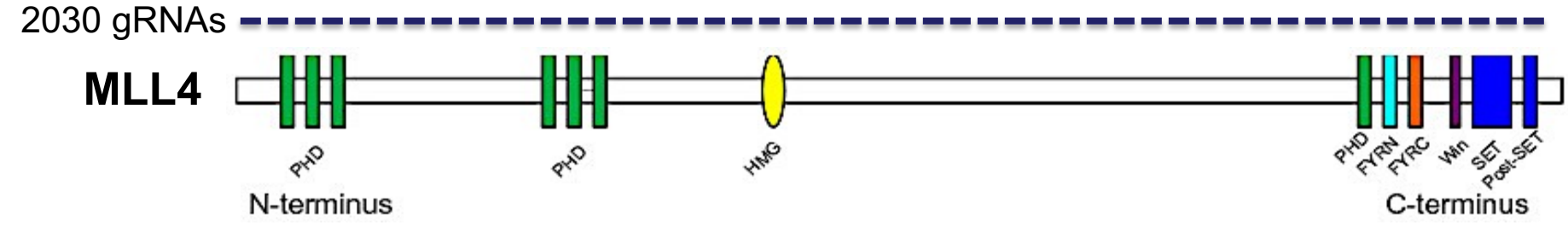


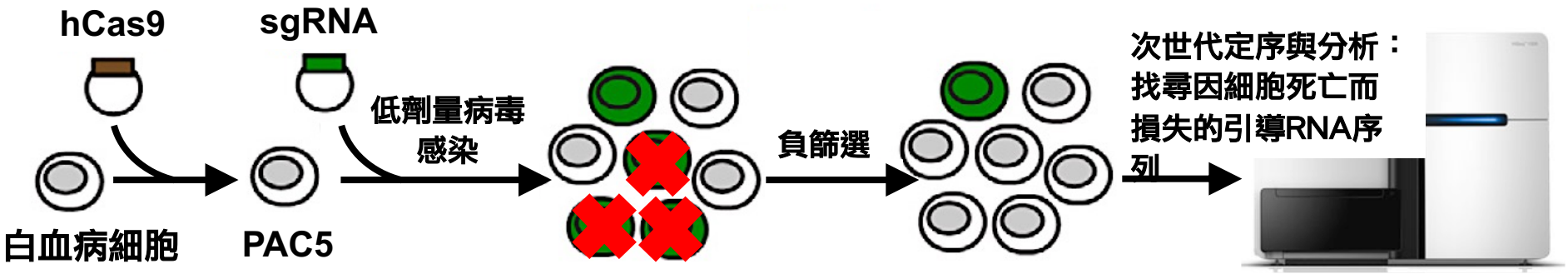


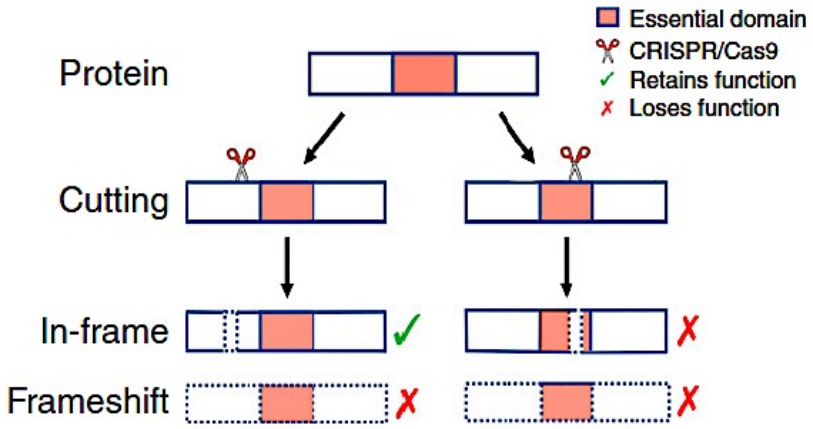
老鼠基因體 20 Kb

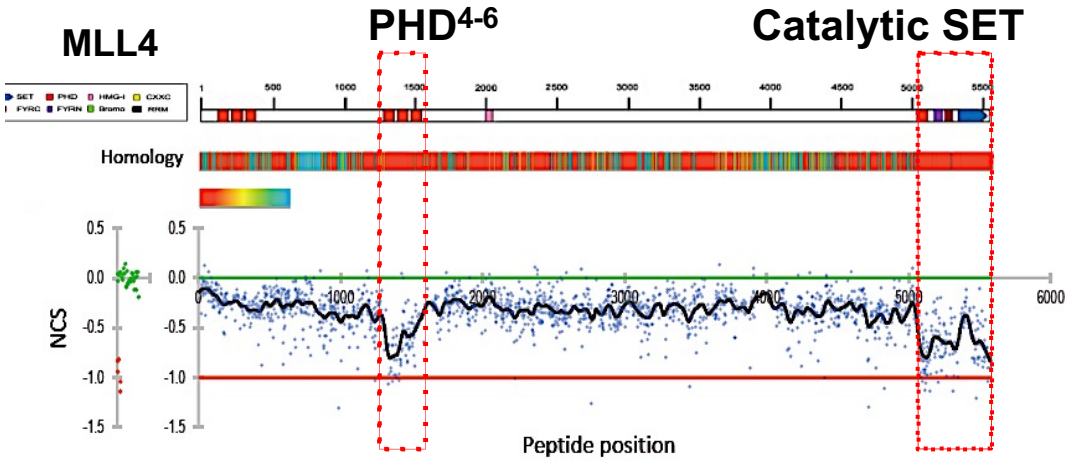


CRISPR技術搜尋功能性蛋白區域



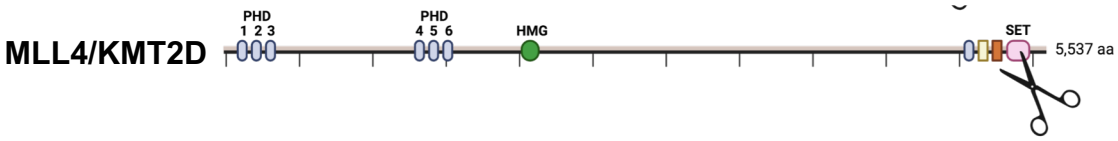


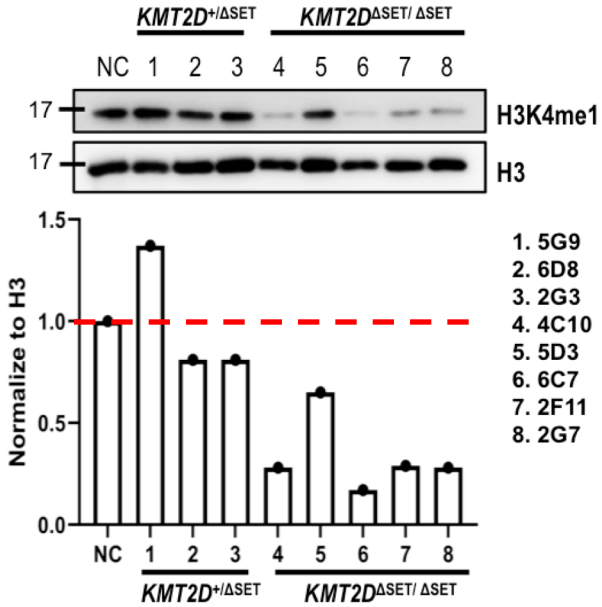




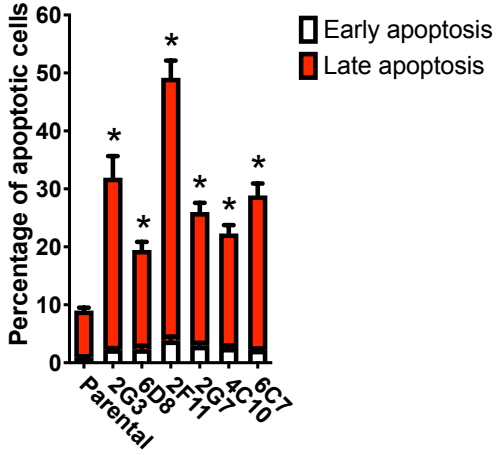
以CRISPR基因編輯破壞MLL4組蛋白修飾功能

CRISPR基因編輯

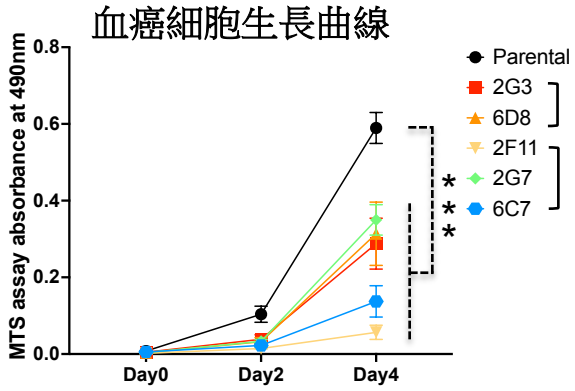




血癌細胞凋亡程度



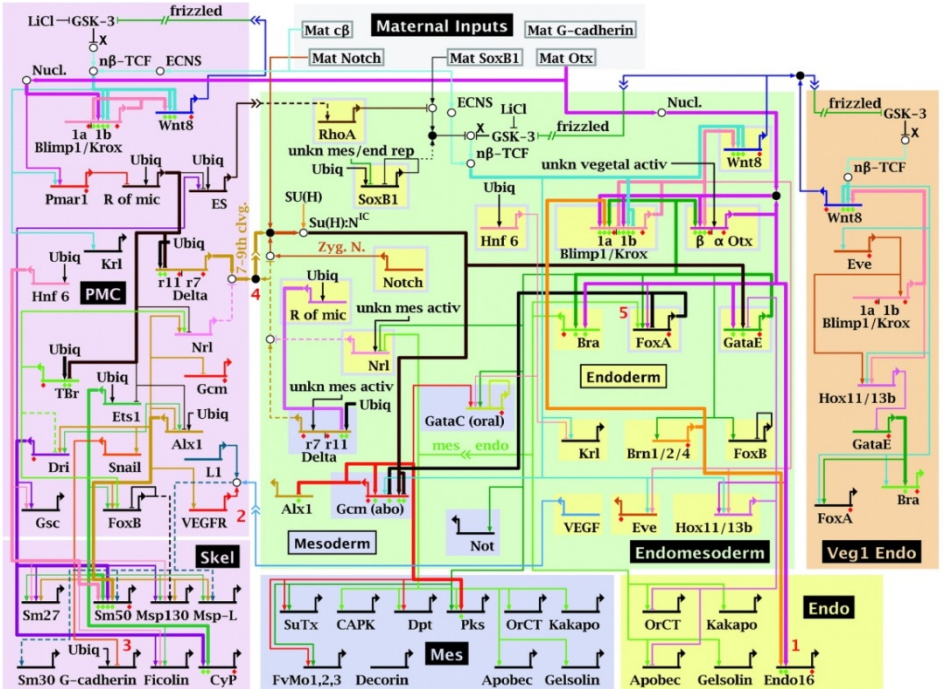
血癌細胞生長曲線



基因調控網路

Gene Regulation Network

基因調控網路如同電路板 circuit



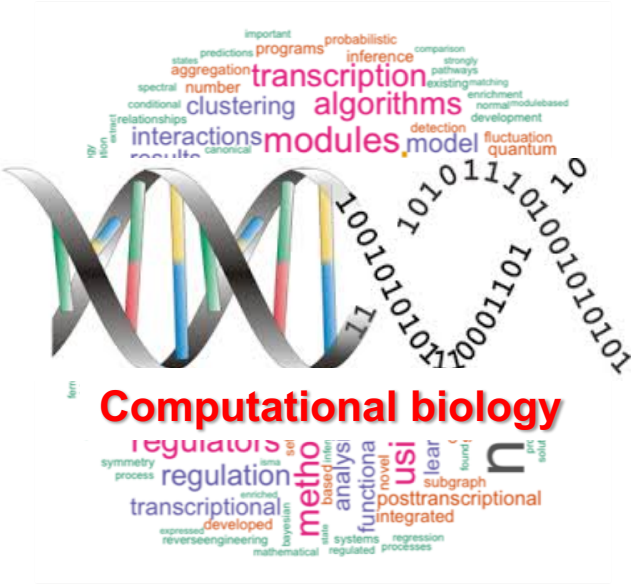
Ann Rev Biophy. and Biomol. Struct. (2007) 36: 191-212

~21000 genes + 無數的 調控 RNA

Systems biology 系統生物學

computational and mathematical modeling of complex biological systems

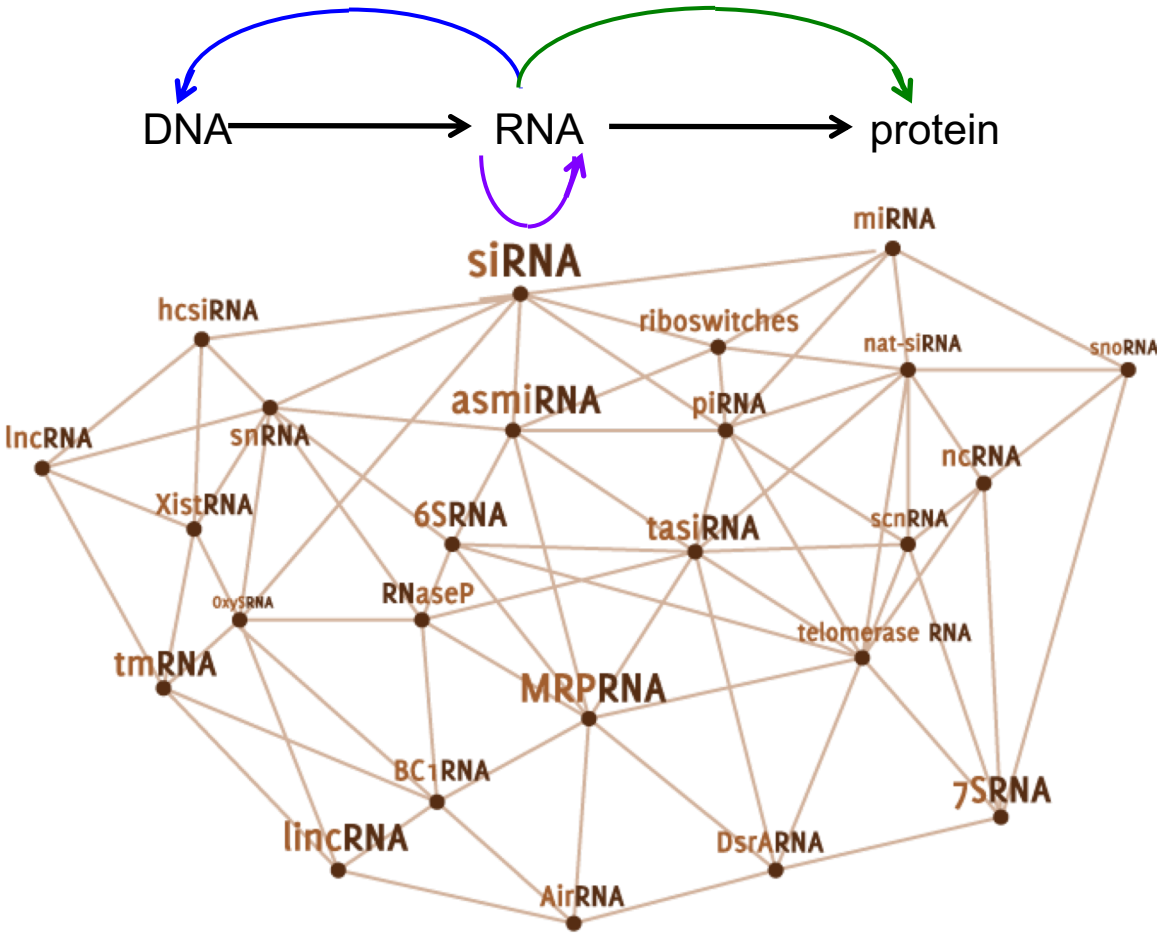
遺傳、分子及細胞生物、分子演化、基因及蛋白體、結構生物、生物資訊(資料庫結構與設計、計算生物演算法、分子模擬)



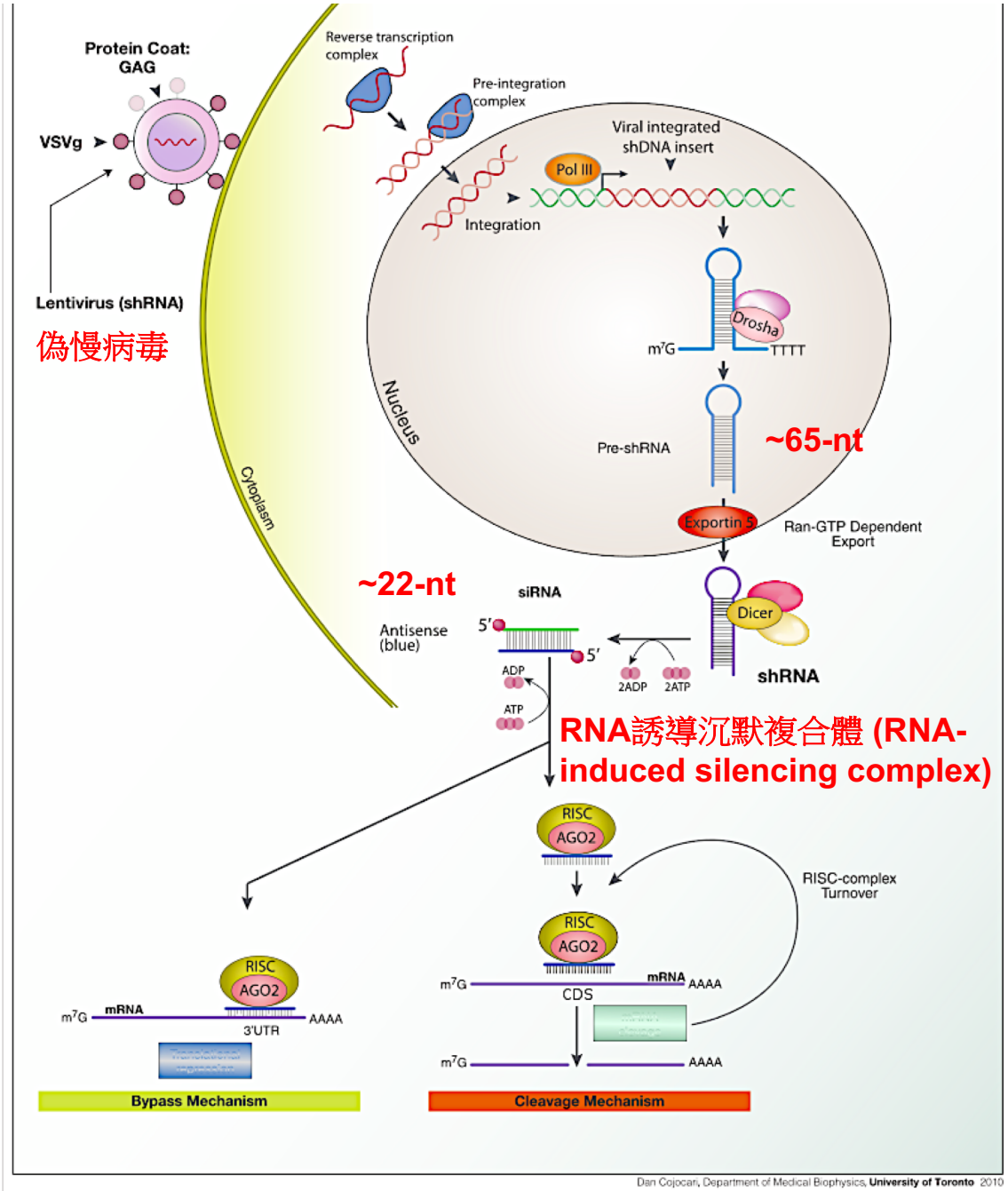
基因表達: 轉錄

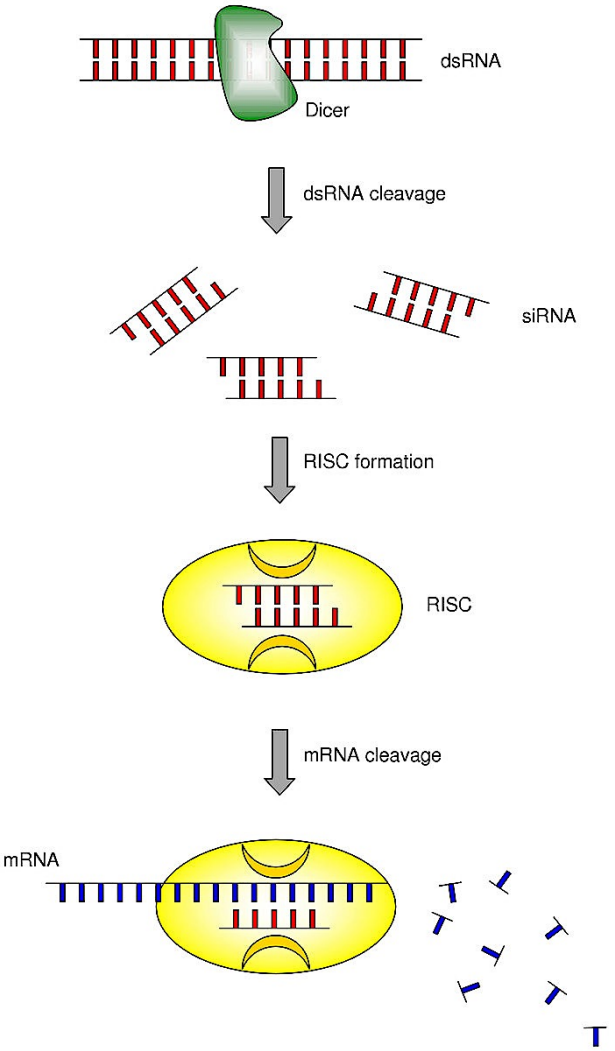
調節性非編碼 RNA

- From bacteria to human
- Influence DNA (epigenetics and transcription), RNA (stability …), protein (function)

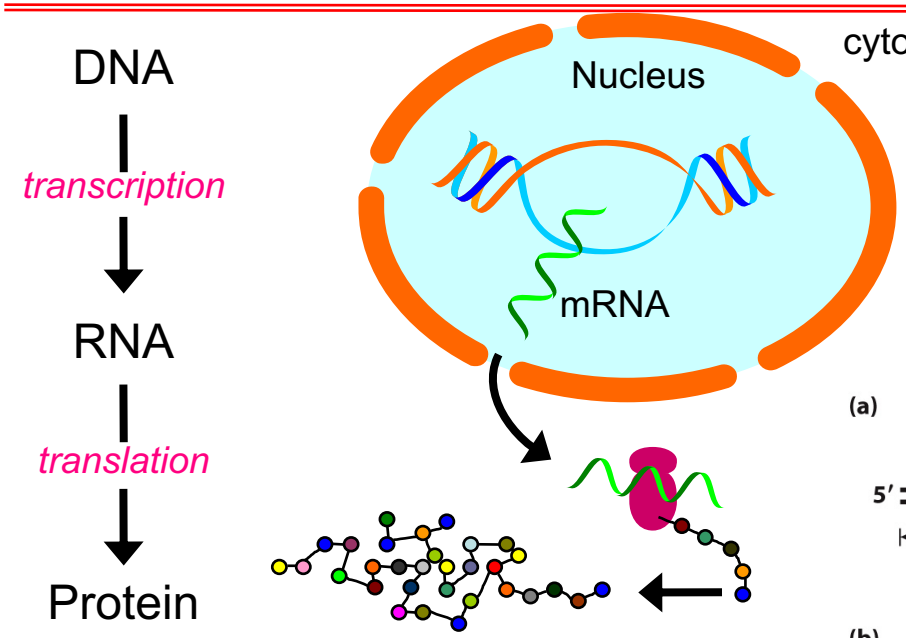


偽慢病毒遞送設計的shRNA和在哺乳動物細胞中的RNA干擾的機制

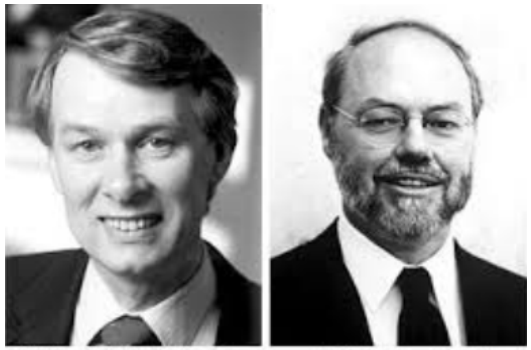




真核生物 mRNA 的剪接 (splicing)



Discovered intron in 1977; Nobel prize in 1993



exon (外顯子): 構成 mRNA 的非轉譯區及蛋白產生 coding 區(轉譯區)
intron (內含子): 在剪接過程中從 RNA 中被移除，不會被送到細胞質

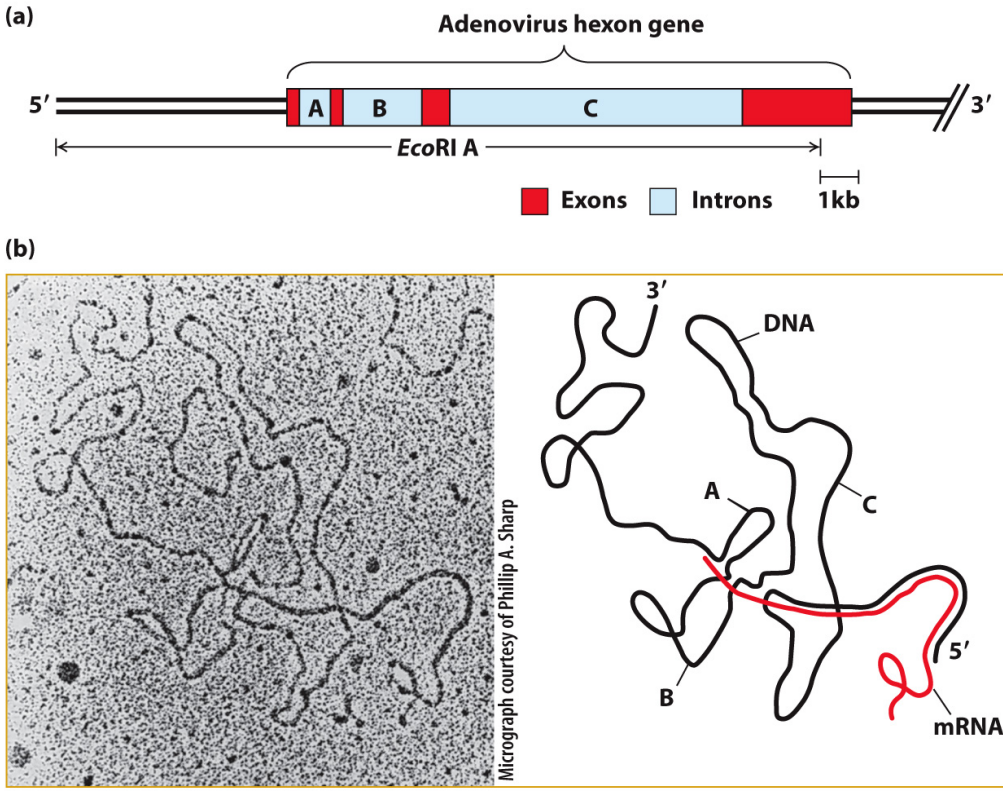
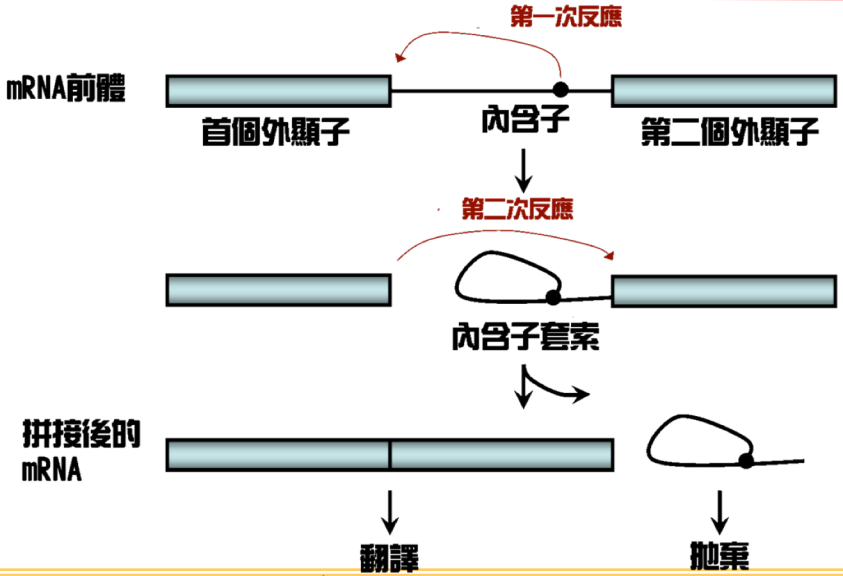


Figure 10-6
Molecular Cell Biology, Eighth Edition
© 2016 W. H. Freeman and Company

RNA選擇性剪接: 增加基因表達複雜度



在不同種類之肌肉細胞中，甲型原肌球蛋白進行不同的剪接型式，產生不同的 mRNA (isoforms)。

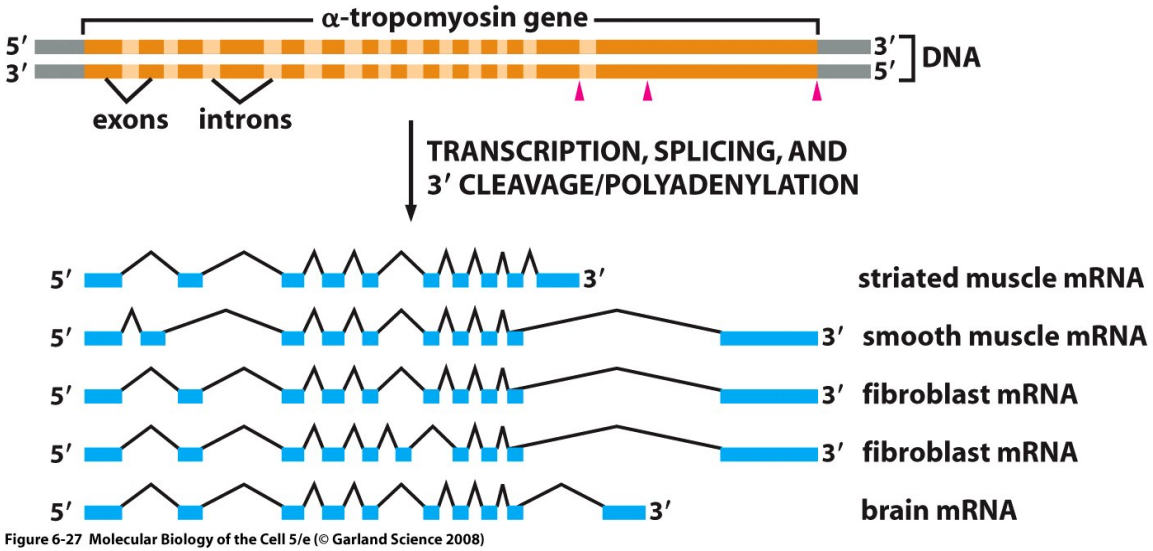
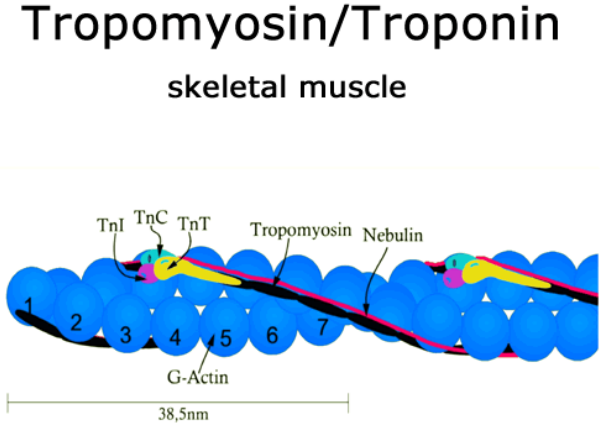


Figure 6-27 Molecular Biology of the Cell 5/e (© Garland Science 2008)



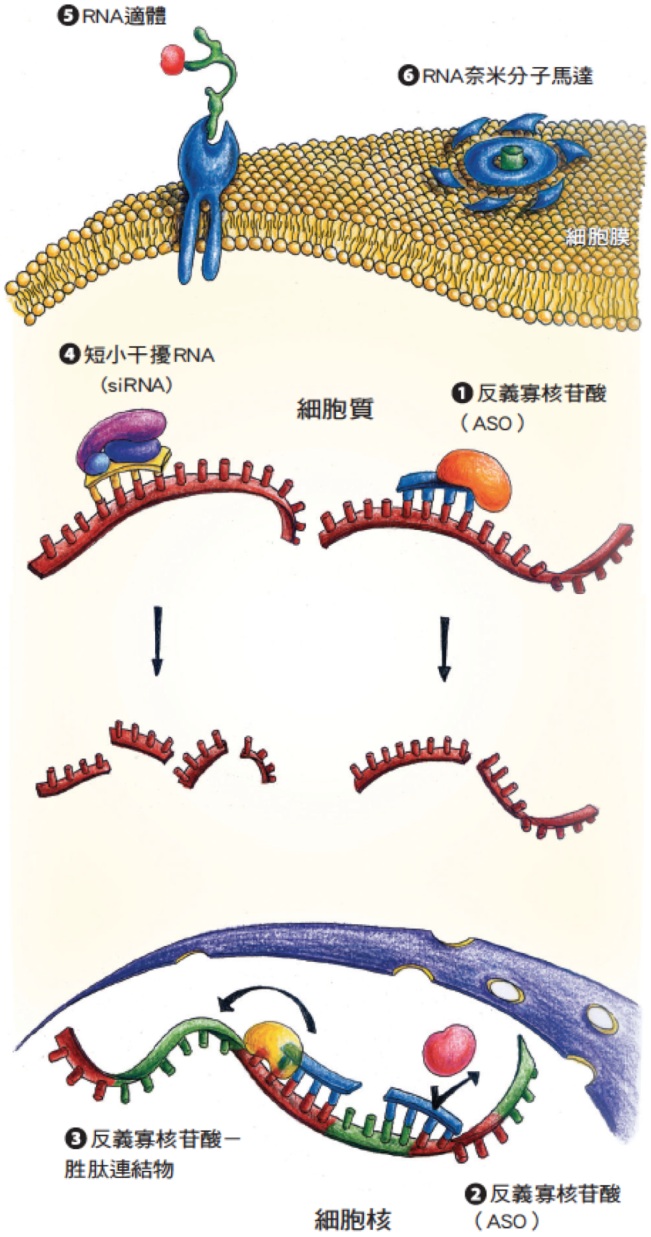
Model of a muscle thin filament showing the localization of laterally aligned tropomyosin in association with the troponin complex.

RNA 藥物

RNA未來醫療新勢力

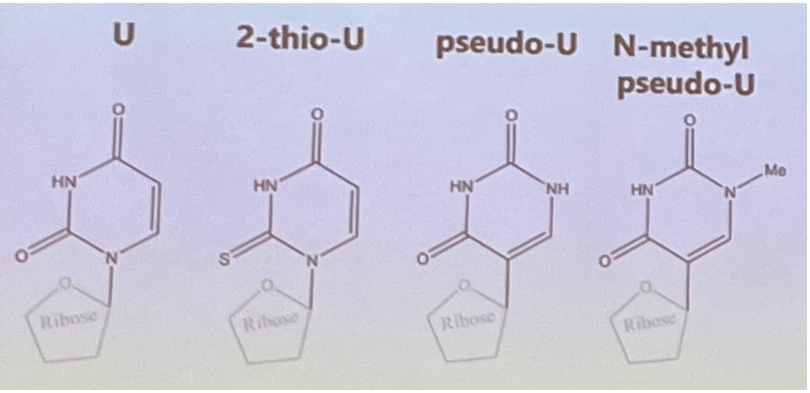
科學人 2014年 9月 (譚婉玉)

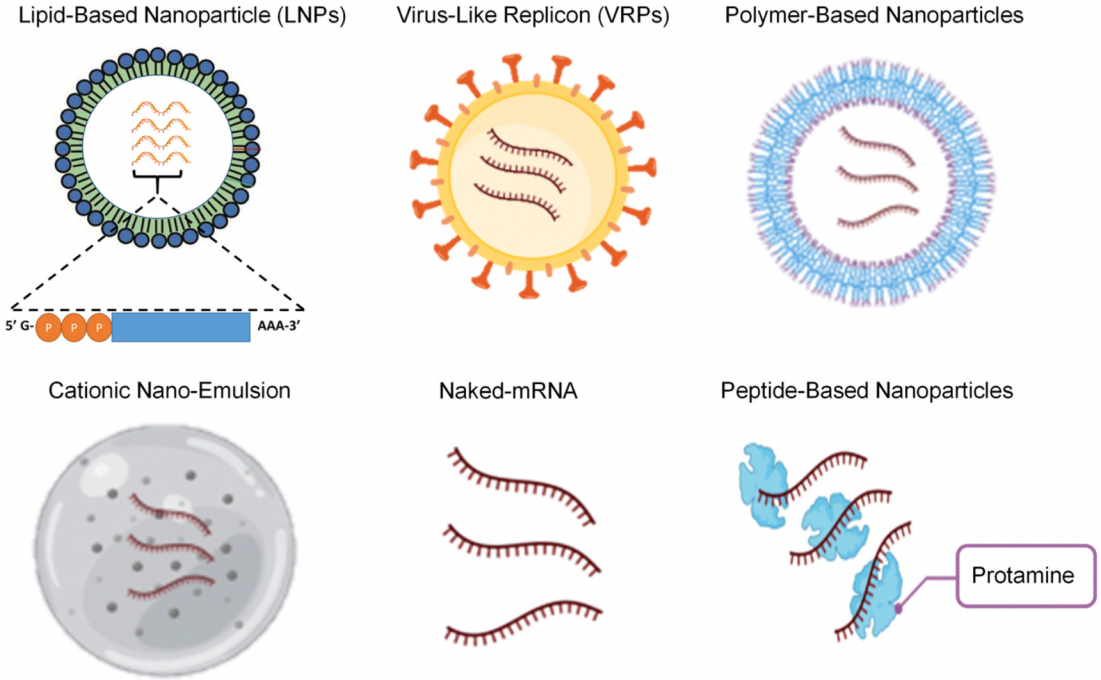
- 反義寡核苷酸 (ASO): antisense oligo 結合到致病蛋白質的mRNA後，分解該mRNA (1)
- ASO 結合到致病mRNA的 precursor 前驅分子，干擾或促進剪接 (2,3)
- 干擾RNA (siRNA) 配對到mRNA上並分解該mRNA (4)
- RNA適體 (aptamer) 可辨識並結合到特定細胞表面抗原: 把一個毒素分子連接上此RNA適體，則有可能把致病細胞摧毀 (5)
- RNA奈米分子馬達 (nano-motor) 可做為載體把藥物攜帶進入致病細胞內 (6)



mRNA疫苗的開發

以修飾過的尿嘧啶取代未修飾的尿嘧啶



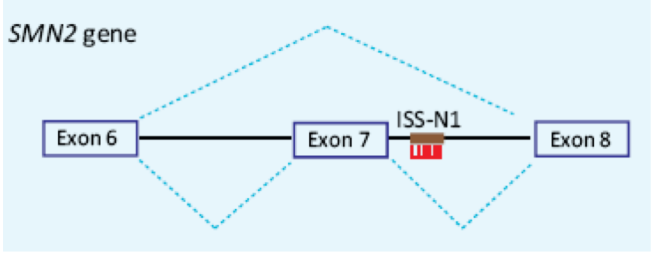


遺傳疾病: mRNA 剪接錯誤會造成的神經退化疾病

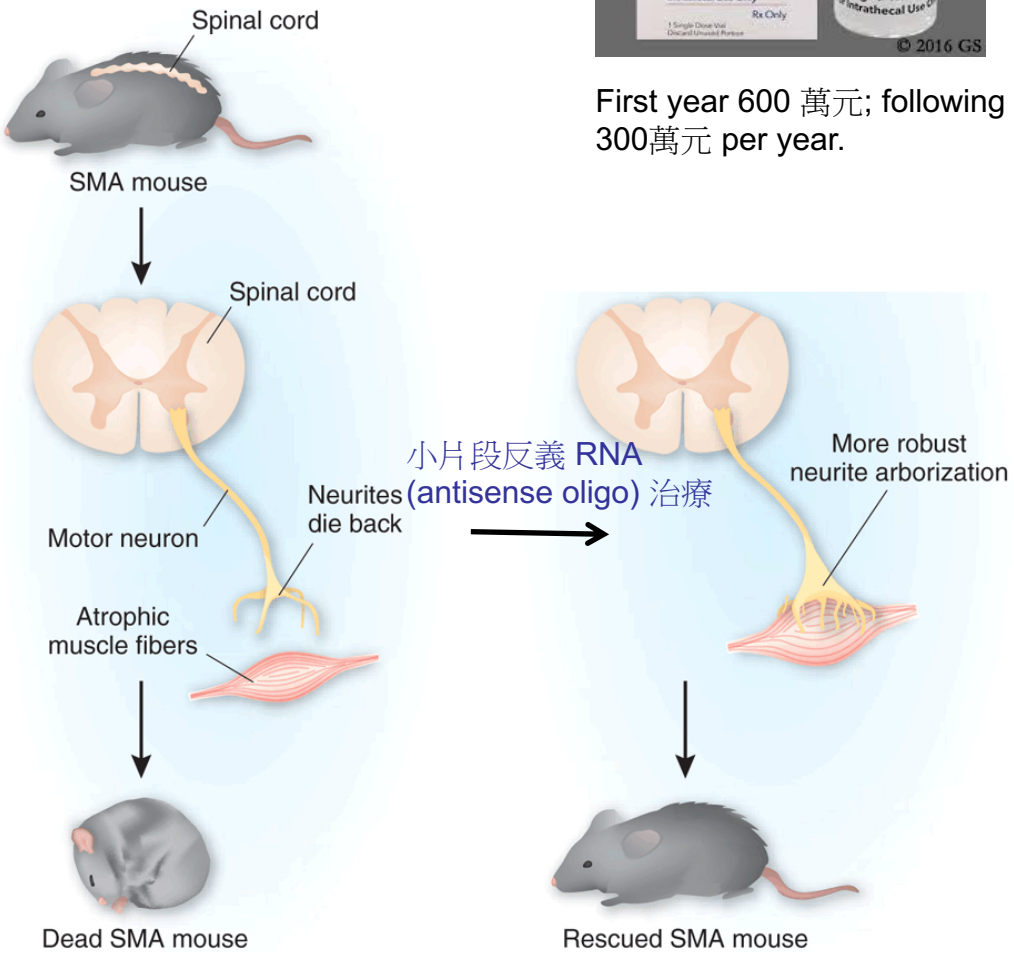
脊髓性肌肉萎縮症 Spinal Muscular Atrophy

隱性遺傳疾病，因先天的基因缺陷，導致脊髓前角運動神經細胞之衰亡與退化，運動神經訊息傳遞中斷，進而造成肌肉逐漸軟弱無力與痲痺，大多數患孩 2歲前即會因呼吸衰竭而死亡。

SMN2 基因的選擇性剪接反應有缺陷造成 exon 7 skipping，恢復 SMN2 的剪接可幫助治療。
antisense oligonucleotide (ASO) therapy: 2016



First year 600 萬元; following 300萬元 per year.



問題與討論