模式生物及模式生物技术

费 俭

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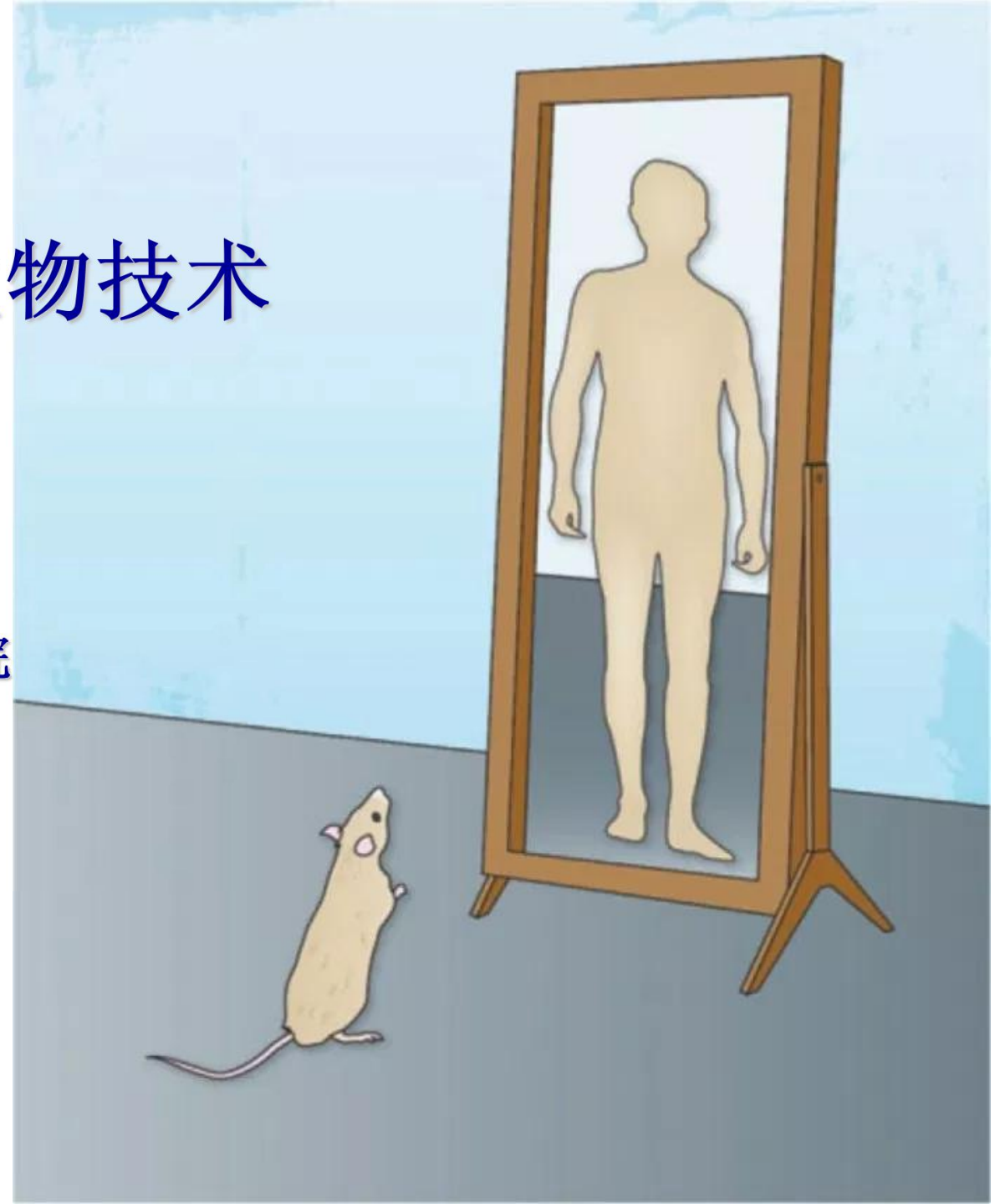
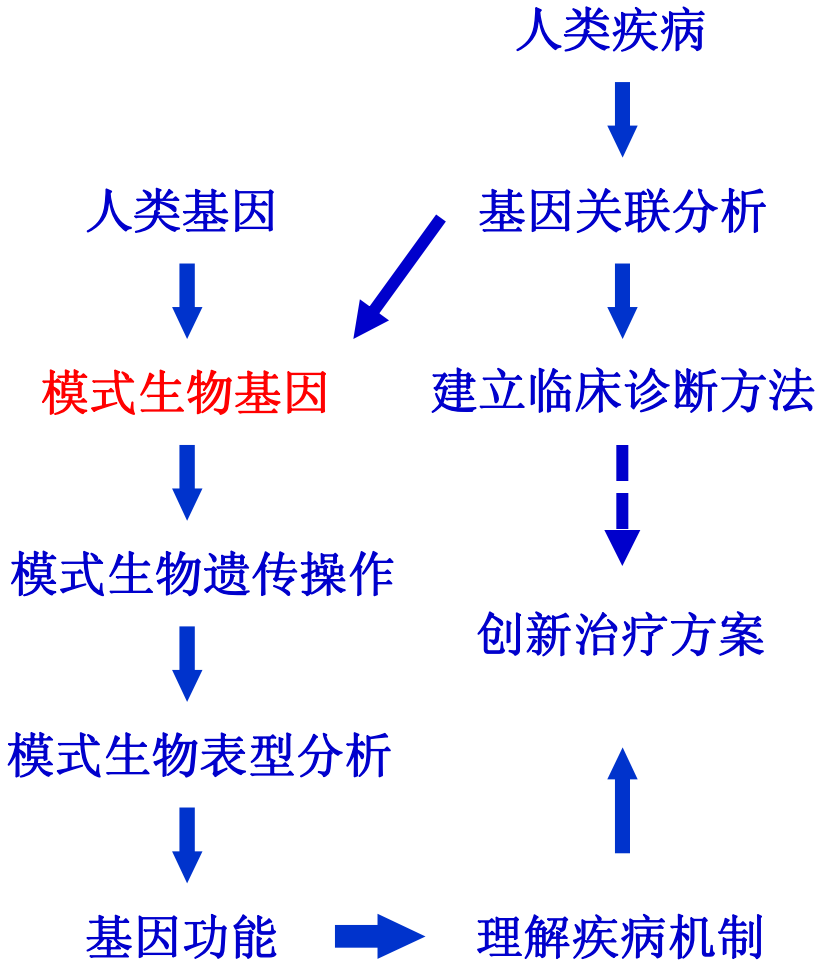


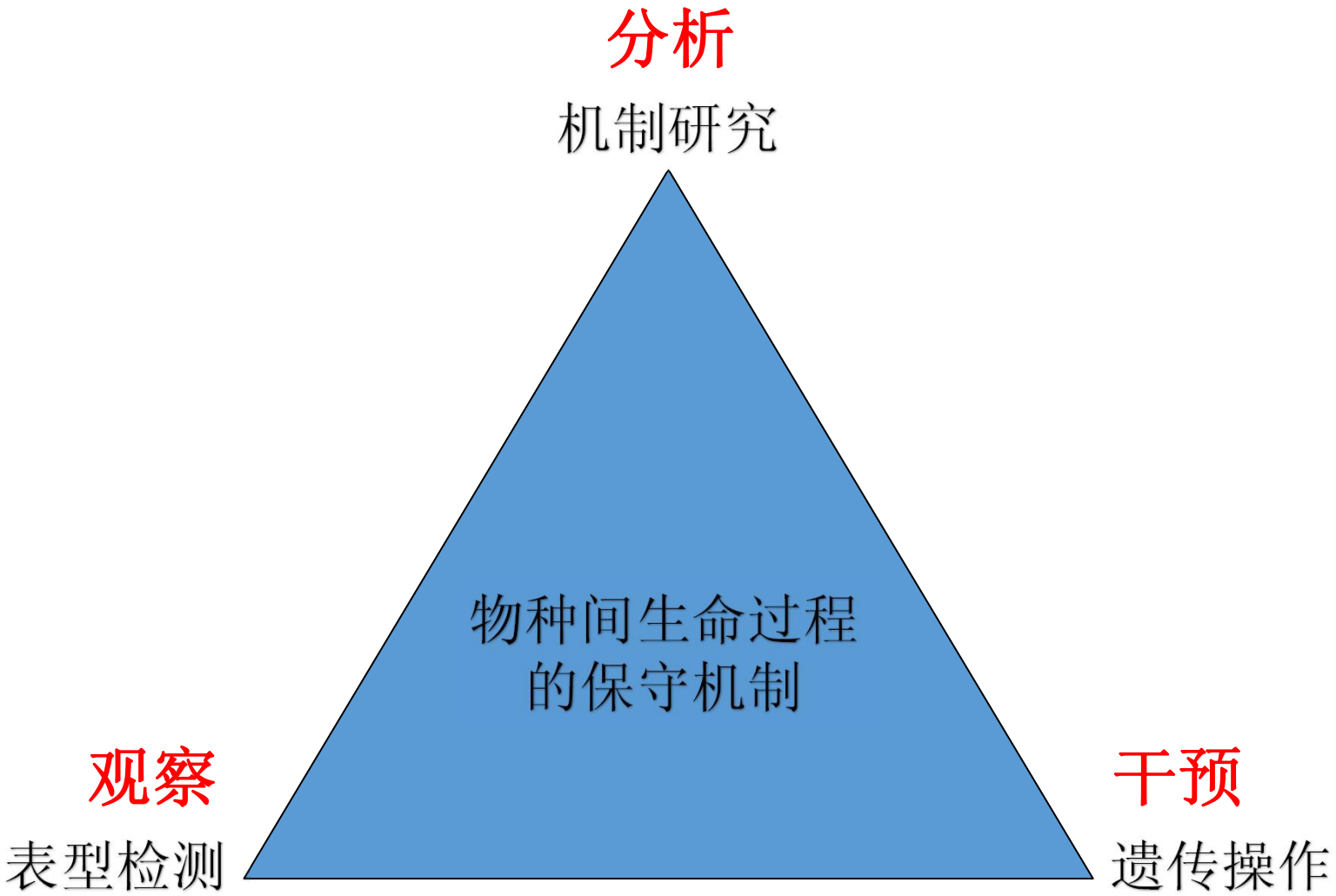
解码生命：后基因组时代”的研究重点

2000年6月26日，六国联合宣布人类基因组草图完成

今天，我们正在学习上帝创造生命时使用的语言，并且正在以前所未有的眼光审视着万物之灵的人类。我们将能够更加细致入微地领略人类自身的复杂和美丽。在获得这深奥的新知识后，人类将获得全新庞大的治疗疾病的力量。



模式生物研究的思想方法



什么叫模式生物（Model organism）

生命科学研究中采用的生物活体模型，用于研究和阐明(一类)生命活动的基本规律或者人类生理病理过程。

生命基本规律在生物进化过程中的保守性，奠定了模式生物研究策略的基础。

豌豆杂交实验奠定了遗传学百年发展的基石

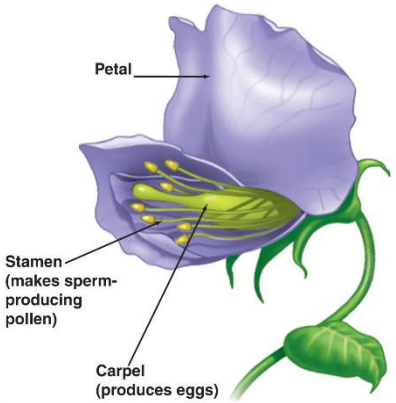
孟德尔(Gregor Johann Mendel)（1822年7月22日-1884年1月6日)是“现代遗传学之父”。他用豌豆杂交实验揭示了生物遗传奥秘的基本规律。人们分别称他的发现为“孟德尔第一定律”和“孟德尔第二定律”。实际上，他用豌豆做研究的形式就是一种利用模式生物的方式。

实验植物的选择

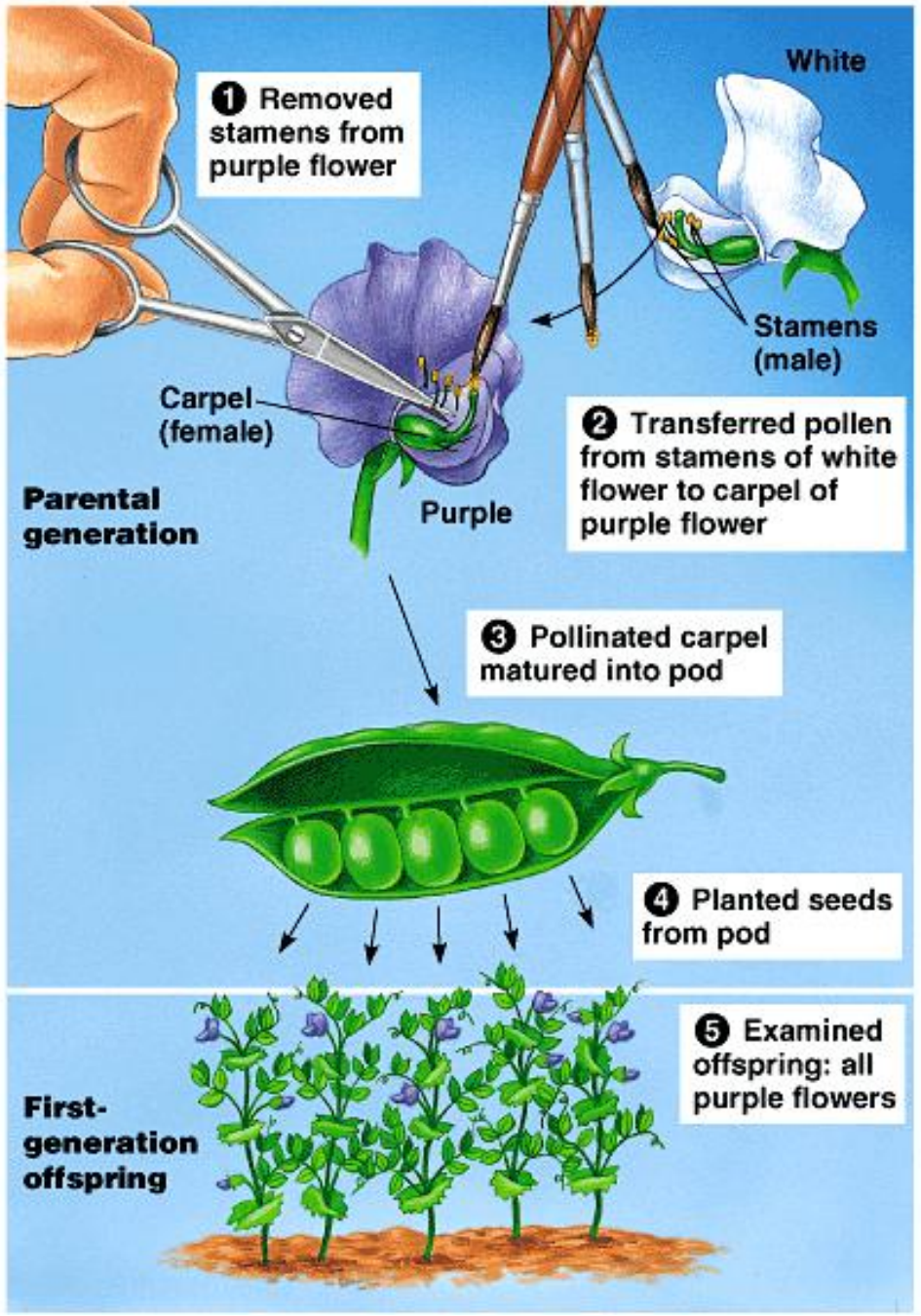
植物具有恒定的分化特征；

进行杂交的时候不会受到外来花粉的污染；

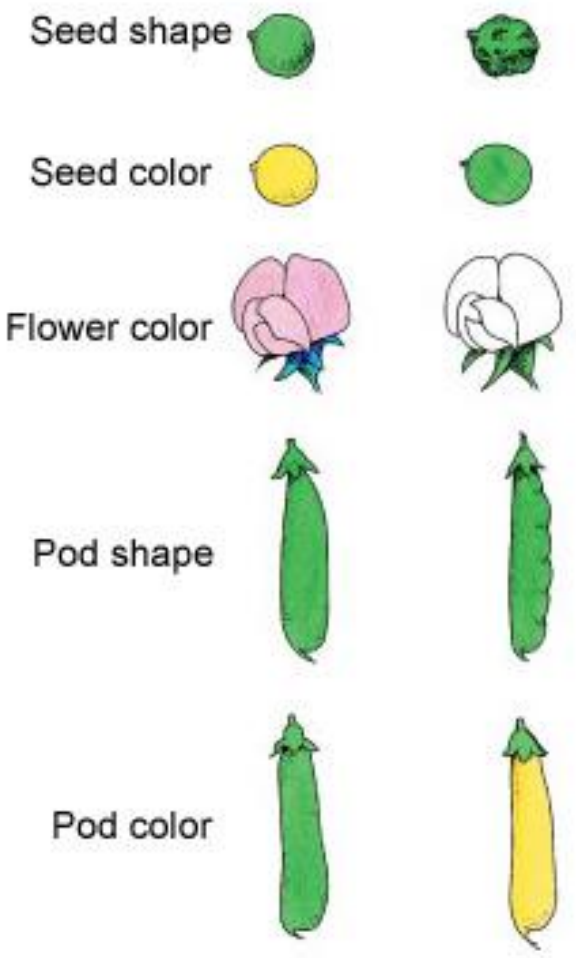
每一代杂交后代生殖力不能变。

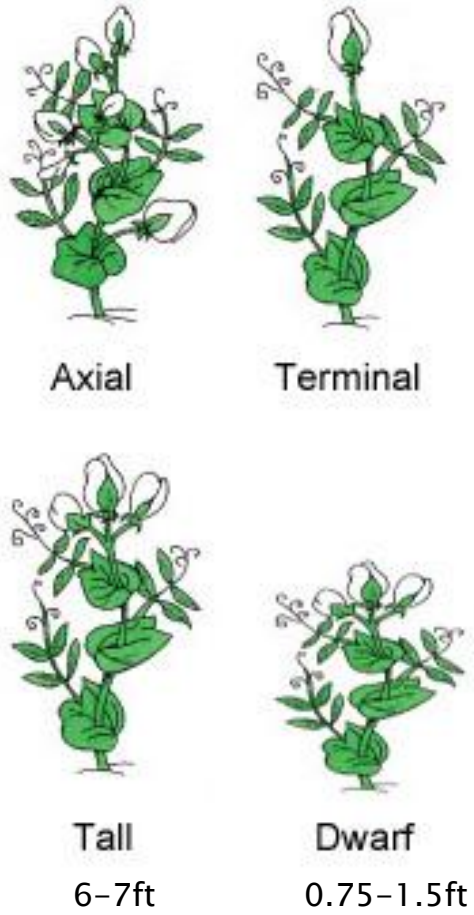


1854 年和1855 年，孟德尔试过34 种不同的豌豆。



实验分组和安排

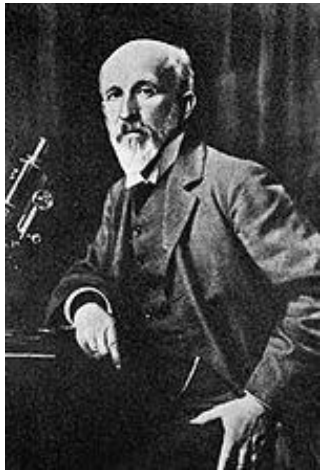




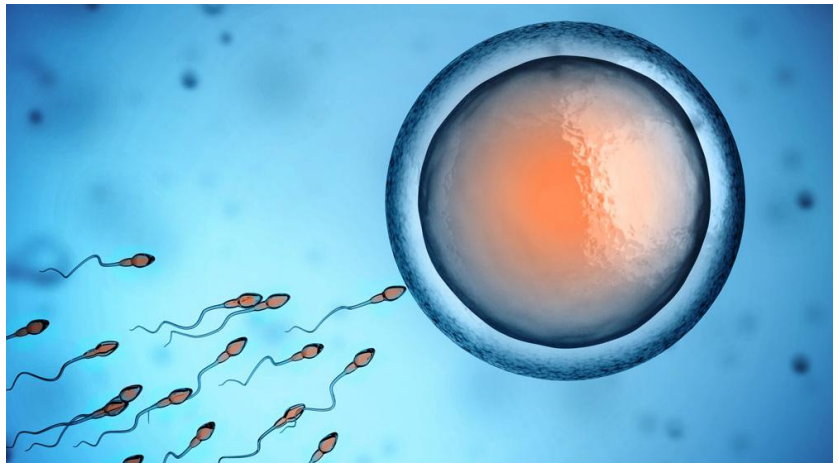
F1代通常更高

120英尺X20英尺（222平方米）

海胆的受精实验成为发育生物学的起点

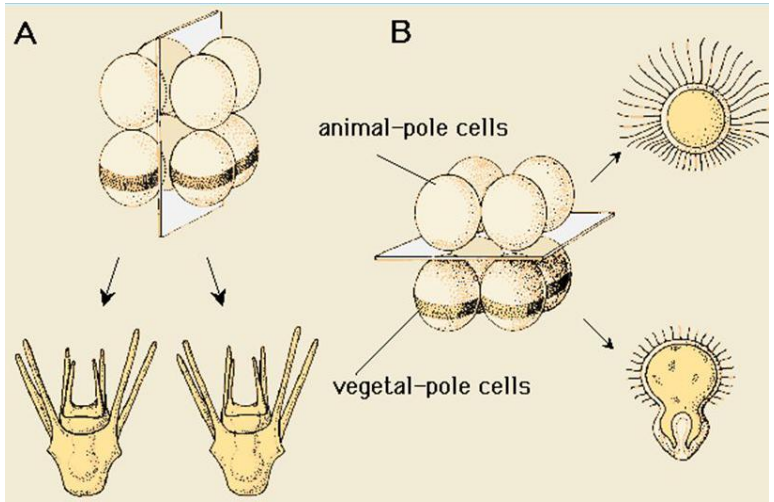


Oscar Hertwig（1849-1922）

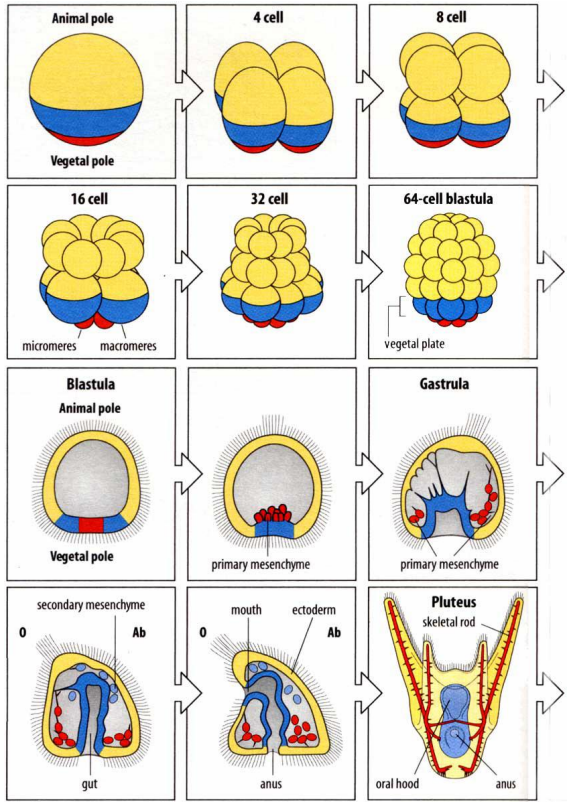


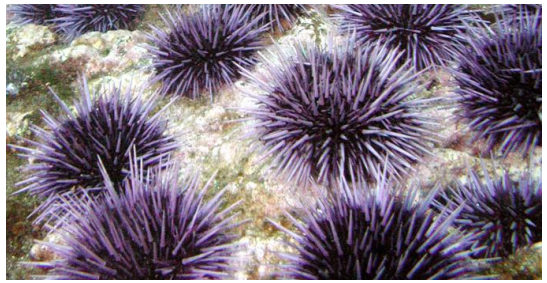
1876年，发现受精是精子进入卵子的过程

Hans Driesch（1876－1941）

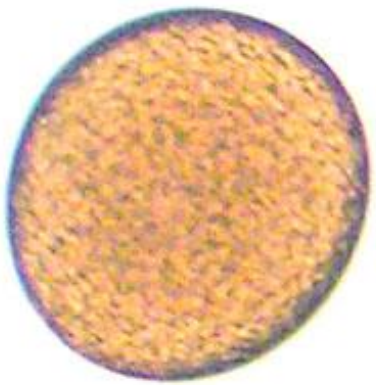


1891年，发现了海胆胚胎的重构

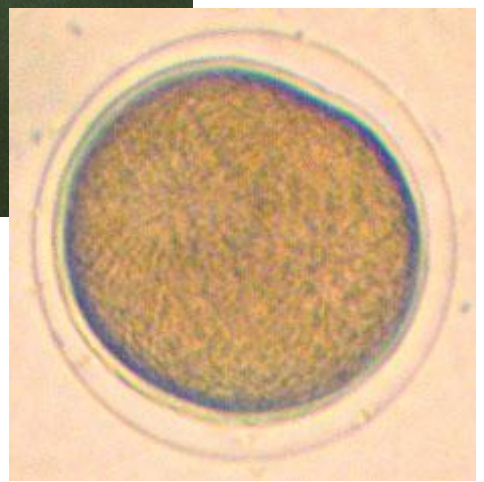




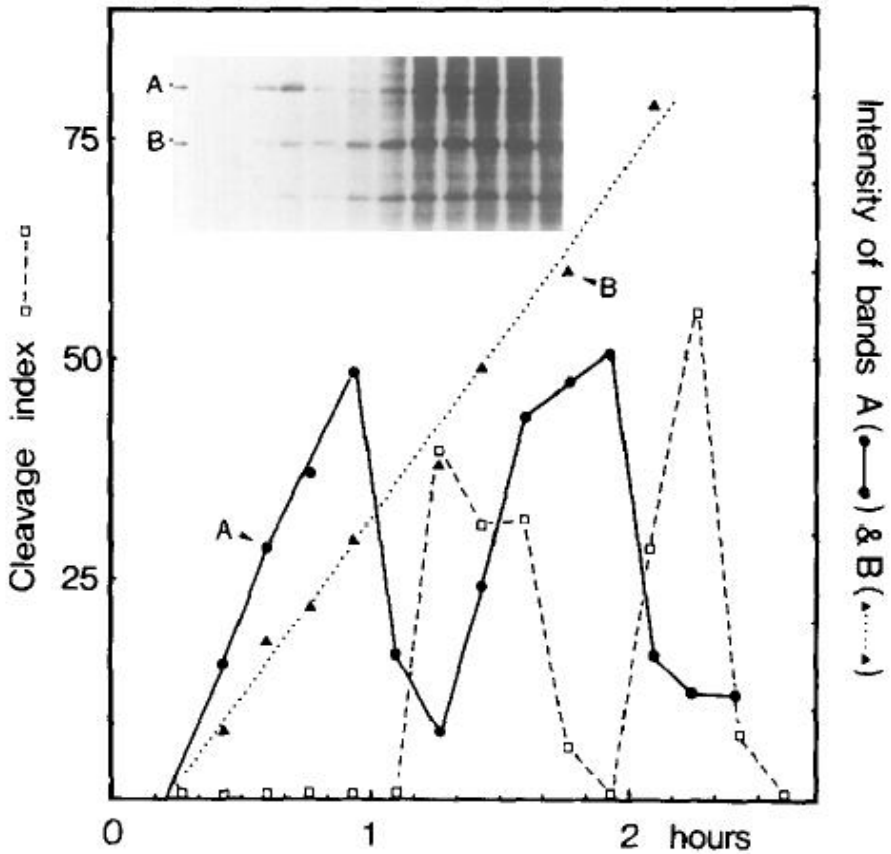
人 工 产 卵 和 受 精

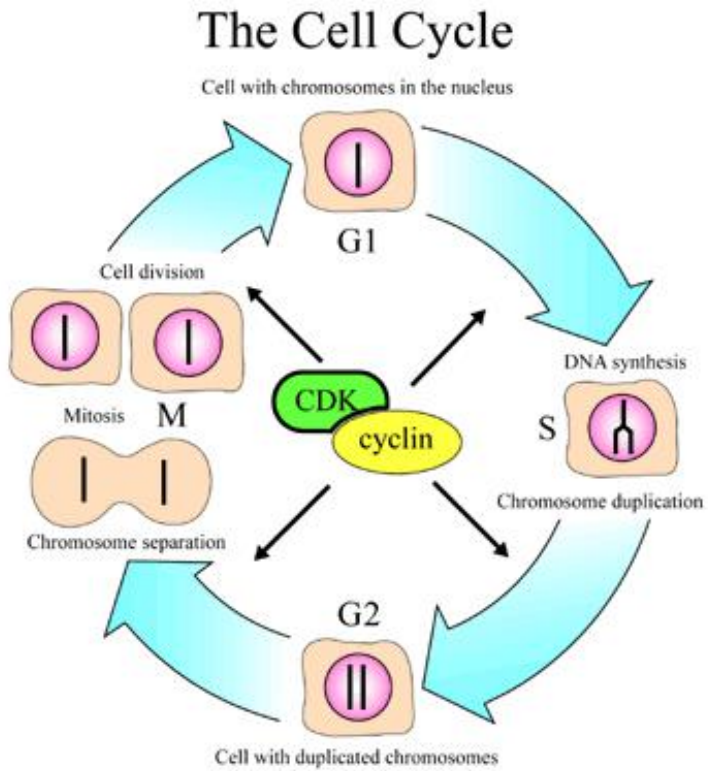


受精

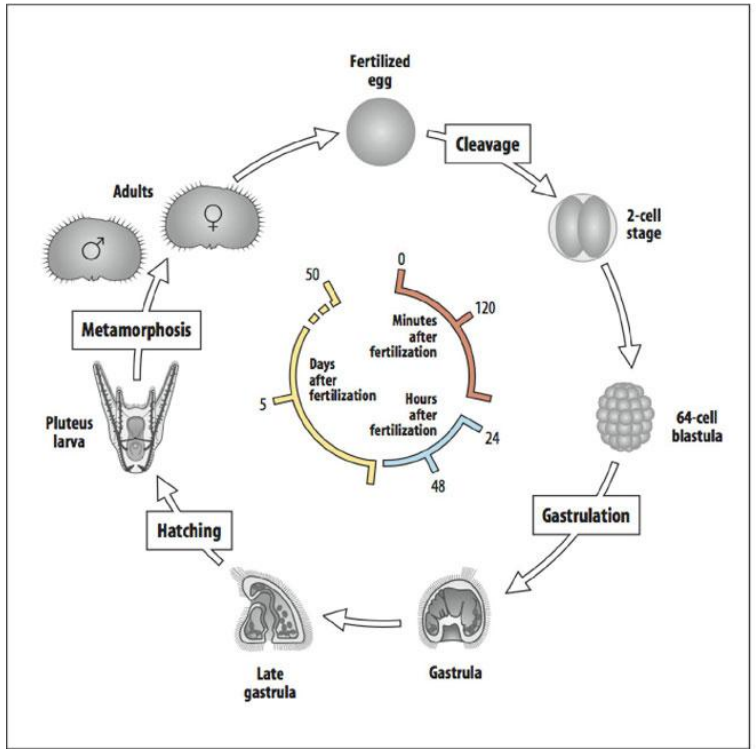


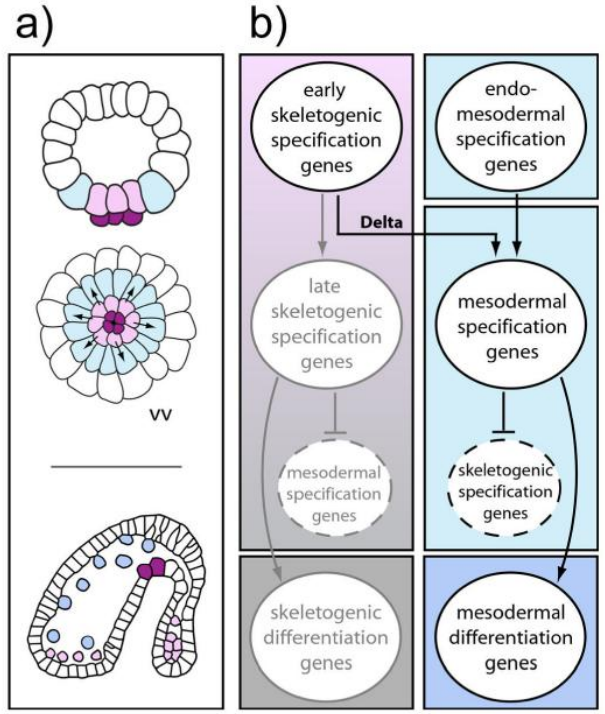
海胆受精实验发现Cyclin





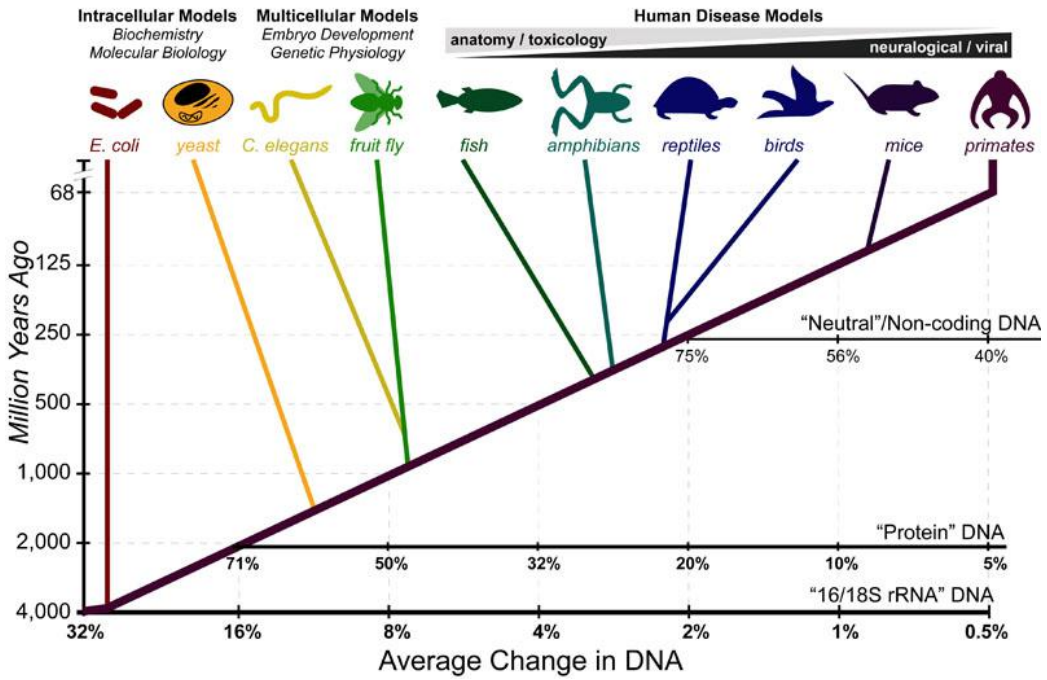
海胆作为早期发育基因调控网络（GNW）的重要模式生物





模式生物的选择

- 有利于回答和解决研究者关注的问题
- 繁殖容易，周期短，研究成本低
- 遗传背景清楚，有遗传操作的手段
- 表型分析容易
- 实验室中常用的模式生物
  - 噬菌体（*Bacteriophages*）
  - 大肠杆菌（*Escherichia coli* ）
  - 酵母（ *Saccharomyces cerevisiae* ）
  - 盘基网柄菌（*Dictyostelium discoideum*）
  - 线虫（ *Caenorhabditis elegans* ）
  - 果蝇（ *Drosophila melanogaster* ）
  - 斑马鱼（*Brachydanio rerio; zebrafish*）
  - 非洲爪蟾（*Xenopus laevis*）
  - 小鼠（*Mus musculus*）
  - 大鼠（*Rattus rattus*）
  - 拟南芥（*Arabidopsis thaliana*）



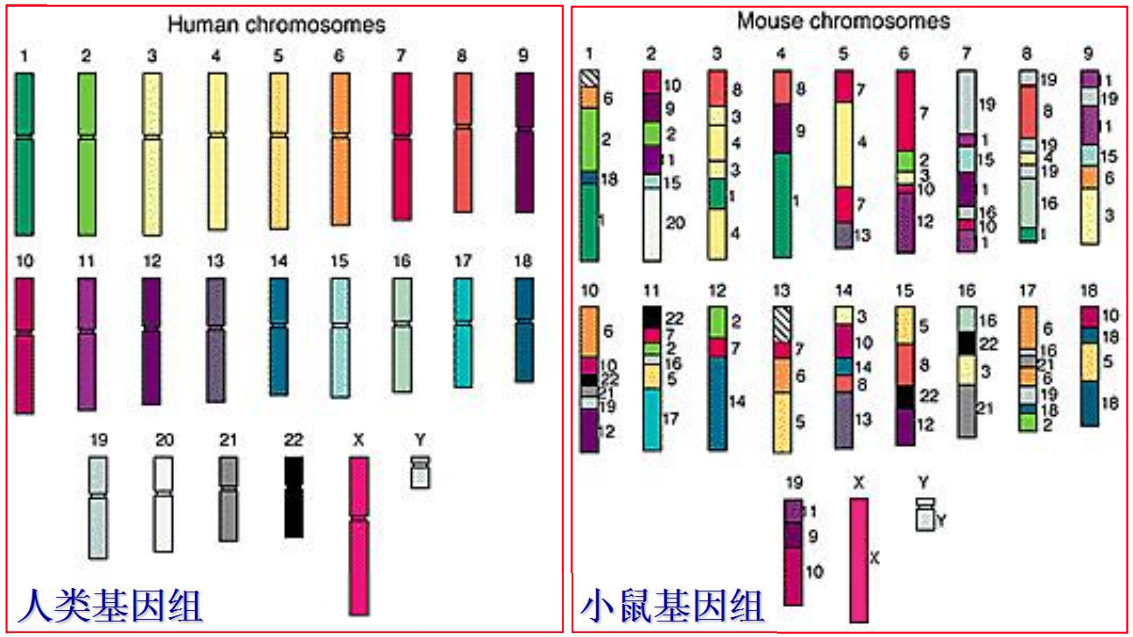
为什么小鼠是研究人类基因和疾病最主要的模式生物

——小鼠与人类是近亲

- 最小的哺乳动物之一（成年鼠体重仅25-40g）
- 生物进化上与人类接近（6000- 7500万年前人鼠可能来自同一个“祖先”）
- 小鼠胎盘形成和早期胚胎发育与人类相近
- 小鼠组织器官结构和细胞功能与人类相似
- 小鼠基因组测序计划已完成
- 人类99%的基因存在于小鼠，基因同源性高达78.5%
- 小鼠基因组中93%的基因排列顺序与人类相同
- 基因组改造的技术手段成熟

Why me?

人类和小鼠基因组的相似性



在模式生物中，小鼠与人类在基因组结构、个体发生和发育过程、组织细胞结构和功能特征等方面非常相似，被广泛应用于解读人类未知功能基因以及人类疾病发病机理与治疗的研究。因此，小鼠被认为是解读“生命天书”的“解码器”；是研究人类疾病的“显微镜”； 是新药或新疗法的“孵化器”，是“代人受过”，为人类健康做出巨大贡献的功臣！

现代实验室小鼠溯源及应用

William Ernest Castle

Clarence Cook Little

1900 Abbie Lathrop 开始饲养小鼠出售

1902 William Ernest Castle 利用小鼠研究肿瘤

1905 W. Castle 和 Clarence Cook Little 报道第一个致死等位基因

1909 Clarence Cook Little 培育了第一个近交系小鼠：DBA

1916 C. Little和Ernest Tyzzer 发现肿瘤移植中的排斥规律

1921 C. Little 建立C57BL

1929 C. Little 建立Jackson Lab

1940 George D Snell 发现组织相容性抗原

1976-1981 转基因小鼠建立

1988-1989 基因剔除技术建立

1998 克隆鼠成功

2002 C57BL小鼠基因组测序完成

Abbie Lathrop
Granby, Massachusetts

human

Base Pairs	3,554,996,726
Coding genes	20,338
Non coding genes	22,521
Gene transcripts	200,310

mouse

Base Pairs	3,482,409,794
Coding genes	22,598
Non coding genes	15,074
Gene transcripts	131,195

小鼠基本生理学特性

- 成年小鼠的食料量：4～8克/天，饮水量4～7毫升/天，排粪量1.4～2.8克/天，排尿量1～3毫升/天
- 实验室小鼠对环境的适应性差，不耐饥饿，不耐冷热，对于多种毒素和病原体具有易感性，反应极为灵敏，对致癌物质也很敏感，自发性肿瘤多
- 性成熟：雌性35～50日龄，雄性45～60日龄；体成熟：雌性为65～75日龄，雄性为70～80日龄。寿命：2 ～ 3年
- 性 周 期：4～5天；妊娠期：19～21天；哺乳期：20～22天；一次排卵10～23个（视品种而定）；每胎产仔数为8～15头；一年产仔胎数6～10胎，属全年、多发情性动物，生育期一般为一年。
- 体温：37.5－38.8℃；心跳次数：480－738次/分钟；呼 吸 数：84－230次/分钟；收缩压113（95～125）mmHg；舒张压81（67～90）mmHg；耗氧量：1530mm2/g活体重；红细胞：9.3（7.7－12.5）万个/立方毫米；白细胞：8.0（4.0-12.0）万个/立方毫米；血红蛋白：14.8（10.0－19.0）克/100毫升血

动物品系分类（按遗传特性）

- **近交系：**
  - 定义：经连续20代（或以上）的全同胞兄妹交配（或者亲代与子代交配）培育而成，近交系数应大于99%，品系内所有个体都可追溯到起源于第20代或以后代数的一对共同祖先
  - 特点：遗传背景均一，实验结果比较准确，可以避免遗传组成不同或个体差异太大等所引起的误差；近交衰退，生活力弱，对生产环境要求高、产仔少、营养要求高。
- **远交系（封闭群）：**
  - 定义：在一定群体内，以非近亲交配方式育成的动物品系，连续15代不从外部引入新的动物种群
  - 特点：遗传背景具有群体均一性，但个体各异，实验能够反映特定群体遗传背景下的情况；繁殖能力强、环境适应能力高。
- **杂交一代：**
  - 定义：两个不同近交系杂交所生的第一代动物称为杂交一代动物或F1代。
  - 特点：个体间遗传均一，个体间遗传变异与近交系一样很小，其基因位点均为杂合型（近交系为纯合型），因此能取得一致的实验结果。表现双亲的显性性状，具有杂种优势，如体质健壮、生长快、易于饲养管理、发育均匀、手术后恢复快等优点。

动物品系分类（按微生物）

- **普通级动物：**没有被疾病控制的动物，排除烈性传染病、人畜共患病，饲养于普通环境下
- **清洁级动物：** 仅对于我国国情而定，微生物控制高于普通普通动物，种子来源于SPF动物（剖腹产），饲养于清洁级环境下，排除规定的寄生虫、微生物（排除对动物危害大和对科学研究干扰大的病原体）
- **SPF动物(Specific pathogen-free)：**无特定病原体动物，没有特定的微生物、寄生虫。但未必没有特定以外的微生物和寄生虫。屏障条件下进行饲养
- **无菌动物(Germ free)：**没有能被检查出微生物、寄生虫的动物。妊娠末期，通过剖腹产、子宫切除手术，将无菌取胎的仔鼠放在隔离器内无菌条件下进行饲养的动物
- **知菌动物(Gnotobiote)：**或称悉生动物，具有已知微生物的动物。隔离器内无菌条件下进行饲养。

常用品系

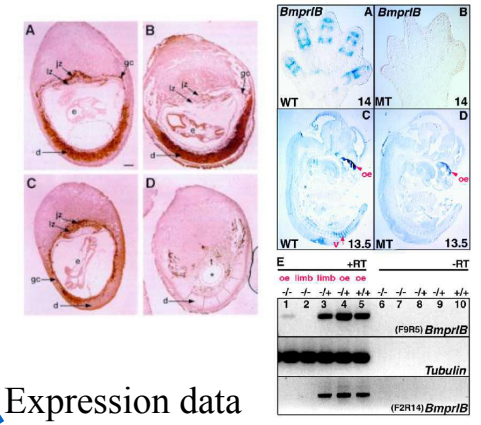
- **C57BL/6小鼠，近交系，黑色。**
  - 自发肿瘤较少，对放射物质耐受力强；补体活性高，较易诱发免疫耐受性；干扰素产量高，对百日咳易感因子（Pertussis HSF）敏感。是肿瘤学、生理学、遗传学研究的常用品系。
- **BALB/cA小鼠，近交系，白色。**
  - 6月龄后出现免疫球蛋白增多症，主要是IgG和IgA量增加；干扰素产量低，对百日咳易感因子敏感，补体活性高；对矿物油诱导浆细胞瘤敏感。。广泛应用于肿瘤学、生理学、免疫学、核医学和单克隆抗体等研究中。
- **DBA/2小鼠，近交系，淡棕色。**
  - 在生理学上，血细胞多，血压低，维生素K缺乏引起死亡率高，对百日咳易感因子敏感，在35日龄左右，听源性癫痫发作率可达100％　。常用于肿瘤学、遗传学、免疫学的研究。

常用小鼠品系

- **昆明小鼠（KM），远交系，白色。**
  - 前身是Swiss小鼠，1946年自印度引进中国。昆明小鼠是我国特有的封闭群小鼠，广泛应用于药理学、毒理学等方面的实验。该鼠适应性强、繁殖能力高，常用于药物毒性、药物筛选、生物效应测定和药效比较的实验。
- **ICR小鼠，远交系，白色。**
  - 起源于美国Fox Chase Cancer Center的Swiss小鼠。该鼠适应能力强，繁殖力强，生长速度快，实验重复性较好；是国际通用的封闭群小鼠，常用于免疫药物筛选、药理、毒理、肿瘤、放射学、食品、生物制品等科研、生产和教学。外周血象和骨髓细胞具有良好的稳定性，是较理想的血液学实验模型。

Strains

Large Datasets



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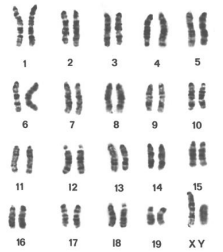
Sequence



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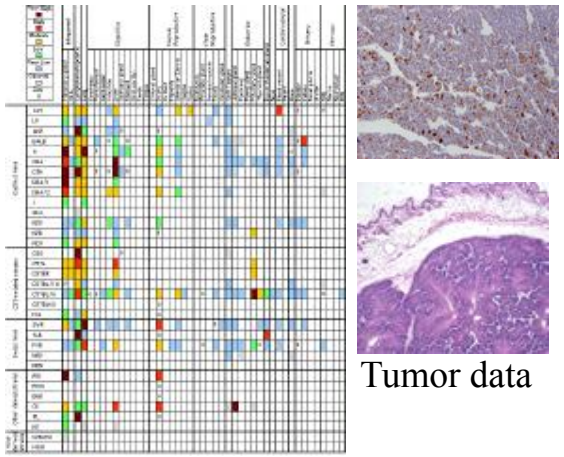
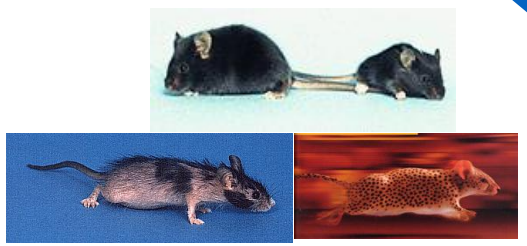
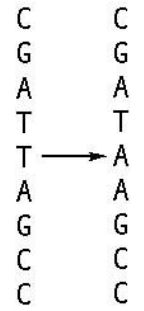


Tumor data



Mutant alleles and Phenotypes

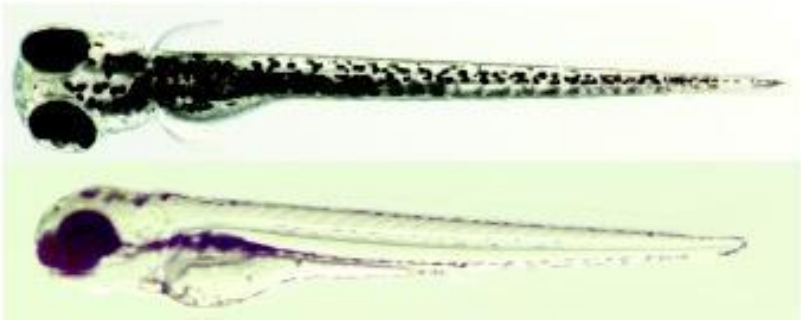


Polymorphisms

斑马鱼（zebrafish； *Danio rerio* ）

George Streisinger
1927–1984

- 1970’s由美国遗传学家乔治 史崔辛格引进模式生物的科学殿堂
- 来源于印度恒河流域
- 个体小（4cm），淡水饲养，繁殖周期短（3个月）
- 产仔频繁，量高，适合实验室养殖
- 脊椎动物，胚胎发育周期短，胚胎透明
- 遗传操作方法成熟（转基因、Morpholino）
- 具有大量突变体，已证明在多方面可以模拟人类疾病
- 25对染色体，168M，30000基因



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People, Labs, Companies
Educational Resources
The Zebrafish Book

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Data Model

Zebrafish International Resource Center
Request: Fish Lines, ESTs/cDNAs, Monoclonal Antibodies, The Zebrafish Book, Paramecia
Submit Fish Lines
Health Services

Genomics
Data mining: ZebrafishMine, BioMart
Browse genome: ZFIN, Ensembl, Vega, GRC, UCSC, NCBI, FishMap
View Genetic Maps
BLAST: ZFIN, Ensembl, Vega, NCBI, MGH
Find cDNAs and ESTs at ZGC
More Zebrafish Genome Resources
Other Fish Genomes and Model Organism Databases

Zebrafish Programs
ZF-HEALTH, Husbandry Resources, more...

News
The Alliance of Genome Resources is Opening its Doors with Version 1.0 Website Release 2017-11-1
19th Australia and New Zealand Zebrafish Meeting (Jan 29 - Feb 1, 2018 - Sydney, Australia) 2017-10-25
ZFIN Newsletters, News Archive

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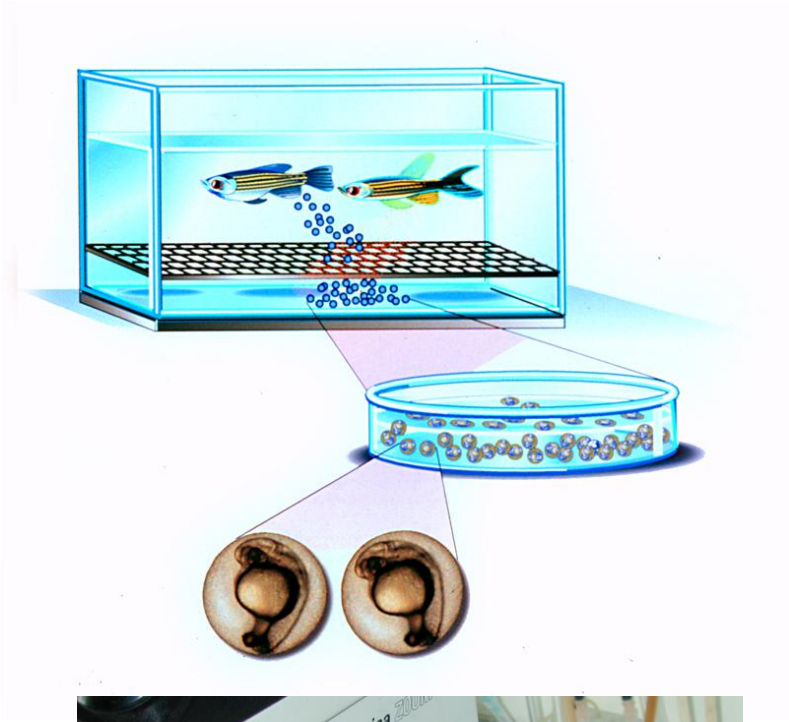
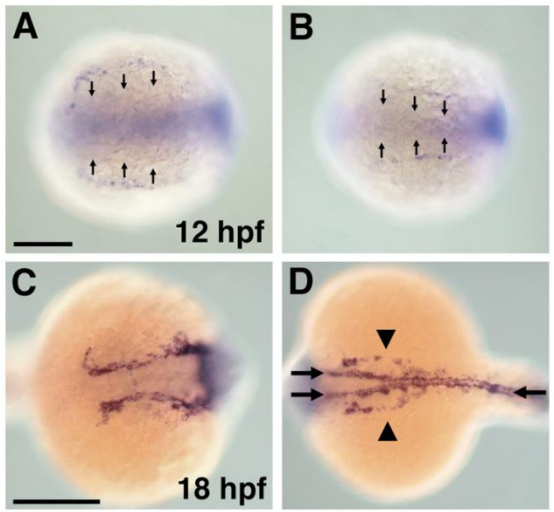
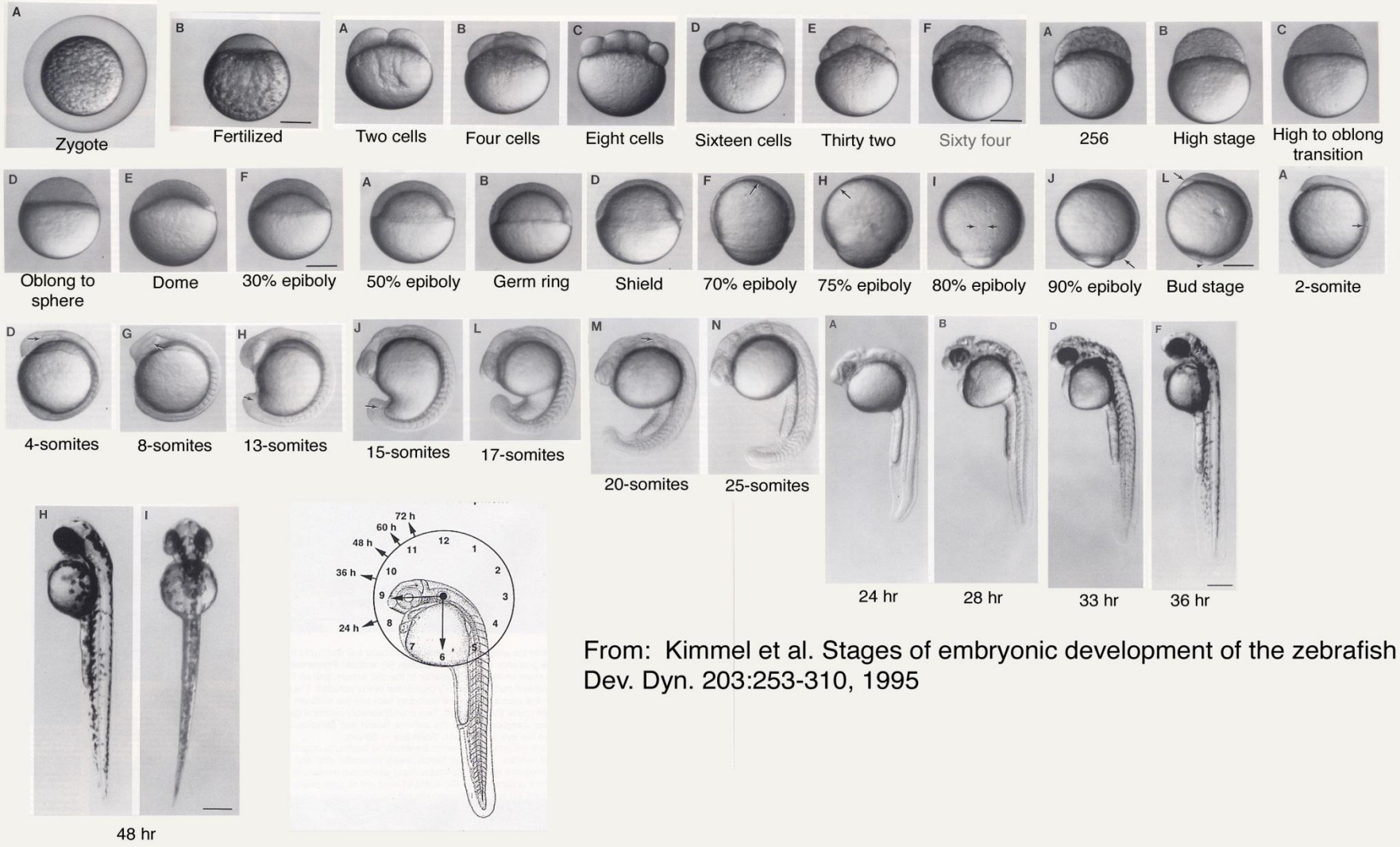



Leica


A
B
12 hpf
C
D
18 hpf

Stages of Embryonic Development of the Zebrafish



From: Kimmel et al. Stages of embryonic development of the zebrafish
Dev. Dyn. 203:253-310, 1995

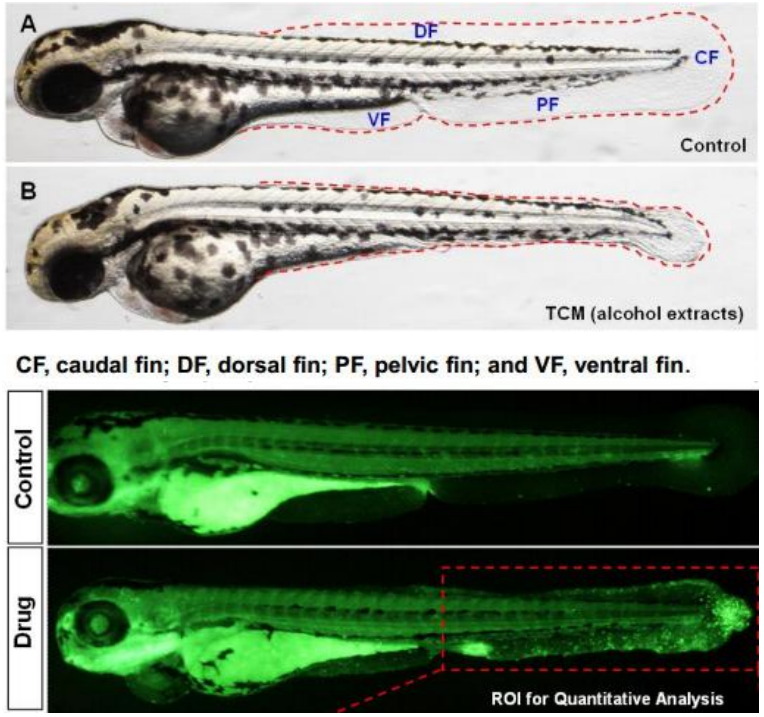
斑马鱼癌症研究模型

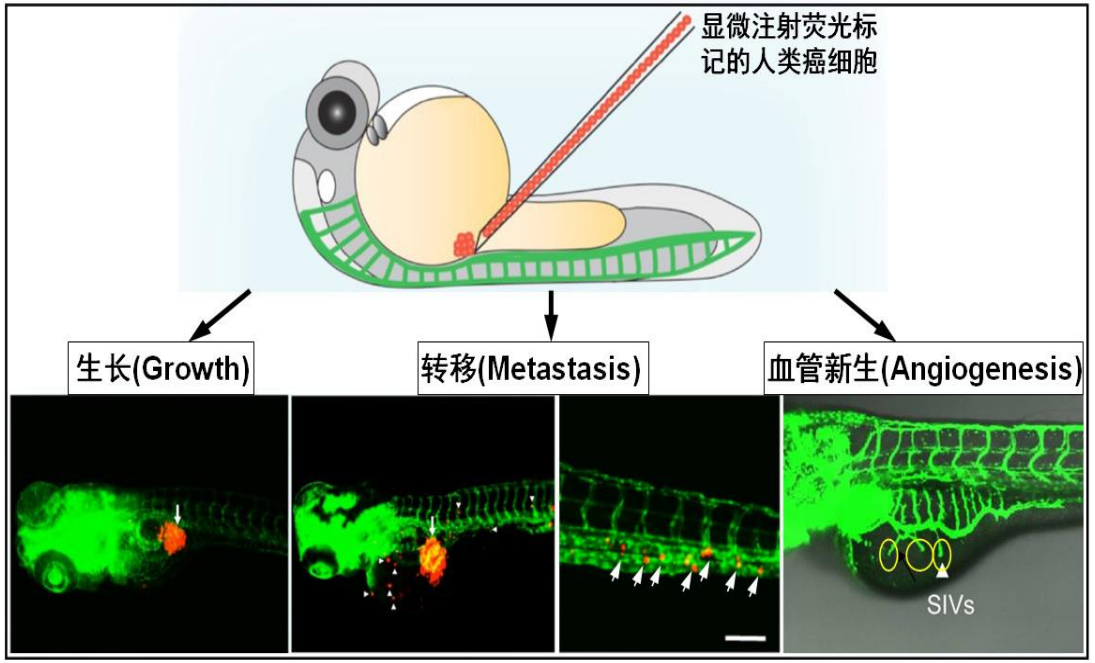
✓抗肿瘤血管药物筛选及药效评估

✓抗细胞增殖药物快速筛选/凋亡模型

✓斑马鱼癌症异种移植模型(超过30种人癌细胞株)

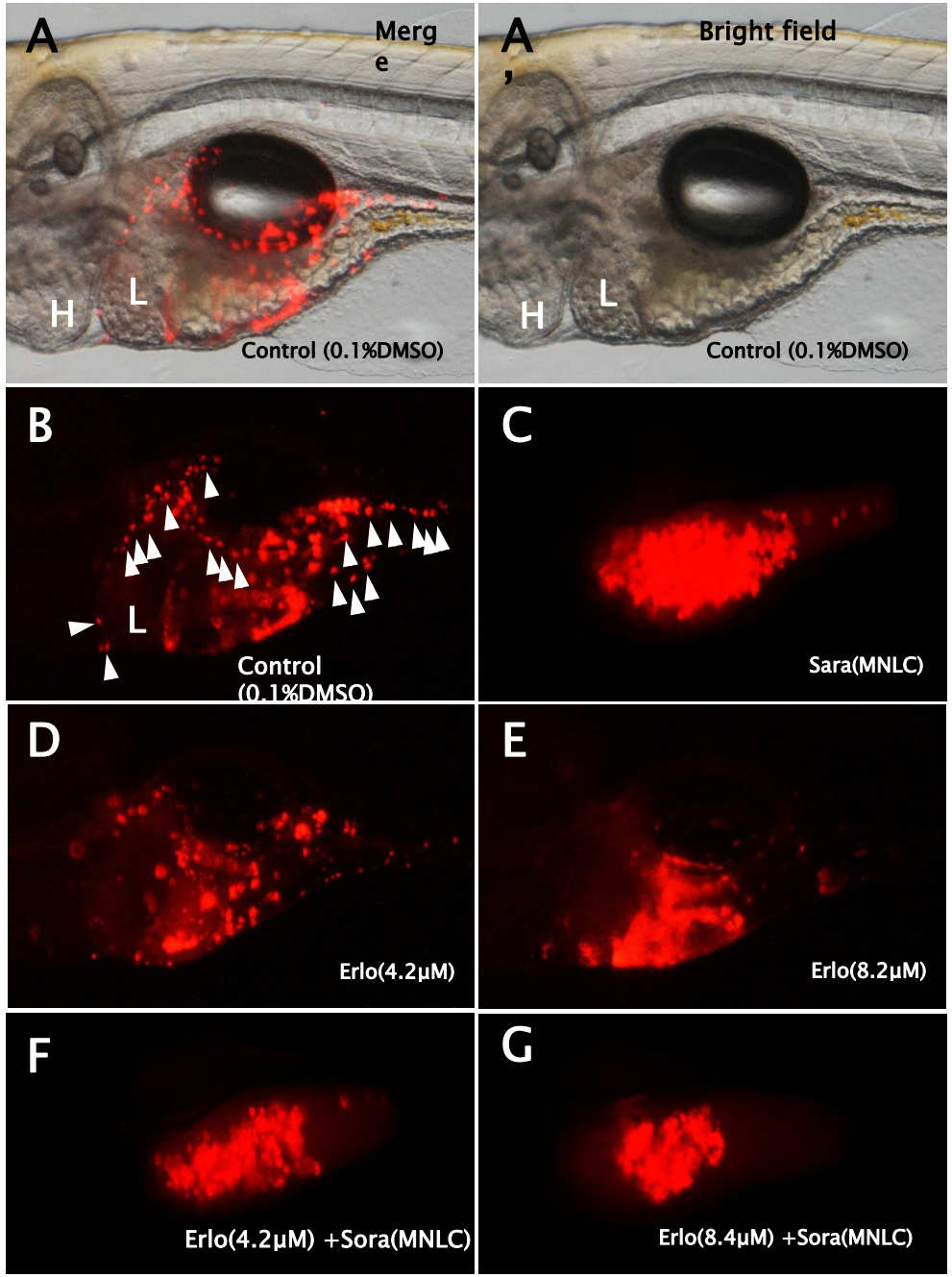
✓靶向抗癌药物筛选及药效评估(VEGF, EGFR, Wnt, Hedgehog, Dll4/Notch, B-RAF, mTOR, BTK……)

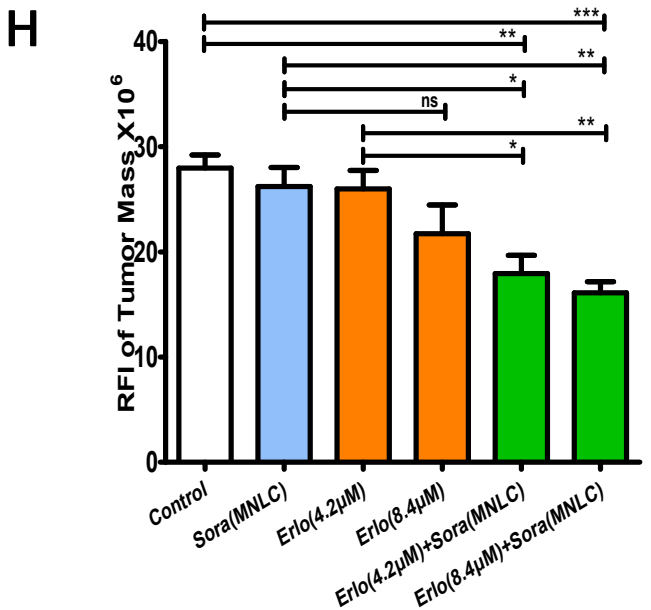


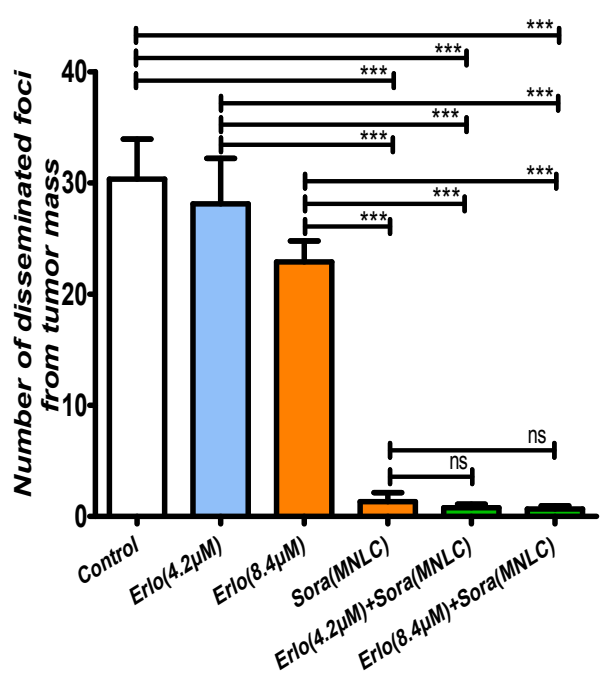


Biomodel Organisms

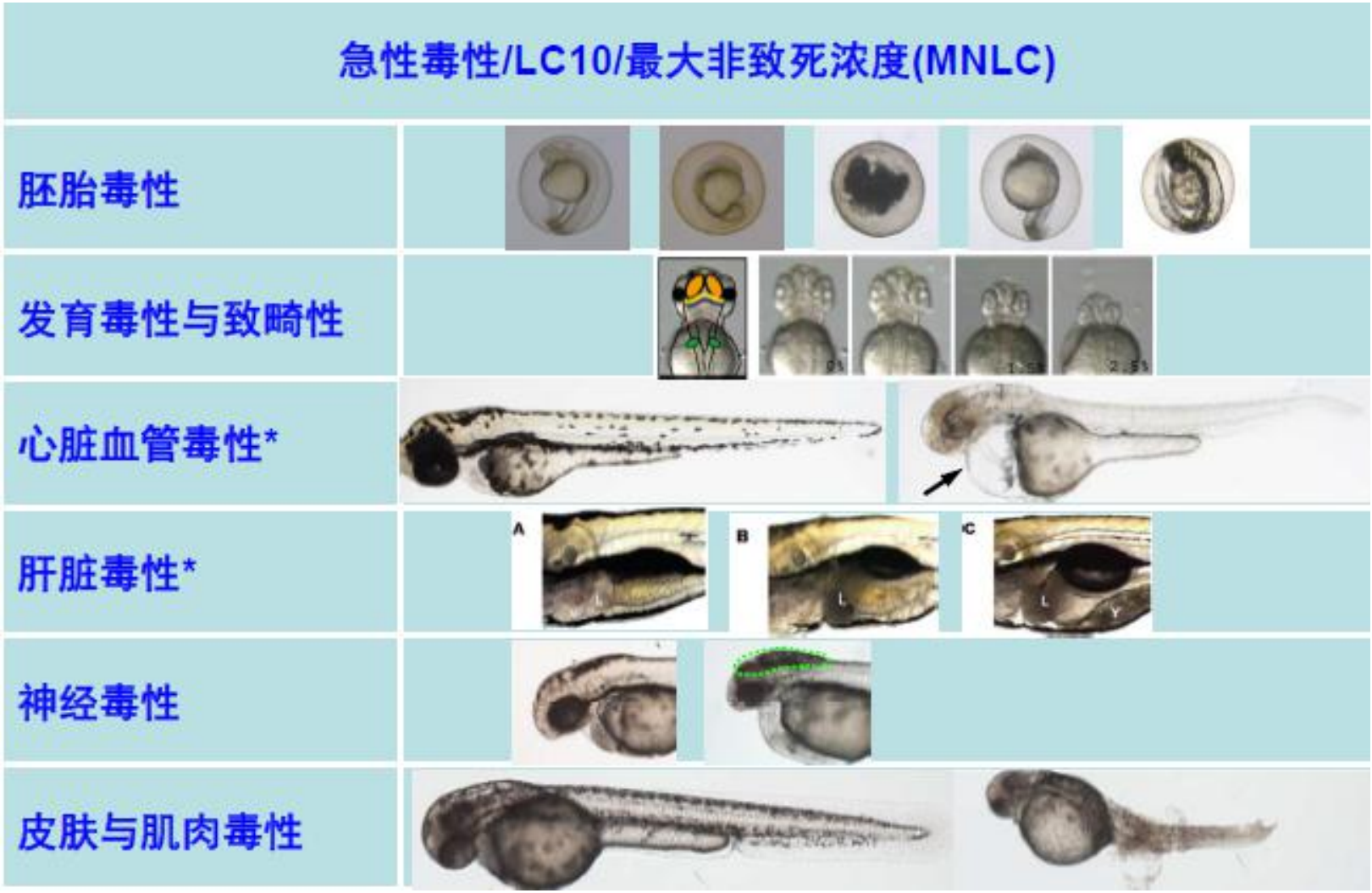
Co-administrations of Erlotinib Sorafenib and inhibited tumor growth and dissemination





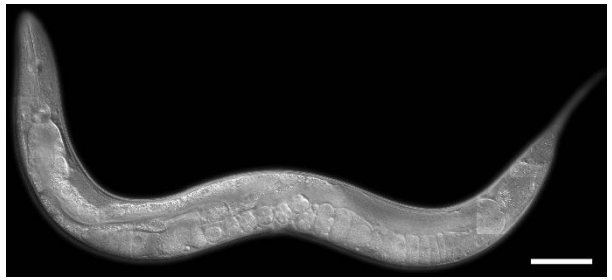


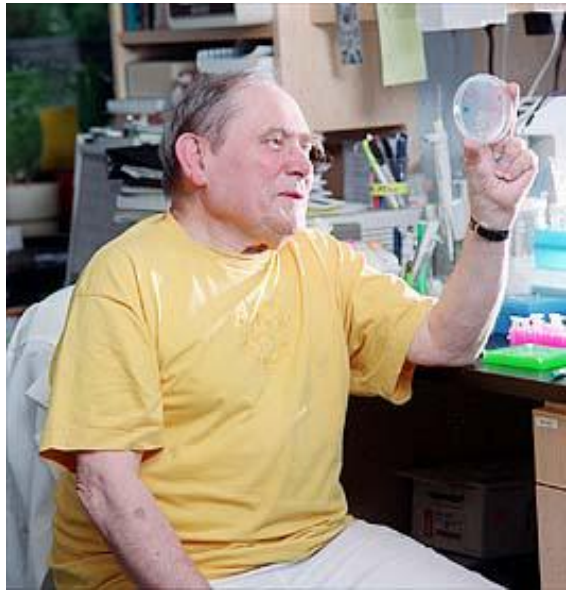
药物毒性与安全性评价系统

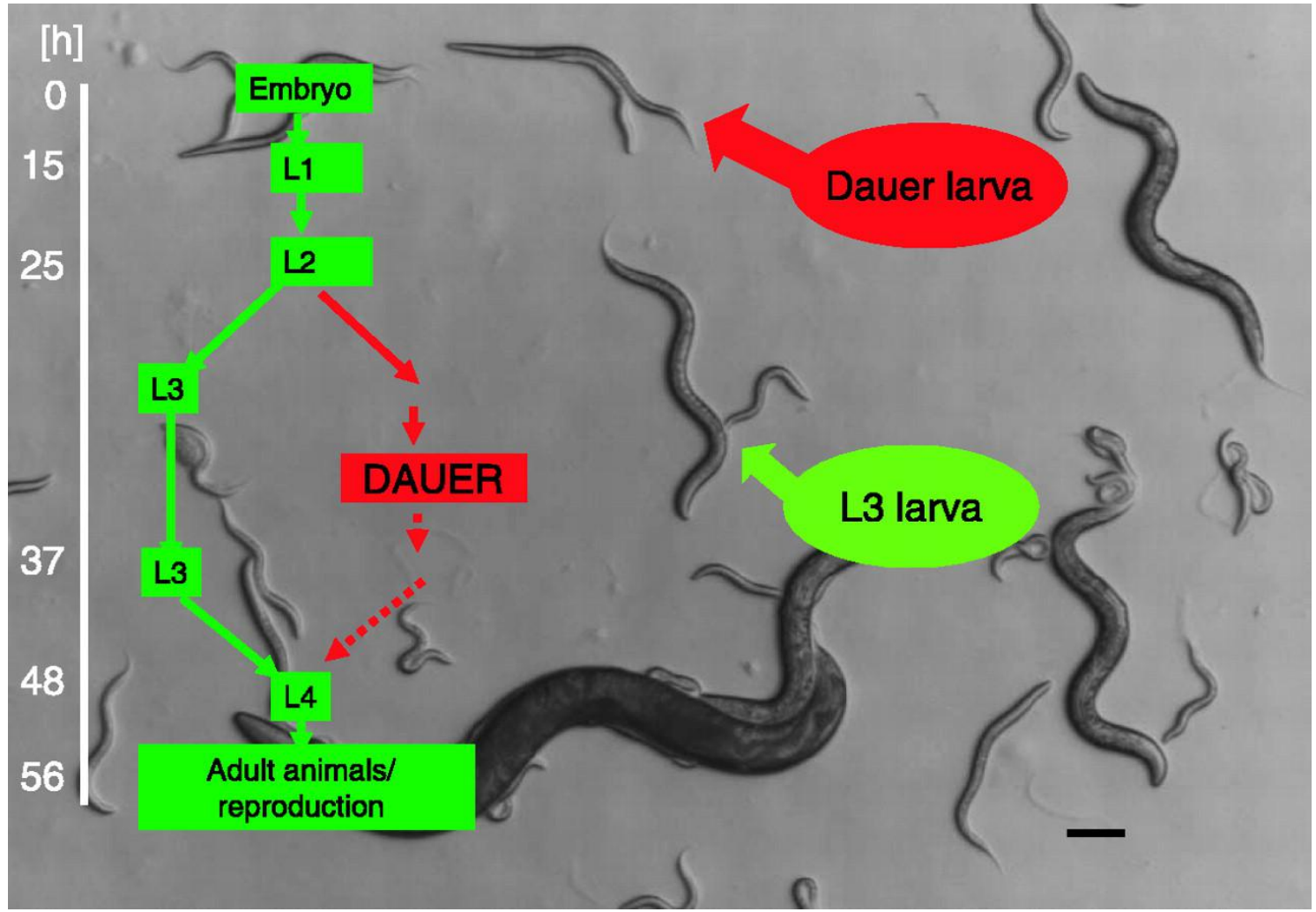


线虫（ *Caenorhabditis elegans* ）

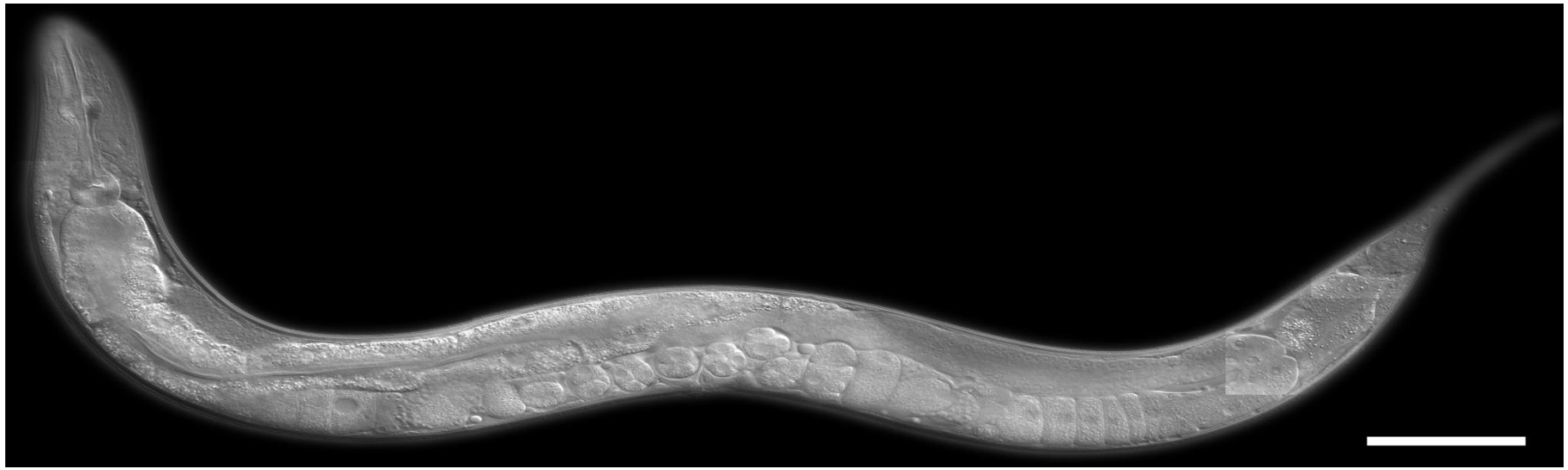
- 成虫大小：1mm，实验室饲养简单，生命周期短，保种方便，大部分为雌雄同体（雄虫占0.1-0.05%），N2是目前实验室常用的野生型品种
- 雌雄同体成虫由959个细胞构成，其中302个细胞为神经细胞；所有的细胞谱系清楚，雌雄同体线虫发育过程中会产生1090个体细胞，但在成虫前有131个细胞经历凋亡而消失。雄虫由1031个细胞构成
- 是研究发育（细胞命运决定）、神经系统的重要模式生物；遗传操作手段成熟（转基因、RNAi、Crispr-cas9）；基因组：5对常染色体，1对性染色体，97M，约20000个基因

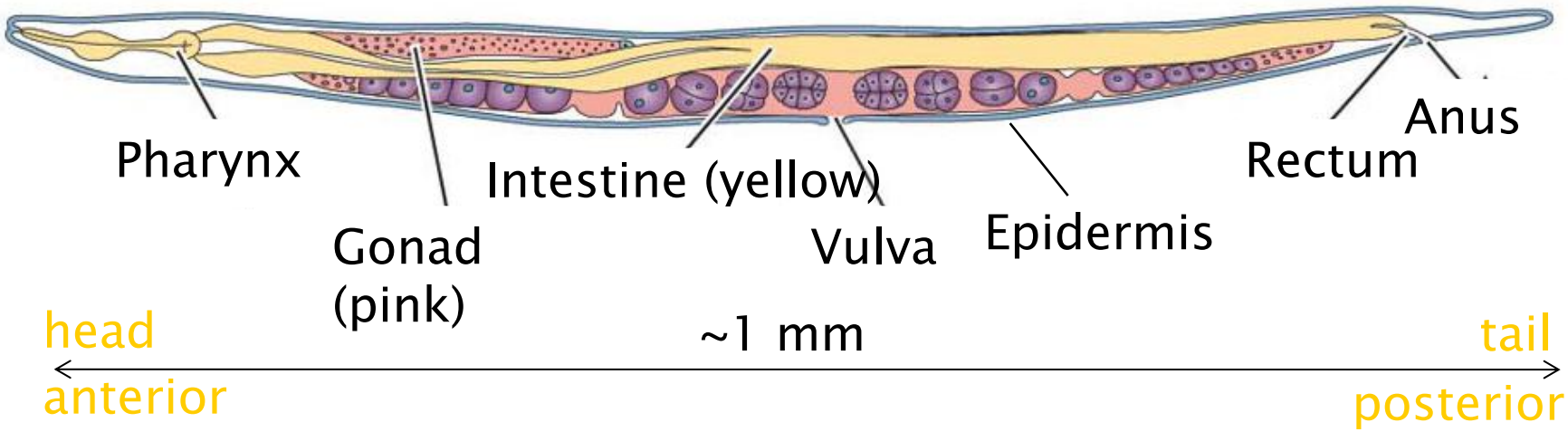





[h]
0
15
25
37
48
56
Embryo
L1
L2
L3
DAUER
L3
L4
Adult animals/
reproduction
Dauer larva
L3 larva

C. elegans 的解剖结构





C. elegans 的生命周期

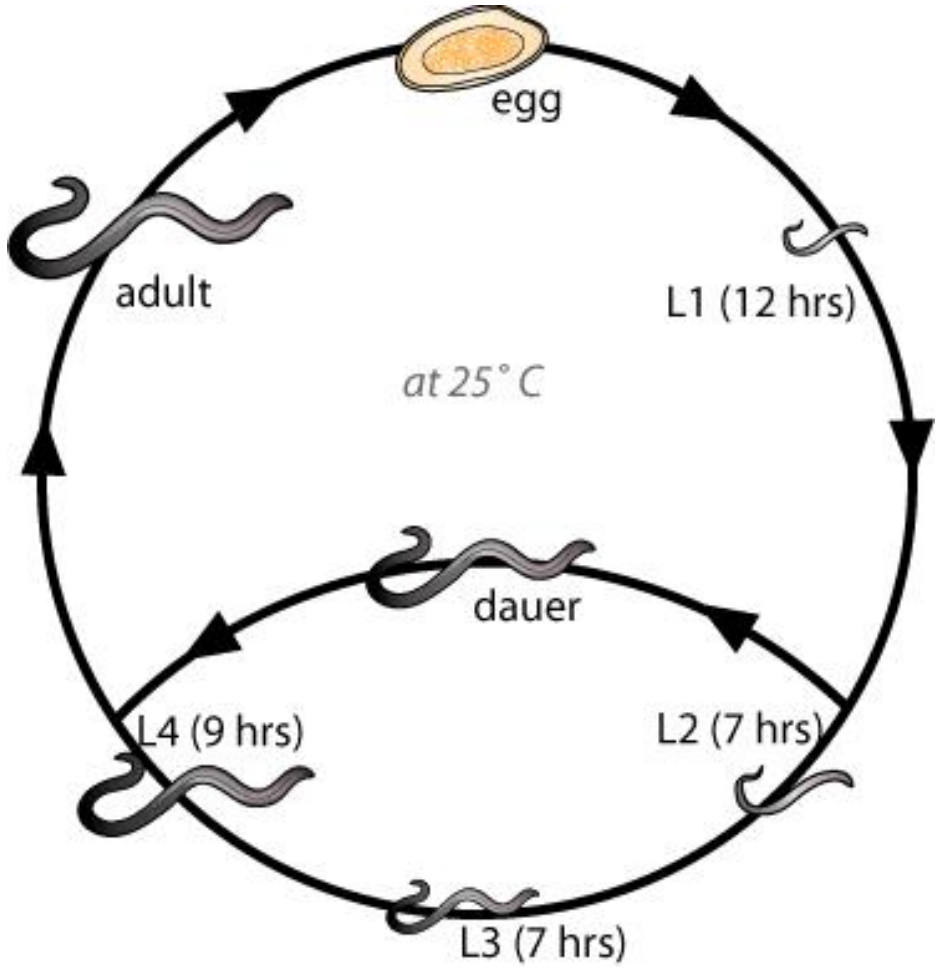


Photo credit: http://www.scq.ubc.ca/genetic-studies-of-aging-and-longevity-in-model-organisms/

雌雄同体和雄虫的生殖系统

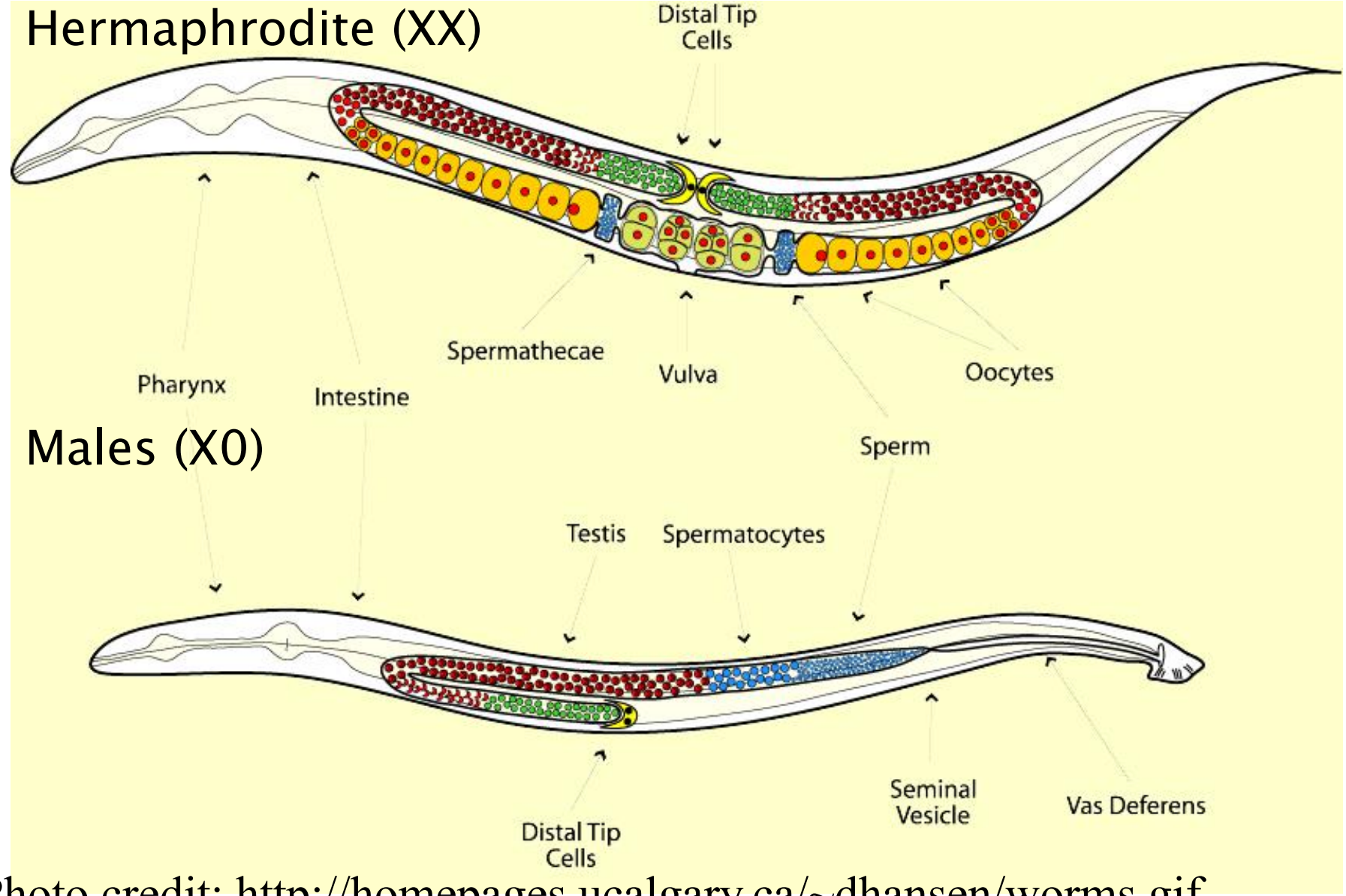
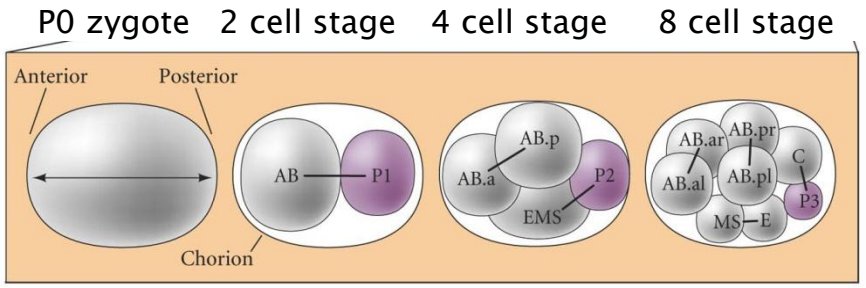
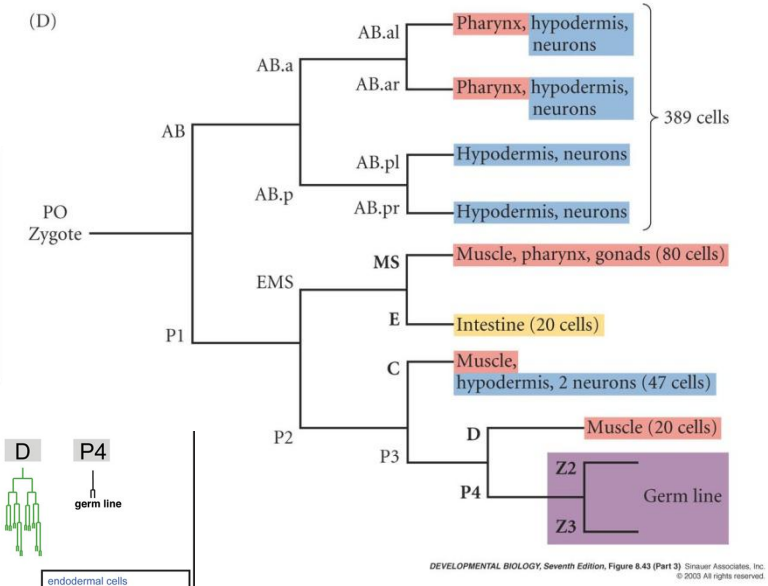
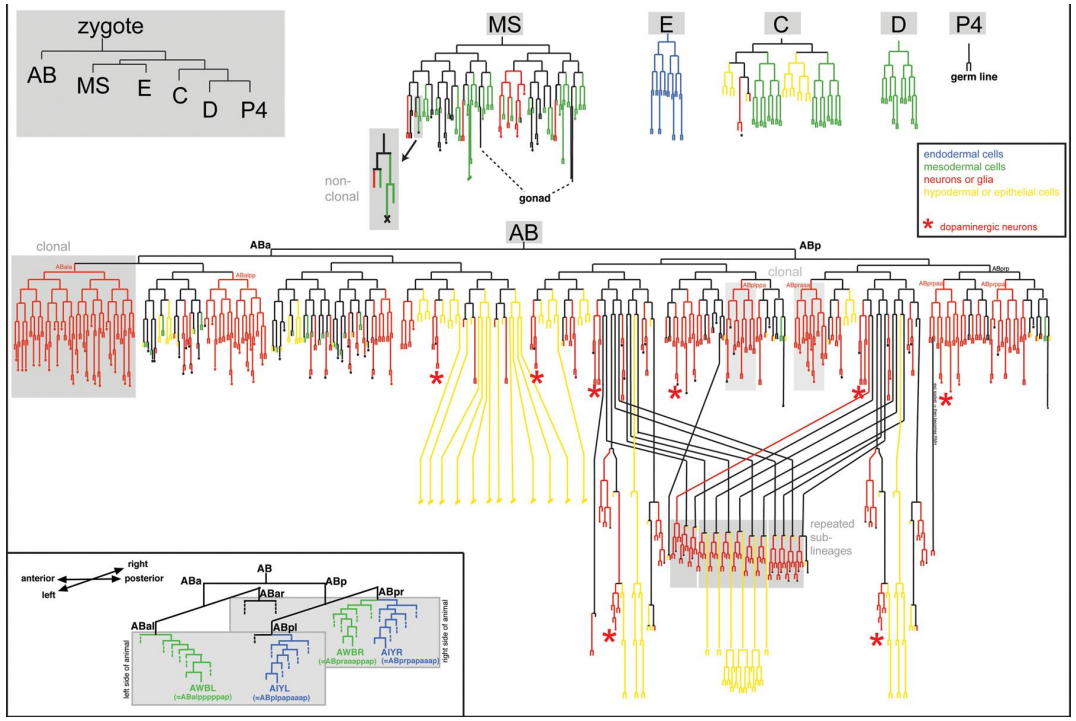


Photo credit: http://homepages.ucalgary.ca/~dhansen/worms.gif

C.elegans的细胞发育谱系

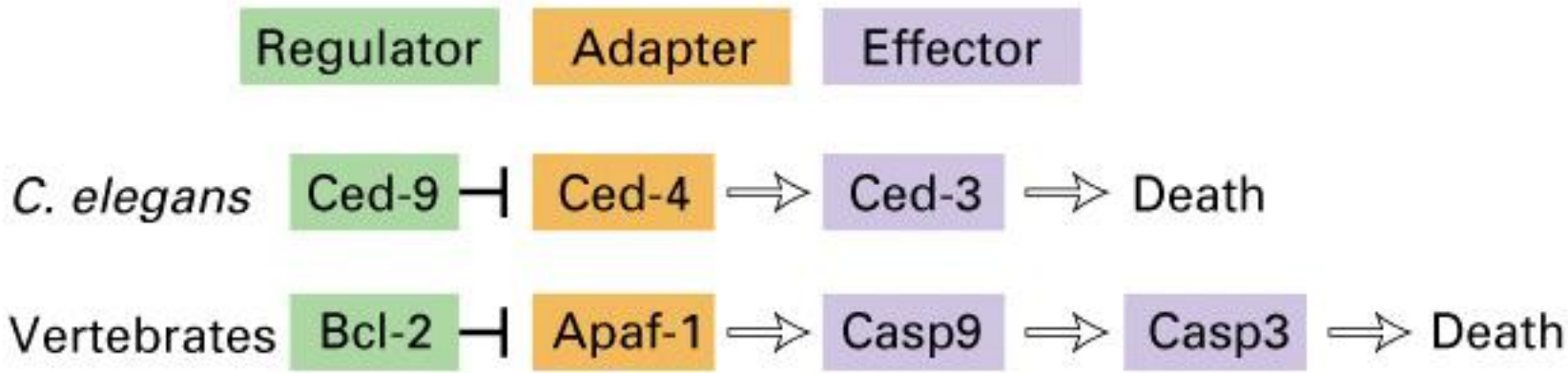




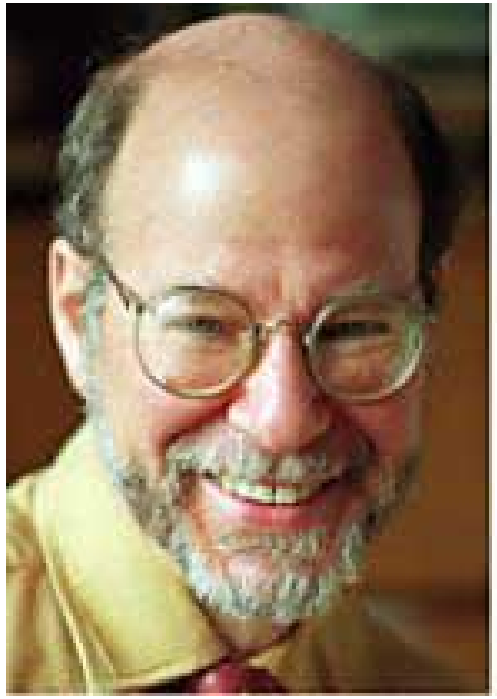
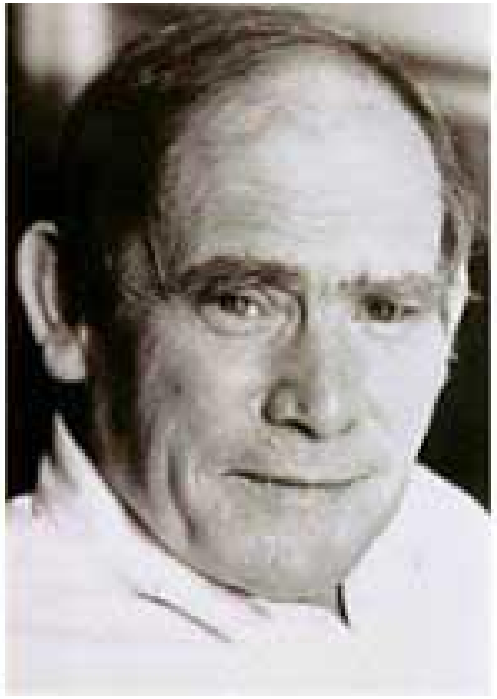
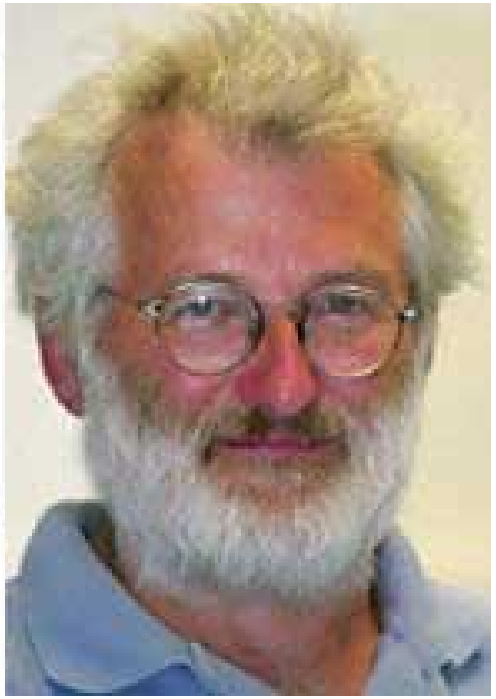


细胞程序性死亡的发现

C. elegans was used to identify the machinery that regulates programmed cell death in vertebrates



线虫已逐渐成为分子生物学、发育生物学等领域耀眼的明星，成为诺贝尔奖的“新宠”，40年来“小虫子”已“催生”出六位诺贝尔奖得主！

罗伯特·霍维茨　　悉尼·布雷内　　约翰·苏尔斯顿

2002年诺贝尔医学或生理学奖

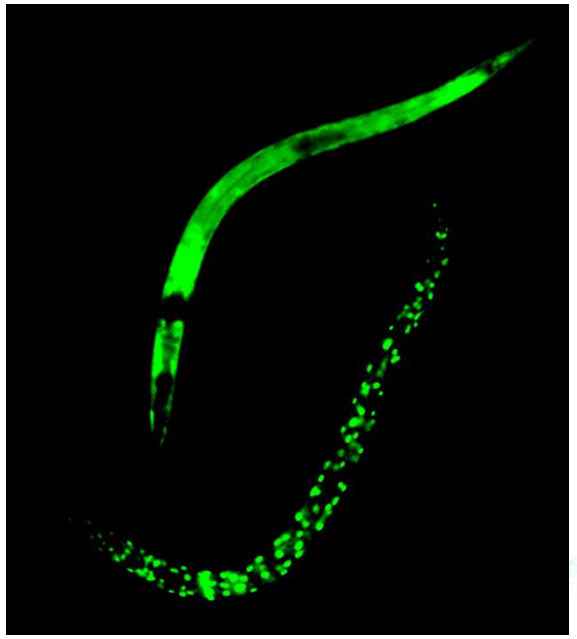
安德鲁·法尔

克雷

格·梅洛

1998年法尔和梅洛分别以线虫为实验材料发现了RNA干扰机制，因而获得了2006年诺贝尔生理学或医学奖。

马丁·沙尔菲
Martin Chalfie
美国科学家，因发现和研究绿色荧光蛋白而获得2008年的诺贝尔化学奖。



Science 11 February 1994:
Vol. 263 no. 5148 pp. 802-805
DOI: 10.1126/science.8303295

Green fluorescent protein as a marker for gene expression

M Chalfie, Y Tu, G Euskirchen, WW Ward, DC Prasher

± Author Affiliations

ABSTRACT

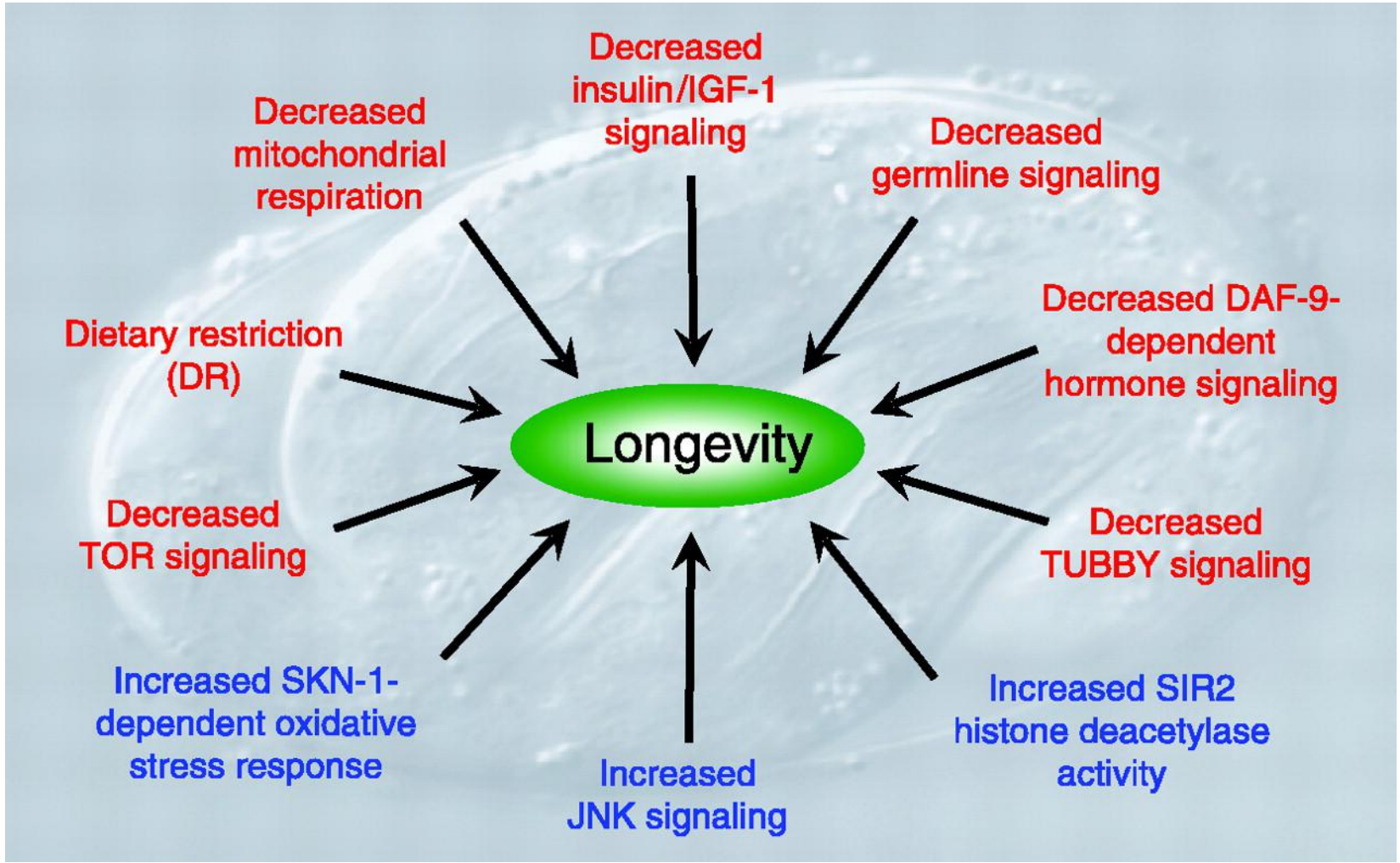
A complementary DNA for the Aequorea victoria green fluorescent protein (GFP) produces a fluorescent product when expressed in prokaryotic (Escherichia coli) or eukaryotic (Caenorhabditis elegans) cells. Because exogenous substrates and cofactors are not required for this fluorescence, GFP expression can be used to monitor gene expression and protein localization in living organisms.

Neurodegeneration models in *C. elegans*

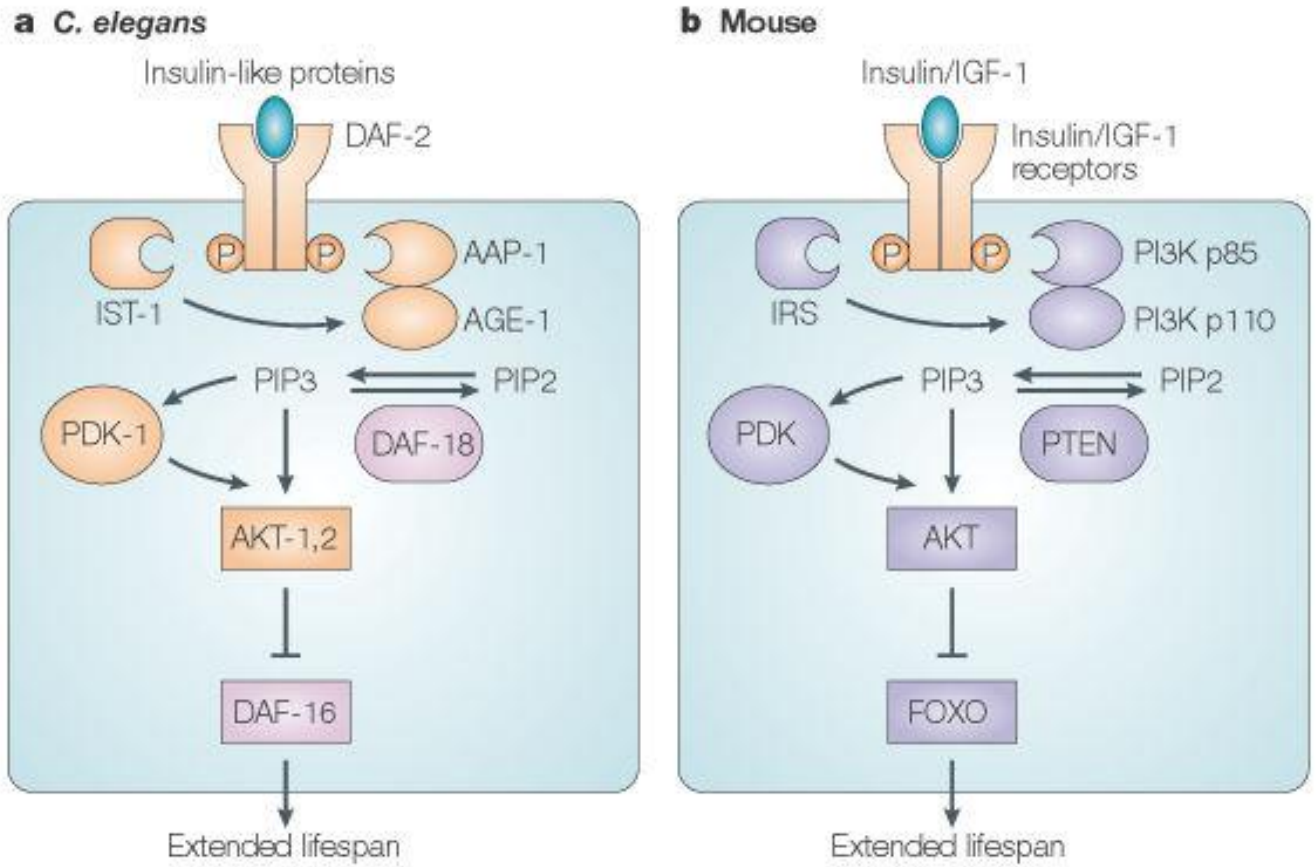
Disease	Pathway or genes (*C. elegans* orthologue)	Model and method of validation
Neurodegeneration		
Alzheimer's disease	Presenilin (*sel-12*)	Genetic egg-laying model and neuronal model: both models were validated by demonstration that human presenilin can substitute for endogenous *C. elegans* presenilin[65,66].
	Aβ	Transgenic model: expression of human β-amyloid (Aβ) peptide in *C. elegans* induced formation of amyloid deposits, which in turn caused paralysis[109–112].
Parkinson's disease	α-Synuclein (no orthologue)	Transgenic model: overexpression of human α-synuclein caused neuronal and dendritic loss in *C. elegans*[113]. Pharmacological model: 6-OHDA (6-hydroxydopamine) a precursor of 1-methyl-4-phenylpyridinium (MPP) induced selective dopamine neuronal death similar to vertebrate models[114].
Huntington's disease	Huntingtin (no orthologue); polyglutamine (polyQ) aggregation	Transgenic model: heterologous expression of a Huntingtin fragment with expanded polyQ in *C. elegans* caused specific polyQ-length-dependent neuronal malfunction and the formation of aggregates[115]. Genome-wide RNAi screen for regulators of polyQ aggregation using fluorescence read-out[116,117].
Neurobiology		
Depression	Serotonin	Behavioural changes after treatment with antidepressants (see text).
Pain, neuronal regeneration	Regeneration	Phenotype model: femtosecond laser operated GFP-labelled axons of *C. elegans* regenerated within 24 hours[118].

Table 1 Some examples of nervous system and neuromuscular disorders modelled in *C. elegans*

Disease	Details	references
Alzheimer's Disease	Transgenic expression of human Aβ	(Drake et al., 2003; Link et al., 2003)
Alzheimer's Disease	Expression of modified tau	(Brandt et al., 2008)
Huntington's Disease	Polyglutamine repeats	(Faber et al., 2002; Satyal et al., 2000)
Spinal muscular atrophy	RNAi of SMN	(Burt et al., 2006; Miguel-Aliaga et al., 1999)
Parkinson's Disease	RNAi modifier screen of synuclein formations	(van Ham et al., 2008)


Decreased insulin/IGF-1 signaling
Decreased mitochondrial respiration
Decreased germline signaling
Dietary restriction (DR)
Decreased DAF-9-dependent hormone signaling
Longevity
Decreased TOR signaling
Decreased TUBBY signaling
Increased SKN-1-dependent oxidative stress response
Increased JNK signaling
Increased SIR2 histone deacetylase activity

胰岛素通路在线虫寿命决定中的作用



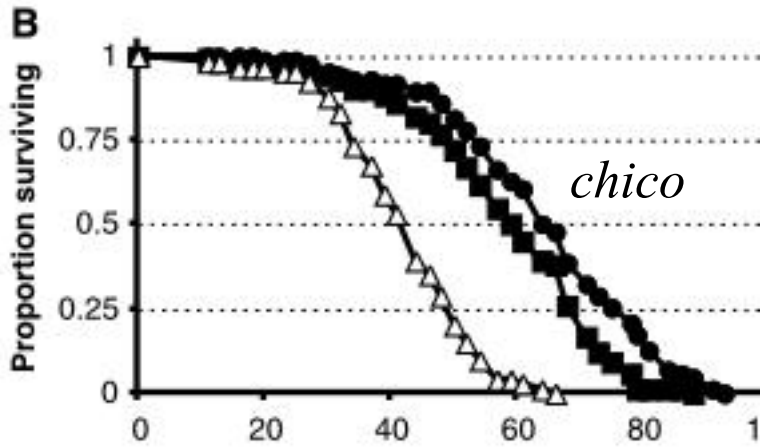
Insulin Receptor Pathway Regulates Lifespan in Flies and Mice

Extension of Life-Span by Loss of CHICO, a *Drosophila* Insulin Receptor Substrate Protein

David J. Clancy,[1] David Gems,[1*] Lawrence G. Harshman,[2] Sean Oldham,[3] Hugo Stocker,[3] Ernst Hafen,[3] Sally J. Leevers,[4,5] Linda Partridge[1]

A Mutant *Drosophila* Insulin Receptor Homolog That Extends Life-Span and Impairs Neuroendocrine Function

M. Tatar,[1*] A. Kopelman,[1] D. Epstein,[1] M.-P. Tu,[1,2] C.-M. Yin,[2] R. S. Garofalo[3]

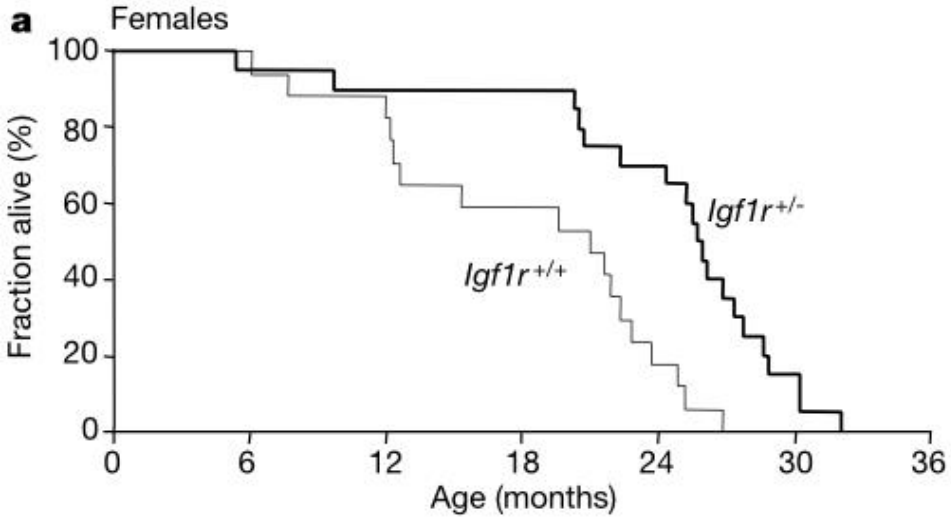


IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice

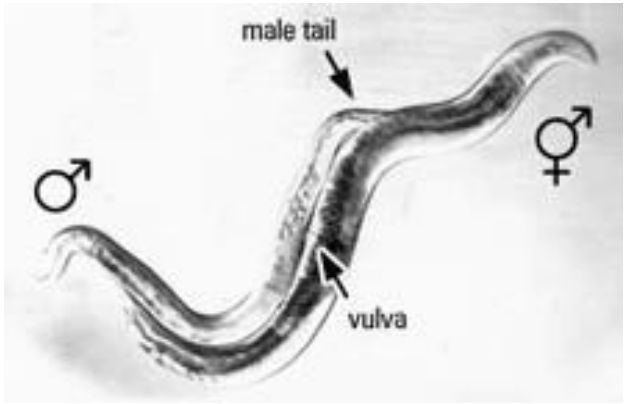
Martin Holzenberger*, Joëlle Dupont†, Bertrand Ducos*, Patricia Leneuve*, Alain Géloën‡, Patrick C. Even§, Pascale Cervera‖ & Yves Le Bouc*

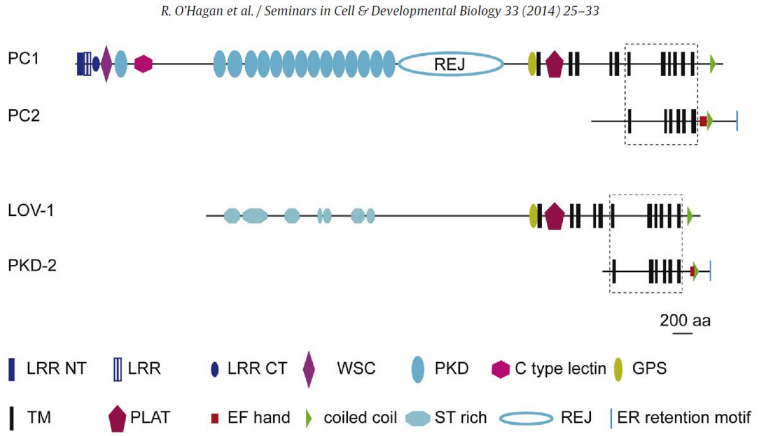
Extended Longevity in Mice Lacking the Insulin Receptor in Adipose Tissue

Matthias Blüher,[1] Barbara B. Kahn,[2] C. Ronald Kahn[1*]

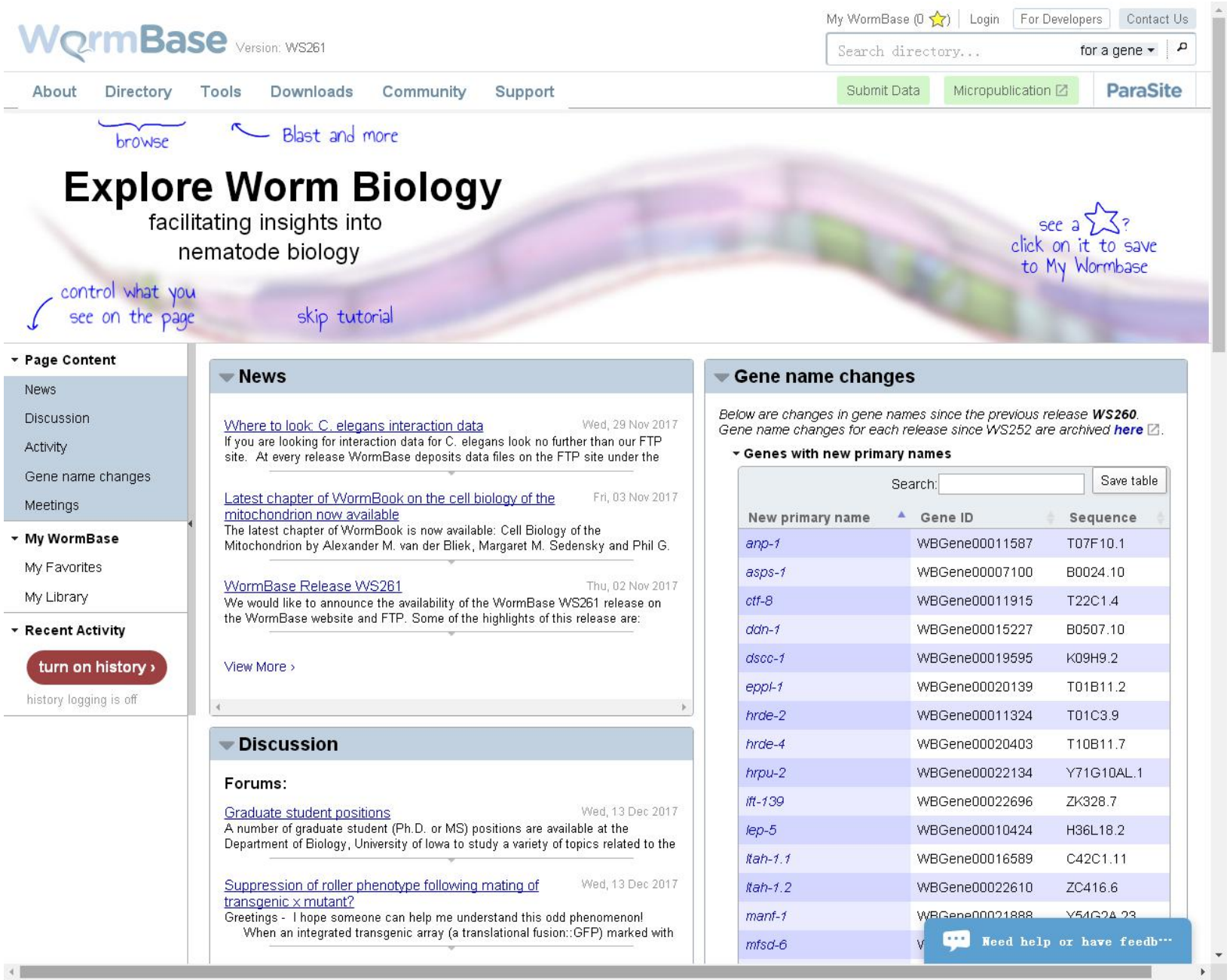


线虫的多囊肾模型　　PKD1,2

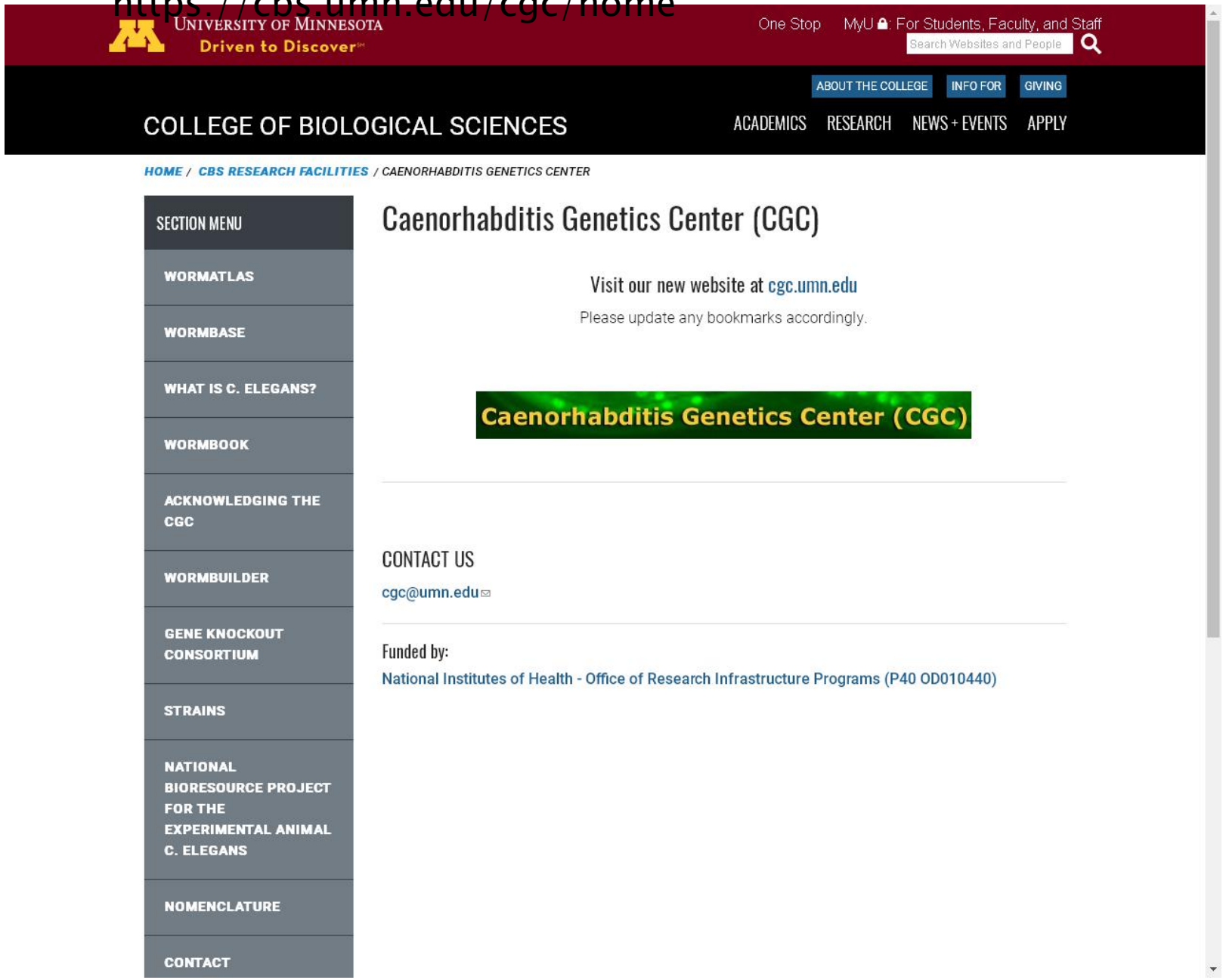




加州理工、冷泉港实验室等　　http://www.wormbase.org/

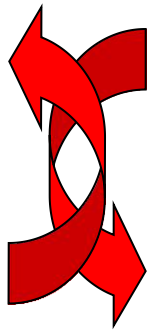


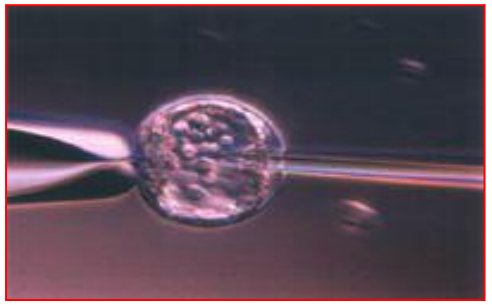
明尼苏达苏达大学
https://cbs.umn.edu/cgc/home

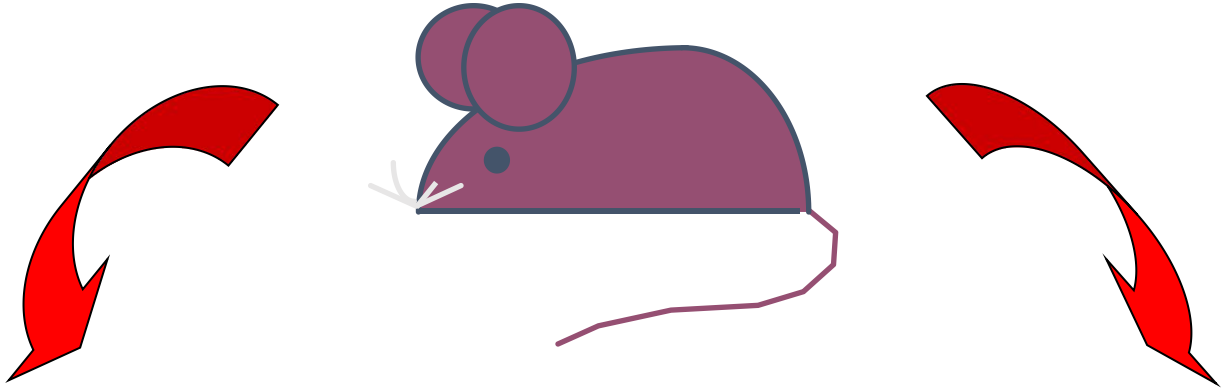


遗传操作技术原理

目的基因

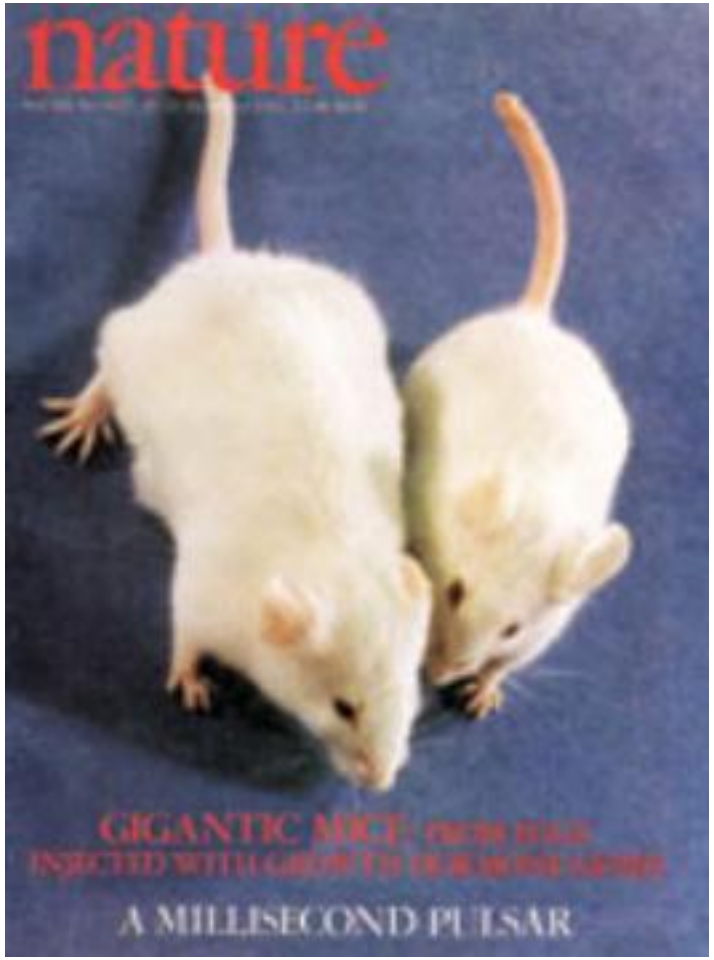






功能获得 ← 转基因及基因定点修饰 → 功能缺失

转基因小鼠技术的发展历史



- 1974 Jaenisch and Mintz：将SV40病毒DNA注射小鼠囊胚，得到整合有SV40病毒的小鼠（Proc Natl Acad Sci U S A. 1974 Apr;71(4):1250-4.）
- 1976 Jaenisch：将逆转录病毒用于小鼠囊胚感染获得转基因小鼠，并能传代（Proc Natl Acad Sci U S A 1976 Apr;73(4):1260-4）
- 1980-81 Gordon et al., Brinster et al., Costantini et al.：DNA小鼠受精卵原核注射建立了转基因小鼠的方法（Science. 1981 Dec 11; 214(4526): 1244-6. Cell. 1981 Nov;27(1 Pt 2):223-31. Nature. 1981 Nov 5;294(5836):92-4.）
- 1982 Palmiter et al., 在小鼠中过度表达大鼠生长激素基因，获得超级小鼠 （Nature. 1982 Dec 6;300(5893):611-5.）

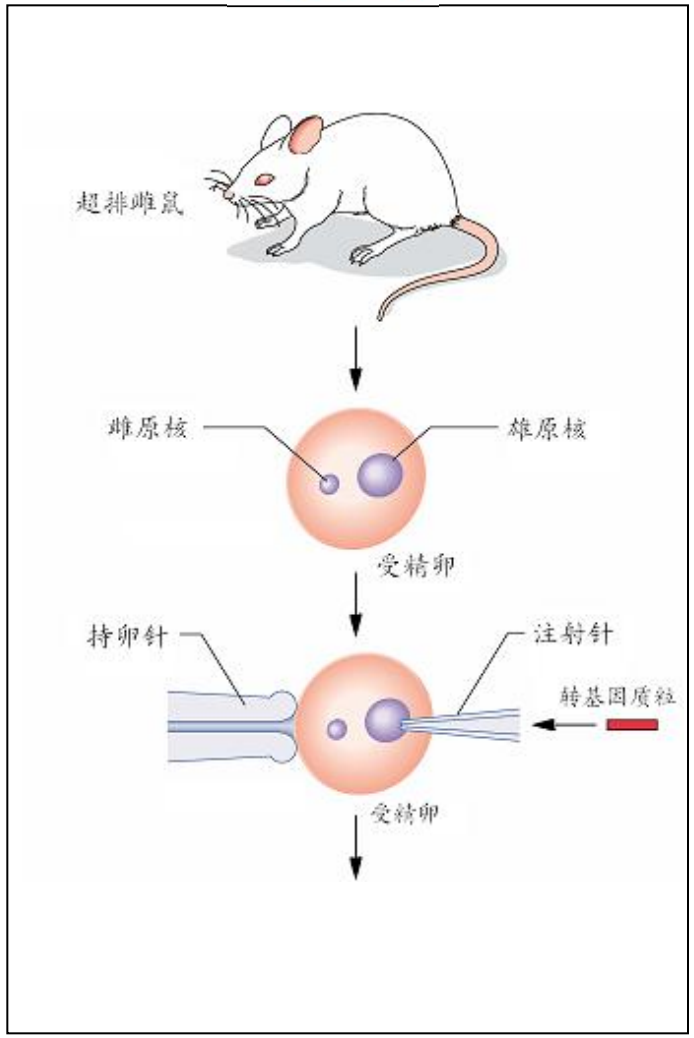
转基因小鼠技术流程

（受精卵显微注射方法）

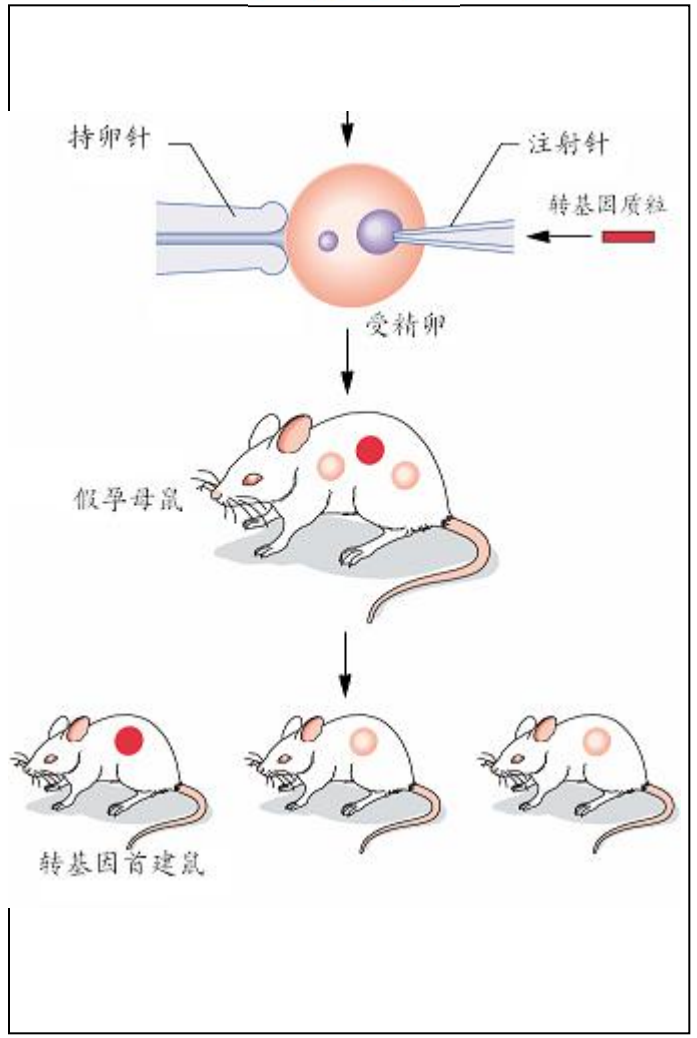
小鼠“基因手术室”

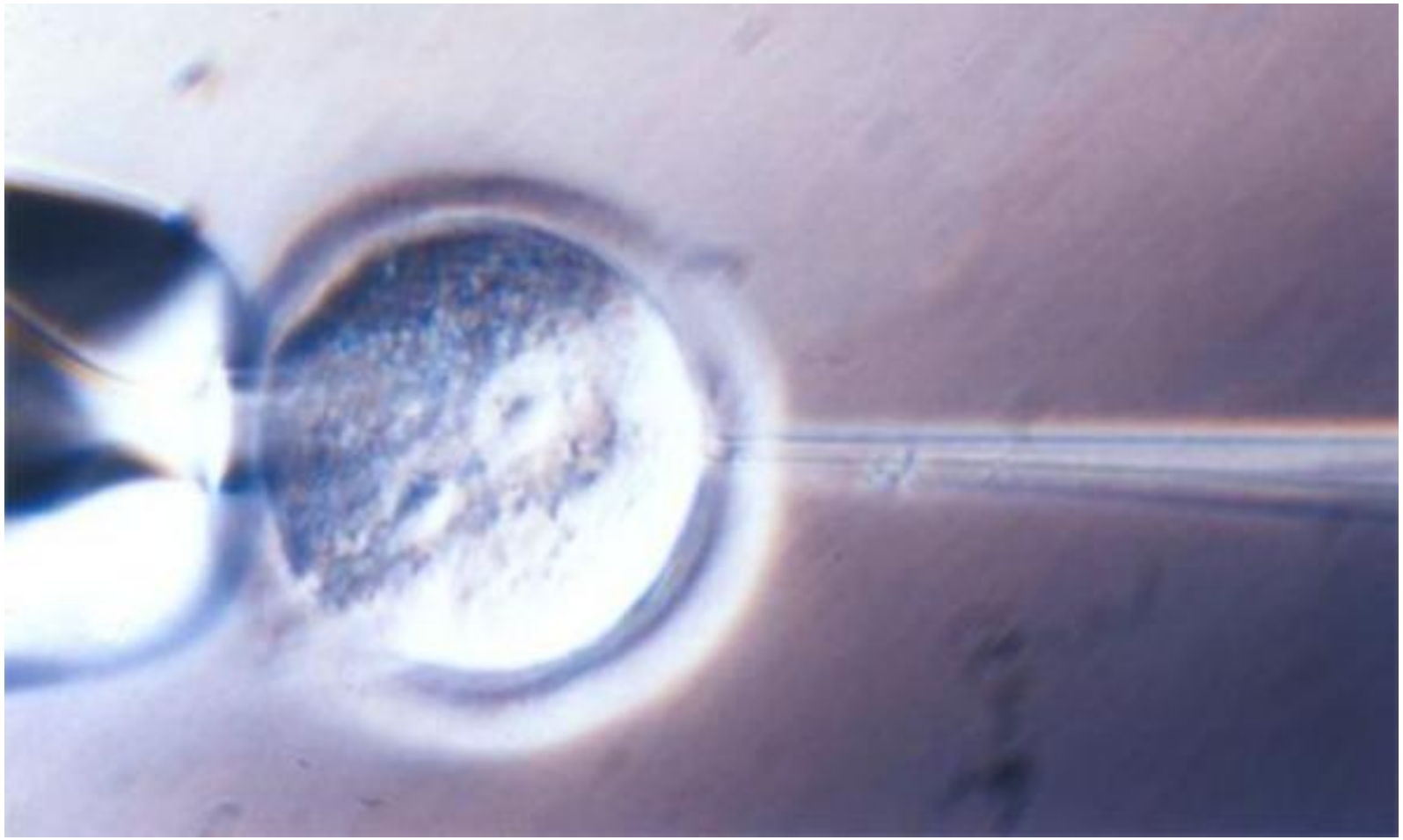
小鼠“病房”

显微注射



胚胎移植





受精卵单细胞原核注射制备转基因小鼠的特点

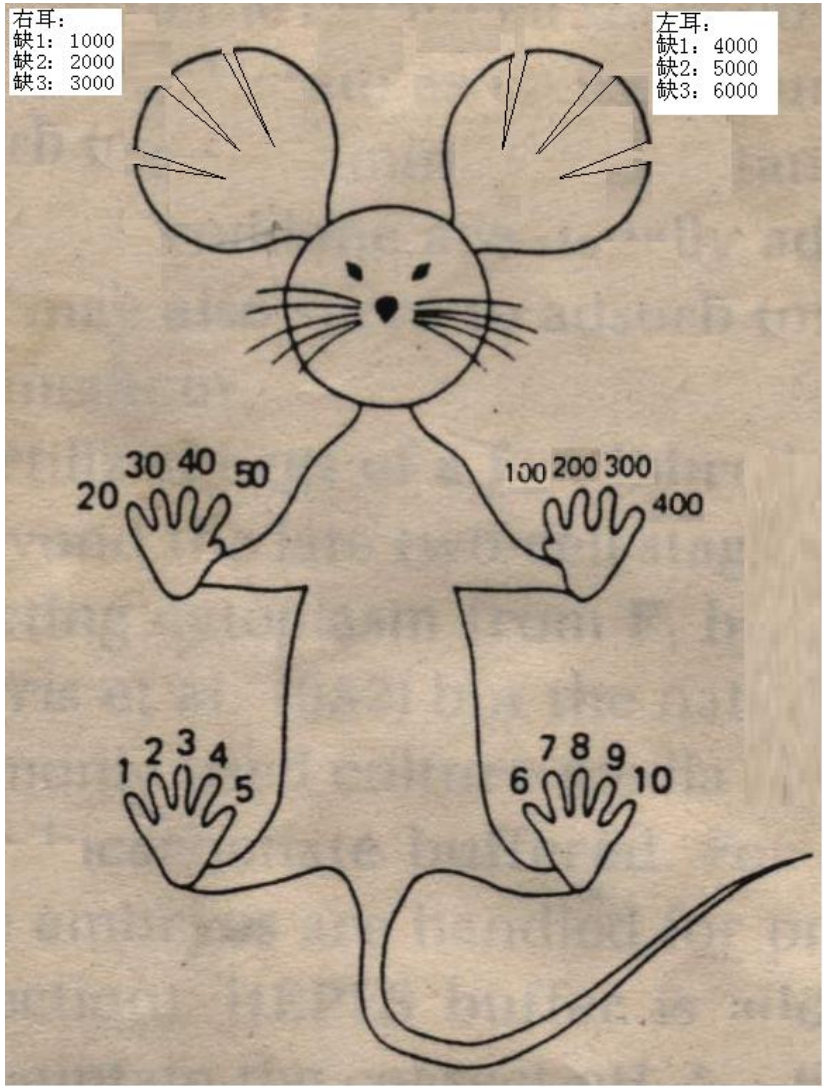
- 随机插入
  - 单位点和多位点插入并存
  - 多拷贝串连
- 插入位点及整合方式影响转基因的表达和表型
  - 插入位点影响
  - 插入序列引发的de novo甲基化
  - 同源性依赖的基因沉默
- 使用前应先建系
- 表型数据需要在两个或以上独立建系小鼠上相互印证

转基因小鼠的繁育 I

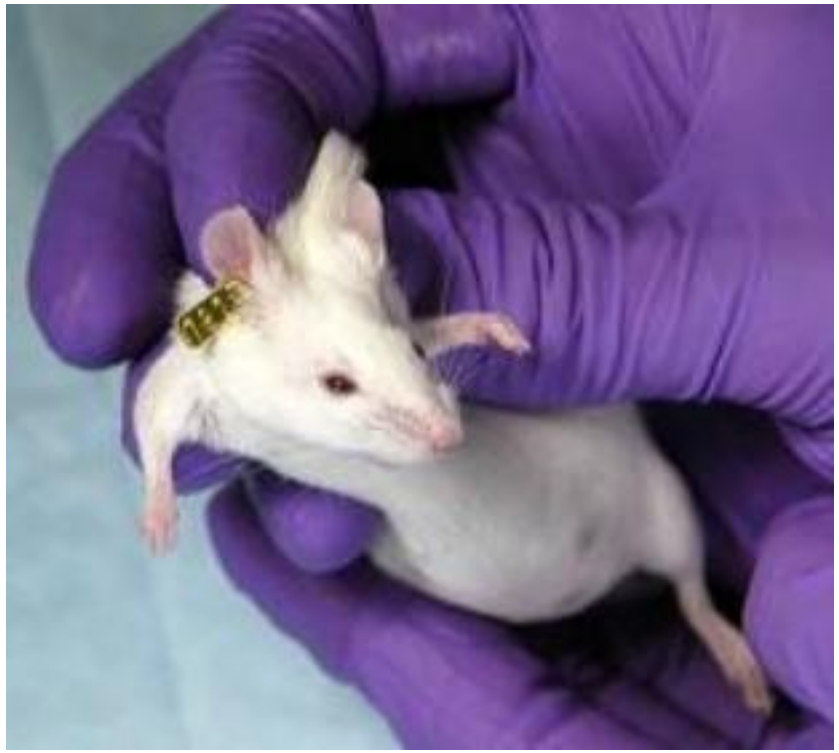
- 决定所需要的小鼠品系背景
- 首建者分别和选定的野生型品系交配繁育
  - 首建者小鼠之间不能进行相互交配
  - 一般杂合子即用来实验
- 同批的实验用鼠尽量为同窝生小鼠
  - 杂合子配野生型，50%野生型，50%为杂合子

转基因小鼠繁育II

- 小鼠的标牌书写（生日，父母）
- 小鼠编号（1-2周）
- 采集小鼠尾部组织或者耳轮组织
- 提取DNA，基因型鉴定
- 小鼠断奶及时雌雄分笼



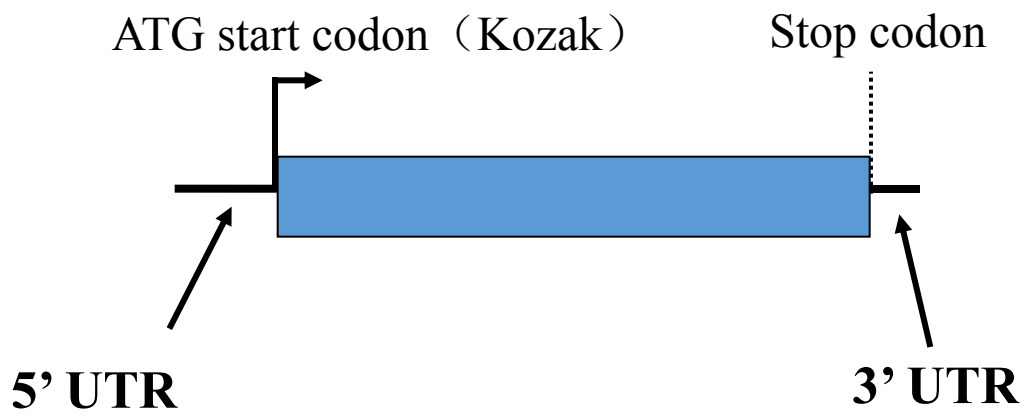

851
851



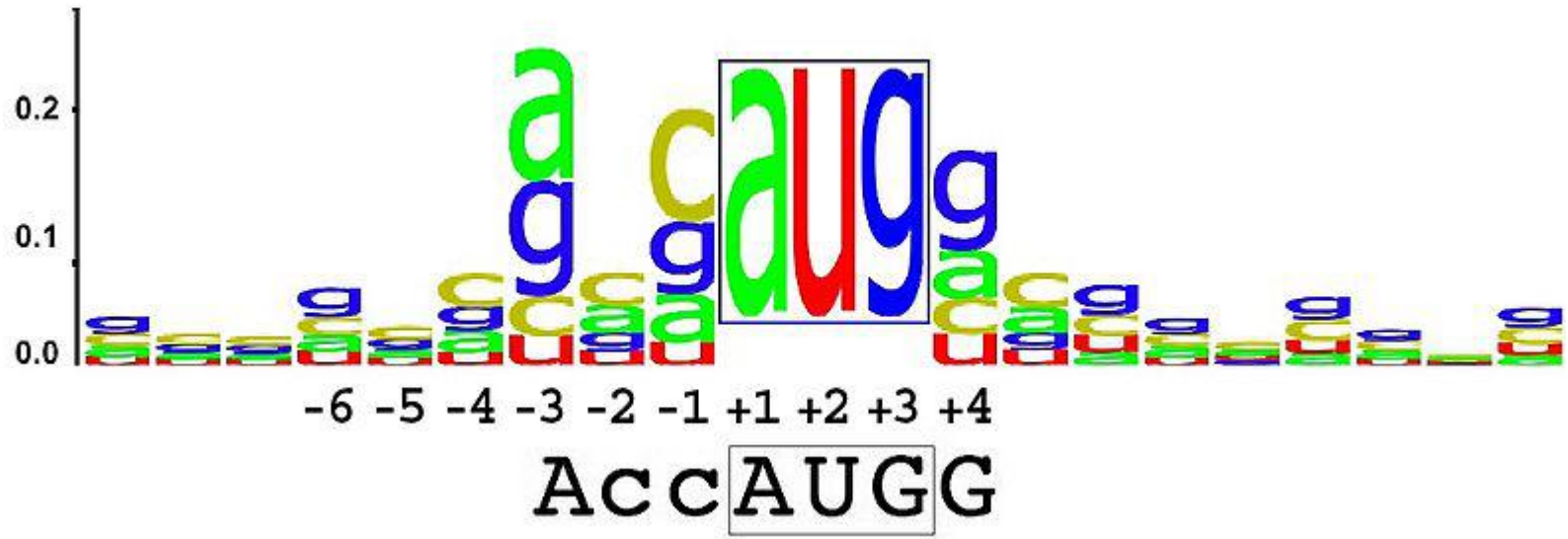

Identification Systems, Inc.

转基因载体构建 Ⅰ

获得所需要表达蛋白的cDNA



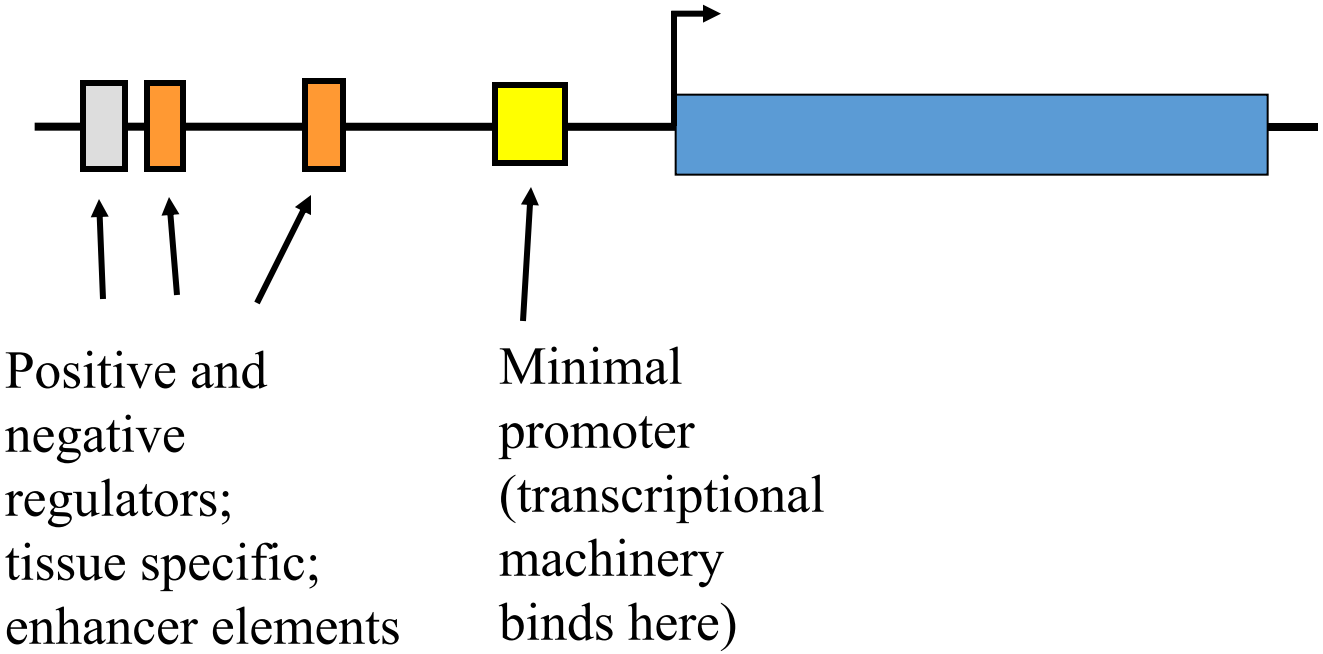
Kozak序列



转基因载体构建 II

获得所需要表达蛋白的cDNA

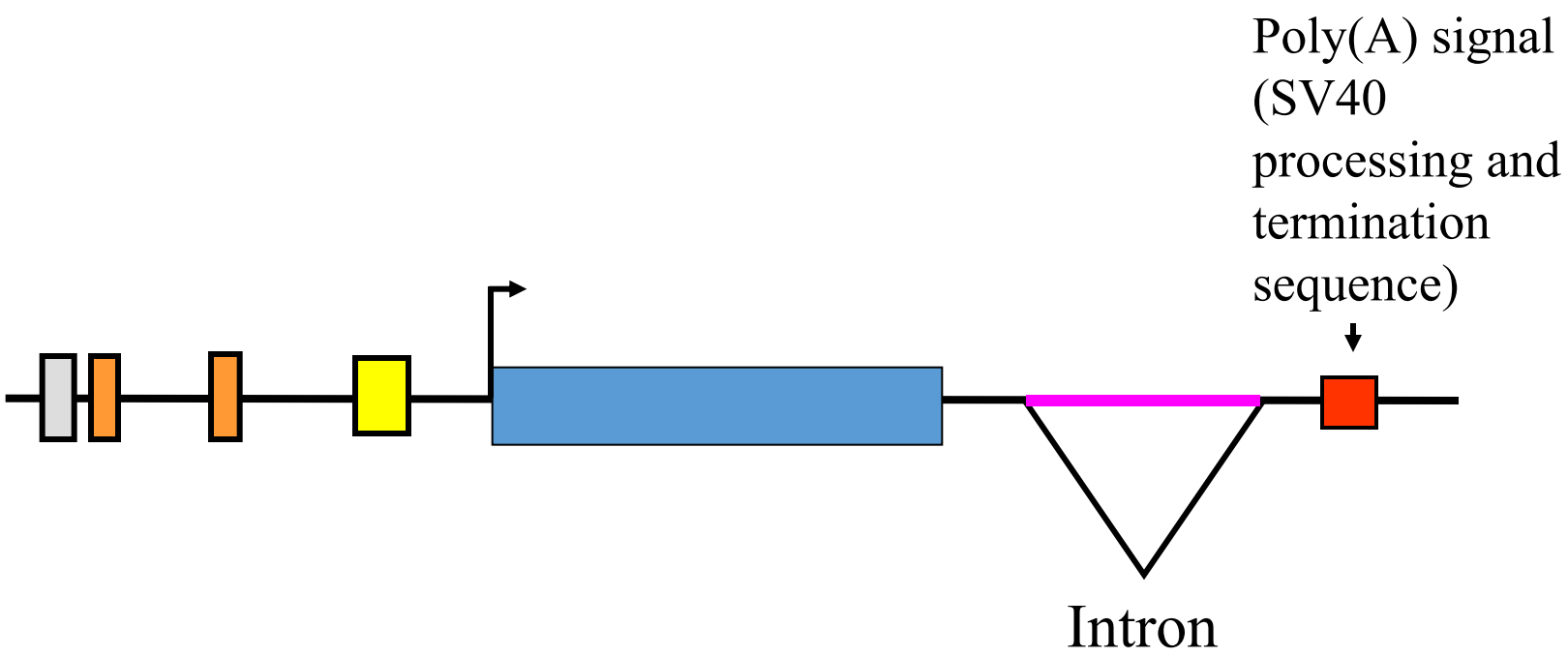
选择合适的基因表达启动子



转基因载体构建III

获得所需要表达蛋白的cDNA
选择合适的基因表达启动子

保证转录后的正确加工



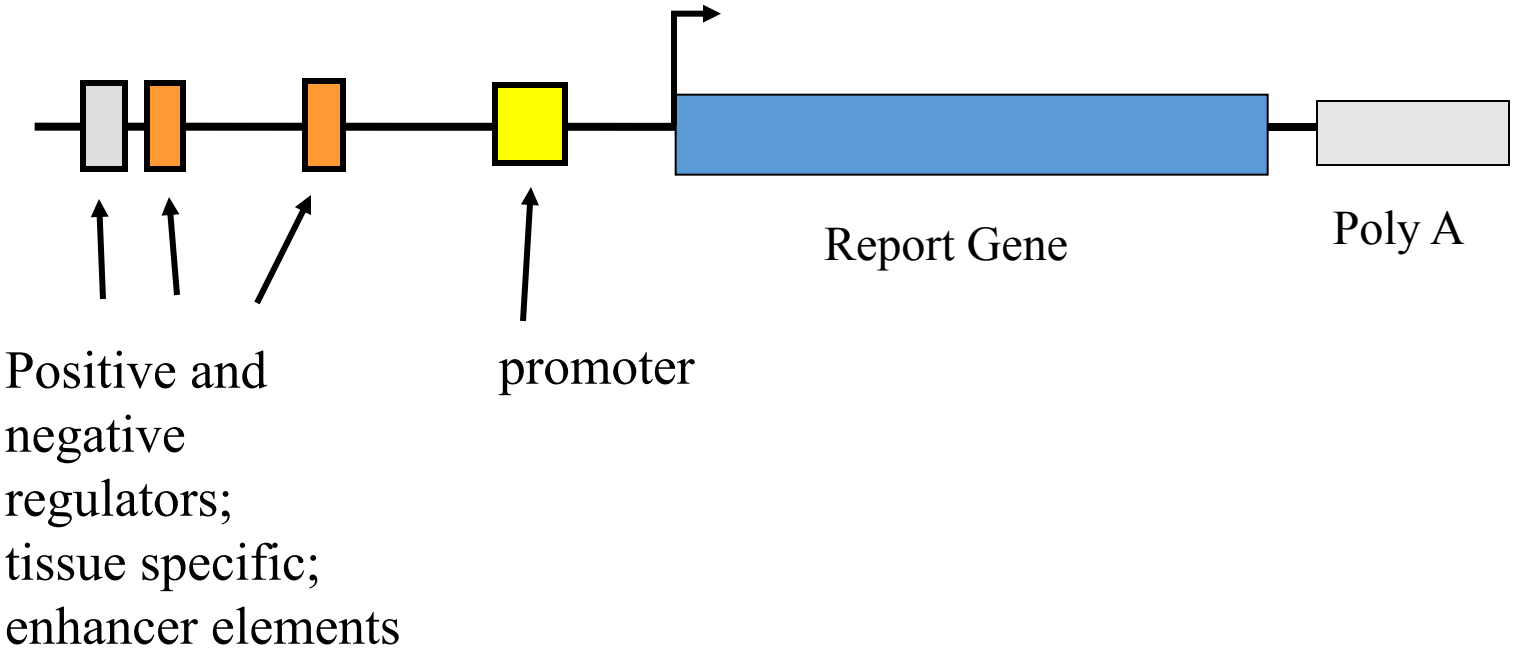
基因调控序列的研究及报告基因

- 启动子；增强子；负调控元件 等等
- 常用的报告基因
  - E.coli β–半乳糖苷酶 (lac Z)
    - 底物:ONPG;MUG;X-gal
  - E.coli 葡糖苷酸酶 (gusA)
    - 底物:X-gluc
  - 荧光素酶(luc)
    - 底物:luciferin
  - 荧光蛋白(GFP)等

报告基因载体构建

Select a report gene

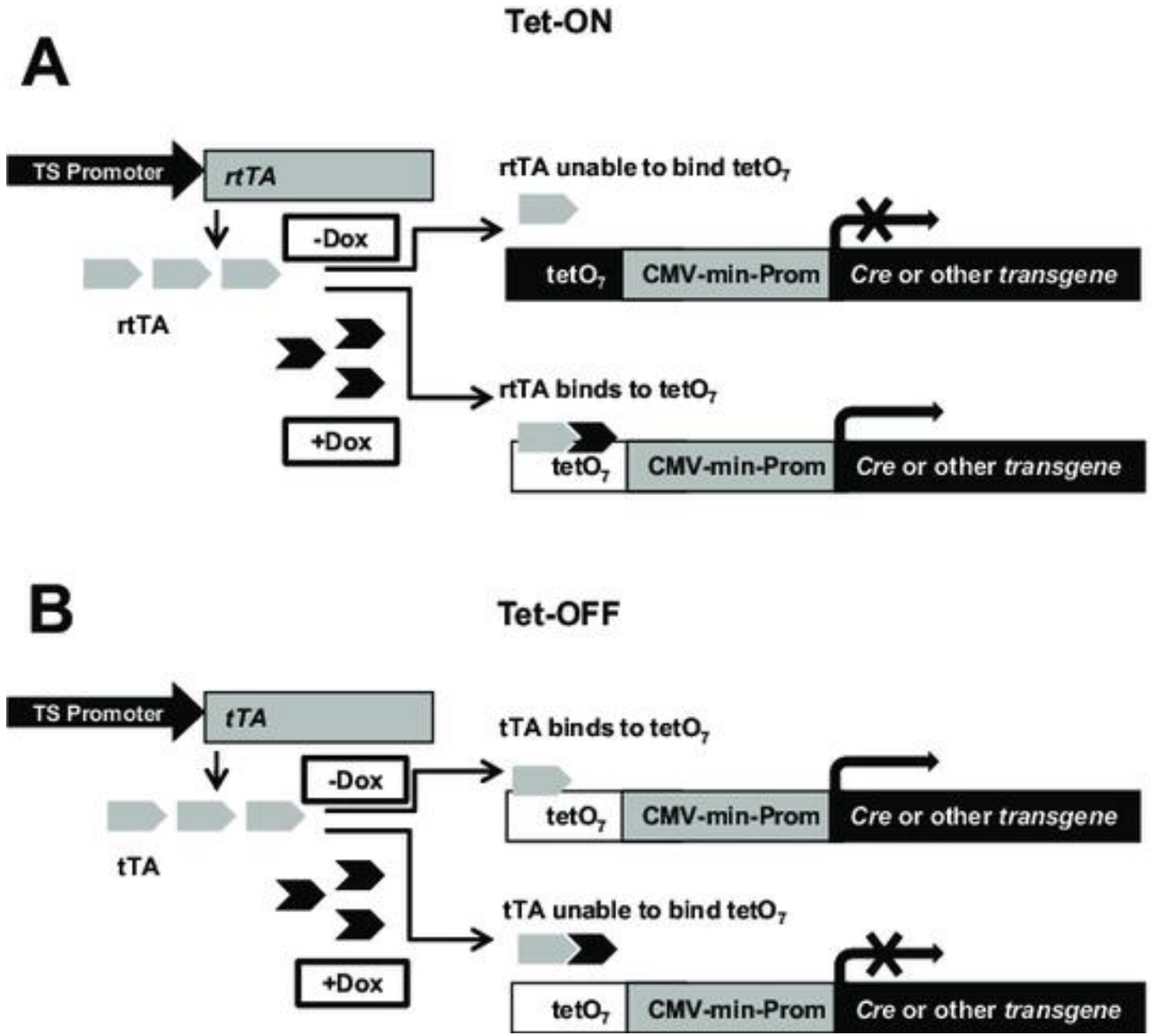
Incorporate the promoter elements you want to drive expression

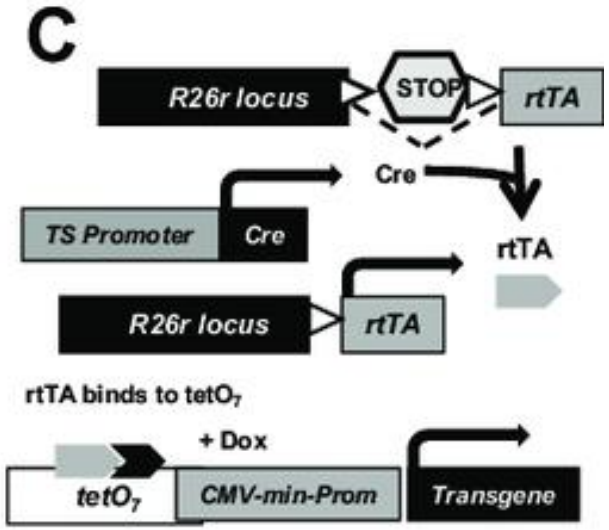


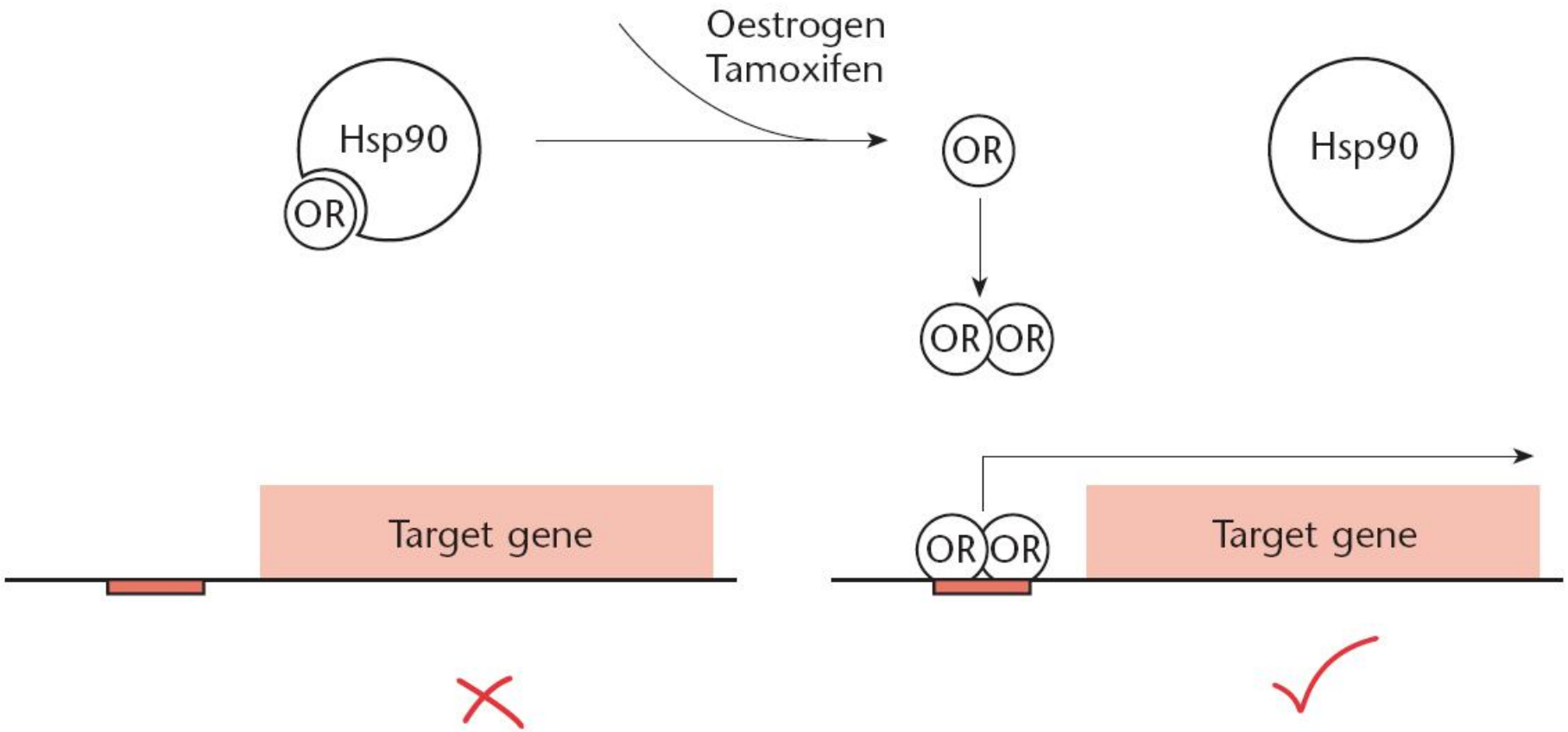
可诱导的基因表达方法

- 利用内源性可诱导的启动子：
  - 热休克蛋白，hsp70 基因
  - 金属硫蛋白基因
- 重组型的可诱导启动子
  - LacI；tet-on or tet-off；蜕皮激素；化合物诱导的二聚化（CID）
- 蛋白水平的诱导
  - ER受体融合蛋白/Tamoxifen；destabilizing domain (DD)
- 重组酶系统

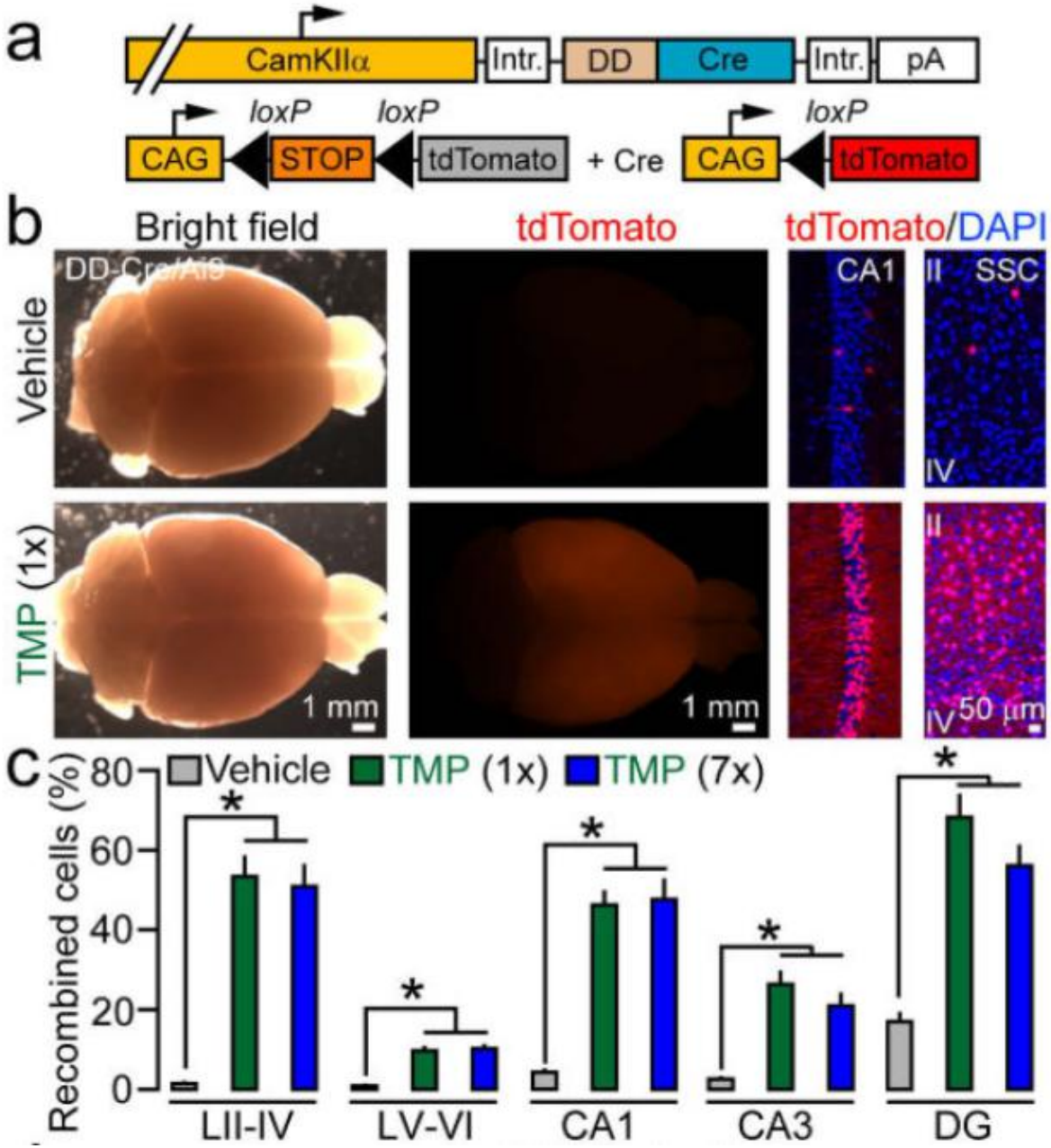
四环素诱导的表达控制系统（Tet-on vs Tet-off）



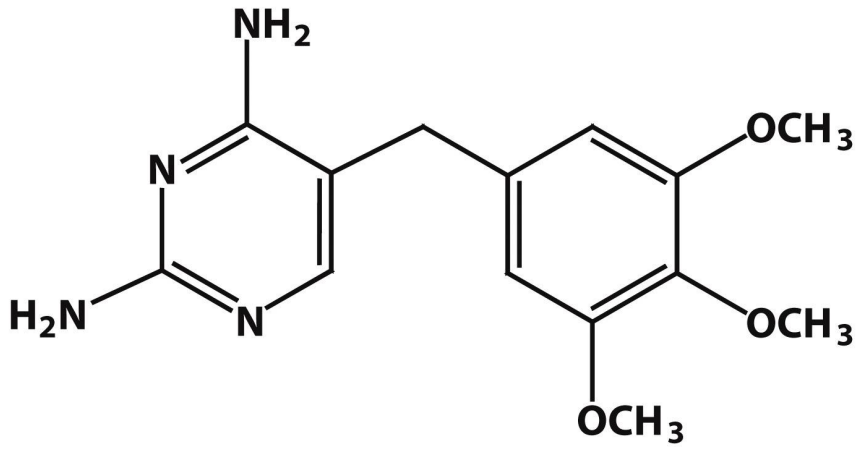




OR蛋白的Hsp90结合区域ET和目标蛋白融合形成人工调控蛋白

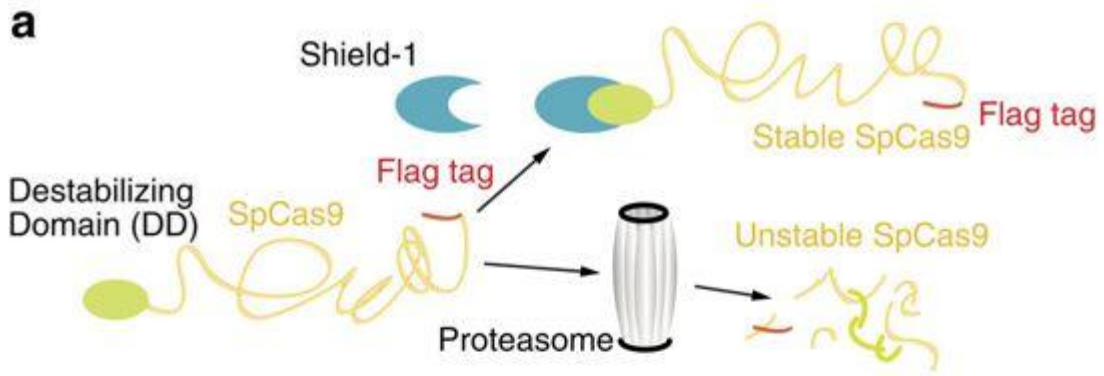


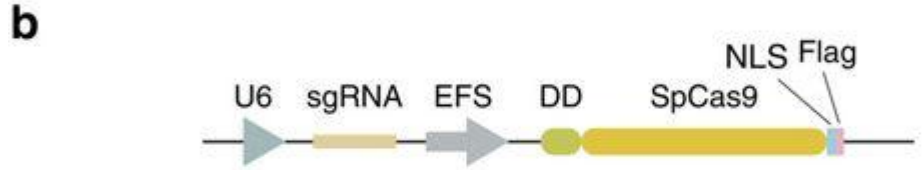
带有来自human FKBP12 或 bacterial dihydrofolate reductase (ecDHFR) 结构域的蛋白在细胞中能迅速被降解,destabilizing domain (DD)。前者可以用Shield-1来稳定，后者可以用Trimethoprim (TMP，甲氧苄氨嘧啶)来稳定融合蛋白。

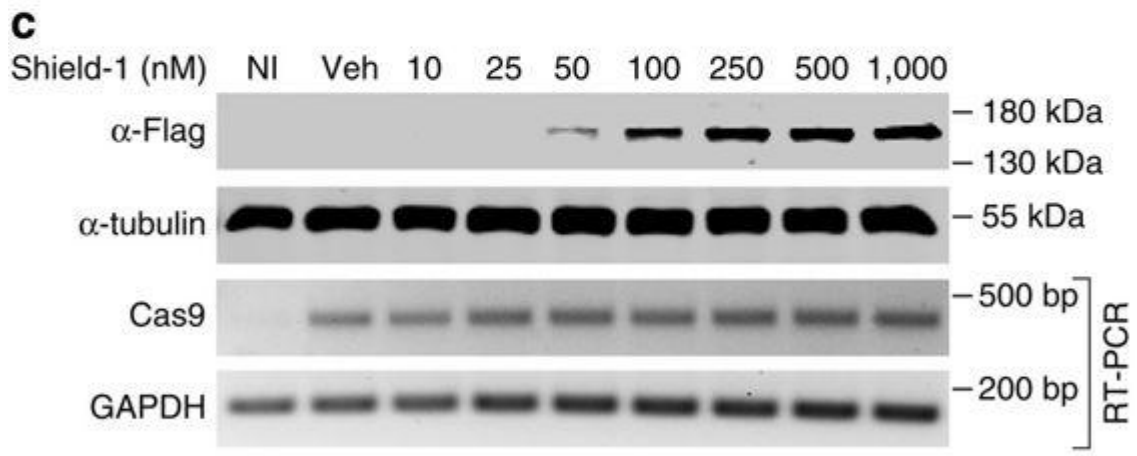


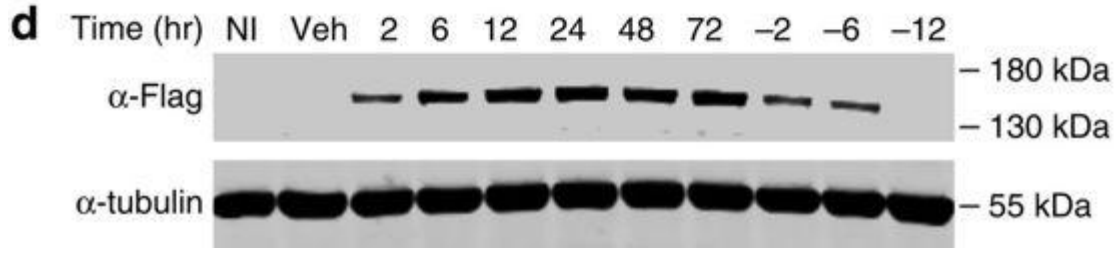
Trimethoprim

Unnumbered 25 p750b
Biochemistry, Seventh Edition

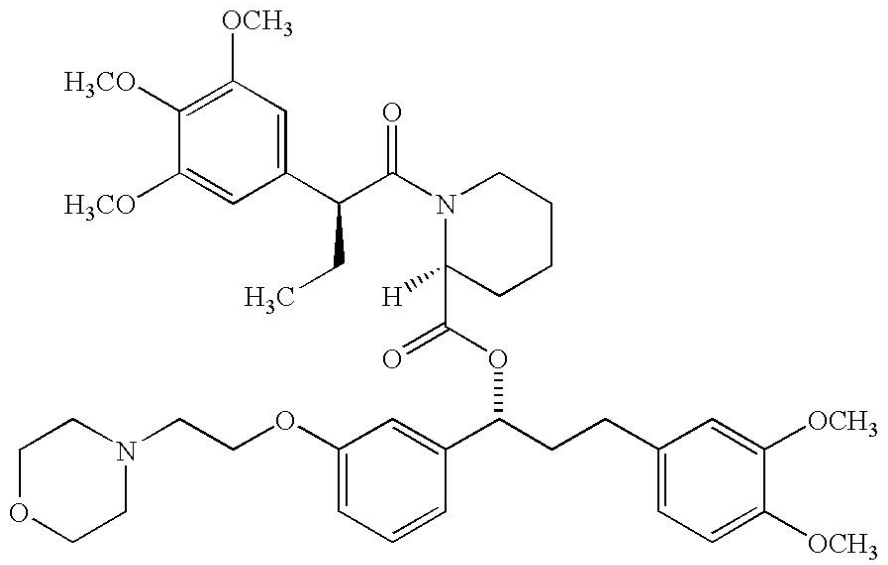

a
Shield-1
Flag tag
Stable SpCas9
Flag tag
Destabilizing
Domain (DD)
SpCas9
Unstable SpCas9
Proteasome


b
NLS Flag
U6
sgRNA
EFS
DD
SpCas9


c
Shield-1 (nM)
NI
Veh
10
25
50
100
250
500
1,000
α-Flag
180 kDa
130 kDa
α-tubulin
55 kDa
Cas9
500 bp
GAPDH
200 bp
RT-PCR

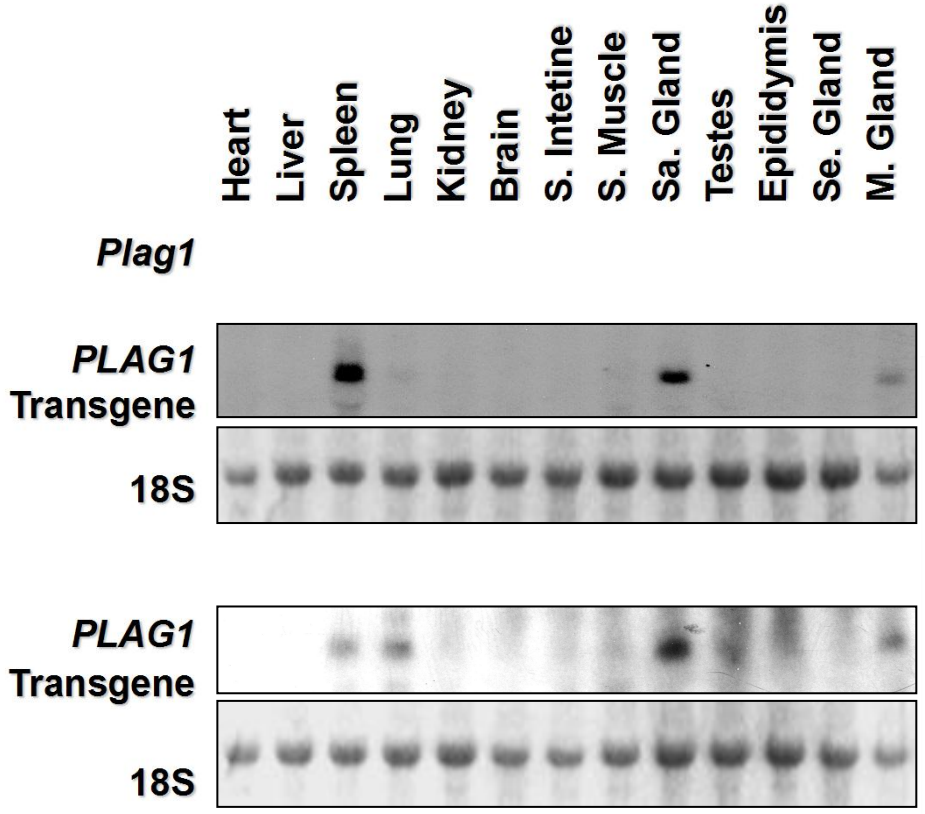

d
Time (hr)
NI
Veh
2
6
12
24
48
72
–2
–6
–12
α-Flag
180 kDa
130 kDa
α-tubulin
55 kDa

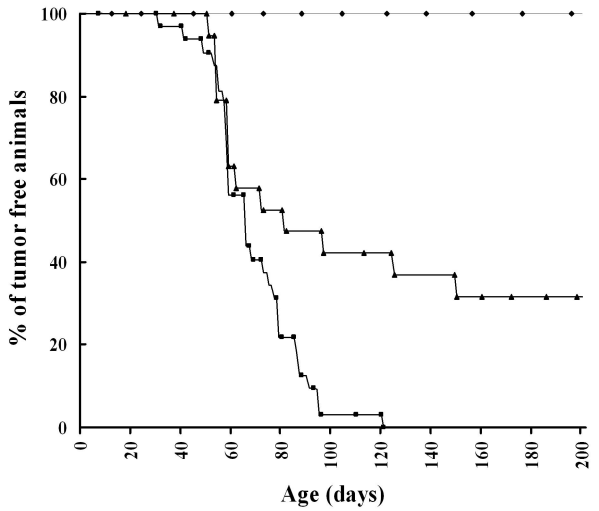
Shield-1


OCH3
H3CO
H3CO
O
N
H3C
H
O
O
N
O
O
OCH3
OCH3

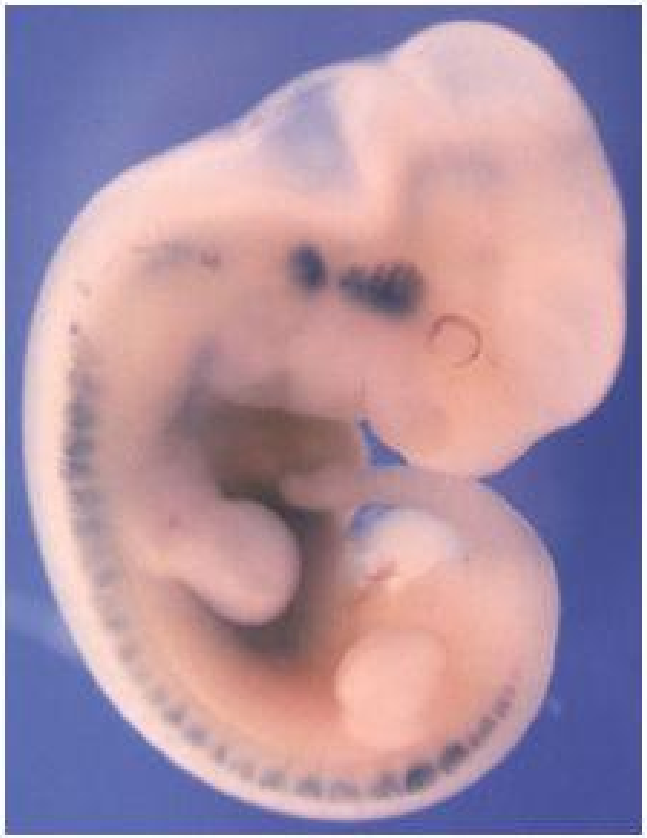
转基因小鼠应用举例：致病基因的确认

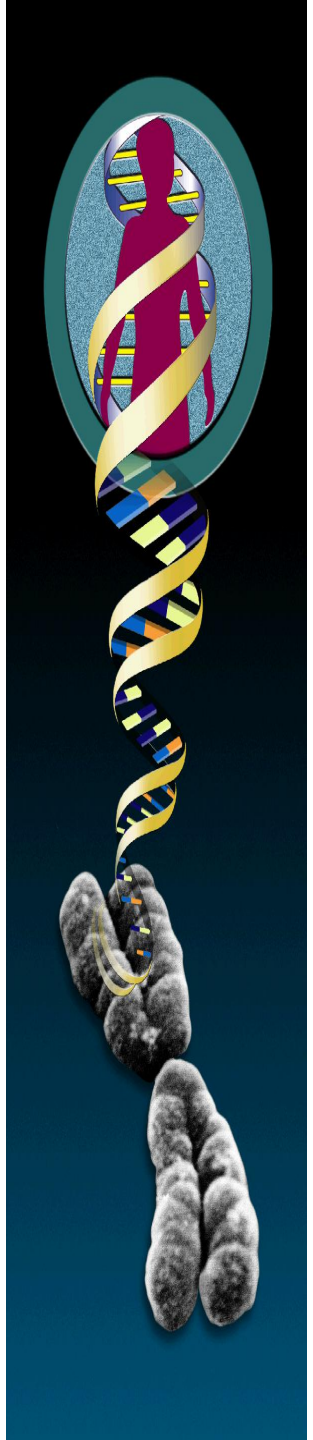
人类唾液腺肿瘤的发病率占头颈部肿瘤的三分之一，其中，唾液腺多形性腺瘤好发于年轻女性(40岁以下)和儿童，其发病率和癌变率均较高。临床研究表明，多形性腺瘤相关基因（PLAG1）在人类唾液腺多形性腺瘤中因染色体互换导致高表达，通过建立转基因小鼠证明了该基因的高表达和肿瘤发生的因果关系。



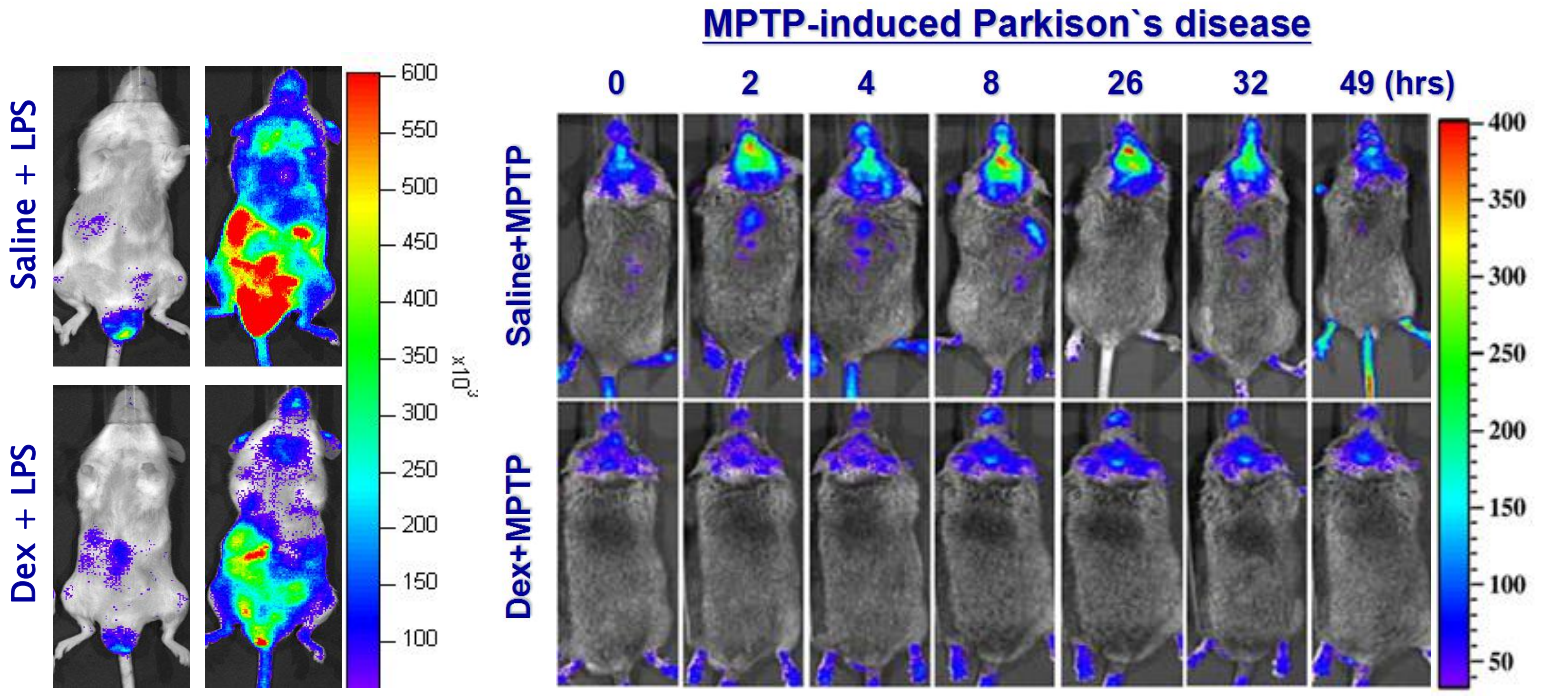


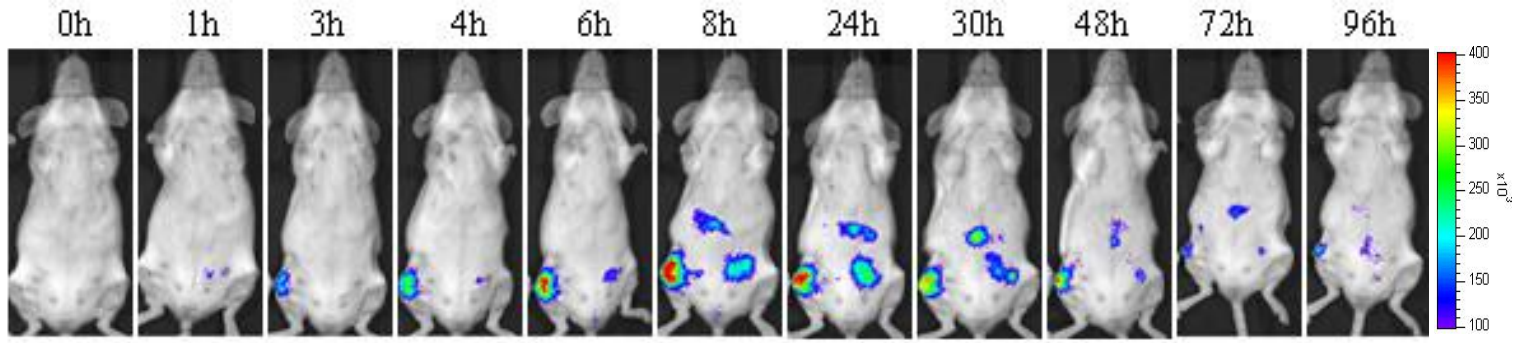
神经前体细胞特性性启动子的研究
（ neurogenin 1 ）





转基因小鼠应用举例：示踪基因的表达



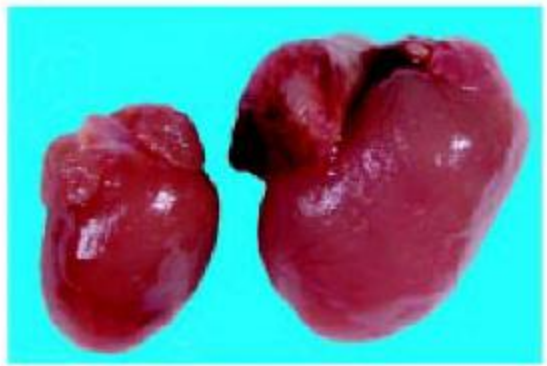


Organism Biomodel

Conditional Expression of an antisense mRNA of the mineralocorticoid receptor (MR) in cardiomyocytes

Cardiomyocyte specific expression of tTA → dox-dependent expression of MR antisense mRNA

Without dox treatment

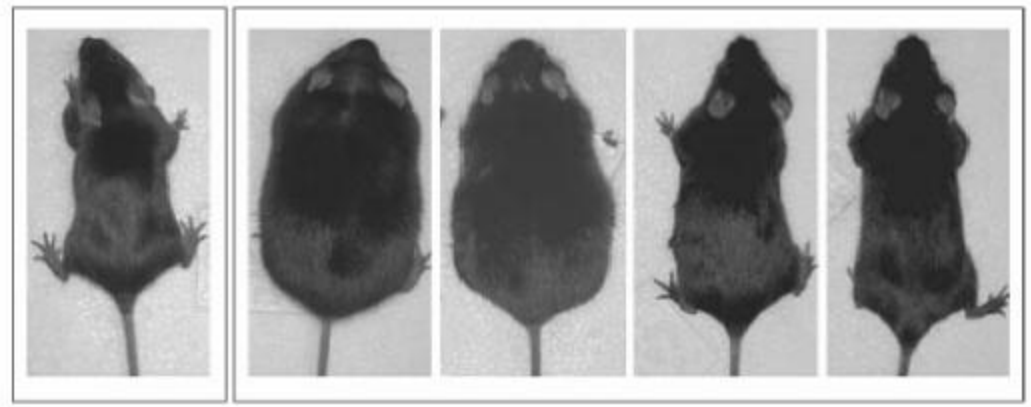


Control: normal

Transgenic: Severe heart disease, rapid weight gain, edema

control **Doxycycline treatment**

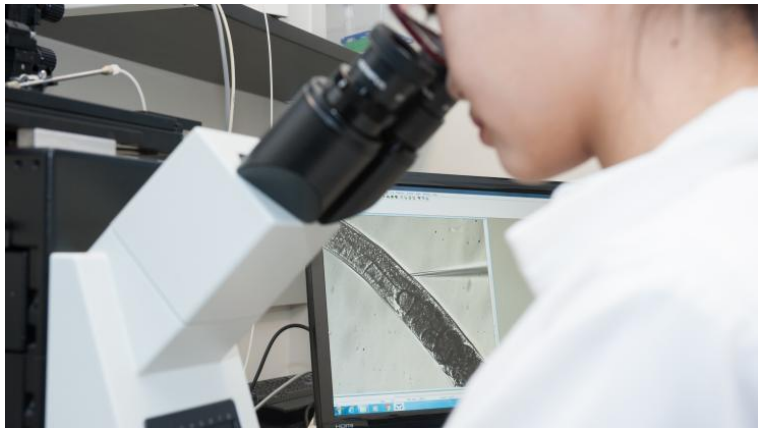
Day 0 **Day 0** **Day 4** **Day 8** **Day 10**

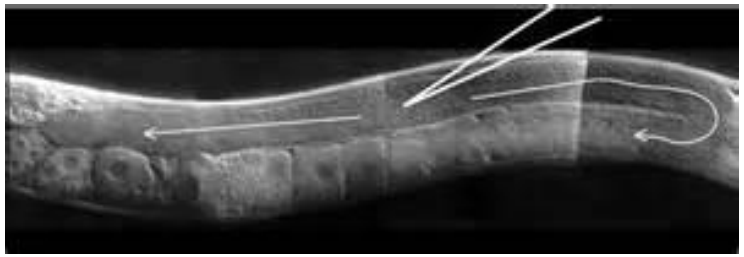


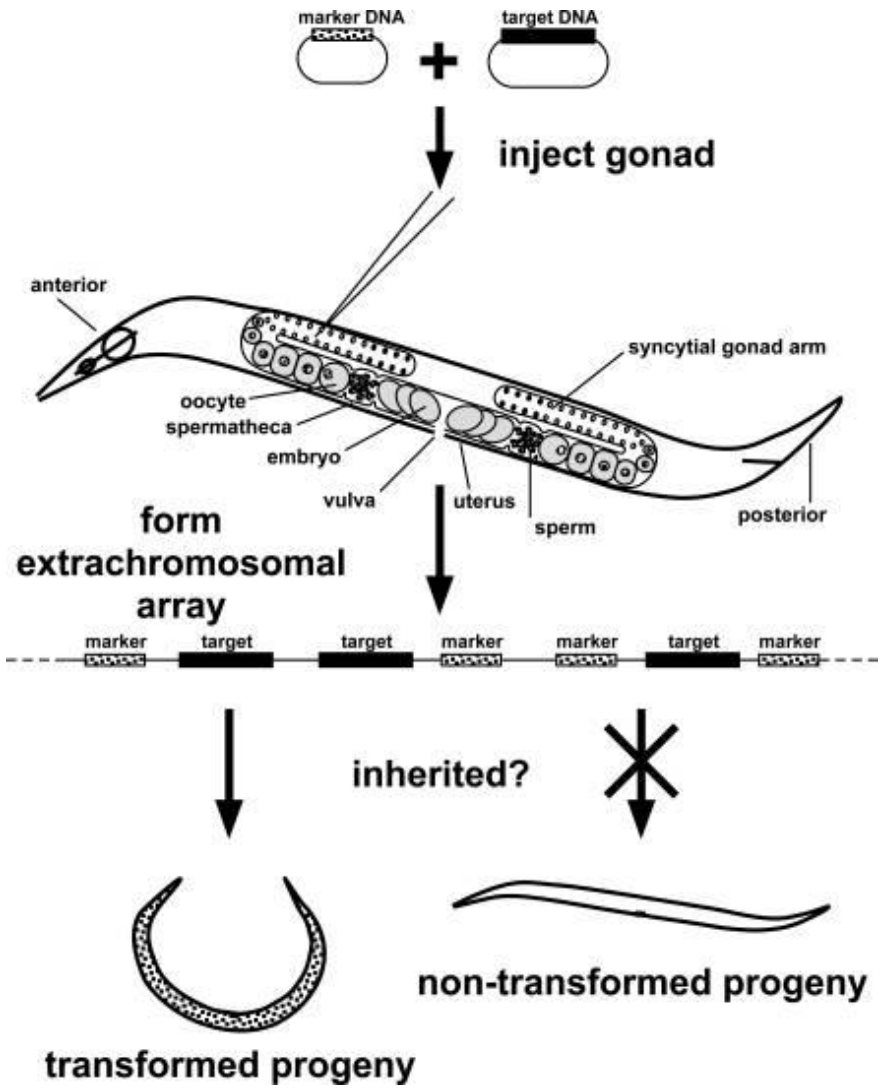
Reversibility of edema

Beggah *et. al.* (2002) *PNAS* **99:**7160

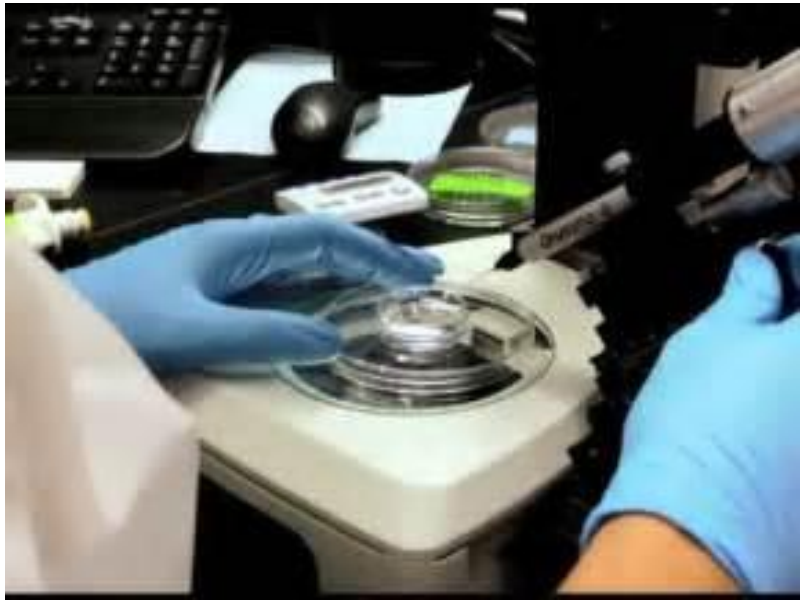
转基因线虫



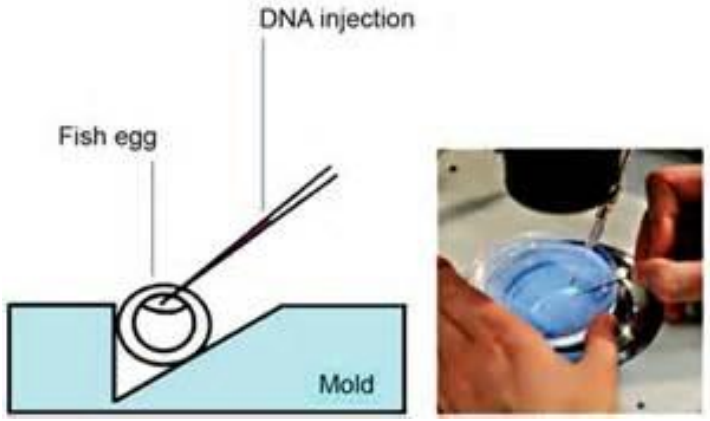


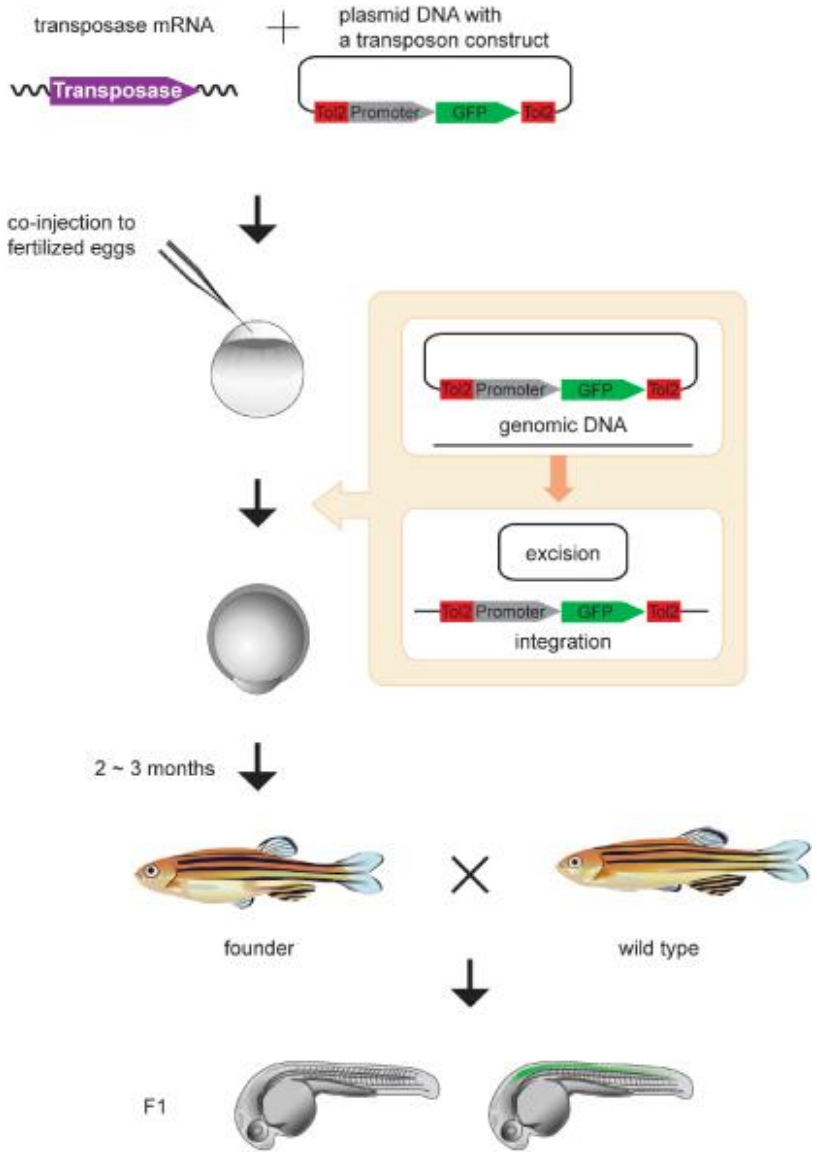

marker DNA
target DNA
inject gonad
anterior
syncytial gonad arm
oocyte
spermatheca
embryo
vulva
uterus
sperm
posterior
form extrachromosomal array
marker
target
target
marker
marker
target
marker
inherited?
non-transformed progeny
transformed progeny

转基因斑马鱼的制备

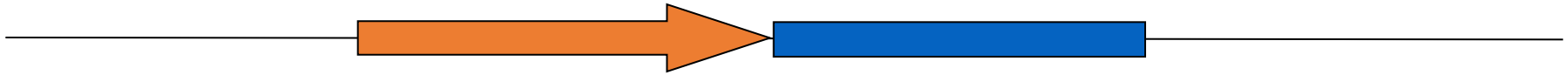


Agarose mold for mounting eggs

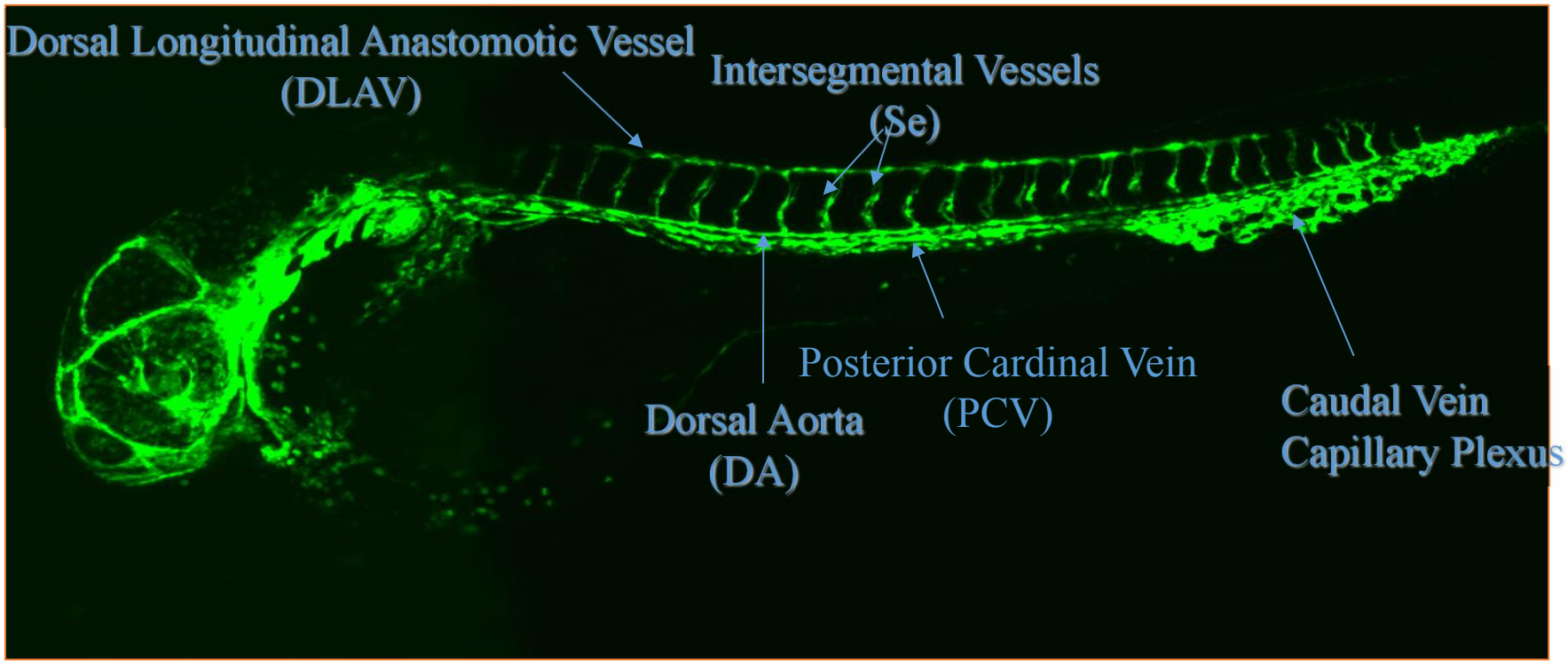




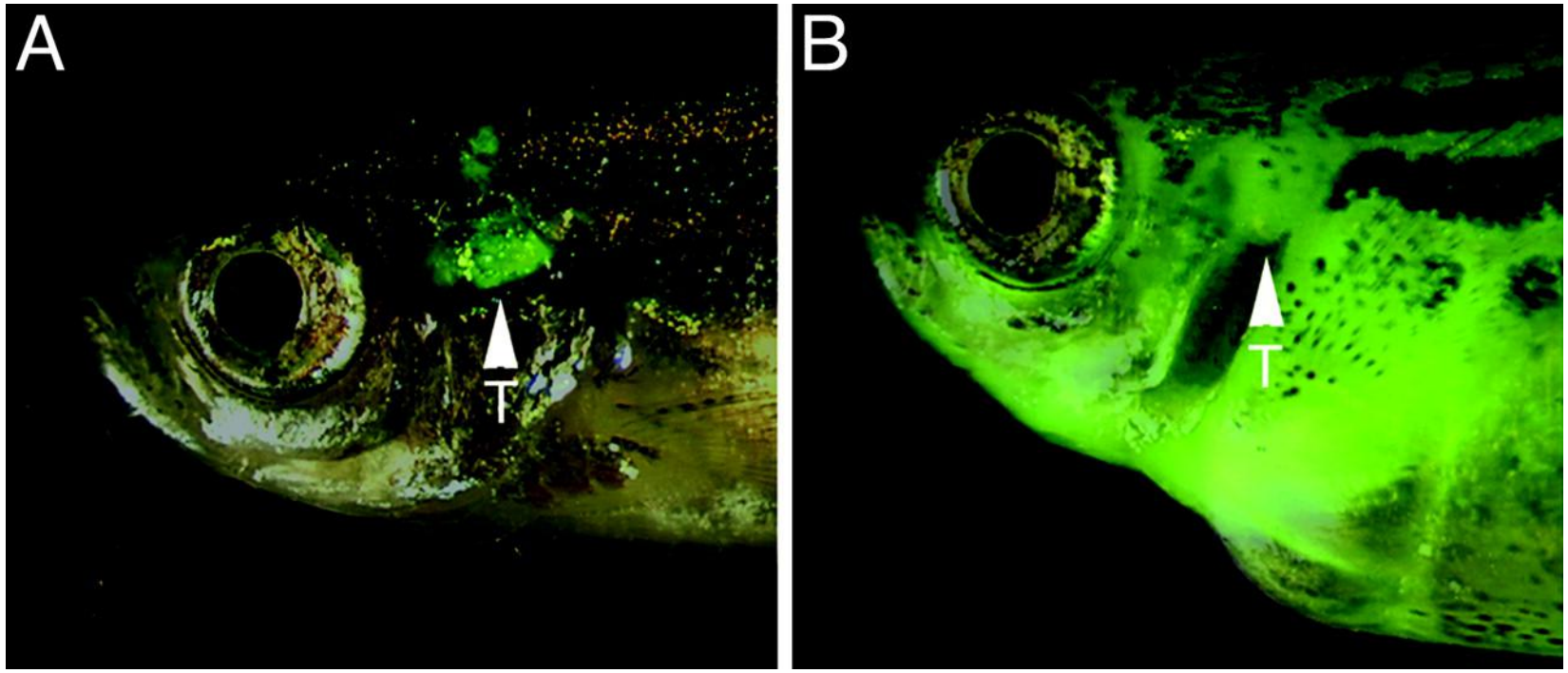
Transgenic zebrafish allow analysis of endothelial cells in living embryos



fli1-egfp transgenic embryo at 2 dpf

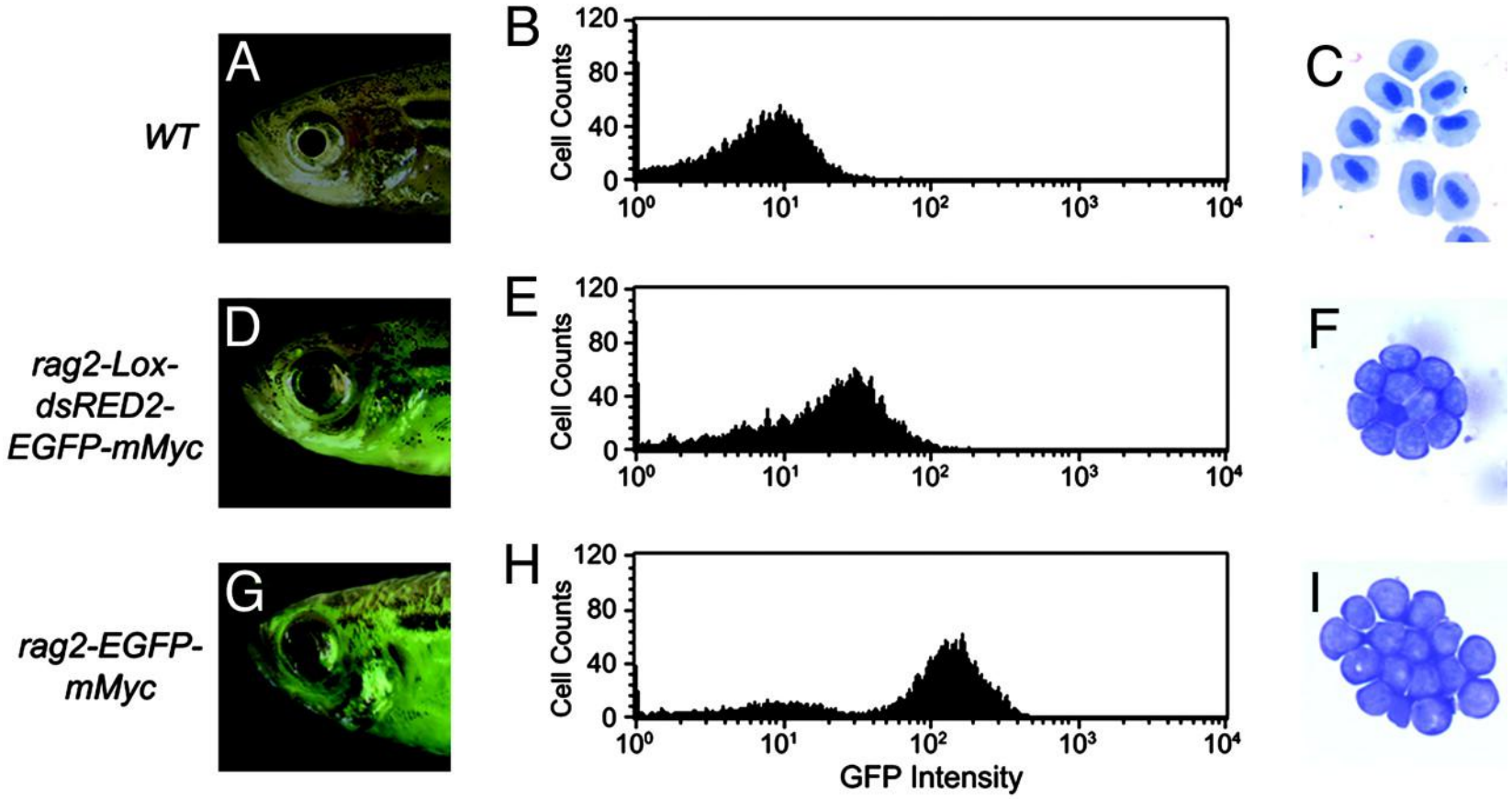


Stable transgenic rag2-EGFP-mMyc zebrafish develop GFP-labeled thymic lymphoma, which progresses to T-ALL.



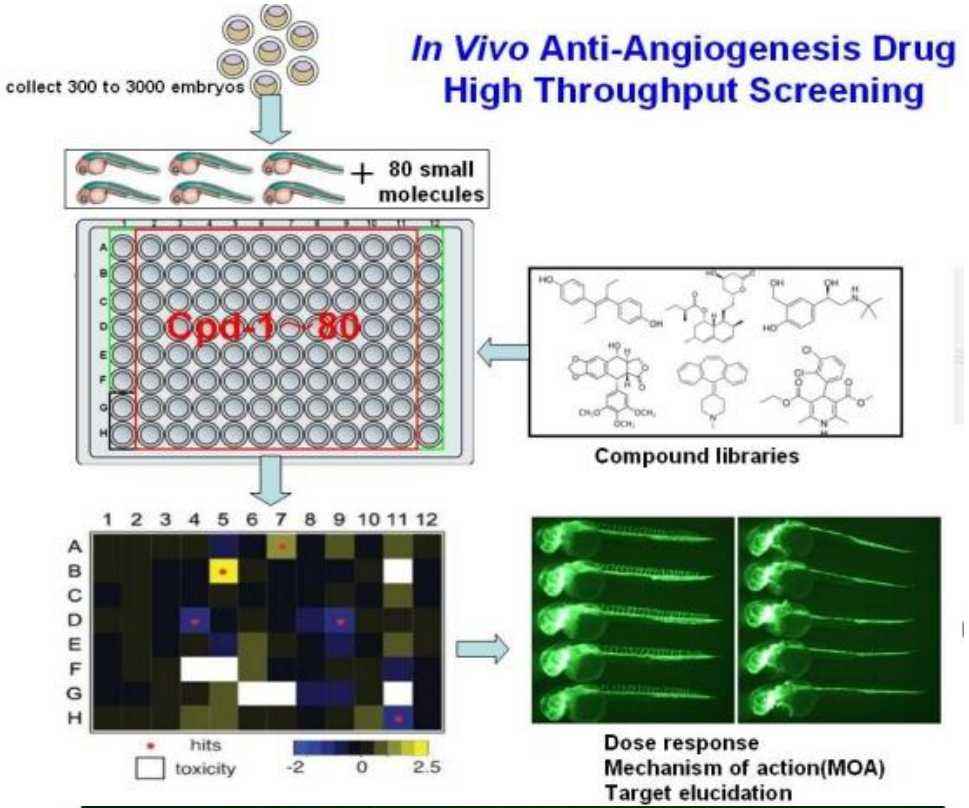
Langenau D M et al. PNAS 2005;102:6068-6073

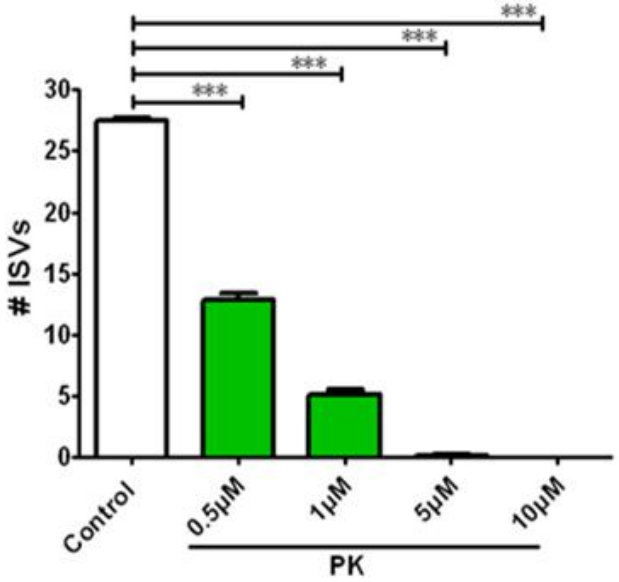
Leukemias developing in Cre-injected rag2-loxP-dsRED2-loxP-EGFP-mMyc transgenic fish are transplantable, express low levels of GFP fluorescence, and have typical lymphoblast morphology.



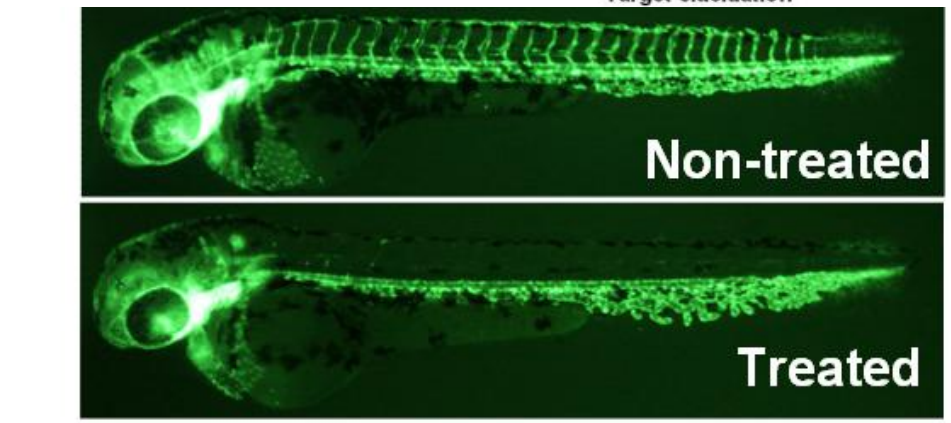
Langenau D M et al. PNAS 2005;102:6068-6073

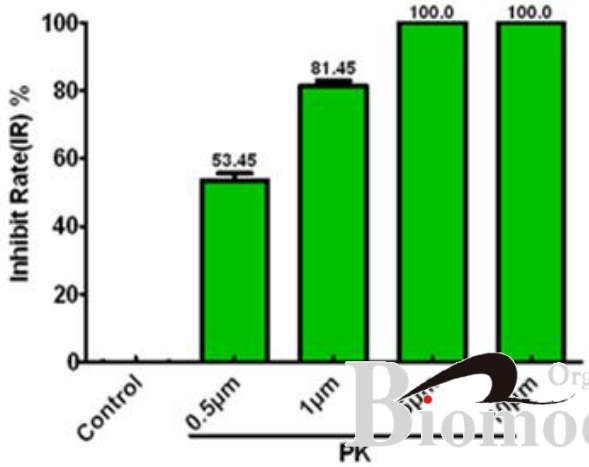
高通量抗血管生成药物筛选模型





*** P<0.001

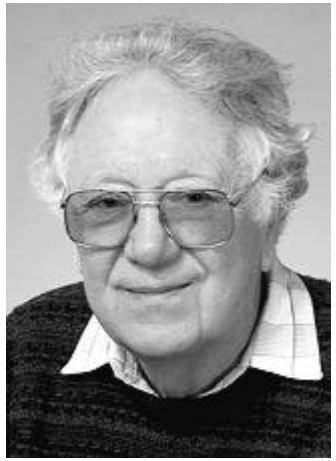




小鼠基因剔除技术的发展历史

Sir Martin J. Evans

Mario R. Capecchi

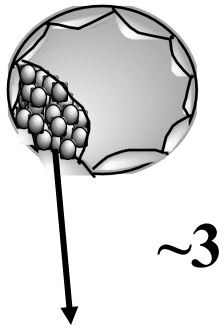


Oliver Smithies

- 1981Evans and Kaufman: （Nature. 1981 Jul 9;292(5819):154-6, Proc Natl Acad Sci U S A. 1981 Dec;78(12):7634-8）建立了ES细胞
- 1986－1987 Robertson et al ，Hooper et al，Kuehn et al，：对ES细胞进行遗传操作，并获得ES细胞来源的小鼠个体（Nature. 1986 Oct 2-8;323(6087):445-8. Nature 1987 Mar 19-25;326(6110):292-5, Nature. 1987 Mar 19-25;326(6110):295-8.）
- 1988 Thomas et al., Doetschman T.et al.在ES细胞中建立基于同源重组的基因打靶技术(Cell. 1987 Nov 6;51(3):503-12.; Nature. 1987 Dec 10-16;330(6148):576-8.)
- 1989 Jaenisch et al: 建立*b2*-microglobulin-deficient基因剔除小鼠(Nature. 1989 Nov 23;342(6248):435-8.)
- 1994 Gu et al， 将Cre-LoxP系统用于ES细胞的打靶，建立条件性基因剔除技术（Science. 1994 . 265:103. ）

Generating ES Cells

Blastocyst 3.5 days



~30 cells

Inner Cell Mass (128 cell stage)

1-totipotent

2-tissue culture

3-Transfectable

4-Selection

5- Differentiation In vitro

Harvest blastocysts on day 3.5 by flushing from the uterine lumem with M2 medium

↓

Plate out blastocysts in individual 10 mm dishes on mitotically inactive MEF or STO feeder cells to provide LIF and other factors

↓

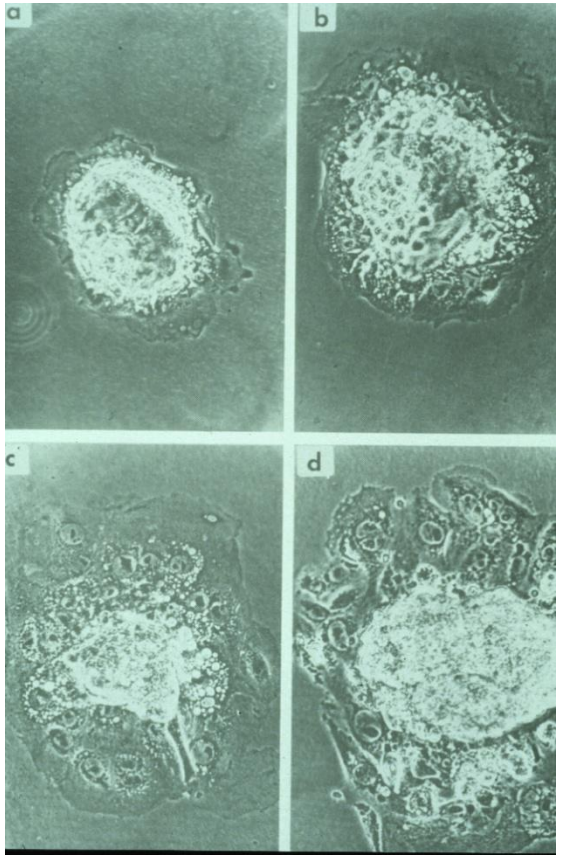
After 1-2 days, blastocysts hatch (A) and attach to the dish by migration of the trophectoderm (TE), while the inner cell mass (ICM) grows (B)

↓

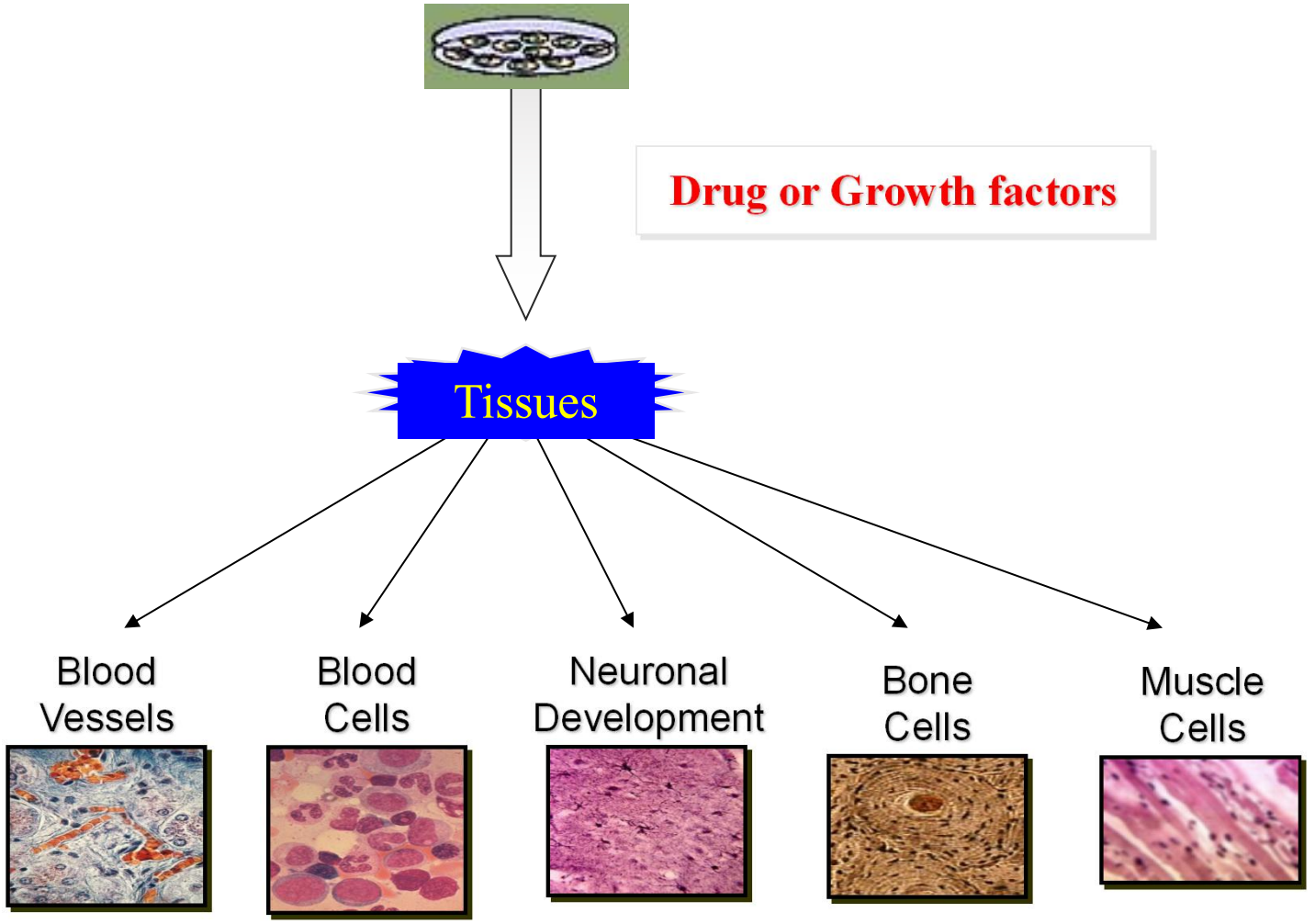
After about 96-120 hours in culture (C, D), the ICM can be dislodged from the TE layer, washed and transplanted to microdrops of medium containing trypsin to disperse the clumps

↓

Transfer disaggregated contents to a fresh feeder cell tissue culture well and inspect daily for signs of differentiation. Primary cell colonies are clearly visible as distinct clumps

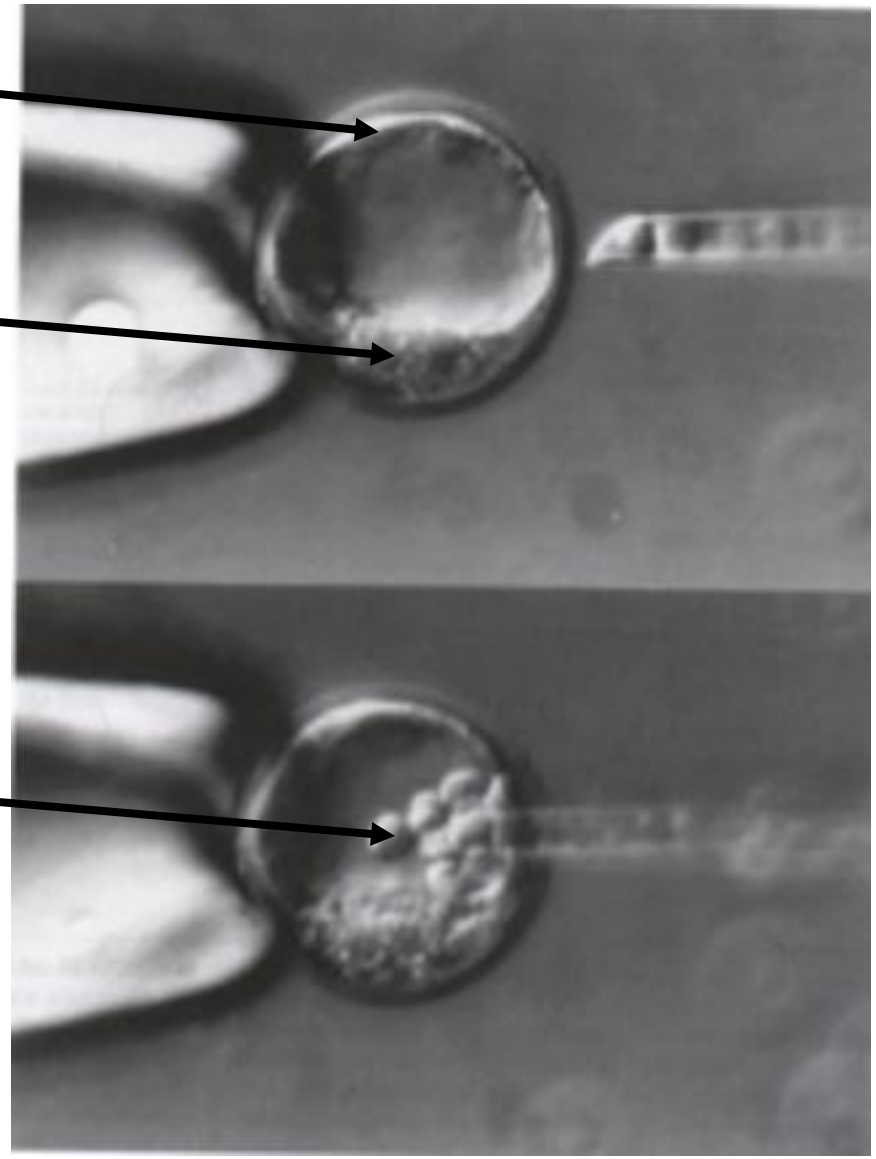


ES Cells Differentiation



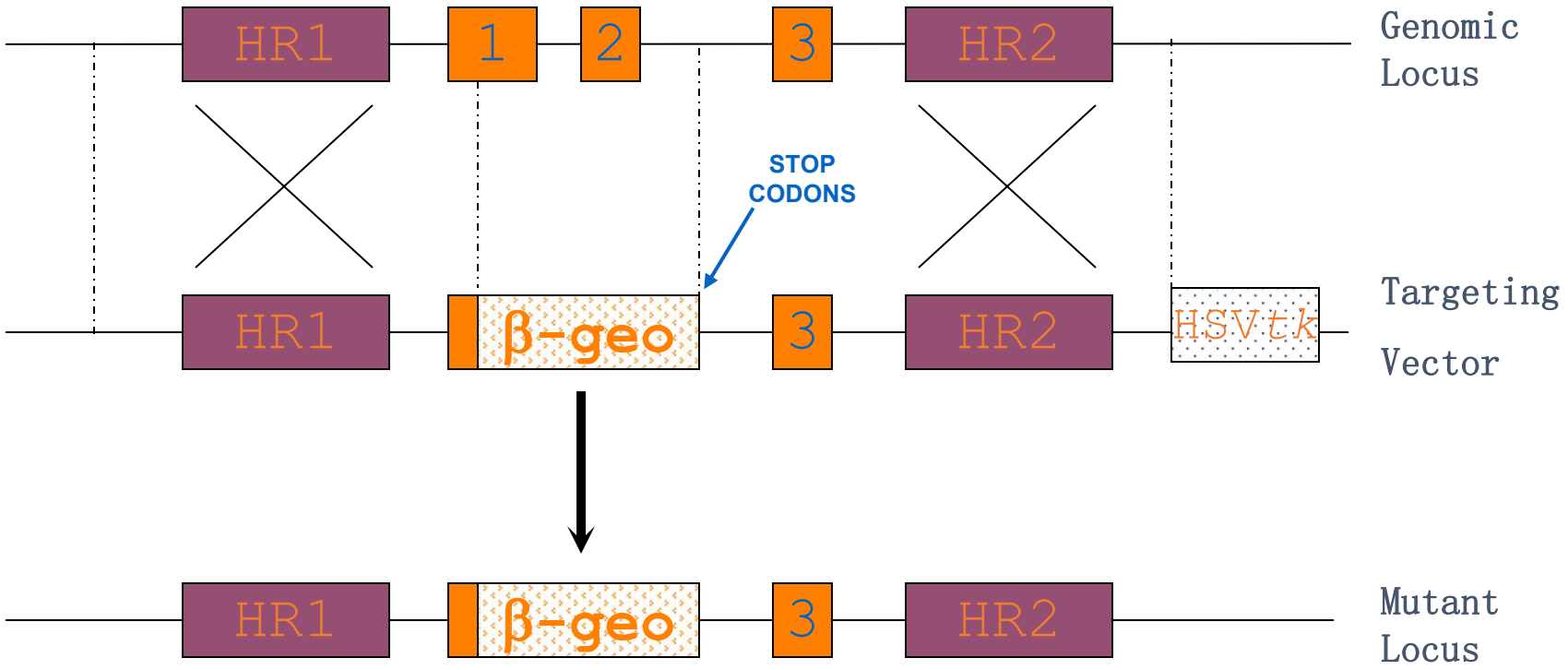
Normal
C57BL/6
Blastocyst
(black)
ICM

ES cells
129/SvJ
(agouti)

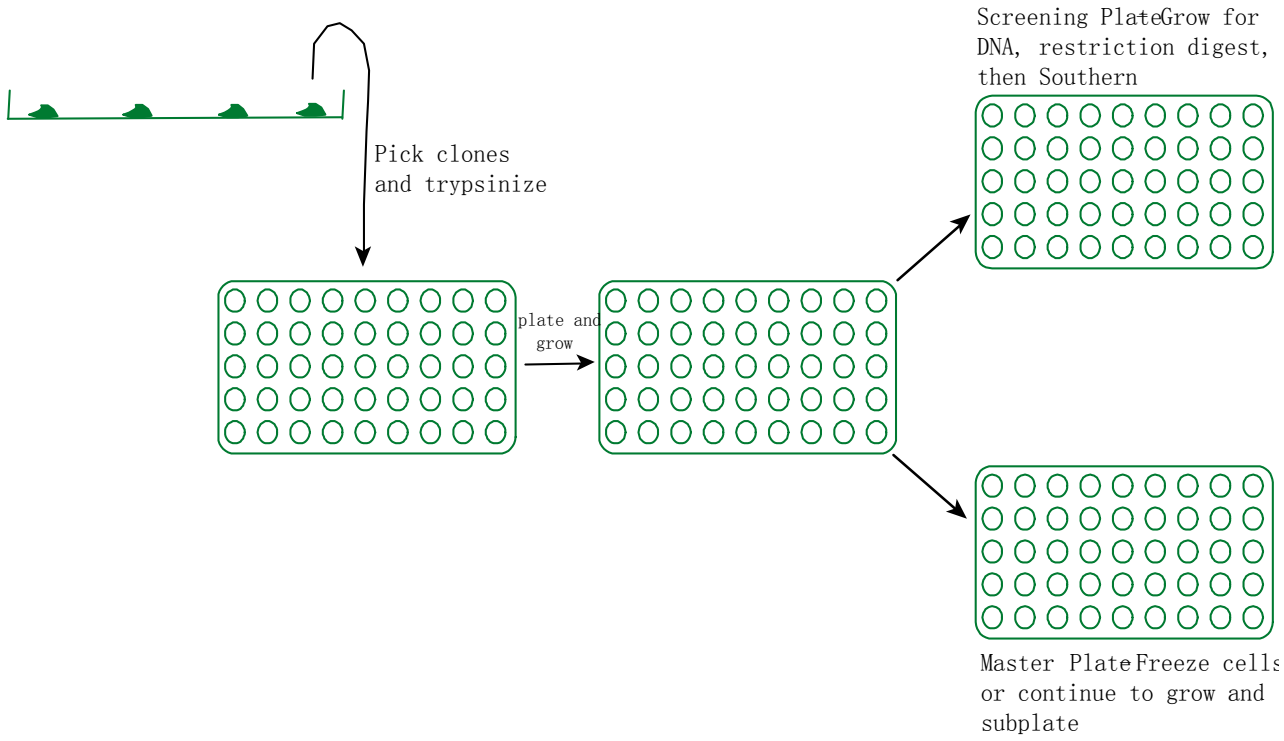


agouti black

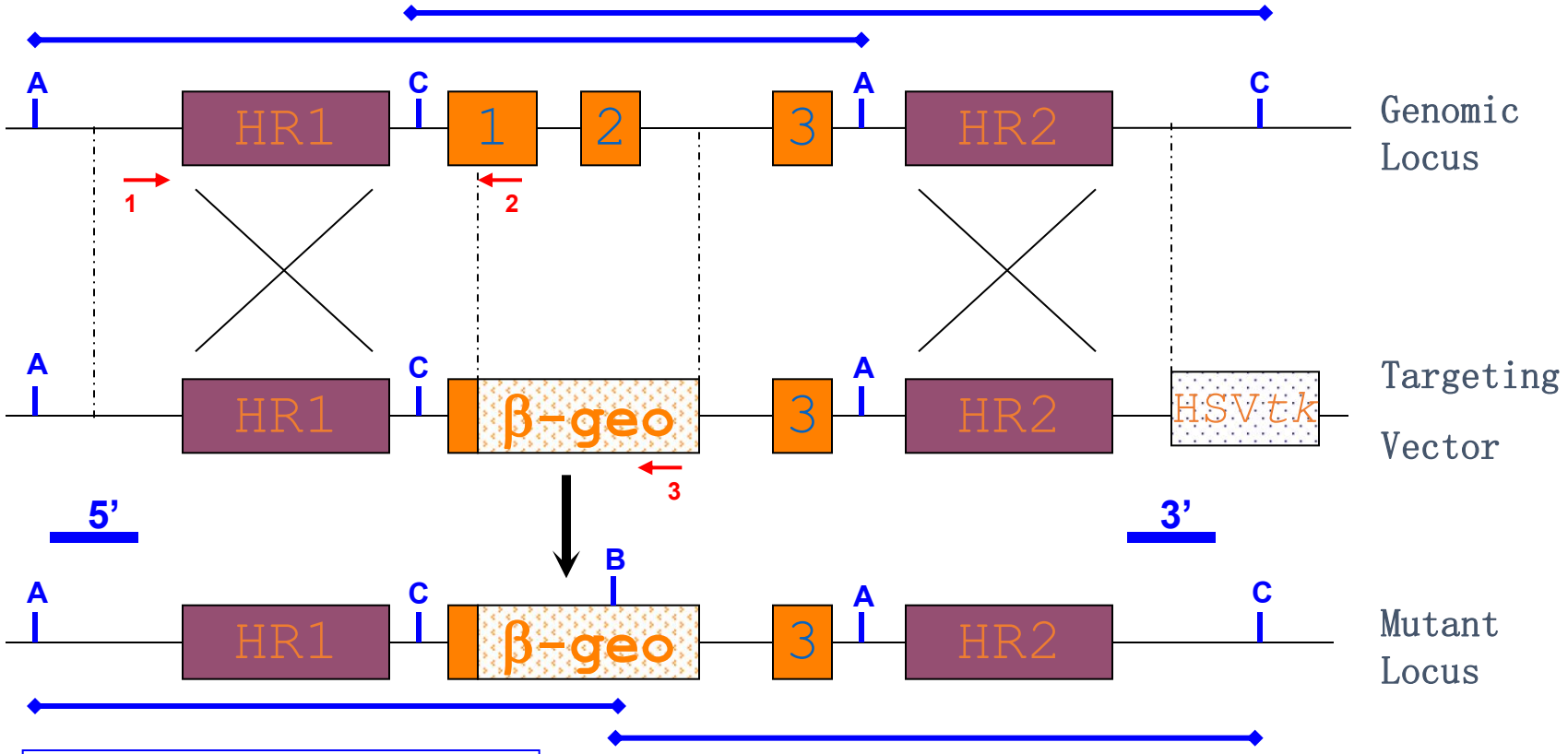
Positive-Negative Selection



Screening ES Cell Clones



Strategies for Screening



SOUTHERN BLOT SCREENING:

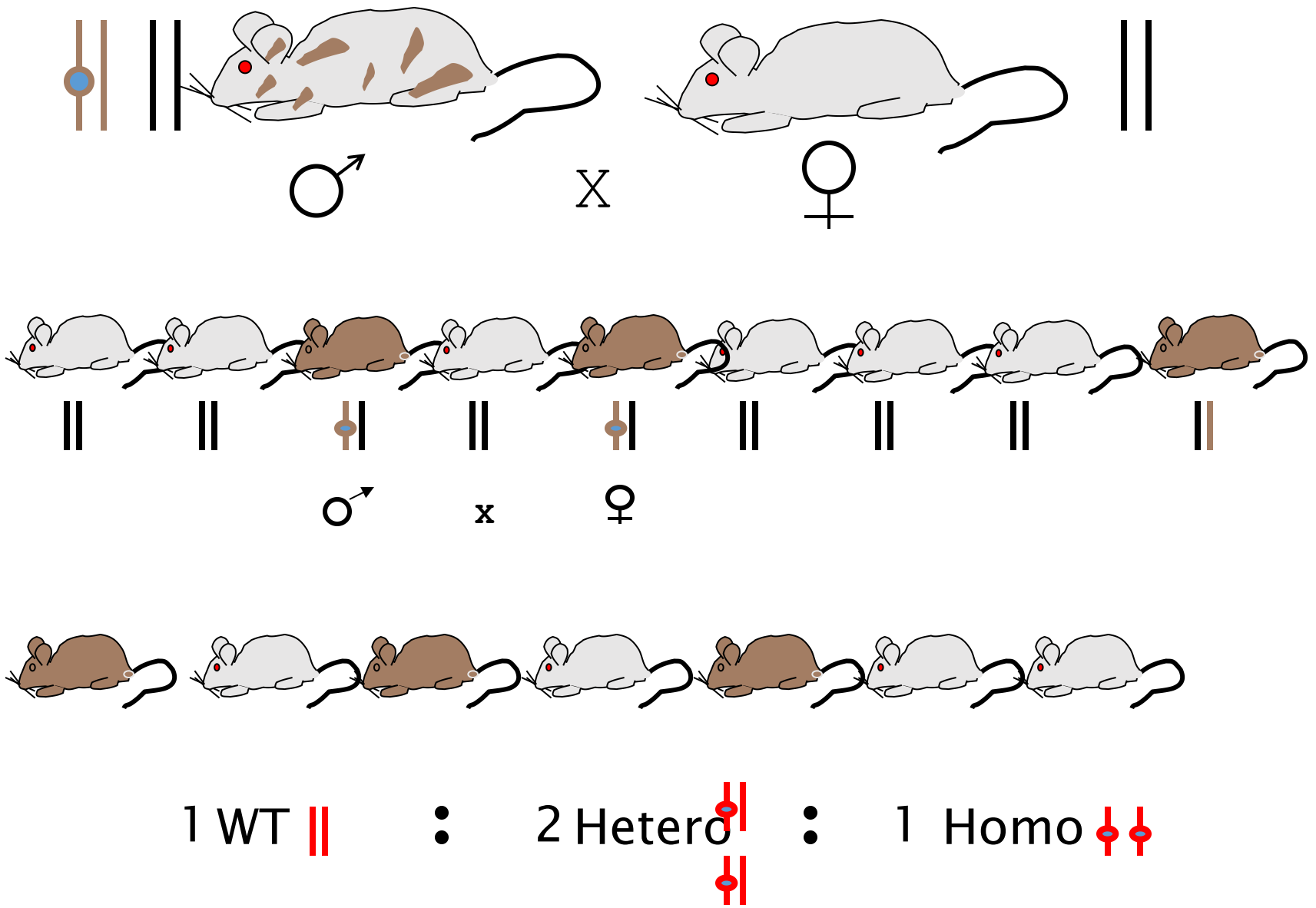
A-A, C-C = both WT and MT, but different sizes, when probed with 5' and 3' probes, respectively

A-B, B-C = Specific to MT when probed with the same probes

PCR SCREENING:

1-2 = WT locus

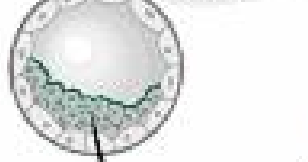
1-3 = MT locus


X
1 WT
2 Hetero
1 Homo

基因剔除小鼠技术流程

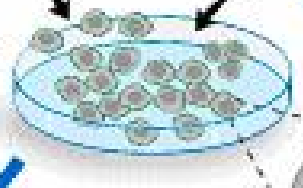
第一步：ES细胞的基因打靶

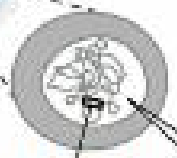
1. ES细胞的分离与培养



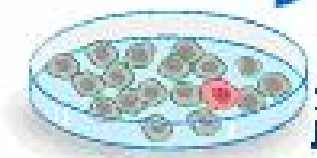
电转

ES细胞



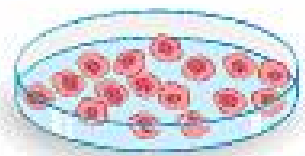


中靶基因



少量的细胞携带中靶基因

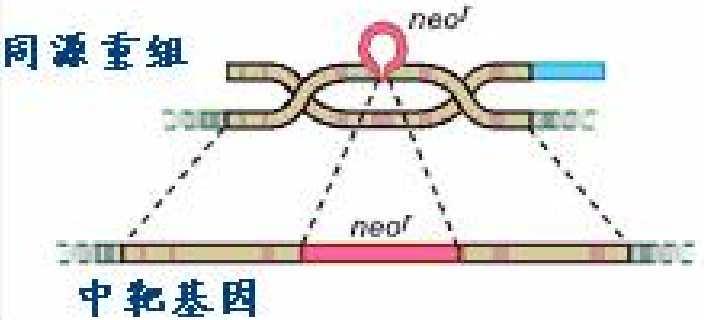
4. 药物筛选



中靶ES细胞的扩增

2. 打靶载体构建

3. ES细胞电转



第二步：从基因敲除ES细胞到基因敲除小鼠

5. ES细胞囊胚腔注射

中靶ES细胞注射到小鼠囊胚腔

注射后的囊胚移植到假孕小鼠子宫内

嵌合的内细胞团

6. 嵌合小鼠的出生与繁育

嵌合小鼠出生

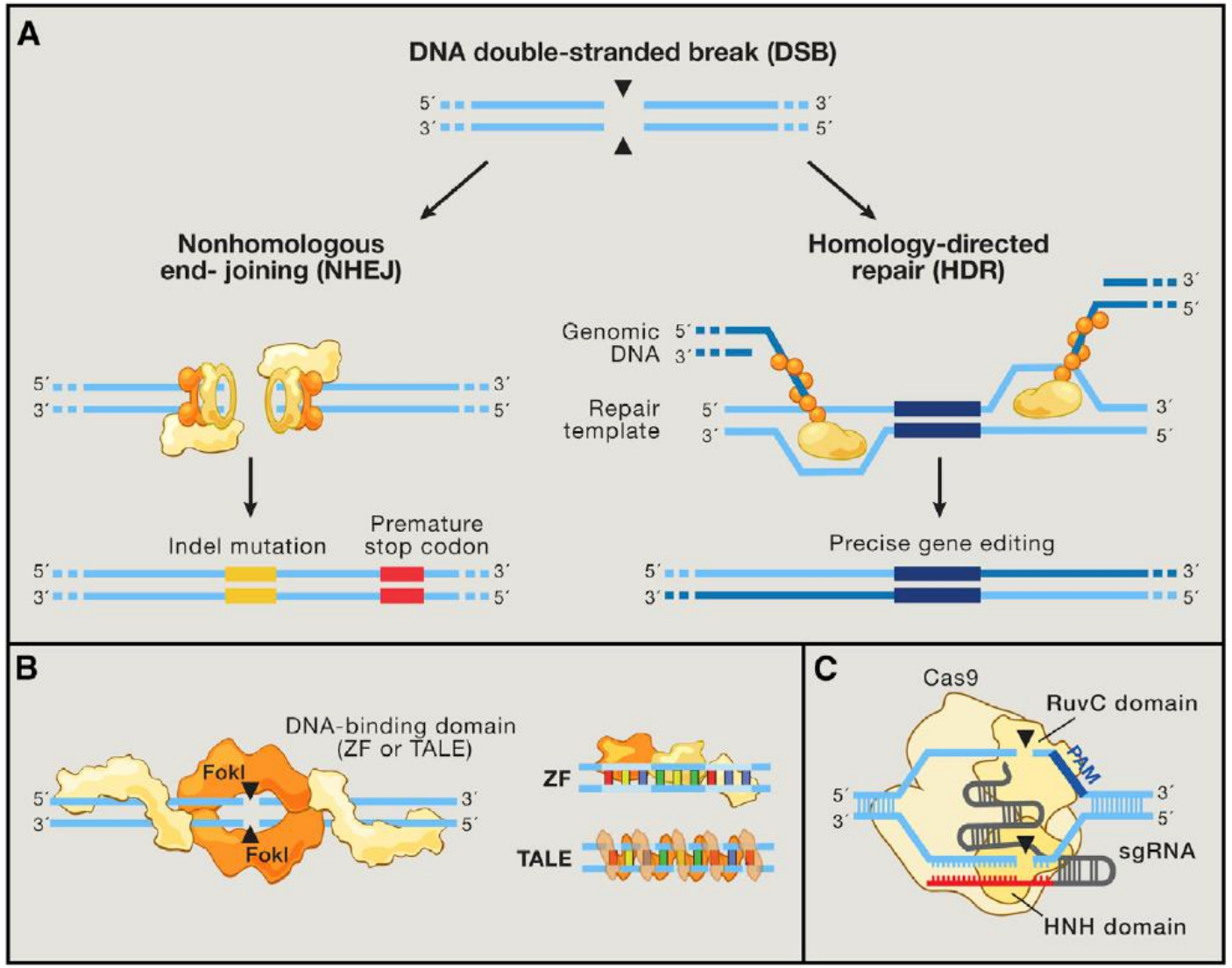
雄性嵌合体小鼠与野生型小鼠交配获得基因敲除小鼠

嵌合体小鼠 ♂

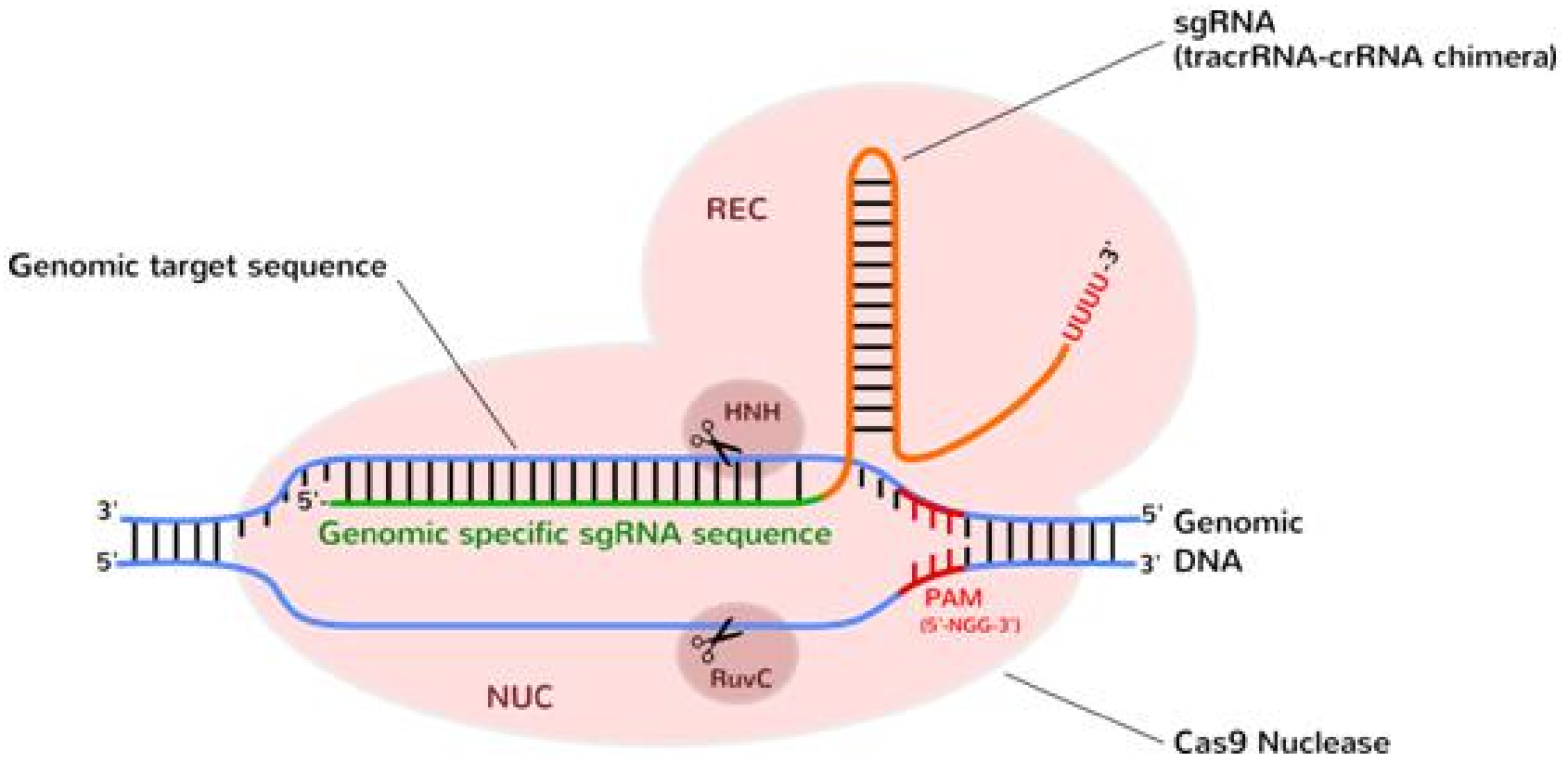
野生型小鼠 ♀

基因敲除小鼠

人工核酸酶系统介导基因组编辑技术



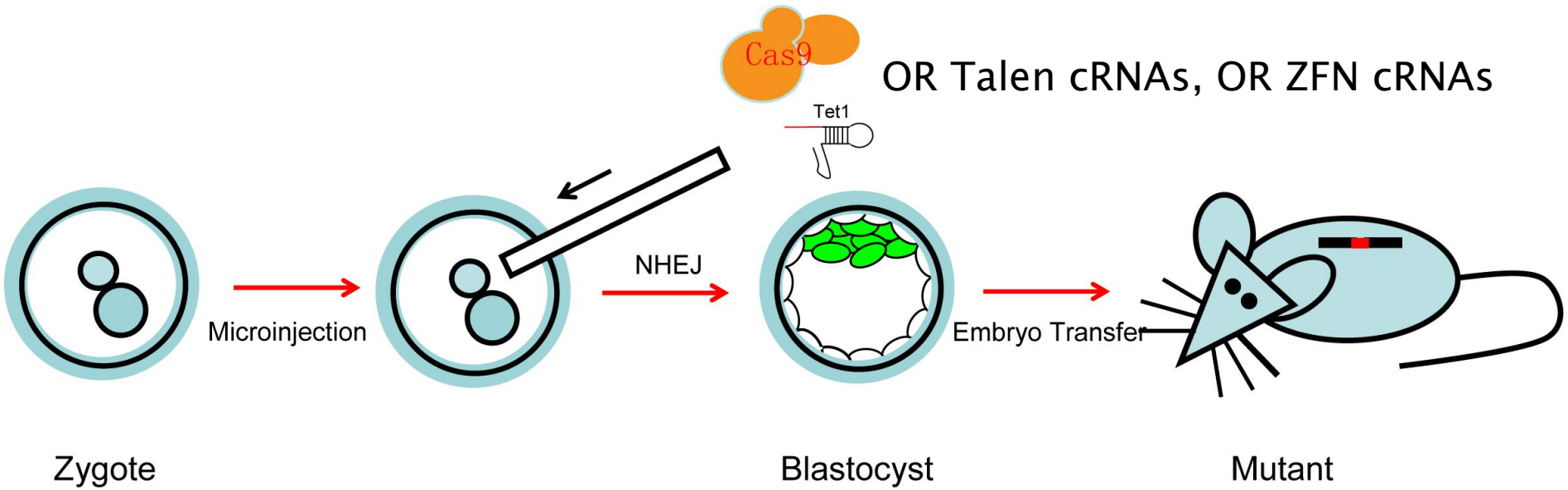
CRISPR/Cas9(From Streptococcus pyogenes)系统基因突变原理

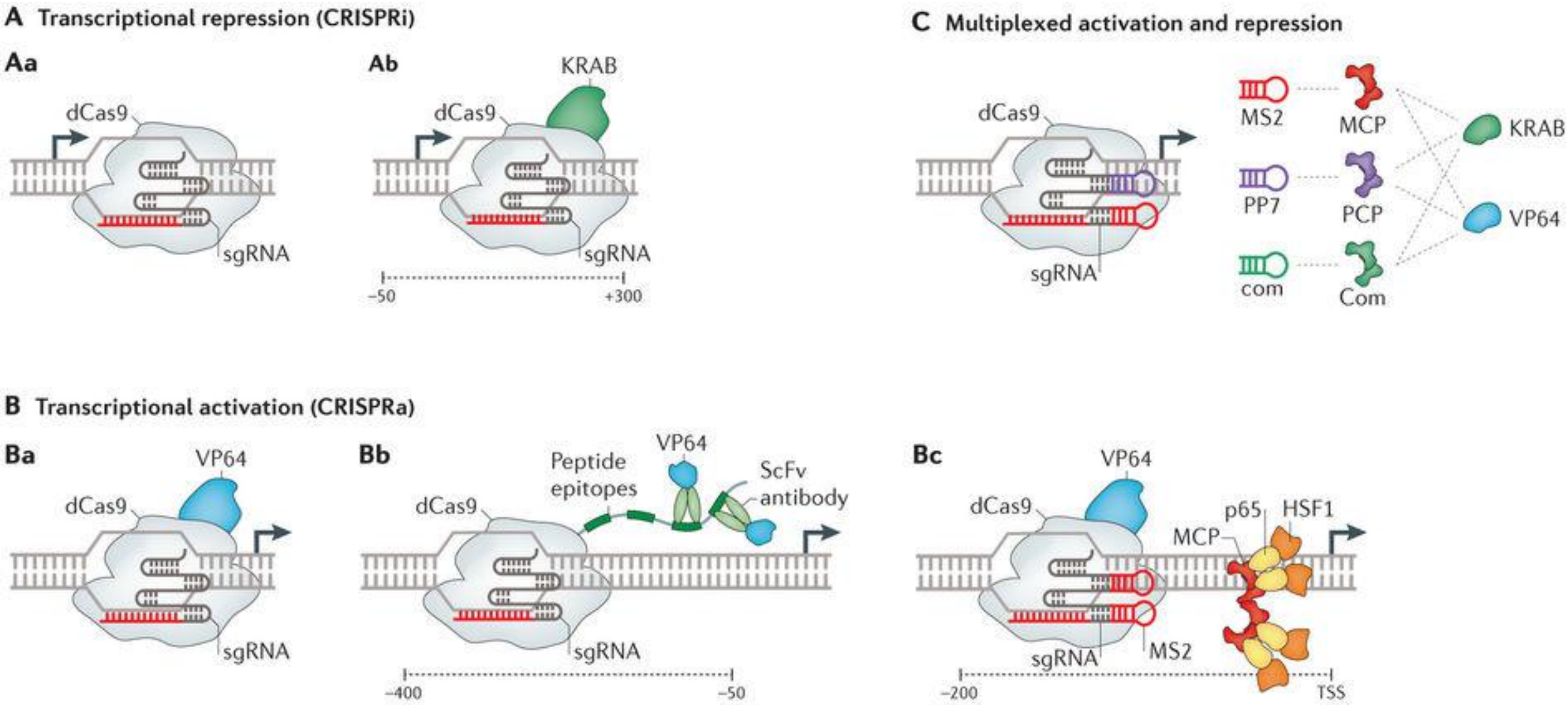


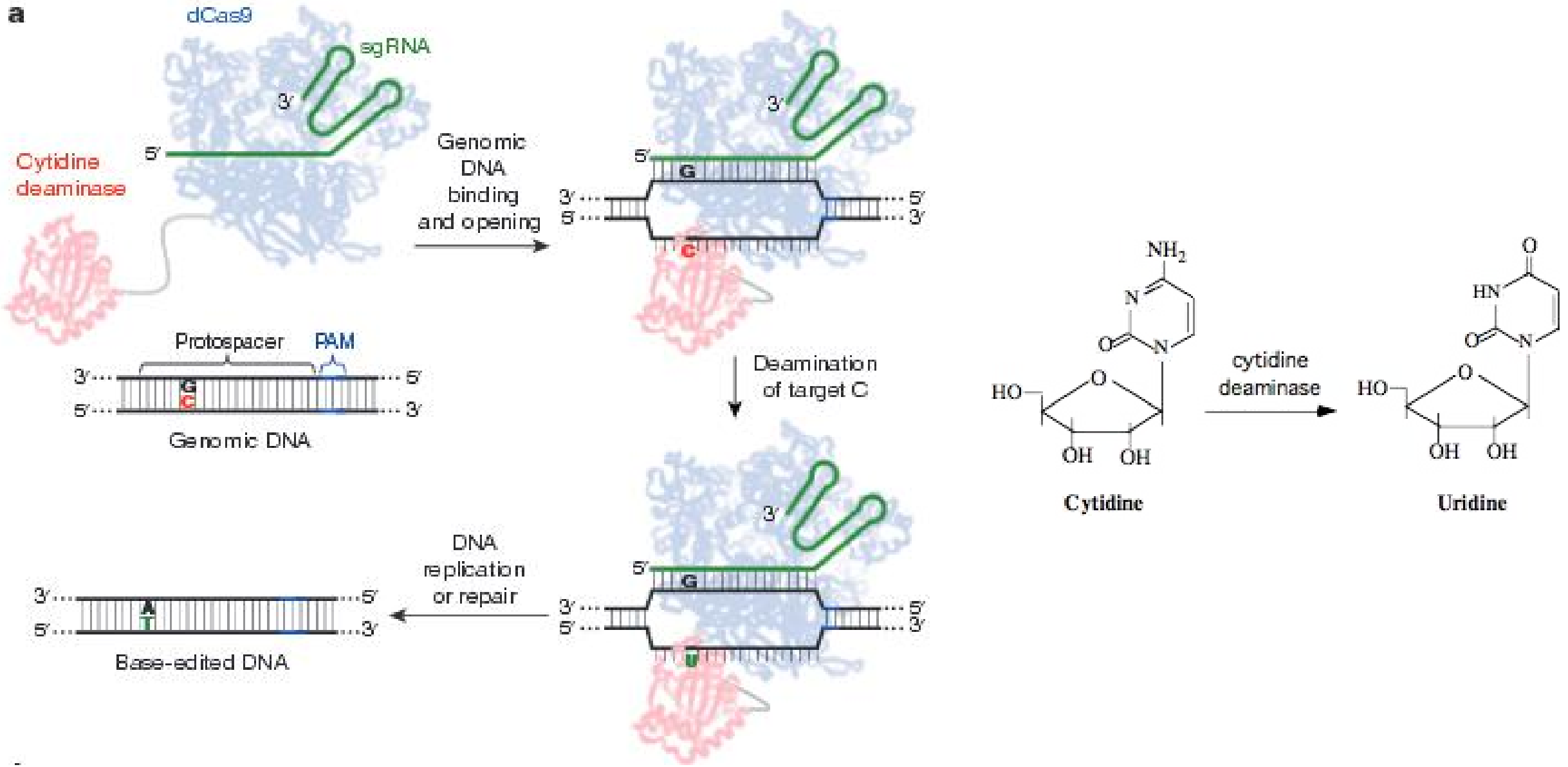
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

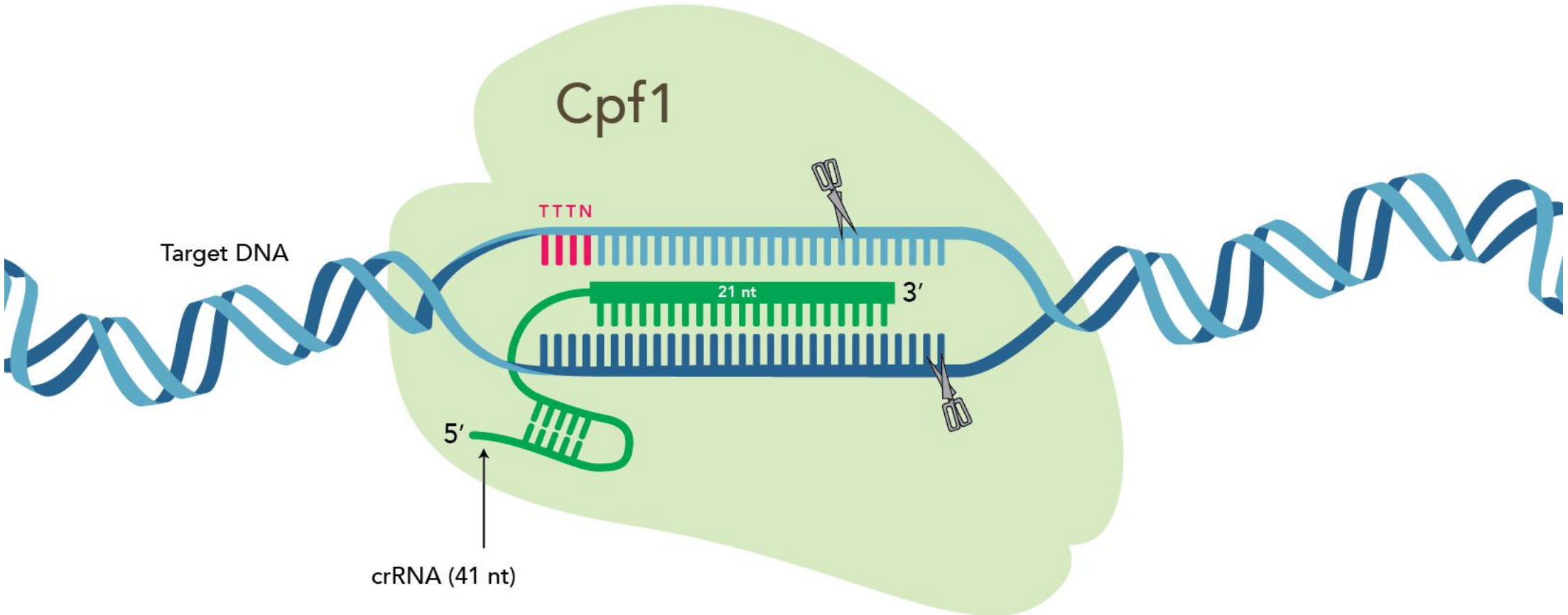
CRISPR-Associated Protein 9 (Cas9) protospacer-adjacent motif (PAM)

人工限制性核酸酶系统介导基因组编辑技术




A Transcriptional repression (CRISPRi)
Aa
dCas9
sgRNA
Ab
KRAB
dCas9
sgRNA
−50
+300
B Transcriptional activation (CRISPRa)
Ba
VP64
dCas9
sgRNA
Bb
VP64
Peptide epitopes
ScFv antibody
dCas9
sgRNA
−400
−50
C Multiplexed activation and repression
dCas9
sgRNA
MS2
MCP
PP7
PCP
com
Com
KRAB
VP64
Bc
VP64
dCas9
p65
HSF1
MCP
sgRNA
MS2
−200
TSS


a
dCas9
sgRNA
3′
5′
Cytidine deaminase
Genomic DNA binding and opening
Protospacer
PAM
3′ ··· 5′
5′ ··· 3′
Genomic DNA
Deamination of target C
DNA replication or repair
Base-edited DNA
NH2
N
O
HO
OH
HN
cytidine deaminase
Cytidine
Uridine

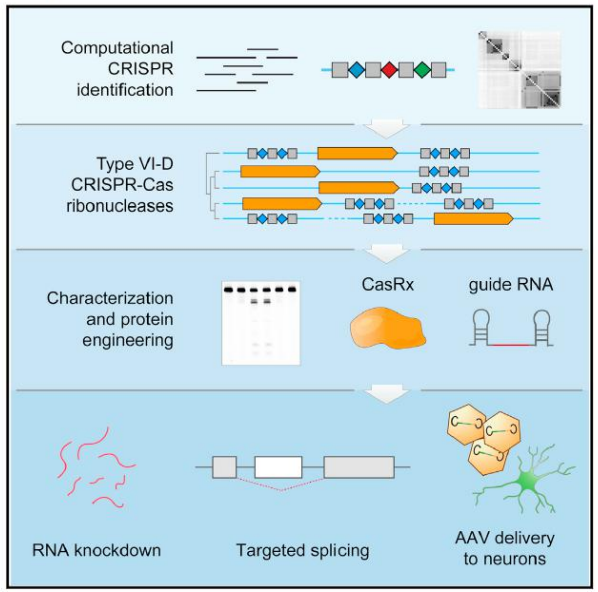


from Prevotella and Francisella bacteria

Cell

Transcriptome Engineering with RNA-Targeting Type VI-D CRISPR Effectors

Graphical Abstract



Authors

Silvana Konermann, Peter Lotfy, Nicholas J. Brideau, Jennifer Oki, Maxim N. Shokhirev, Patrick D. Hsu

Correspondence

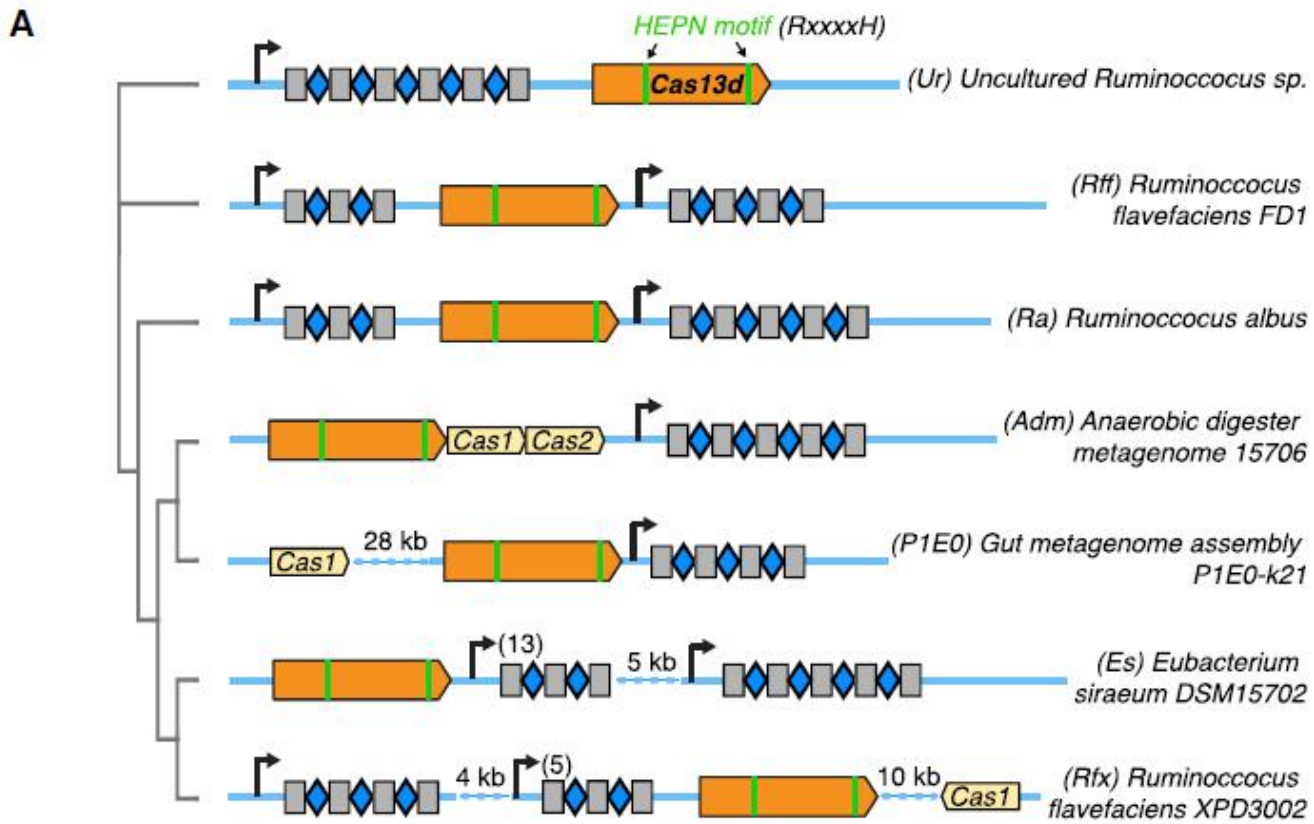
patrick@salk.edu

In Brief

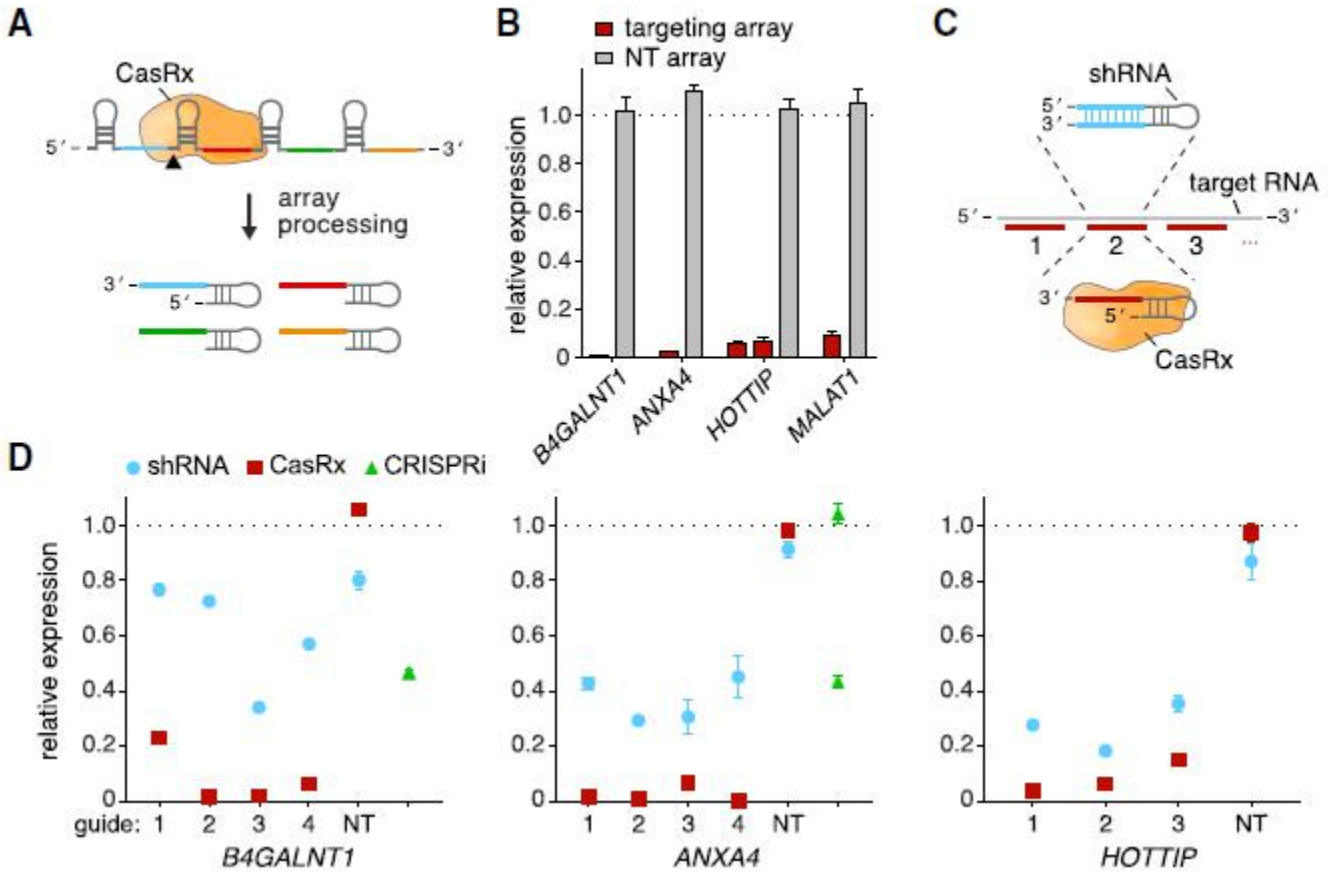
A new family of RNA-targeting CRISPR-Cas systems allows specific modulation of splicing events to reduce pathological tau isoforms in a neuronal model of frontotemporal dementia.

Highlights

- Computational pipeline identifies the RNA-targeting type VI-D CRISPR-Cas family
- Ortholog screen and protein engineering yields the programmable ribonuclease CasRx
- CasRx RNA knockdown exhibits favorable efficiency and specificity relative to RNAi
- Neuronal AAV delivery of dCasRx splice effectors alleviates tau mis-splicing

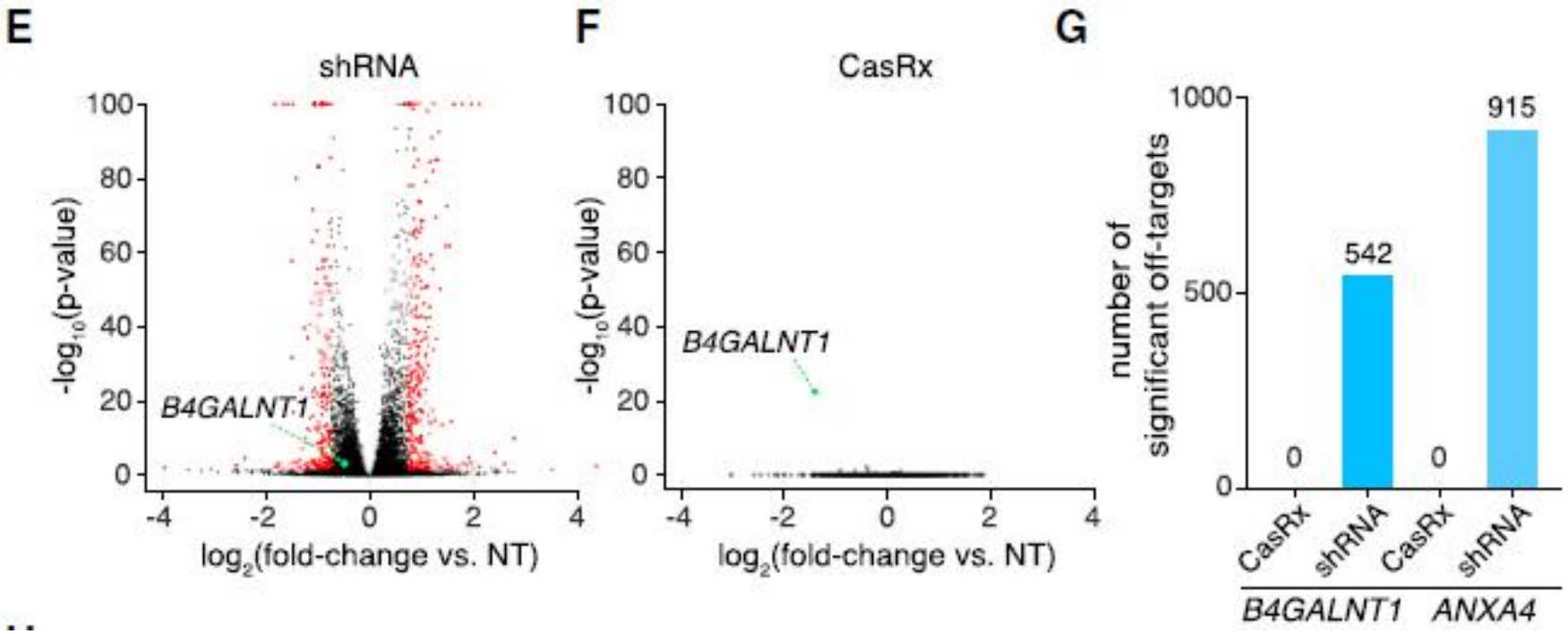


Konermann et al., 2018, Cell *173*, 1–12
April 19, 2018
https://doi.org/10.1016/j.cell.2018.02.033



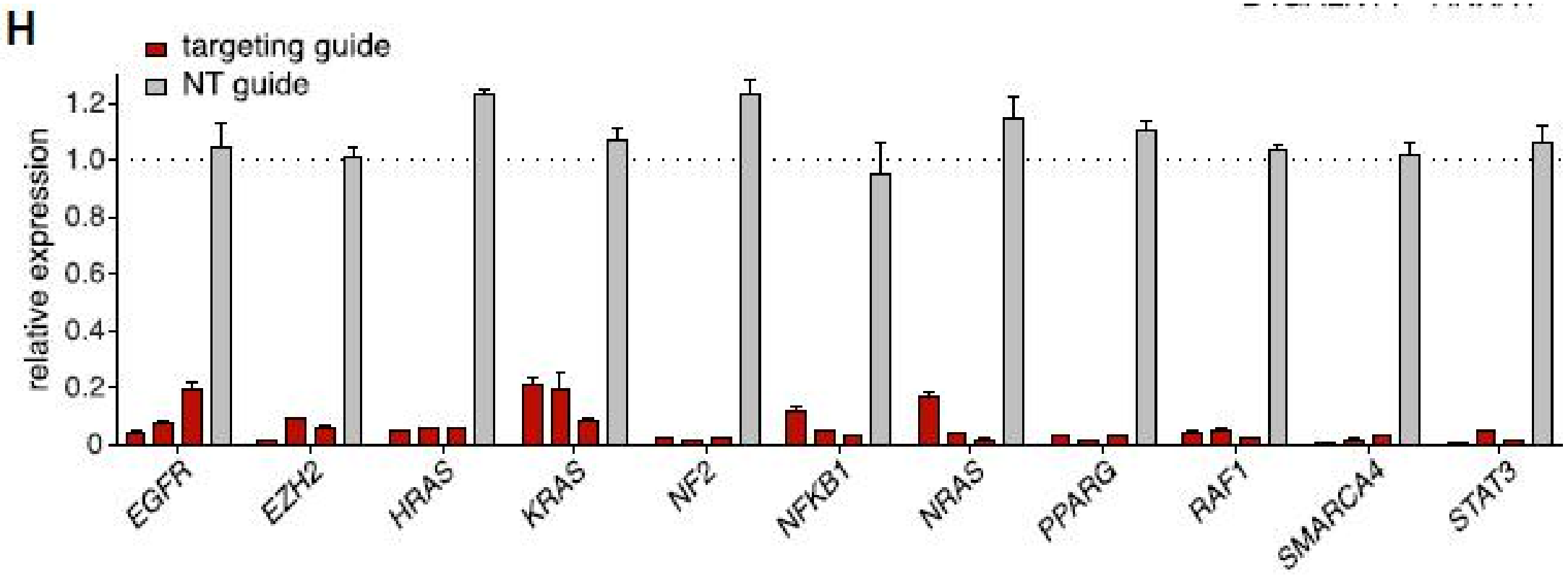
Across 3 endogenous transcripts, CasRx outperformed shRNAs (11/11) and CRISPRi (4/4) in each case, exhibiting a median knockdown of 96% compared to 65% for shRNA and 53% for CRISPRi after 48 hr.

同时针对多个细胞内源基因的转录本　　和RNAi及Crispri相比，CasRx的效果显著



This suggests that the moderate bystander cleavage observed in vitro (Figure 2C) may not result in observable off-target transcriptome perturbation in mammalian cells.

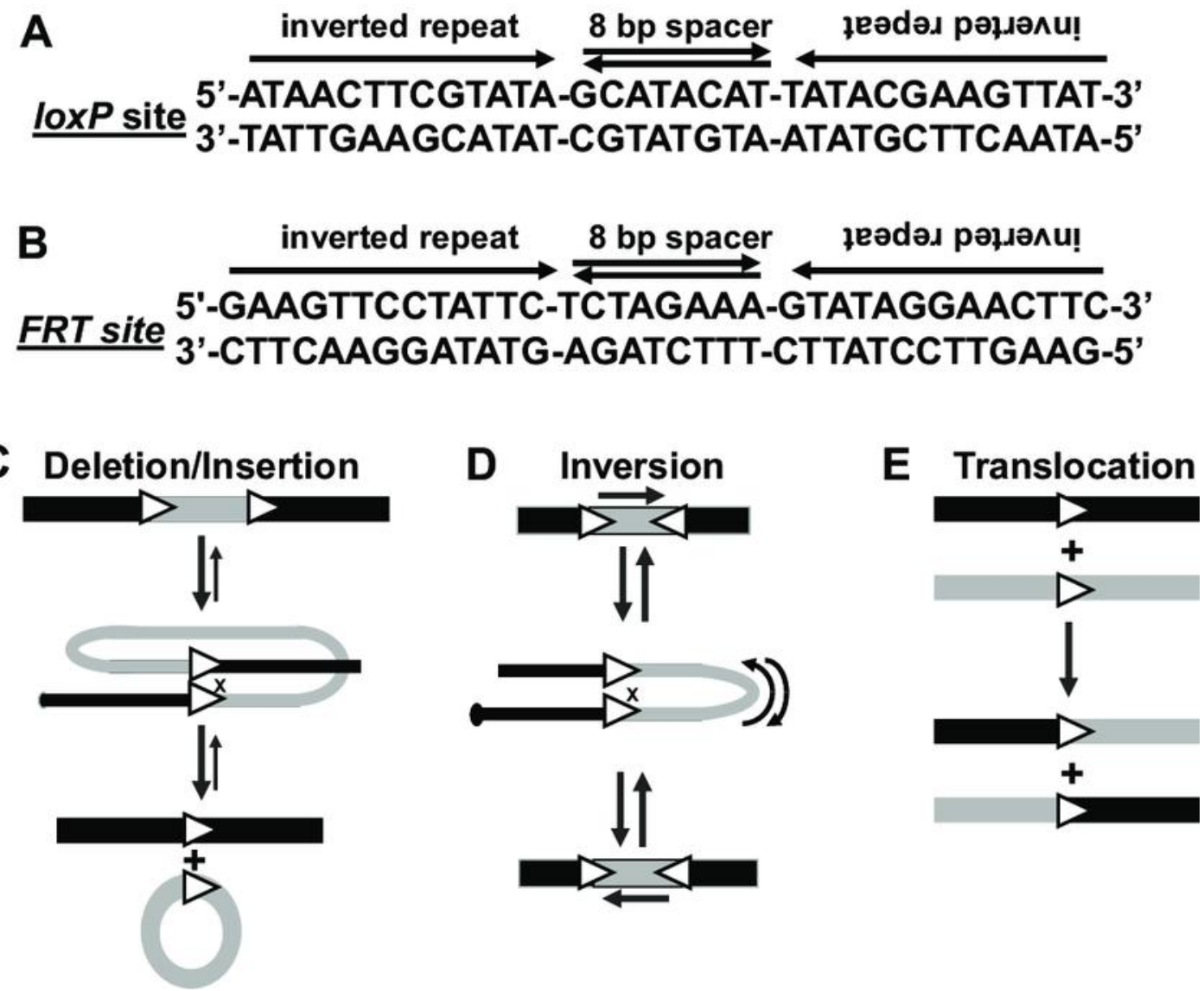
和RNAi相比，CasRx方法几乎没有脱靶现象

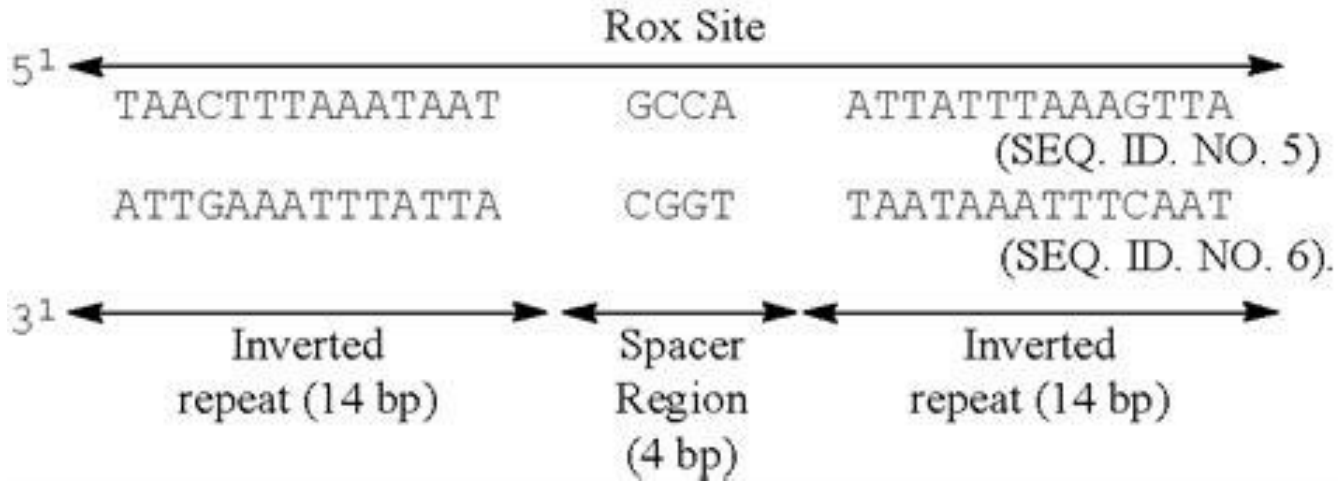


尝试了更多的内源基因，Cas13d可以普遍适用，最差也能达到80%的抑制效率

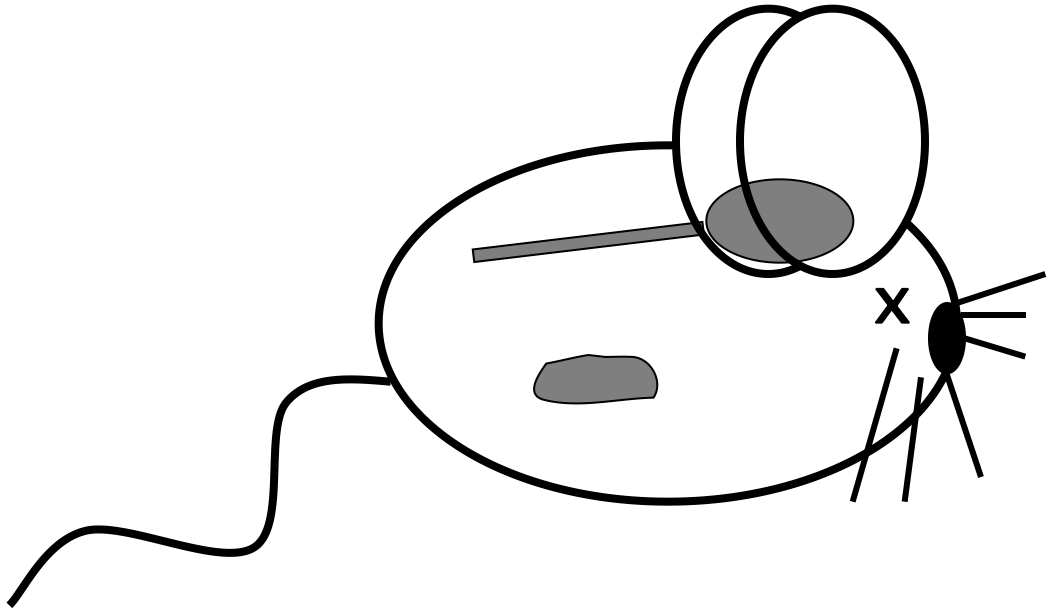
条件性基因打靶技术

- 常规基因打靶技术的局限性
  - 所有细胞都改变
  - 胚胎致死
  - 功能代偿
  - 功能冗余
- 重组酶系统在基因打靶中的应用
  - Cre/loxP recombinase
  - FLP/FRT recombinase
  - Dre/rox recombinase

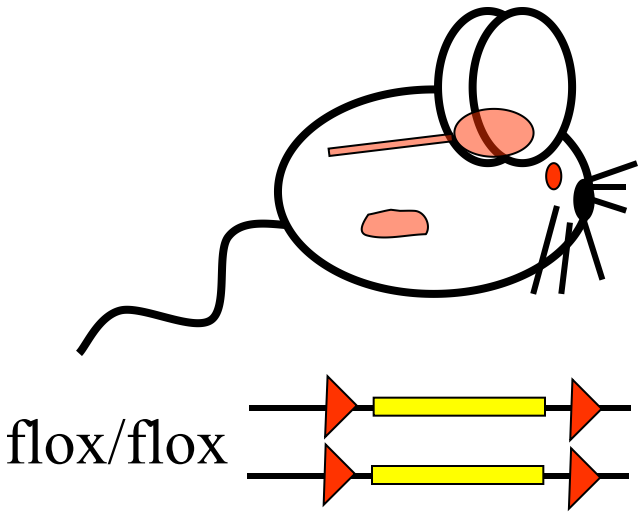

A
inverted repeat
8 bp spacer
inverted repeat
loxP site
5’-ATAACTTCGTATA-GCATACAT-TATACGAAGTTAT-3’
3’-TATTGAAGCATAT-CGTATGTA-ATATGCTTCAATA-5’
B
inverted repeat
8 bp spacer
inverted repeat
FRT site
5'-GAAGTTCCTATTC-TCTAGAAA-GTATAGGAACTTC-3’
3’-CTTCAAGGATATG-AGATCTTT-CTTATCCTTGAAG-5’
C
Deletion/Insertion
D
Inversion
E
Translocation


Rox Site
5'
TAACTTTAAATAAT
GCCA
ATTATTTAAAGTTA
(SEQ. ID. NO. 5)
ATTGAAATTTATTA
CGGT
TAATAAATTTCAAT
(SEQ. ID. NO. 6).
3'
Inverted repeat (14 bp)
Spacer Region (4 bp)
Inverted repeat (14 bp)

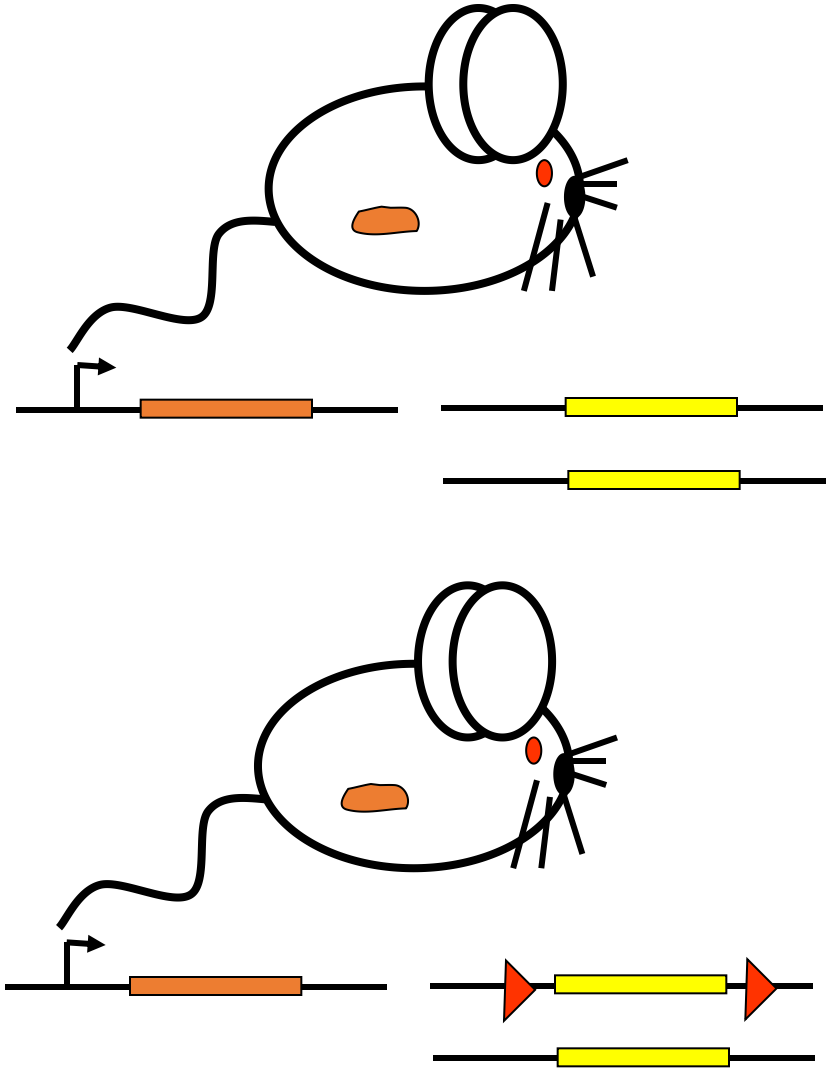
目标基因全身敲除导致死亡



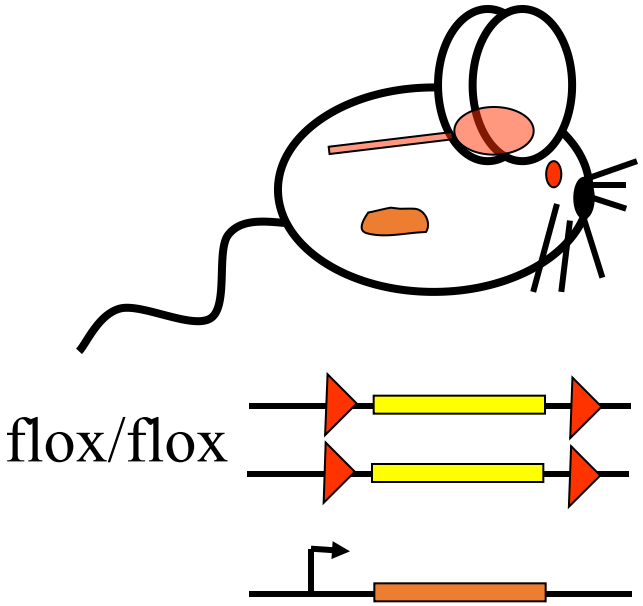
在目标基因两侧插入loxP位点

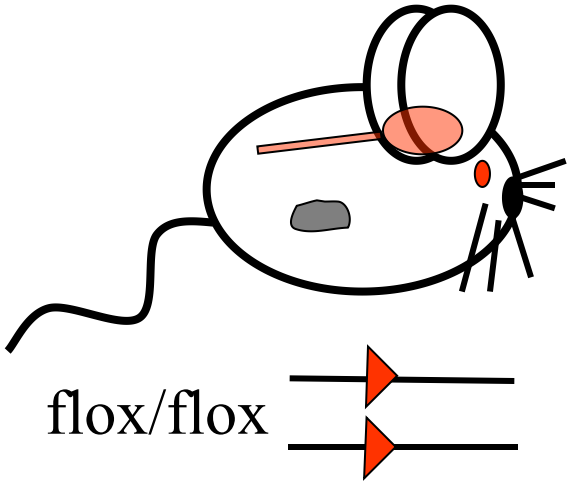


建立或选定一个表达Cre的小鼠



经过杂交育种，最后得到Cre;abc$^{flox/flox}$小鼠

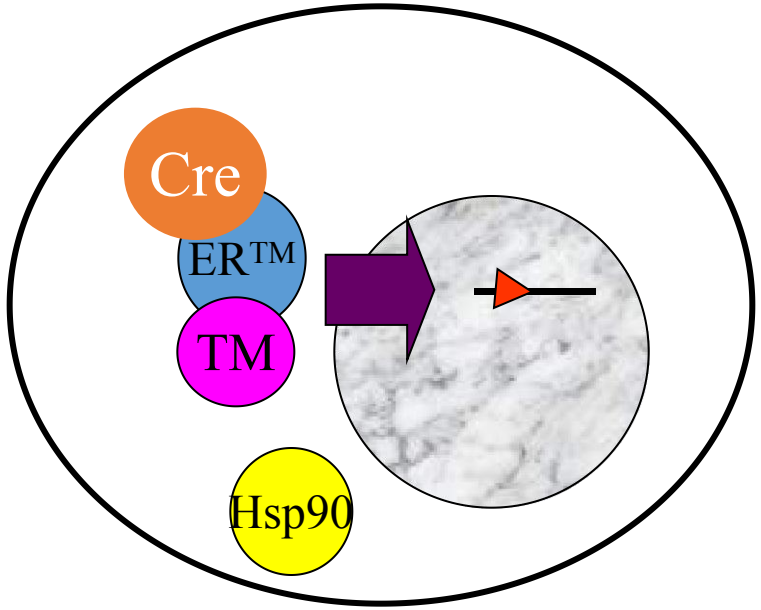




Tamoxifen-inducible system

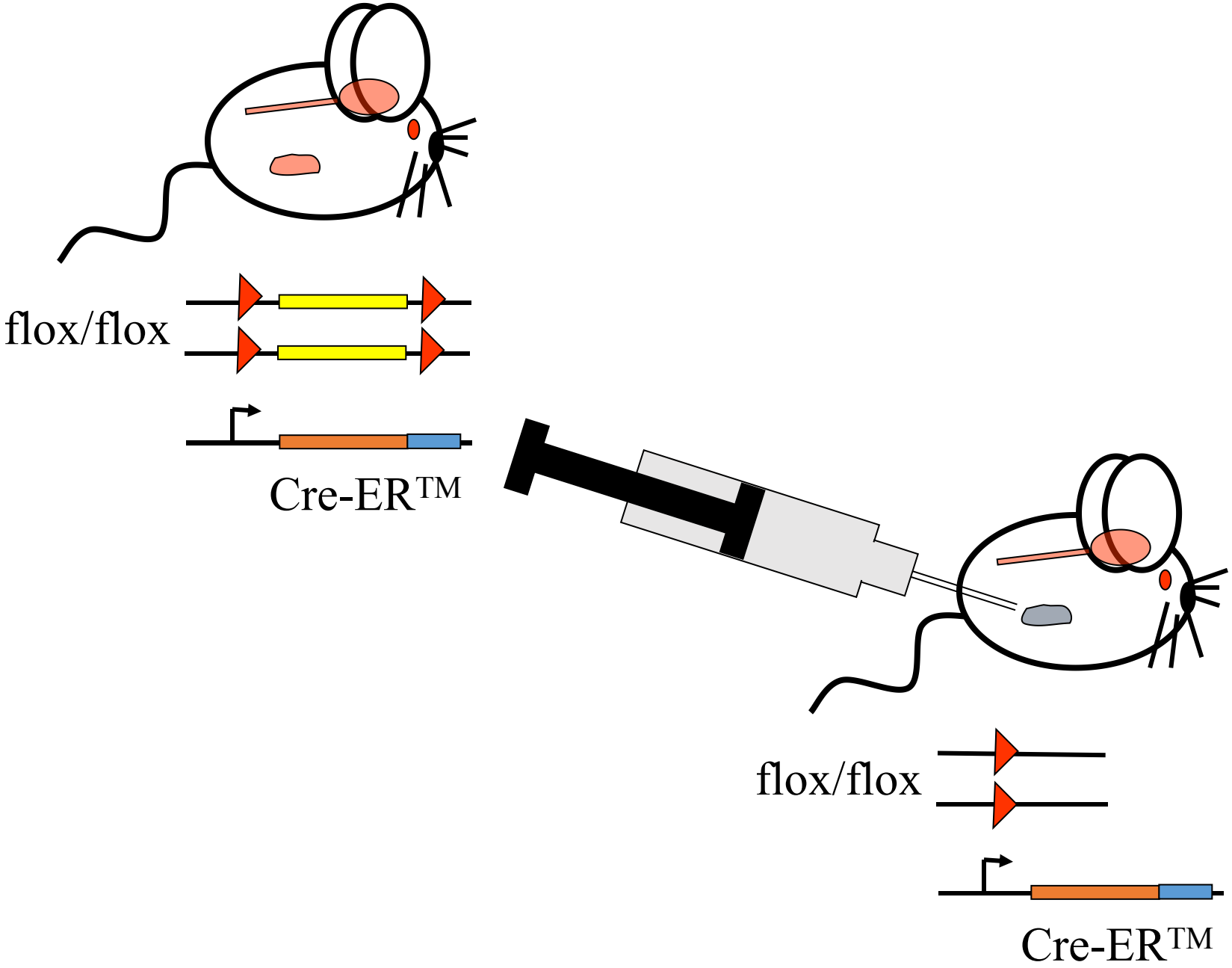
Uses a fusion protein combining activity of Cre and a mutant form of the ligand binding domain of the estrogen receptor (ER^{TM})

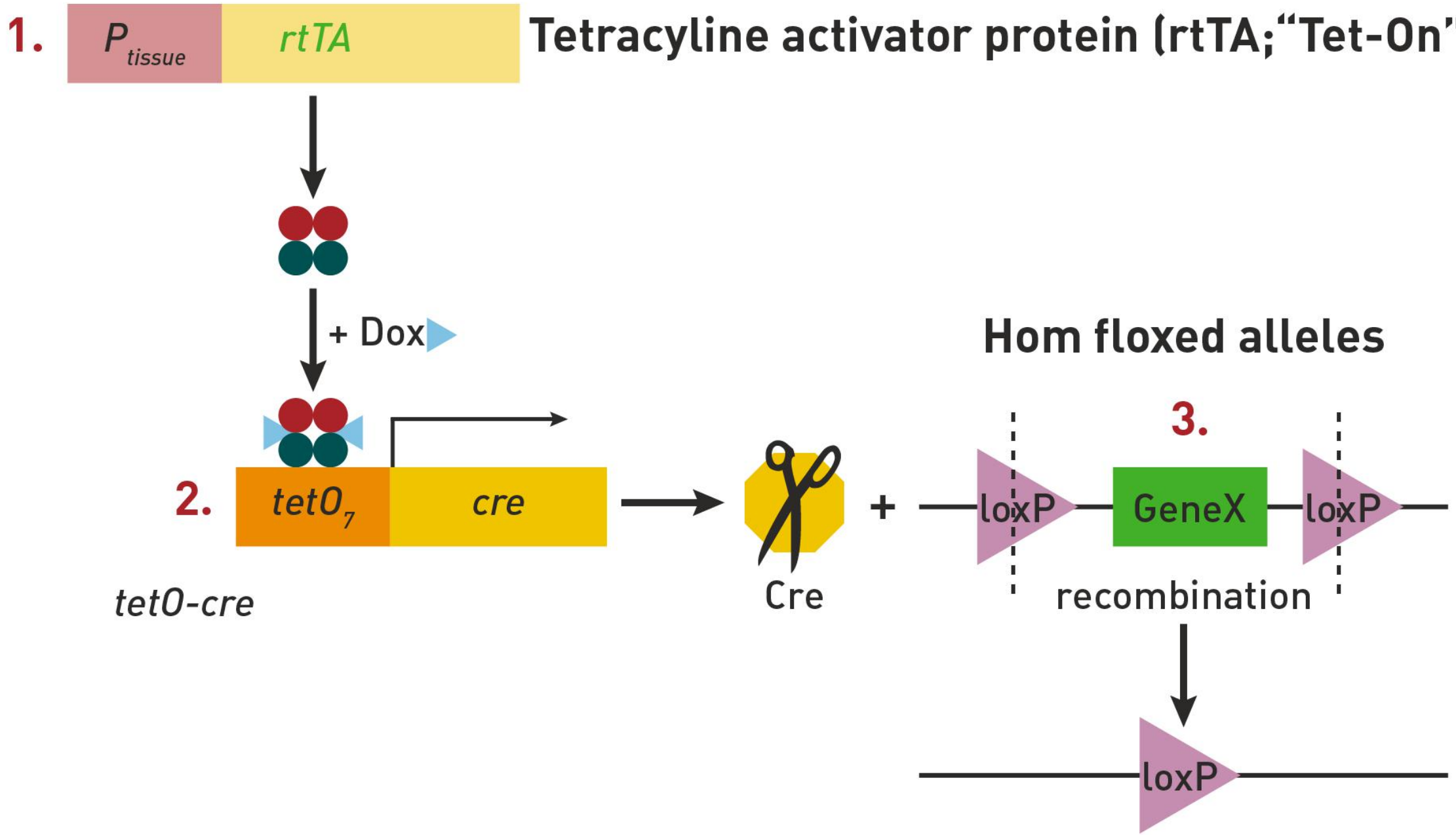
Cre-ER^{TM} does not bind estrogen, but does bind 4-OH-tamoxifen



cell

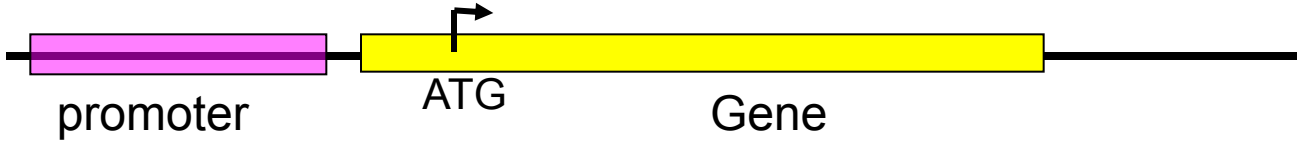
Upon addition of tamoxifen, Cre-ER^{TM} is released from Hsp90, allowing access to the nucleus and Cre-mediated recombination

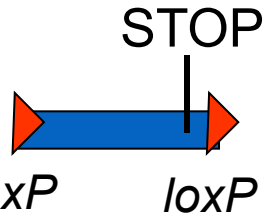

flox/flox
Cre-ER™
flox/flox
Cre-ER™


1.
P_{tissue}
rtTA
Tetracyline activator protein (rtTA;"Tet-On")
+ Dox
2.
$tetO_7$
cre
tetO-cre
Cre
+
Hom floxed alleles
3.
loxP
GeneX
loxP
recombination
loxP

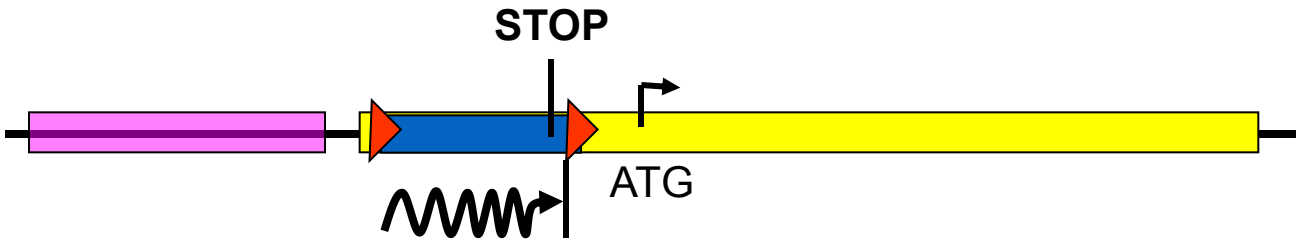
Expression of Cre can be used to turn on a transgene in temporally regulated manner.

Transgene: want to express the gene of interest (in yellow) off the promoter of interest (in purple), but not until late in development.

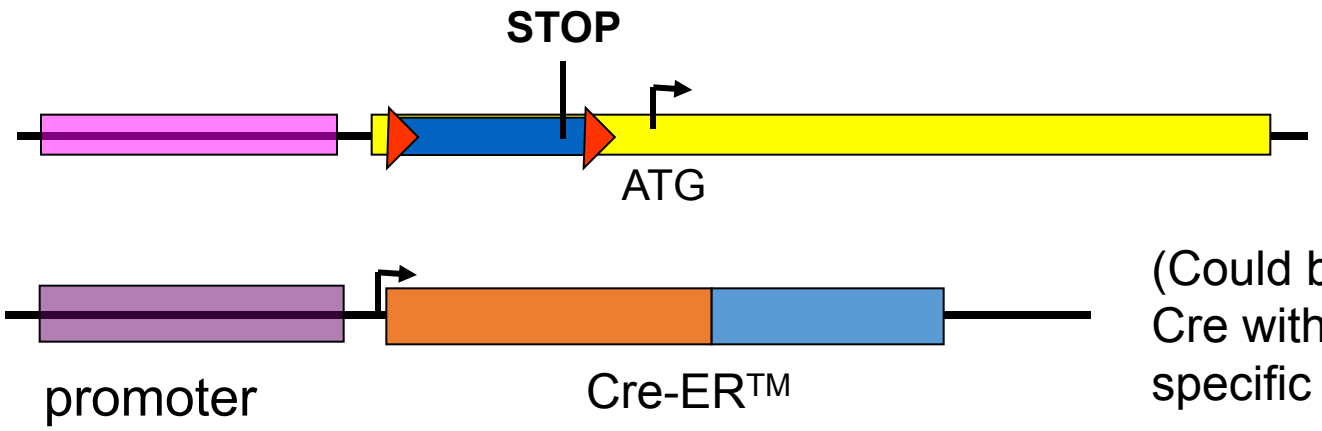




Clone a short bit of floxed sequence near the front of the construct such that when the transgene turns on it produces only a short bit of nonsense.

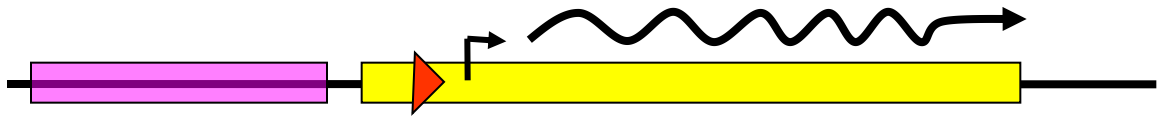


Breed the mice carrying your transgene with another transgenic line expressing endogenous Cre-ER^{TM}.



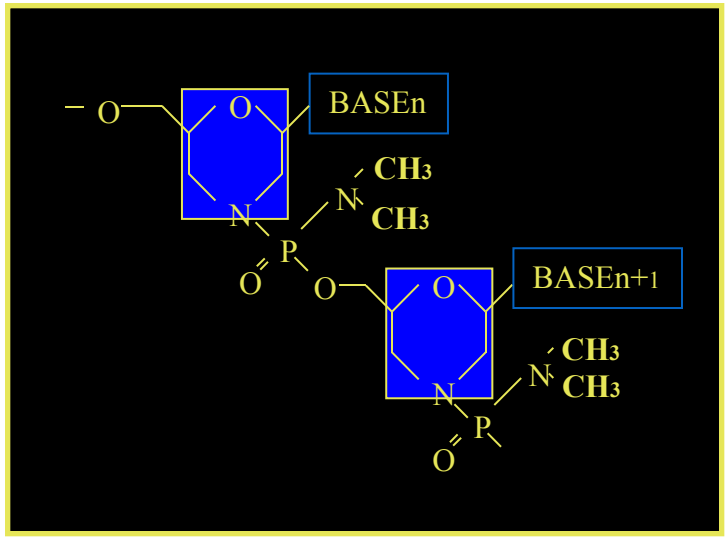
(Could be normal Cre with tissue-specific promoter)

Upon injection of tamoxifen at required stage of development, Cre is free to recombine *loxP* sites and remove translation STOP codon.



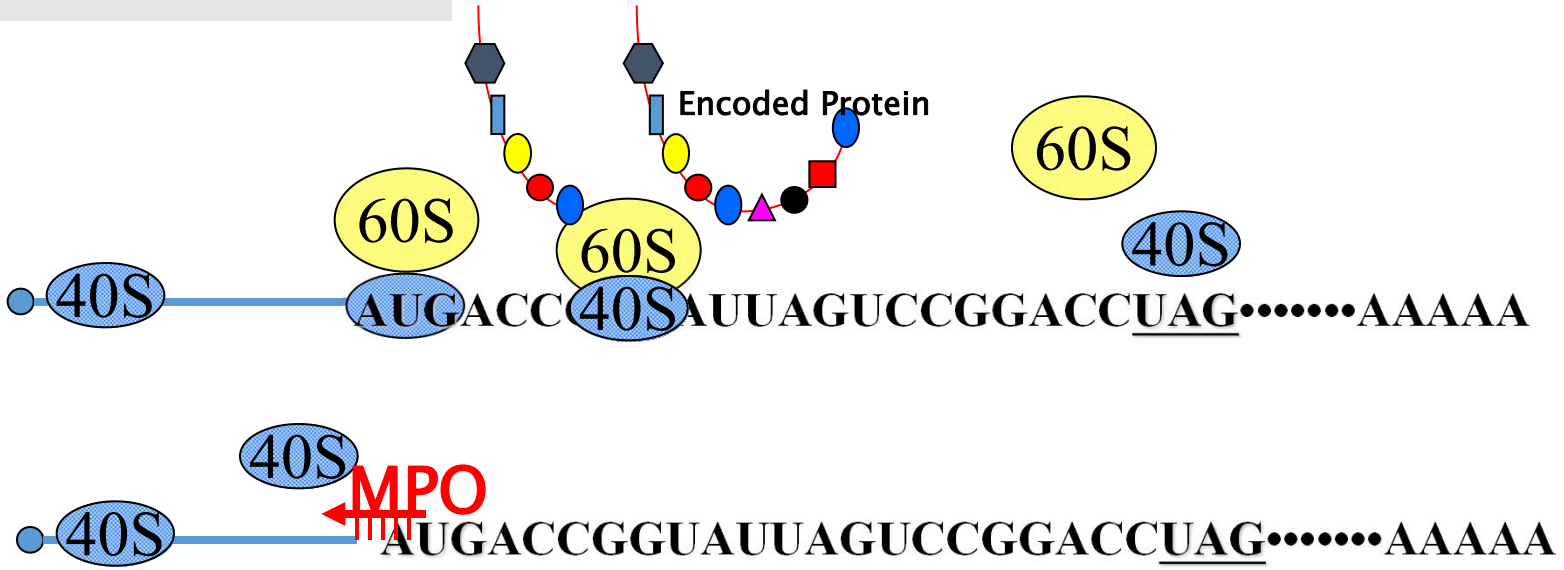
This allows transgene to produce full length mRNA.
NB. Have to check all still in frame AND hope retained loxP site does not affect function of transgene

吗啉修饰的反义核酸技术（Morpholino）：抑制蛋白翻译

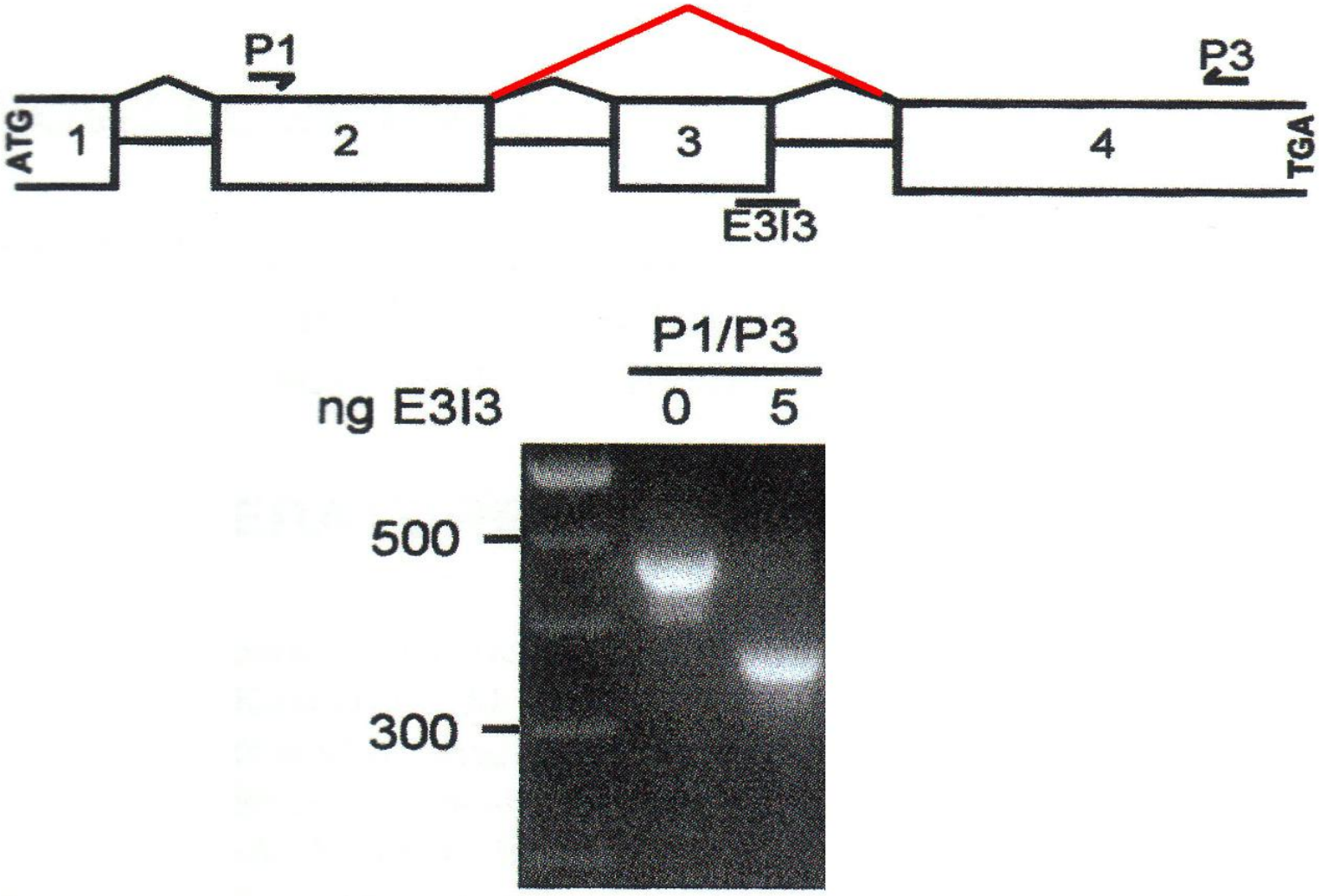


Antisense oligonucleotides
Designed as 25 mers
Bind tightly
Resistant to digestion
Low toxicity
Not RNAseH mediated

Inhibition of Translation

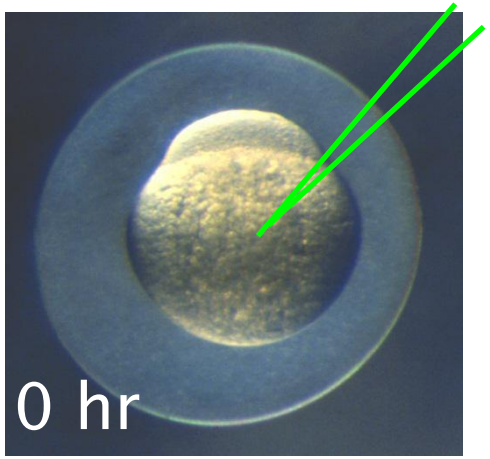


吗啉修饰的反义核酸技术（Morpholino）：抑制RNA剪接



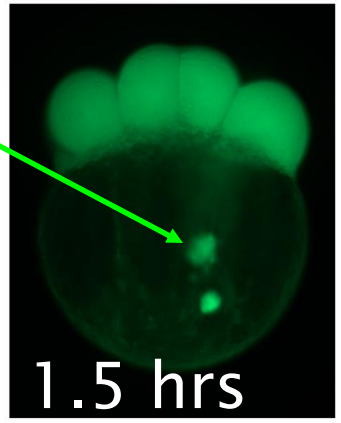
Draper BW, Morcos PA, Kimmel CB. Inhibition of zebrafish fgf8 pre-mRNA splicing with morpholino oligos: A quantifiable method for gene knockdown. Genesis. 2001 Jul;30(3):154-6

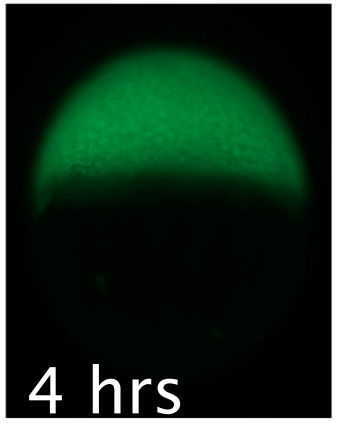
吗啉修饰的反义核酸技术（Morpholino）：显微注射

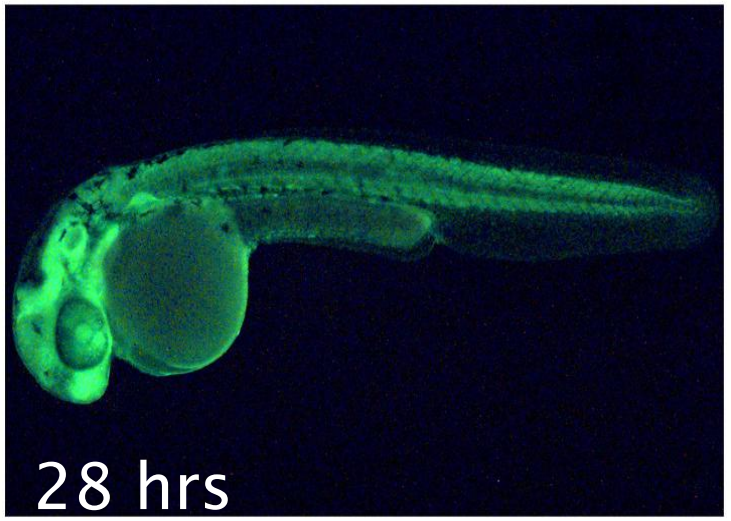


显微注射

注射点





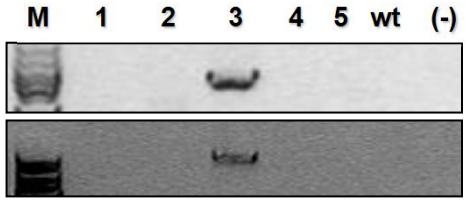


Nasevicius and
Ekker (2000, 2001)

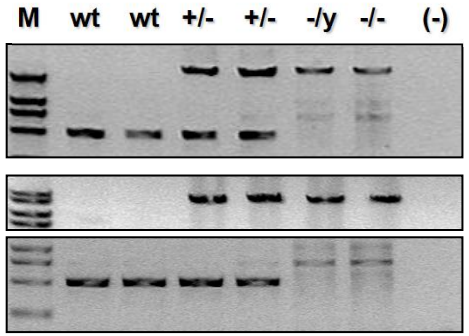
基因剔除小鼠应用举例：疾病模型构建

血友病A是由凝血因子VIII（FVIII）缺乏和(或)其功能障碍所引起的X连锁隐性遗传性出血病。我们利用基因剔除技术建立了FVIII基因剔除小鼠模型（简称“血友病A小鼠模型”），该小鼠模型具有明确的血友病A症状。

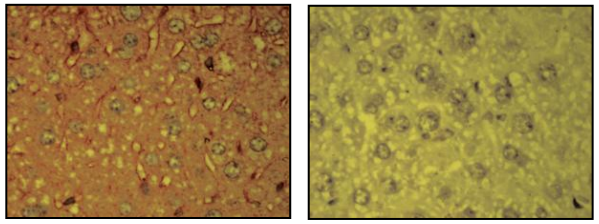
胚胎干细胞重组



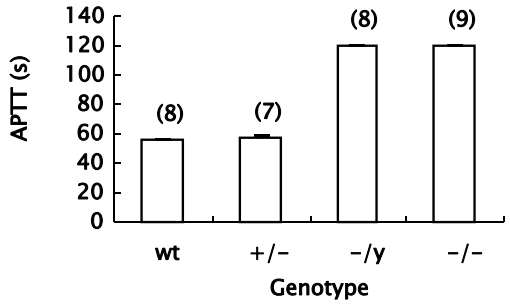
小鼠基因型鉴定



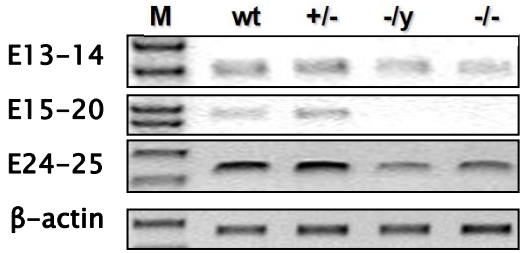
肝脏VIII因子蛋白检测



活化部分凝血活酶时间检测

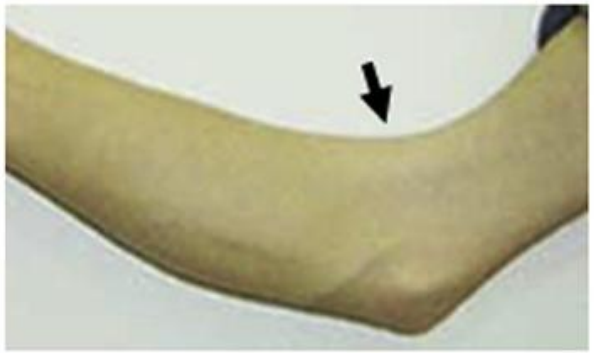


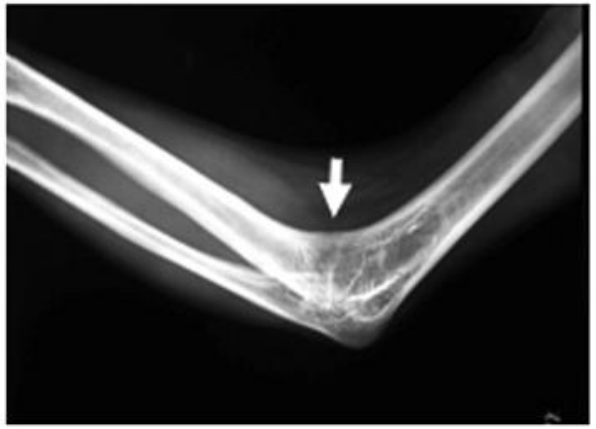
VIII因子表达检测

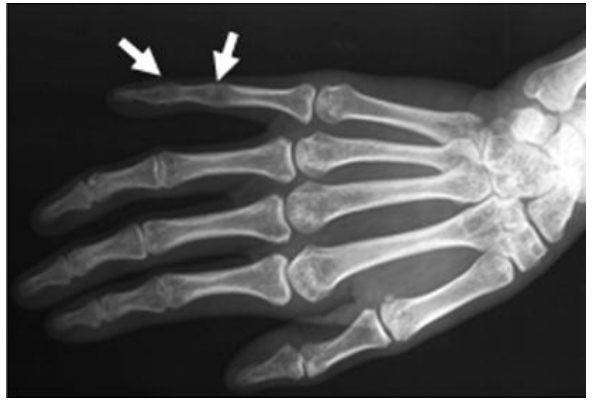


致病基因确认：单基因遗传病突变基因（ *Fgf9*$^{wt/S99N}$ mice）

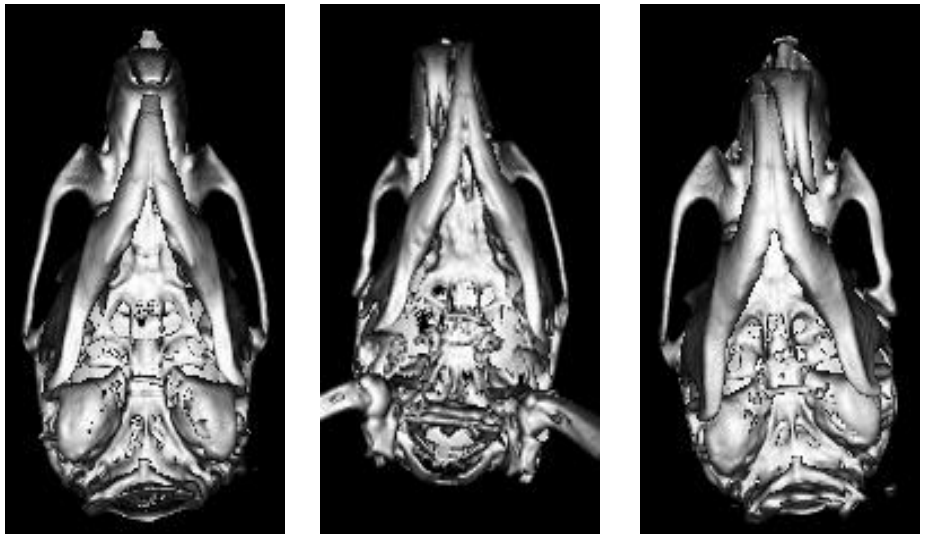
SYNS

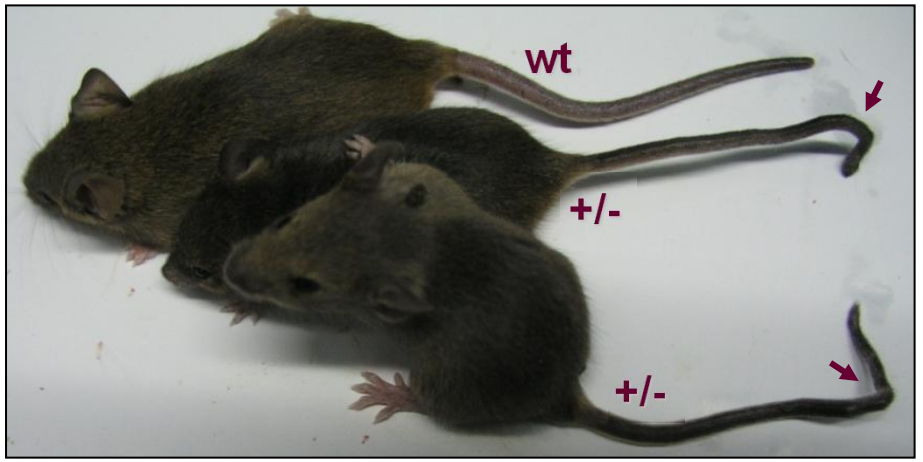






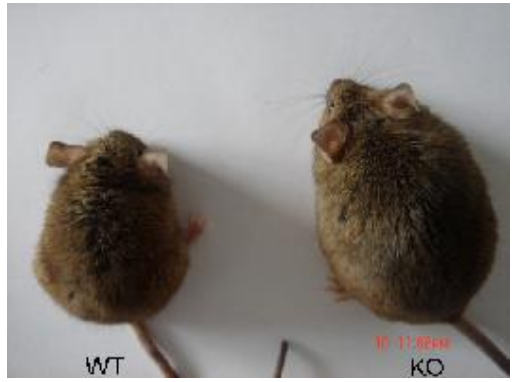
Fgf9$^{wt/wt}$ *Fgf9*$^{wt/S99N}$ *Fgf9*$^{wt/S99N}$

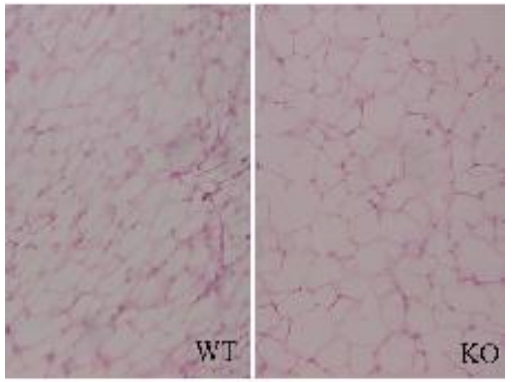


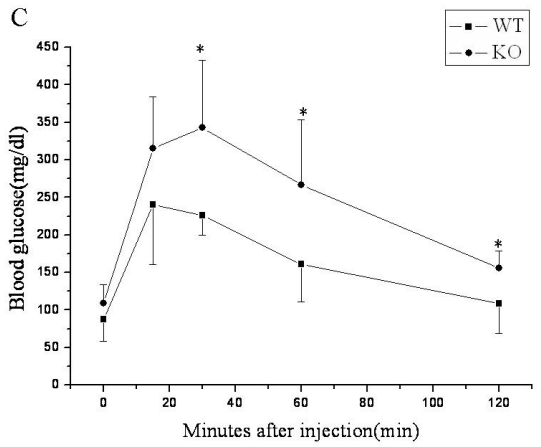


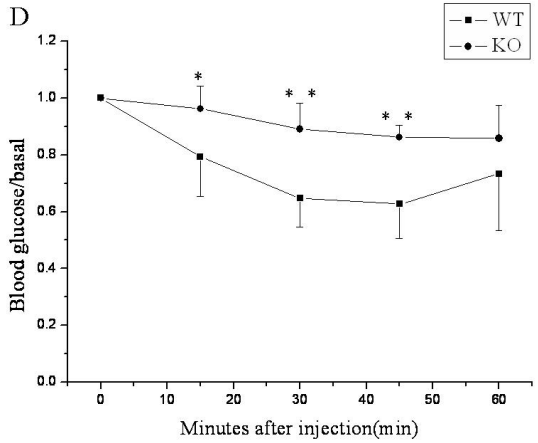
未知基因功能的发现：肥胖小鼠模型

利用基因打靶技术，建立了未知功能分泌肽基因（plrp1）敲除小鼠。结果发现，缺失这个基因的小鼠成年后发生肥胖，并表现出糖尿病早期的某些症状，从而将这个基因的功能和肥胖及糖尿病联系起来。

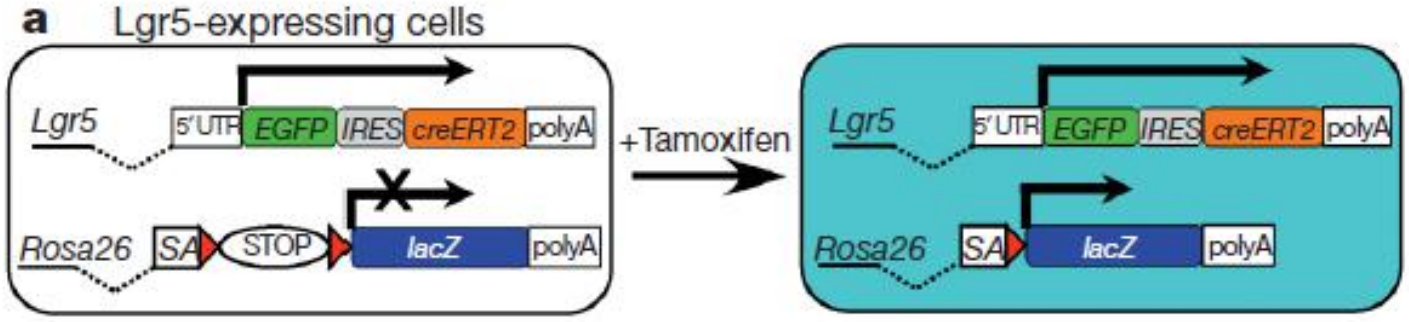


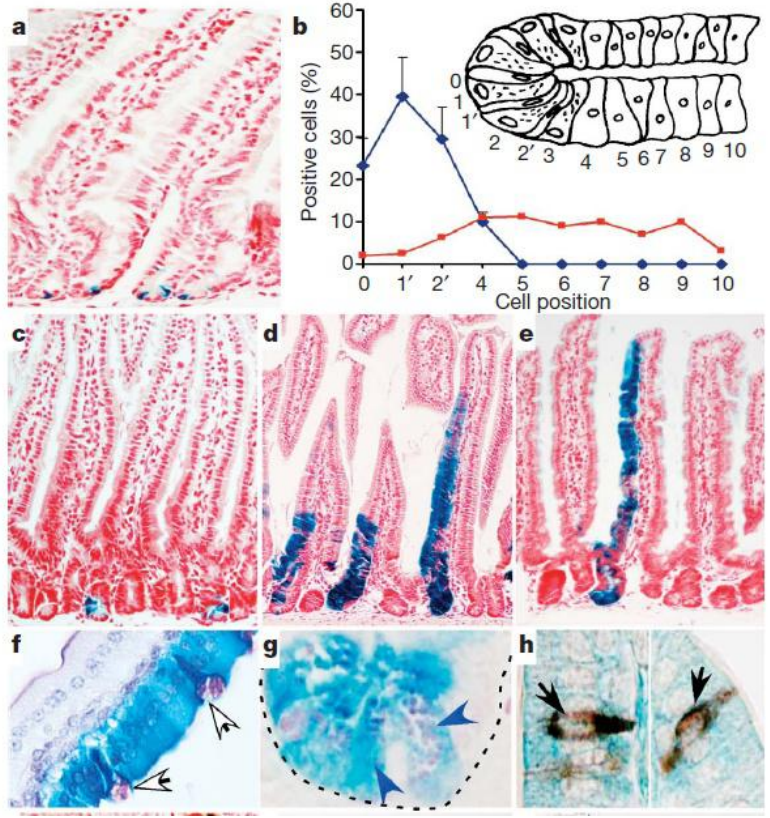






基因工程小鼠应用举例：细胞谱系的遗传标记



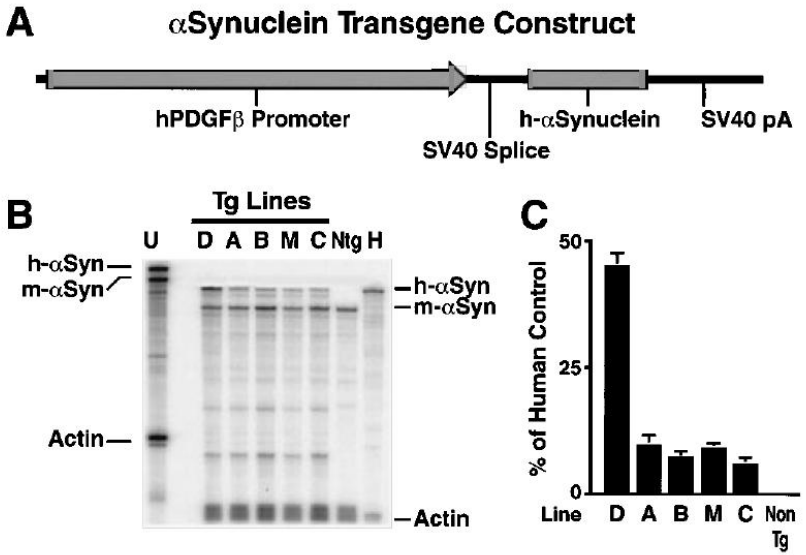


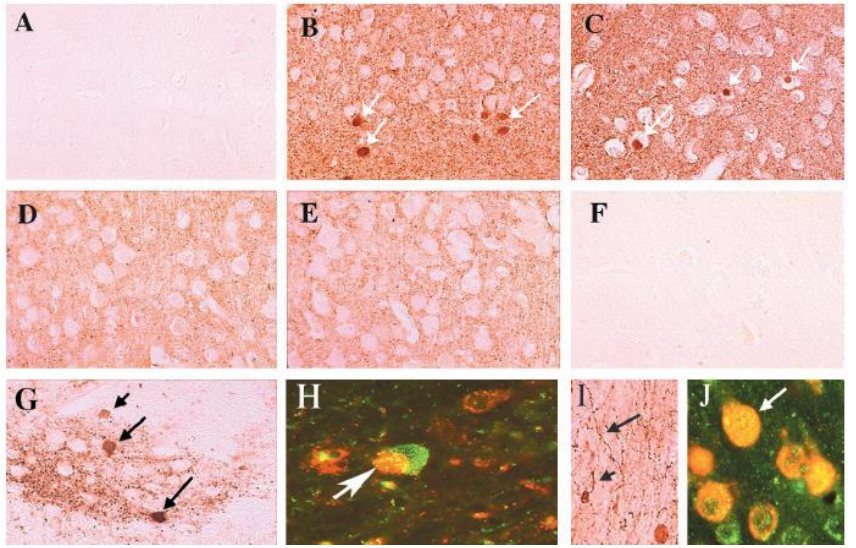
Science
AAAS

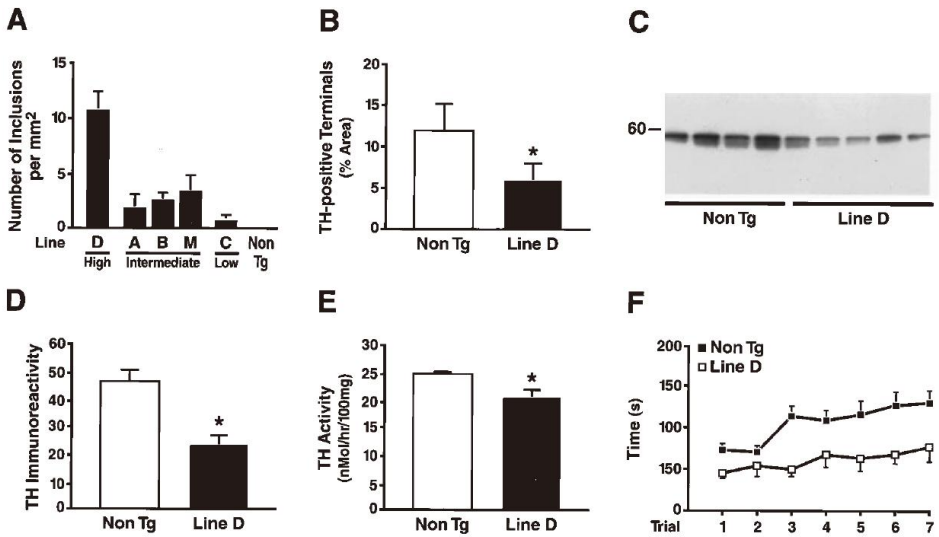
Dopaminergic Loss and Inclusion Body Formation in α-Synuclein Mice: Implications for Neurodegenerative Disorders

Eliezer Masliah *et al.*

Science **287**, 1265 (2000);
DOI: 10.1126/science.287.5456.1265







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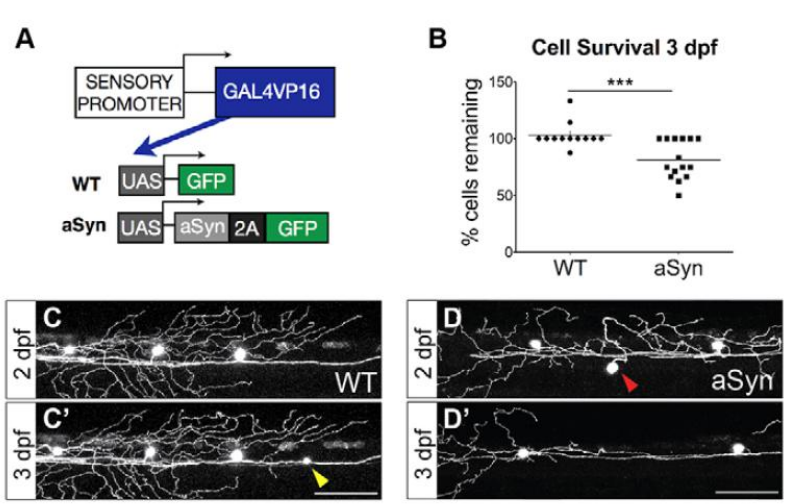
RESEARCH ARTICLE

Axon degeneration and PGC-1α-mediated protection in a zebrafish model of α-synuclein toxicity

Kelley C. O'Donnell[1], Aaron Lulla[2], Mark C. Stahl[2], Nickolas D. Wheat[1], Jeff M. Bronstein[2,3] and Alvaro Sagasti[1,*]



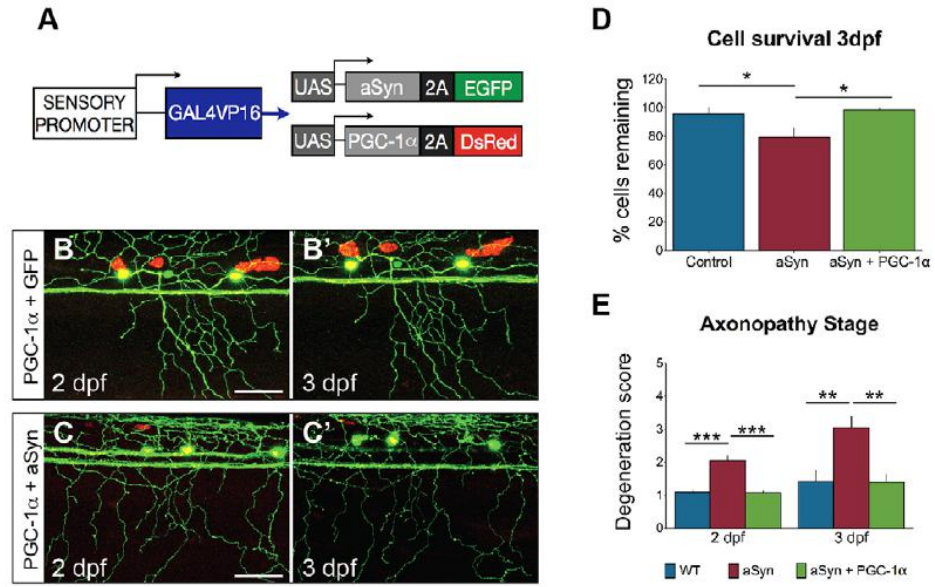


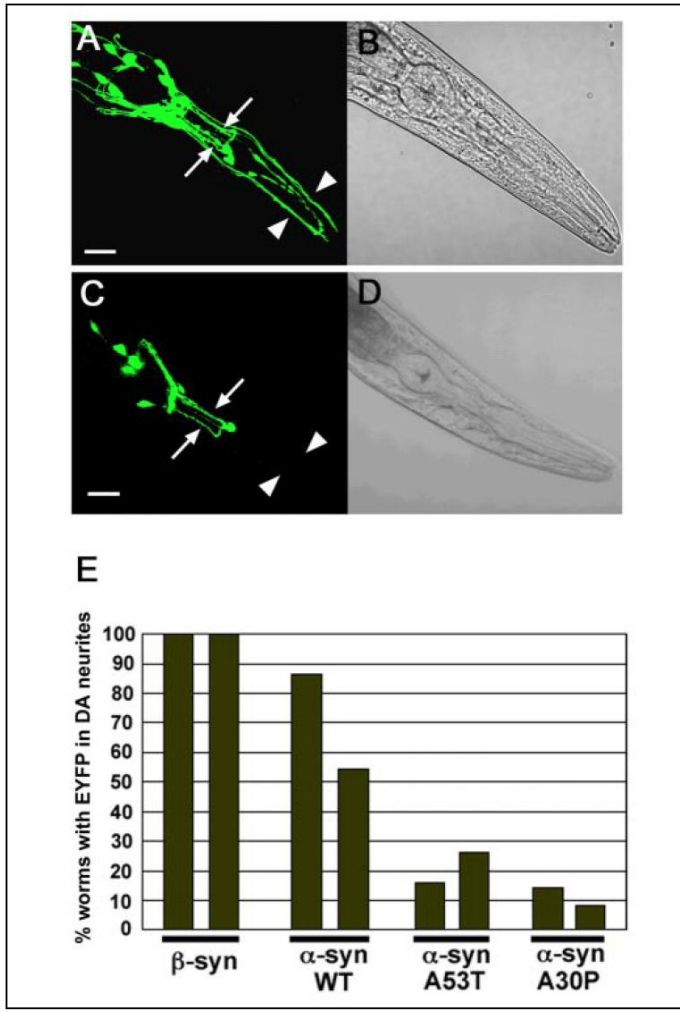
Fig. 1. Alpha-synuclein is moderately toxic to zebrafish sensory neurons between 2 and 3 dpf. (A) Transgenes to express GFP (WT) or

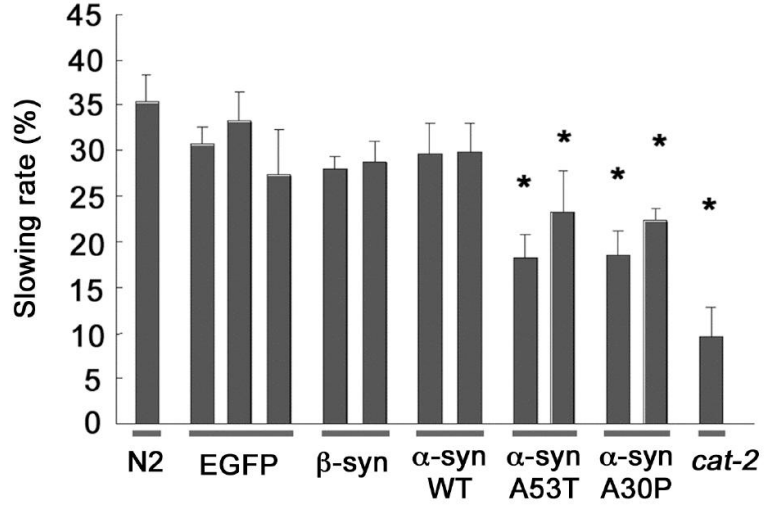
Molecular Basis of Cell and Developmental Biology:

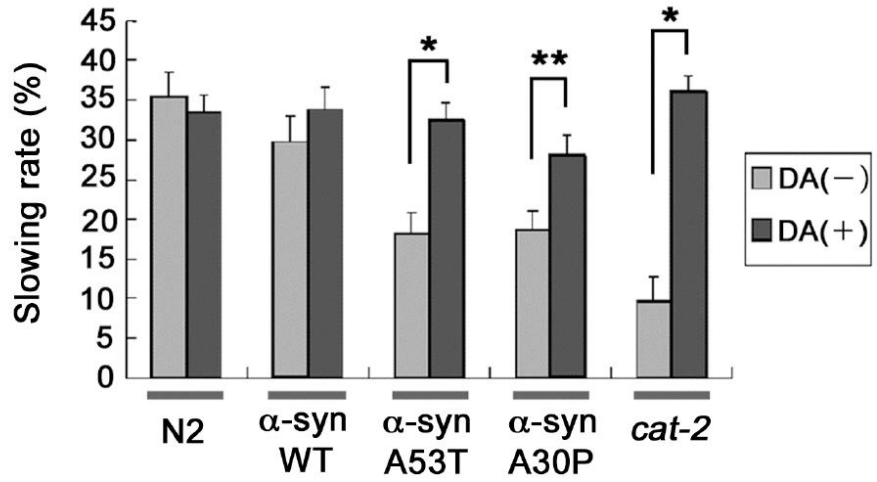
Familial Parkinson Mutant α-Synuclein Causes Dopamine Neuron Dysfunction in Transgenic *Caenorhabditis elegans*

Tomoki Kuwahara, Akihiko Koyama, Keiko Gengyo-Ando, Mayumi Masuda, Hisatomo Kowa, Makoto Tsunoda, Shohei Mitani and Takeshi Iwatsubo

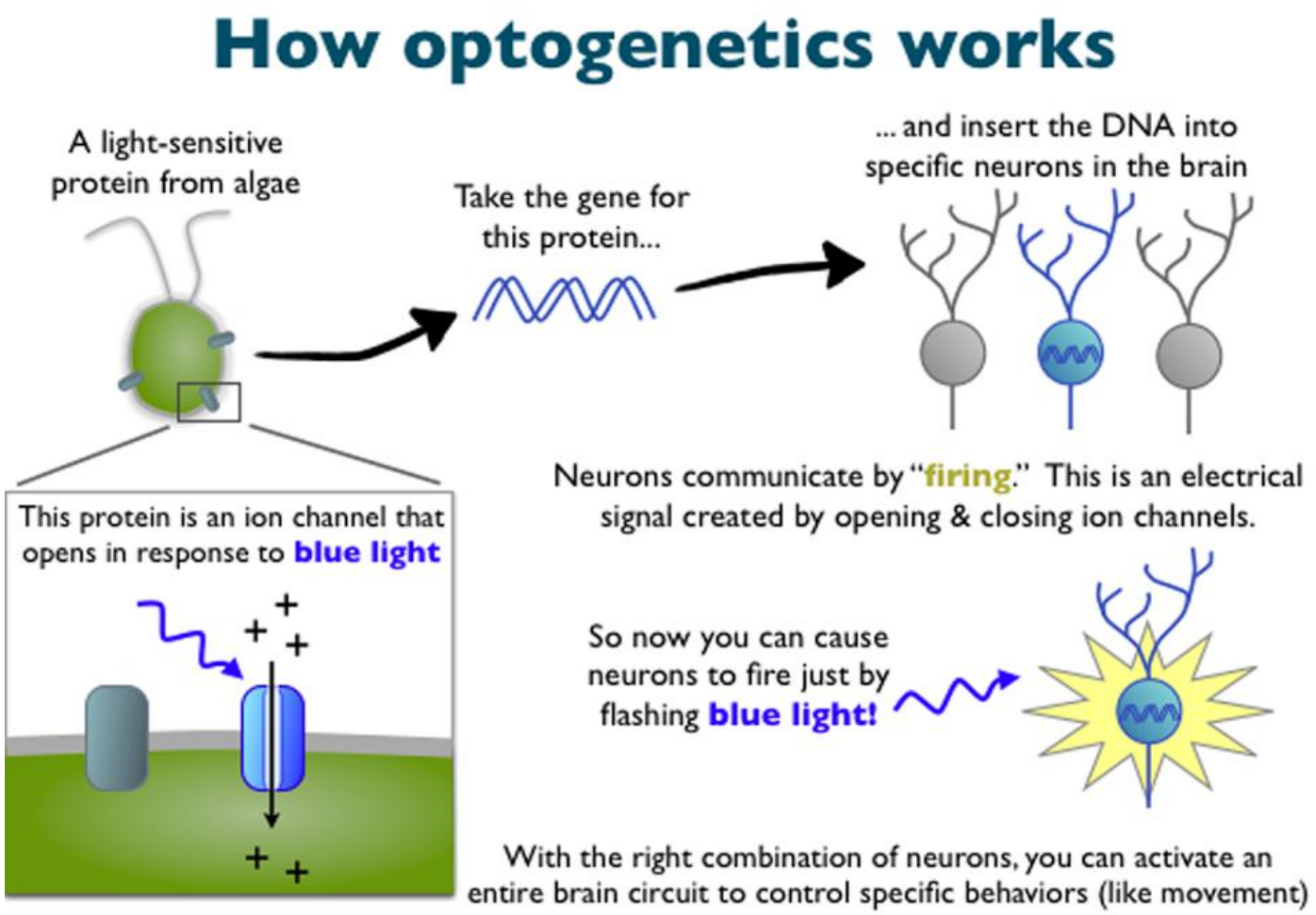
J. Biol. Chem. 2006, 281:334-340.
doi: 10.1074/jbc.M504860200 originally published online October 31, 2005

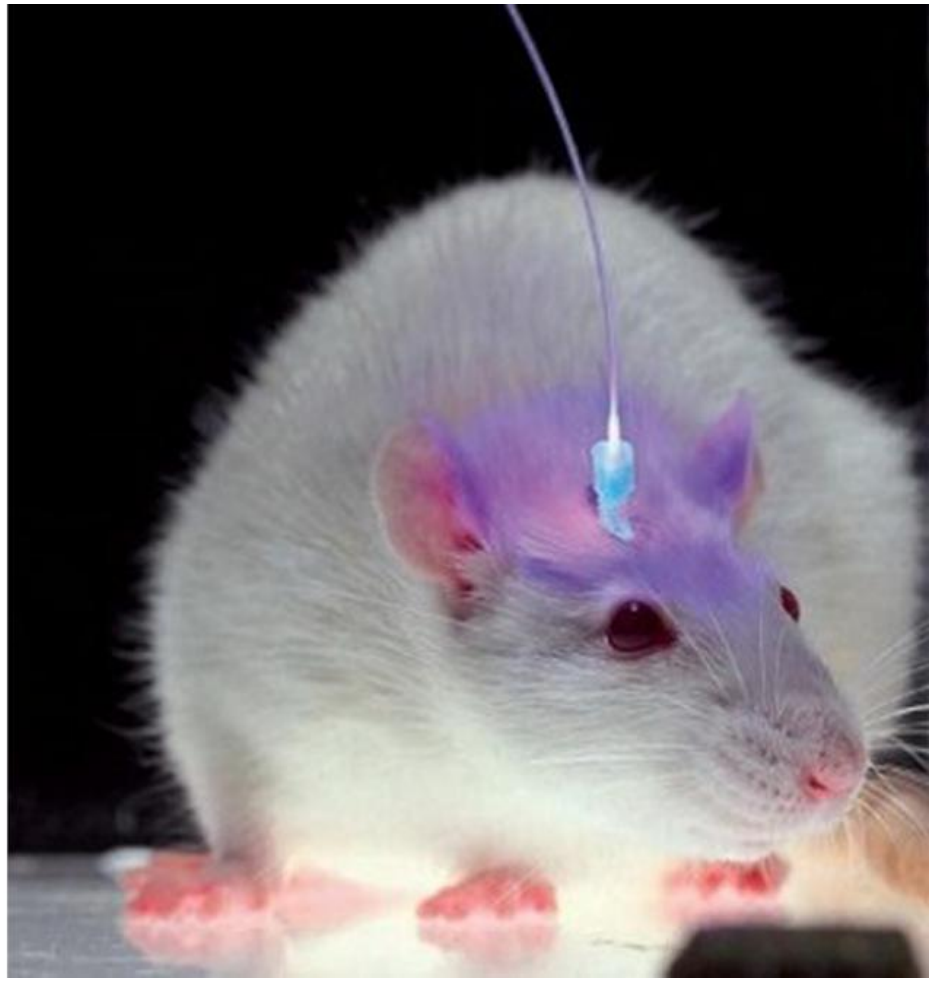

A
B
C
D
E
% worms with EYFP in DA neurites
100
90
80
70
60
50
40
30
20
10
0
β-syn
α-syn WT
α-syn A53T
α-syn A30P


Slowing rate (%)
45
40
35
30
25
20
15
10
5
0
N2
EGFP
β-syn
α-syn WT
α-syn A53T
α-syn A30P
cat-2

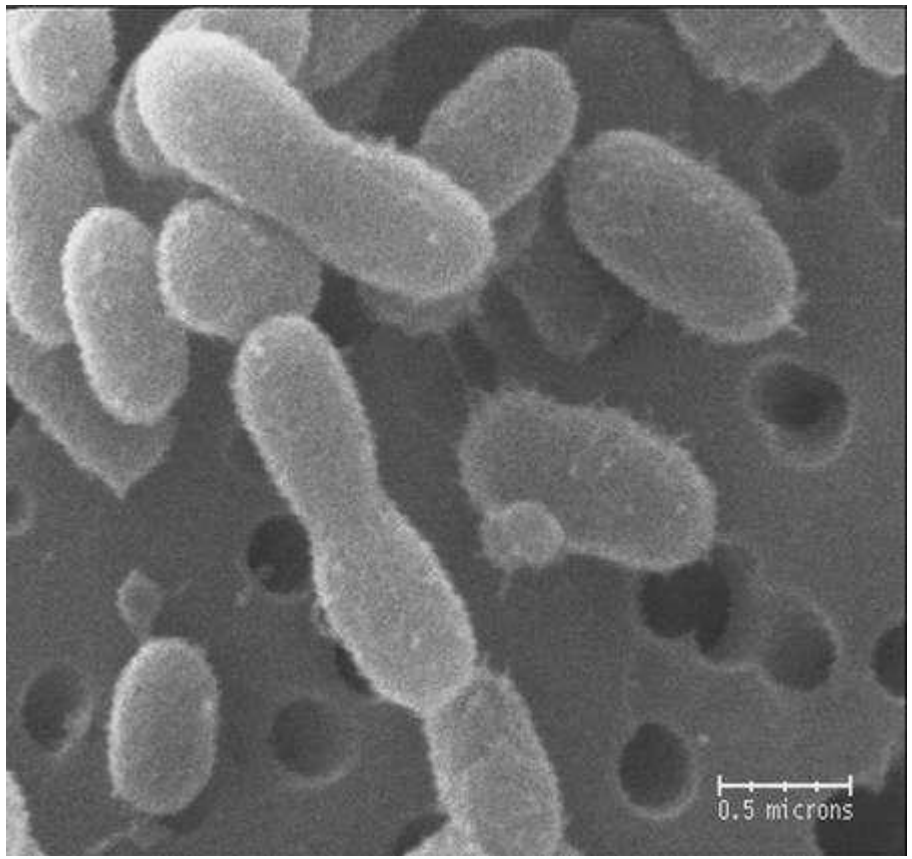

Slowing rate (%)
45
40
35
30
25
20
15
10
5
0
N2
α-syn WT
α-syn A53T
α-syn A30P
cat-2
DA(−)
DA(+)

光遗传学技术

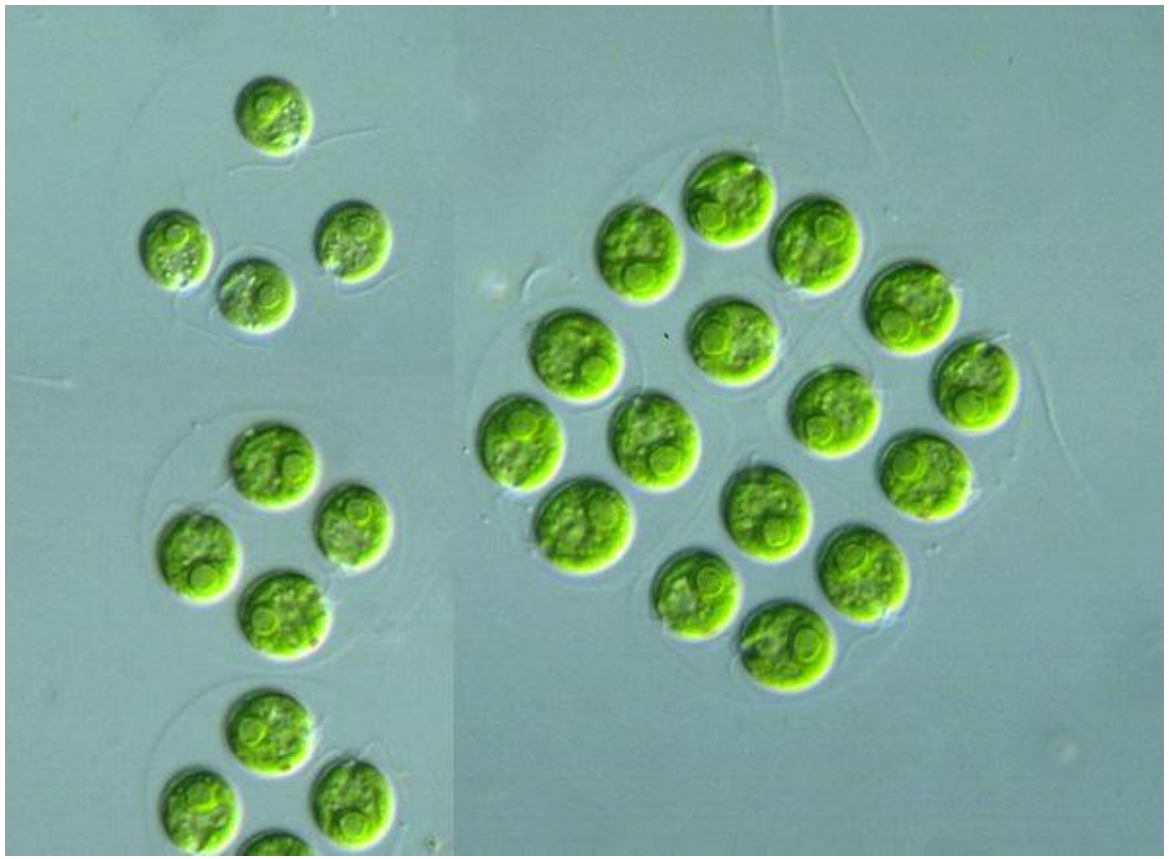




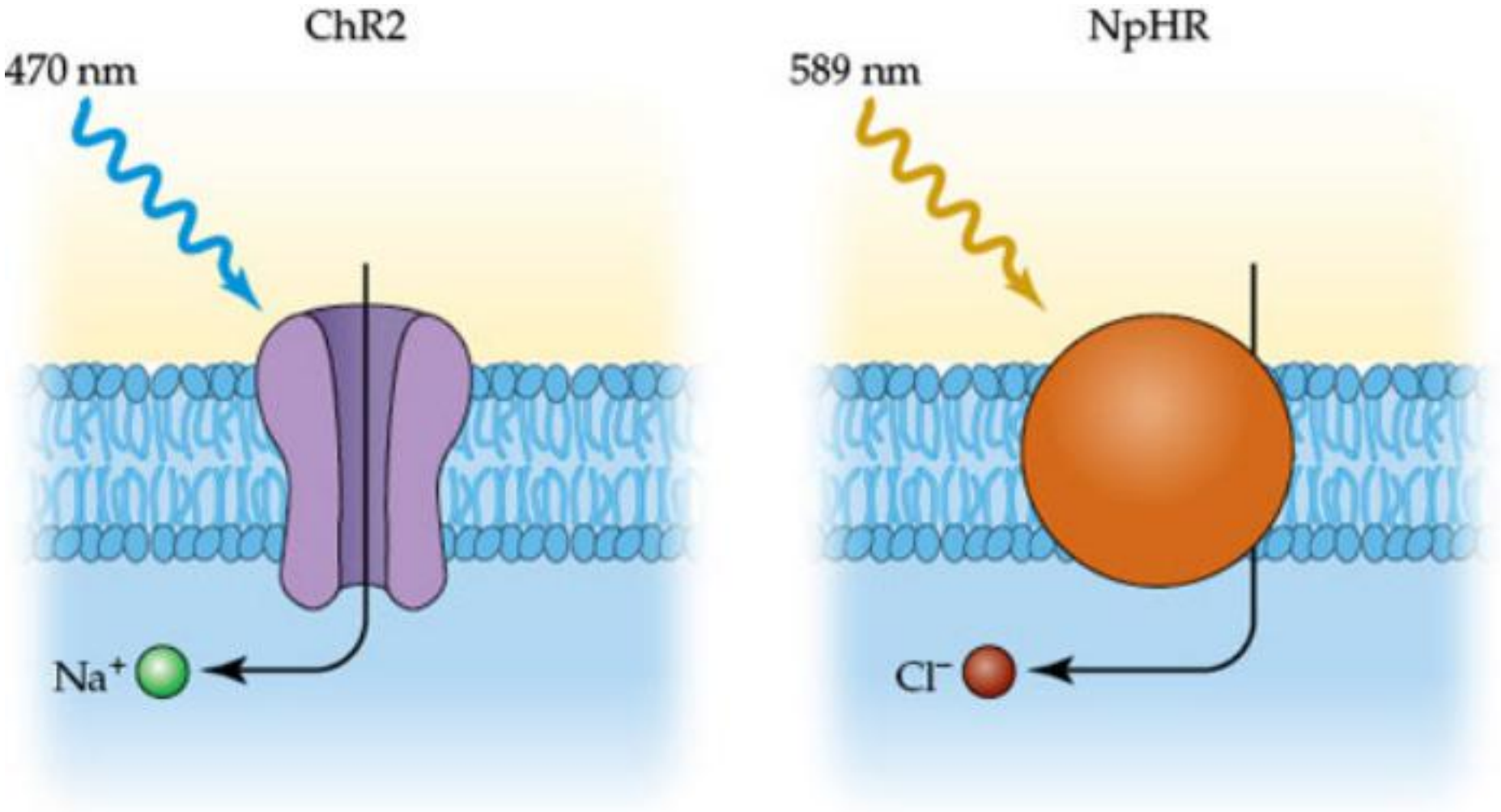
发明人：Karl Deisseroth of Stanford University and the Howard Hughes Medical Institute and Ed Boyden of Massachusetts Institute of Technology



Halobacterium halobium，盐生盐杆菌
bacteriorhodopsin，光感氢离子通道；
halorhodopsin (NpHR)，光感氯离子泵

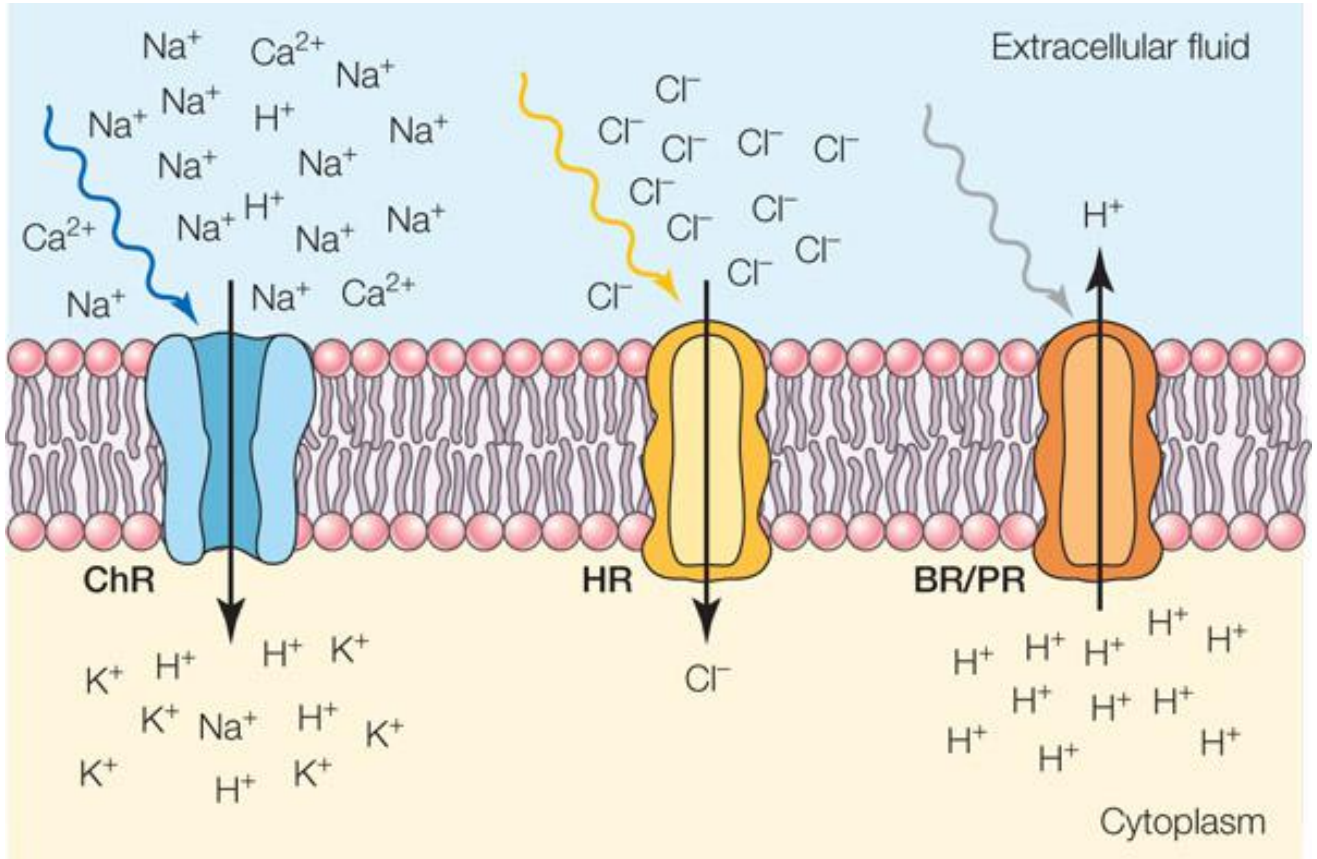


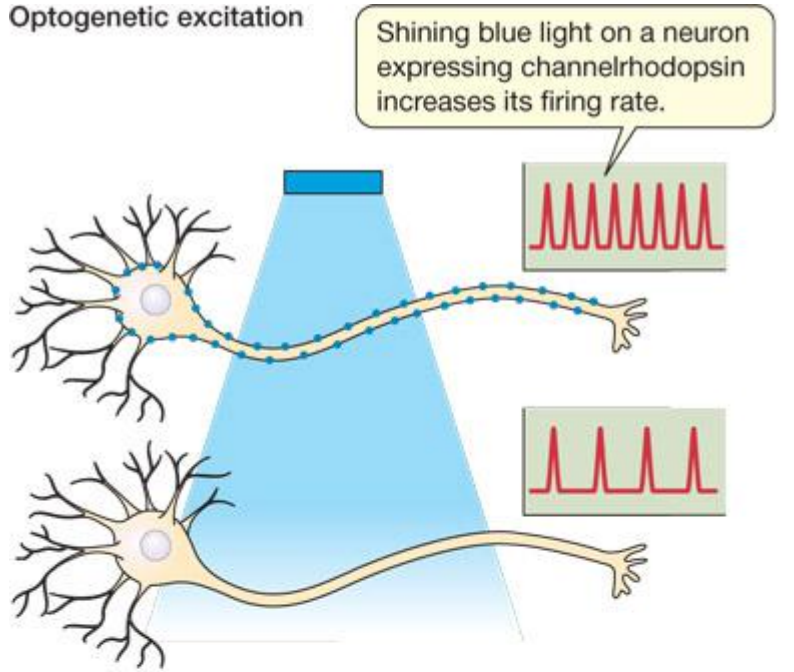
Chlamydomonas reinhardtii，莱茵衣藻
channelrhodopsin1，2：ChR1，ChR2，光感阳离子通道

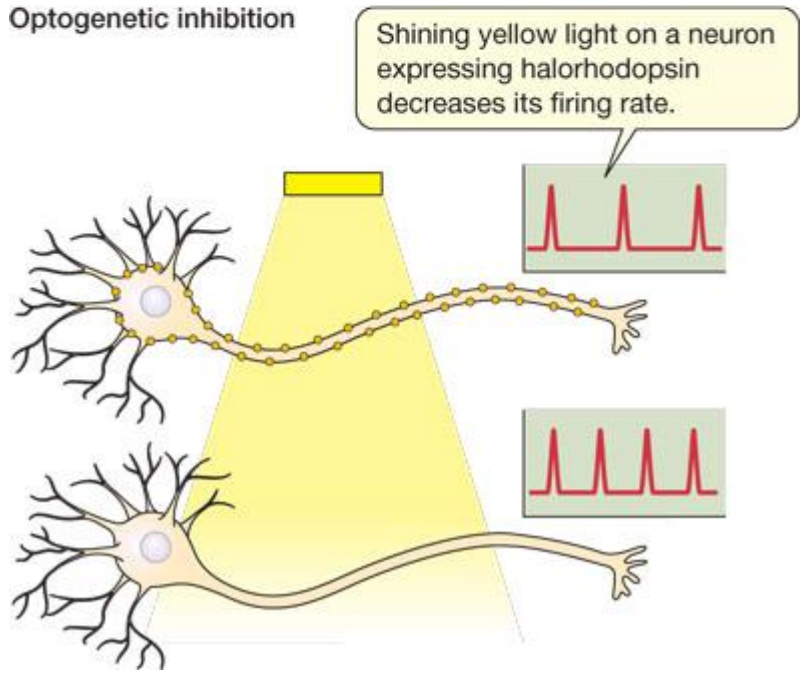


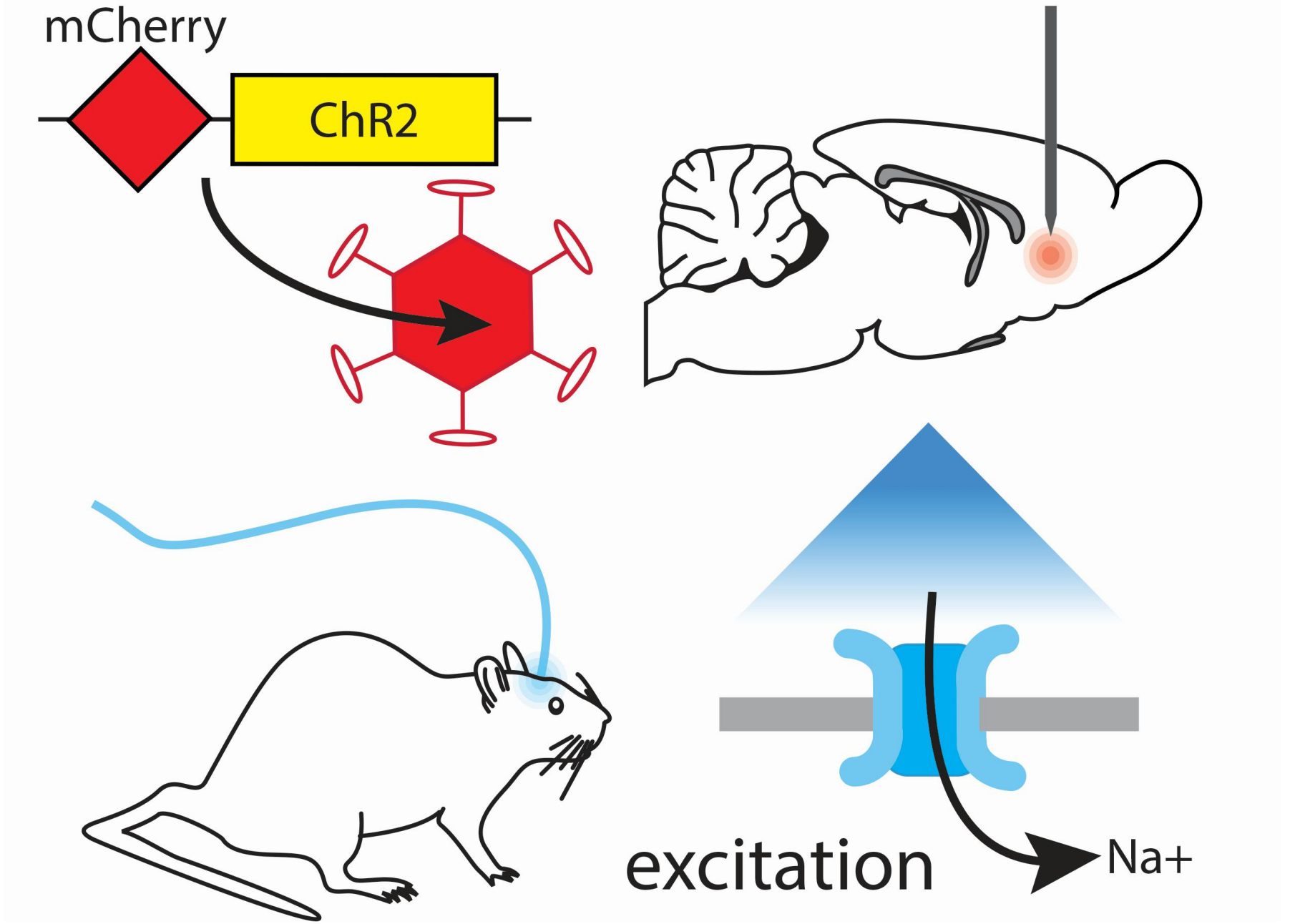
来自绿藻*Chlamydomonas reinhardtii*

来自细菌*Natromonas pharaonis*

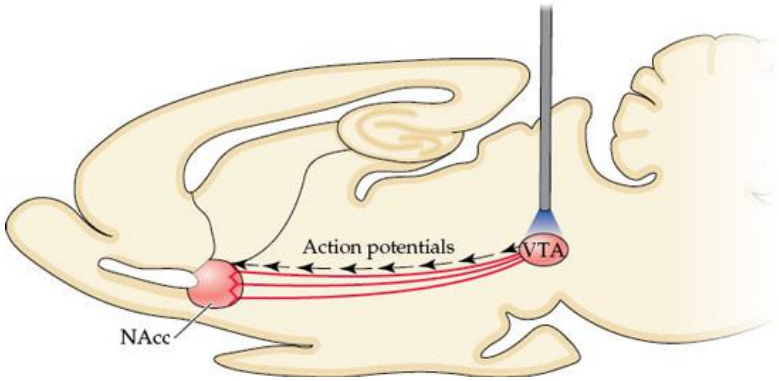

Extracellular fluid
Na+
Ca2+
Cl−
H+
K+
ChR
HR
BR/PR
Cytoplasm

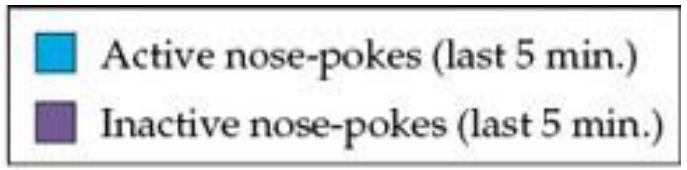

Optogenetic excitation
Shining blue light on a neuron expressing channelrhodopsin increases its firing rate.

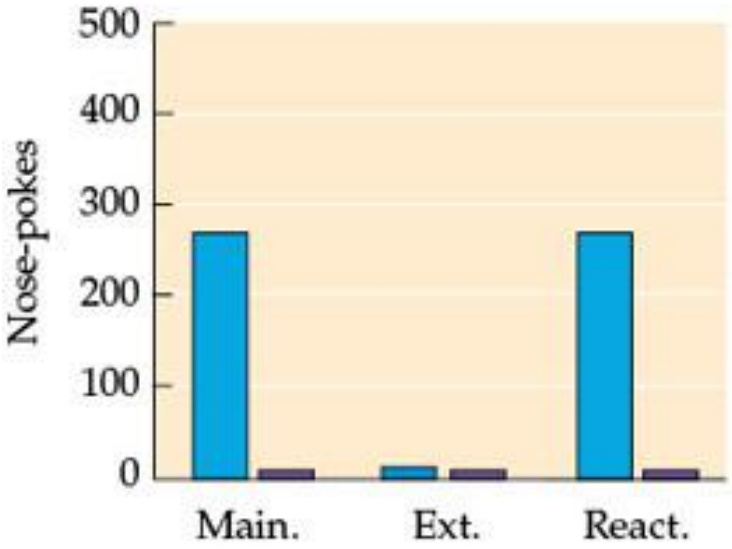

Optogenetic inhibition
Shining yellow light on a neuron expressing halorhodopsin decreases its firing rate.

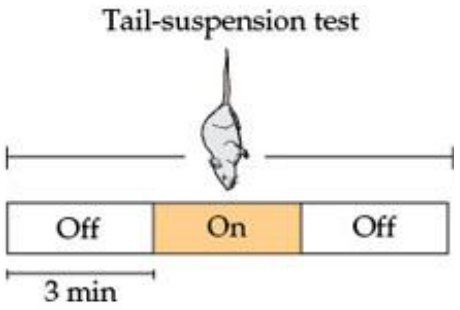

mCherry
ChR2
excitation
Na+

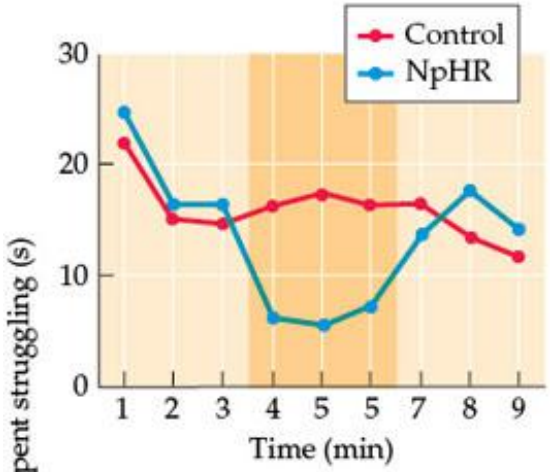
VTA多巴胺神经元激活或抑制参与奖赏和抑郁样调控

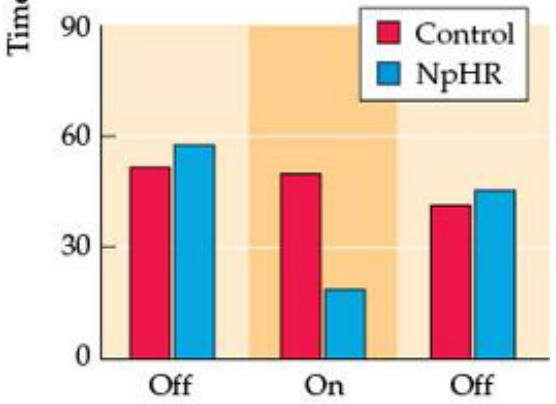


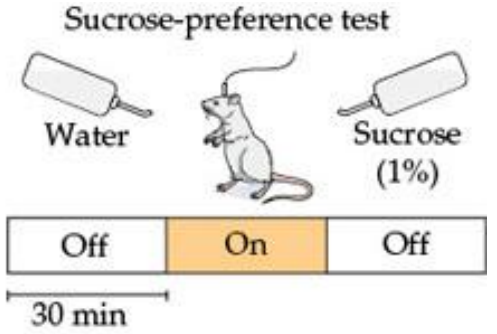


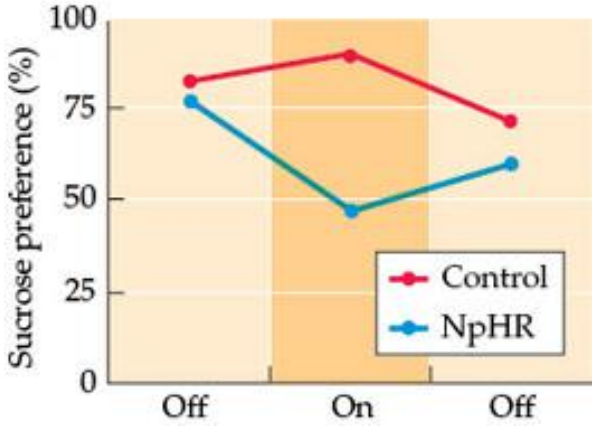






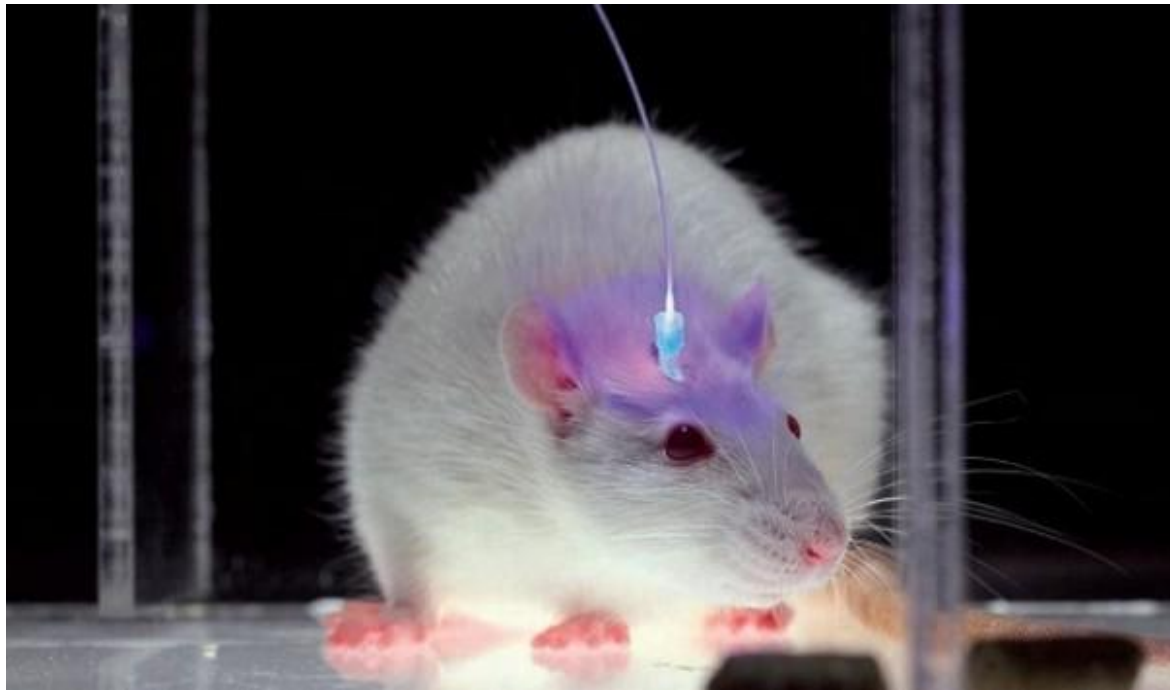






Nat. Rev. Neurosci., 13, 251–266.

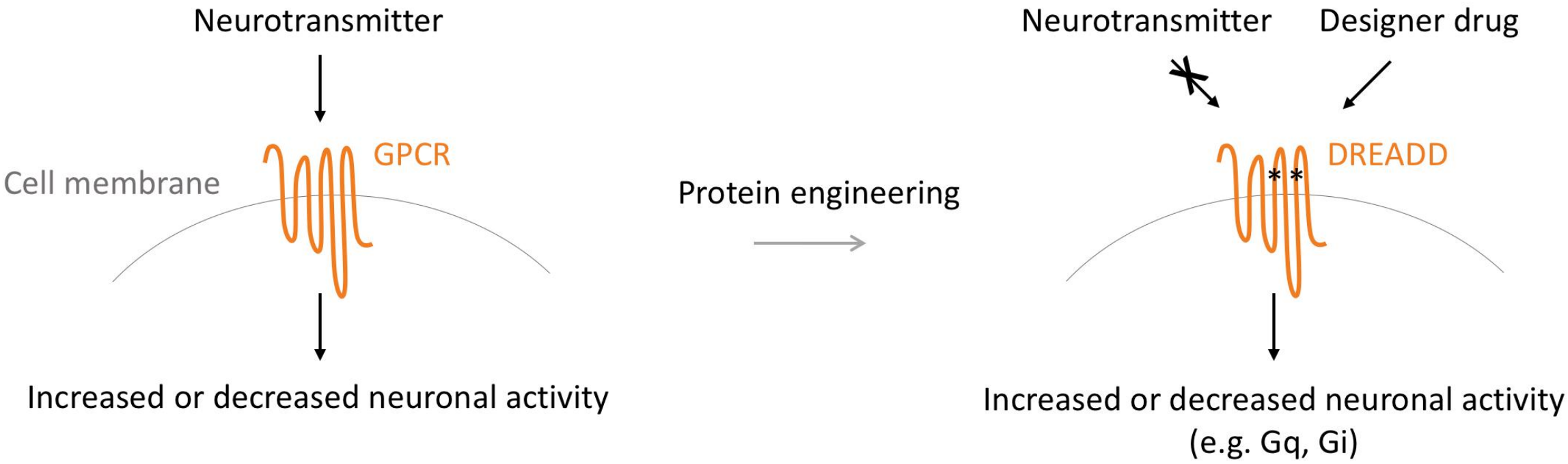
Neuron, 72, 721–733.

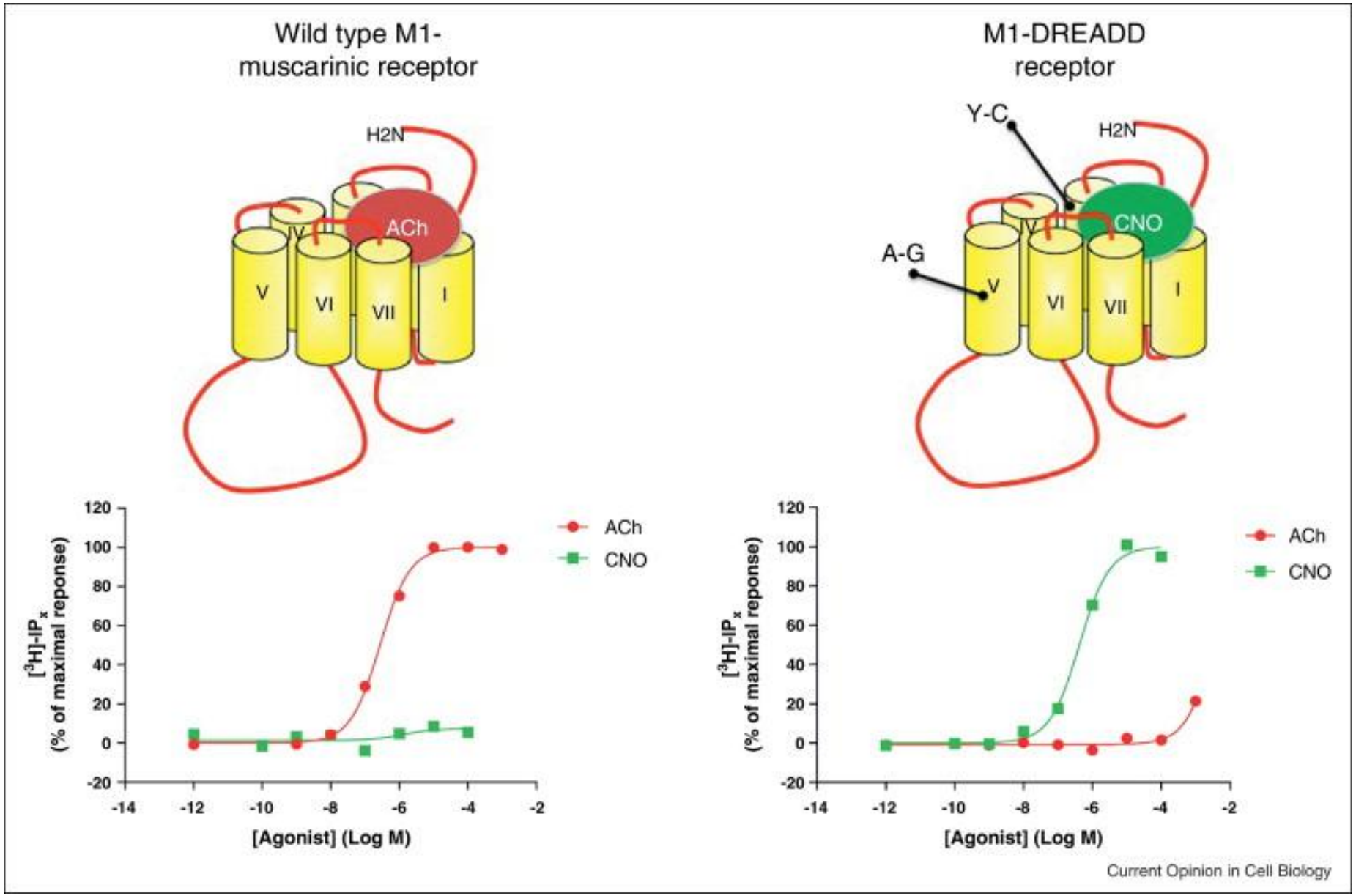



Teleopt
EXT
TRG
OPTION
I²C

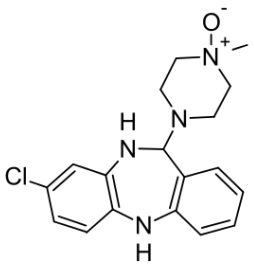
化学遗传学技术

Designer receptors exclusively activated by designer drugs (DREADDs)

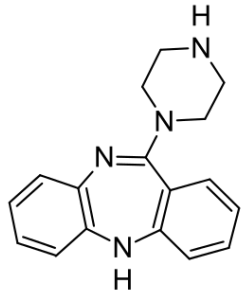



Wild type M1-
muscarinic receptor
H2N
ACh
V
VI
VII
I
[3H]-IPx
(% of maximal reponse)
120
100
80
60
40
20
0
-20
-14
-12
-10
-8
-6
-4
-2
[Agonist] (Log M)
ACh
CNO
M1-DREADD
receptor
Y-C
H2N
CNO
A-G
V
VI
VII
I
[3H]-IPx
(% of maximal reponse)
120
100
80
60
40
20
0
-20
-14
-12
-10
-8
-6
-4
-2
[Agonist] (Log M)
ACh
CNO
Current Opinion in Cell Biology

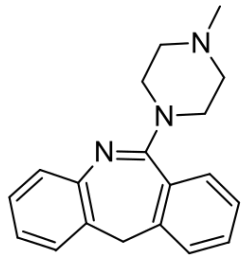
CNO



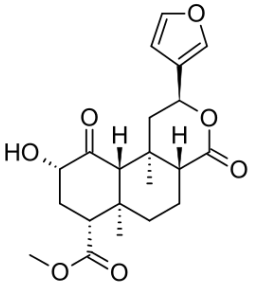
PERLAPINE



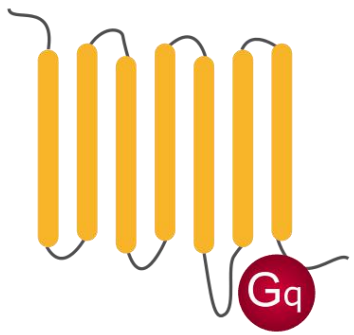
COMPOUND 21



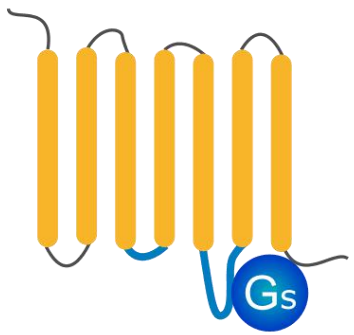
SALVINORIN B



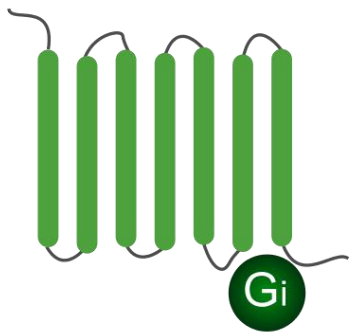
hM3Dq



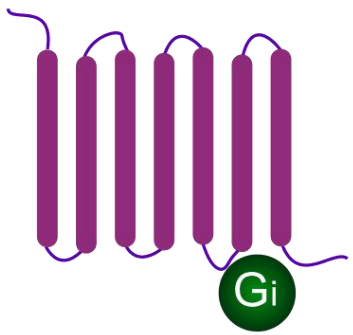
GsD



hM4Di



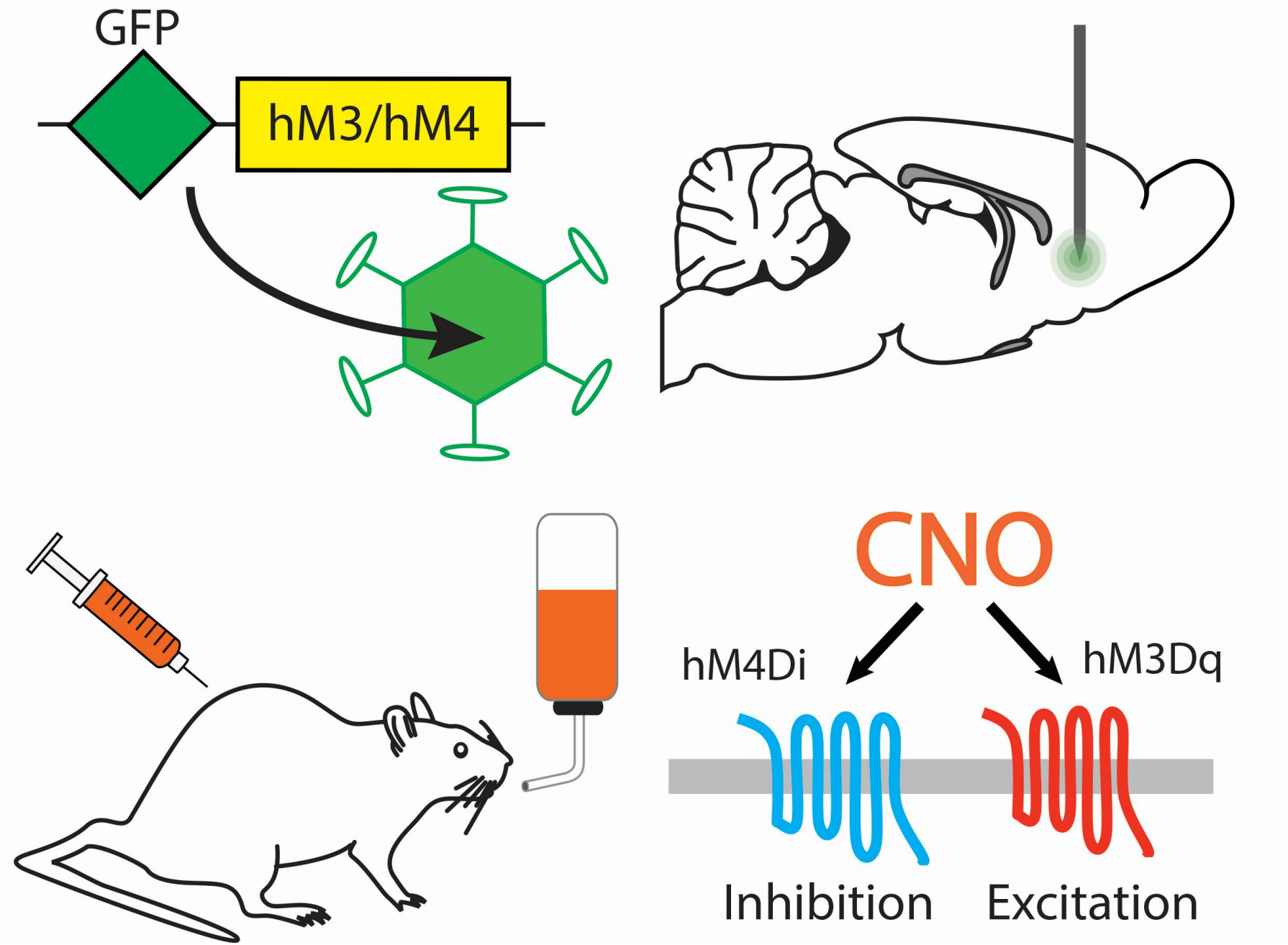
KORD

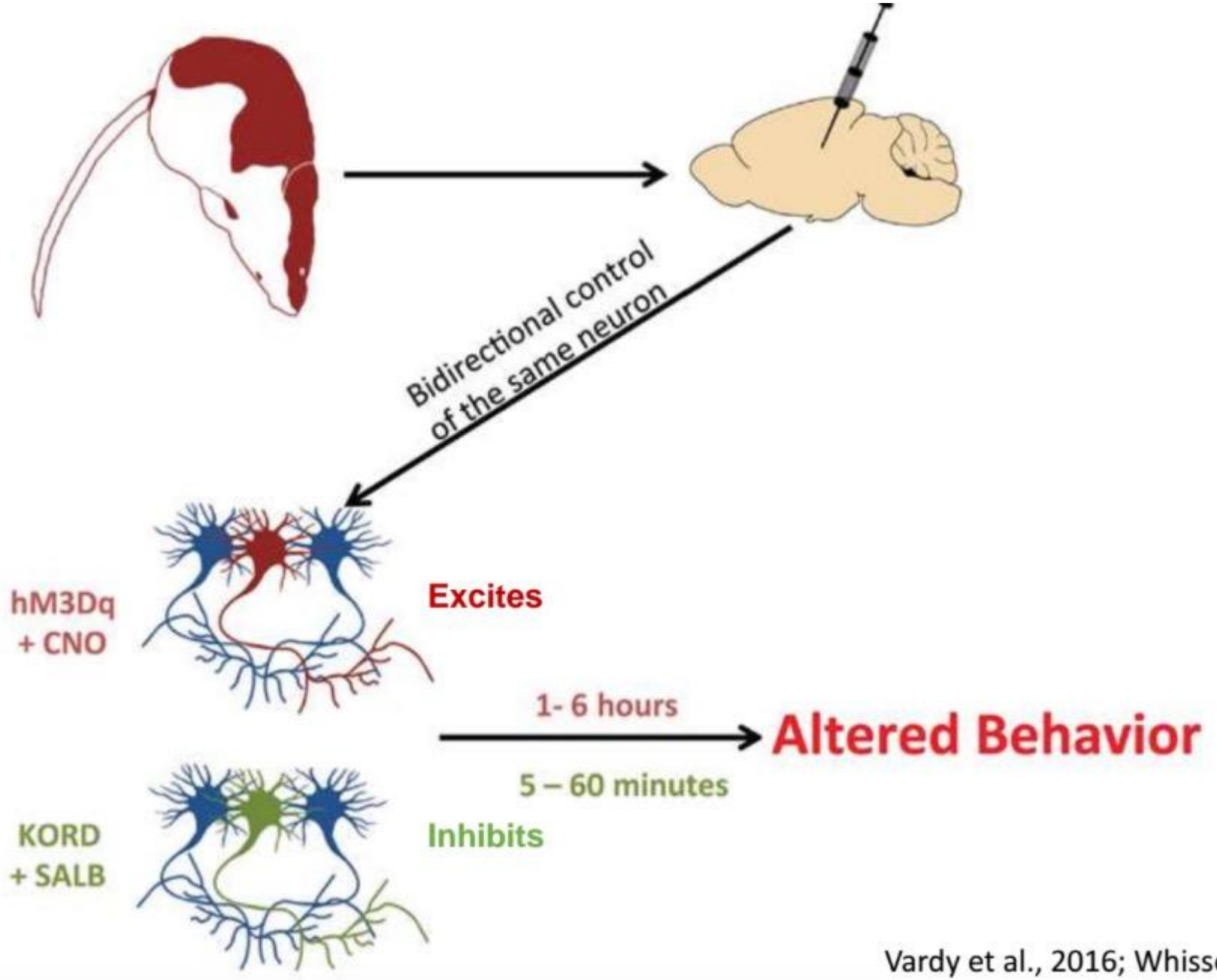


Increase intracellular calcium
(Depolarization)
Increase cell excitability

Activate adenylyl cyclase
Increase cAMP

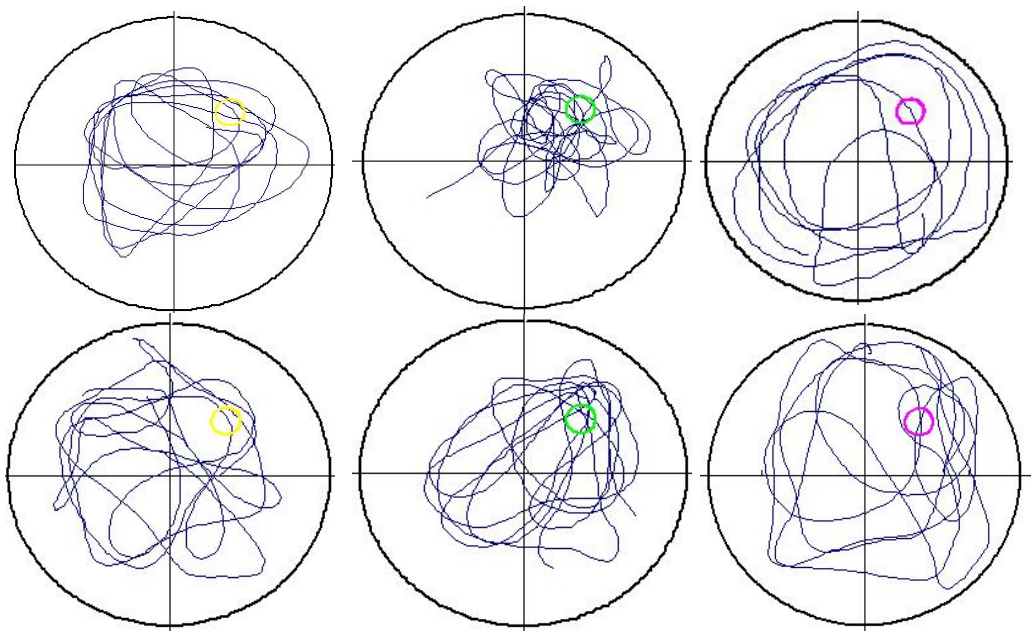
Activate K^+ channels (GIRKs)
(Hyperpolarization)
Inhibit neurotransmitter release
(Neuronal silencing)


GFP
hM3/hM4
CNO
hM4Di
hM3Dq
Inhibition
Excitation

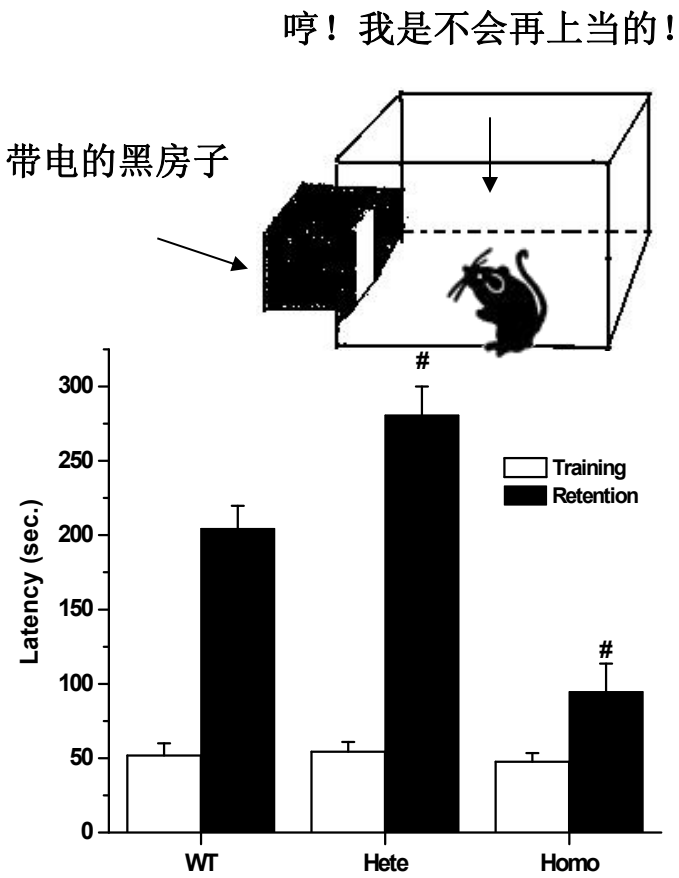


Vardy et al., 2016; Whissel et al., 2016

天才与白痴，一个拷贝的差异：GAT1在学习记忆中的作用



正常小鼠（WT）　　杂合小鼠（Hete）　　纯合小鼠（Homo）



小鼠在受电击后，再次进入暗室所需要的时间

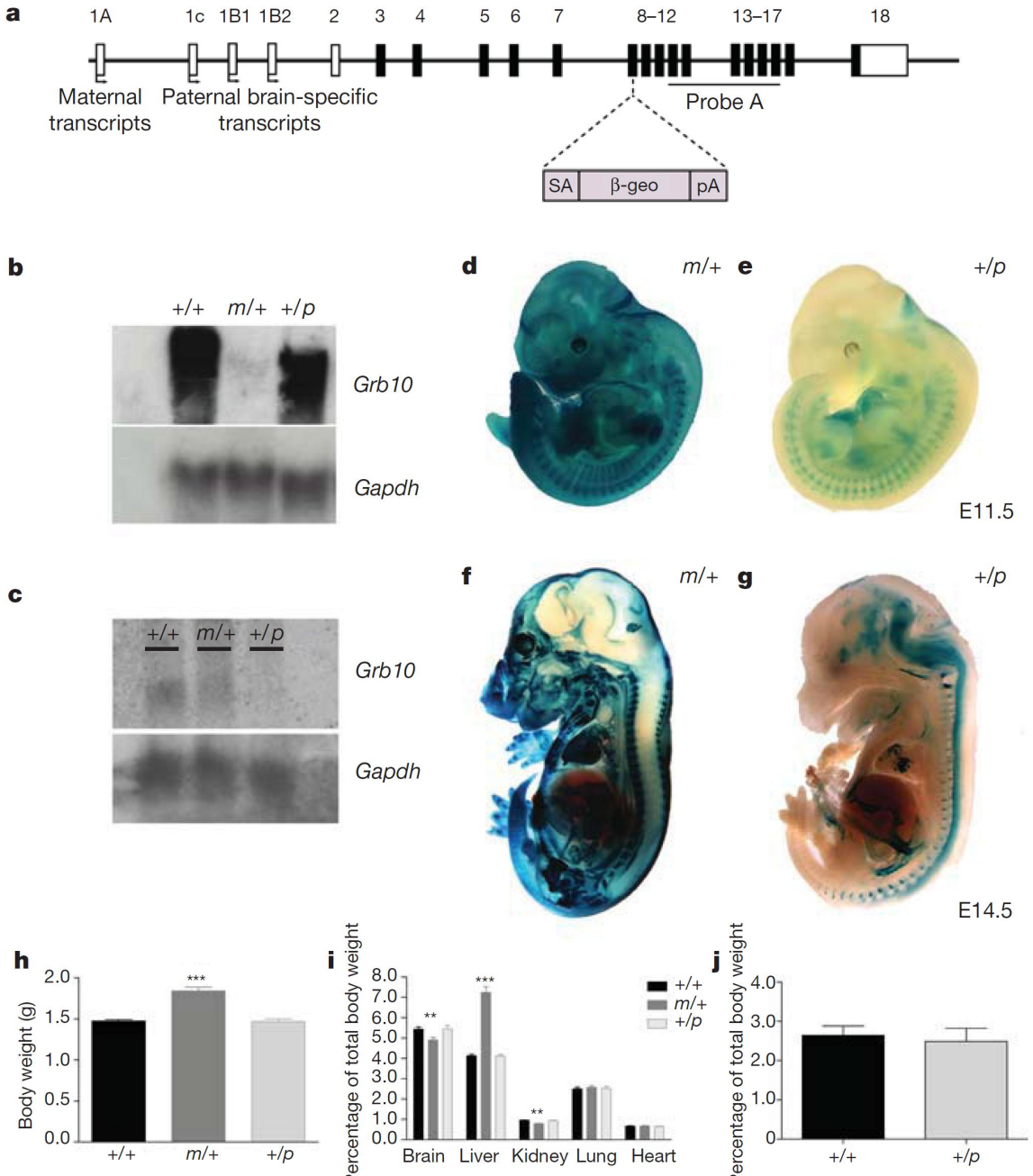
男女有别：等位基因的不等位表达

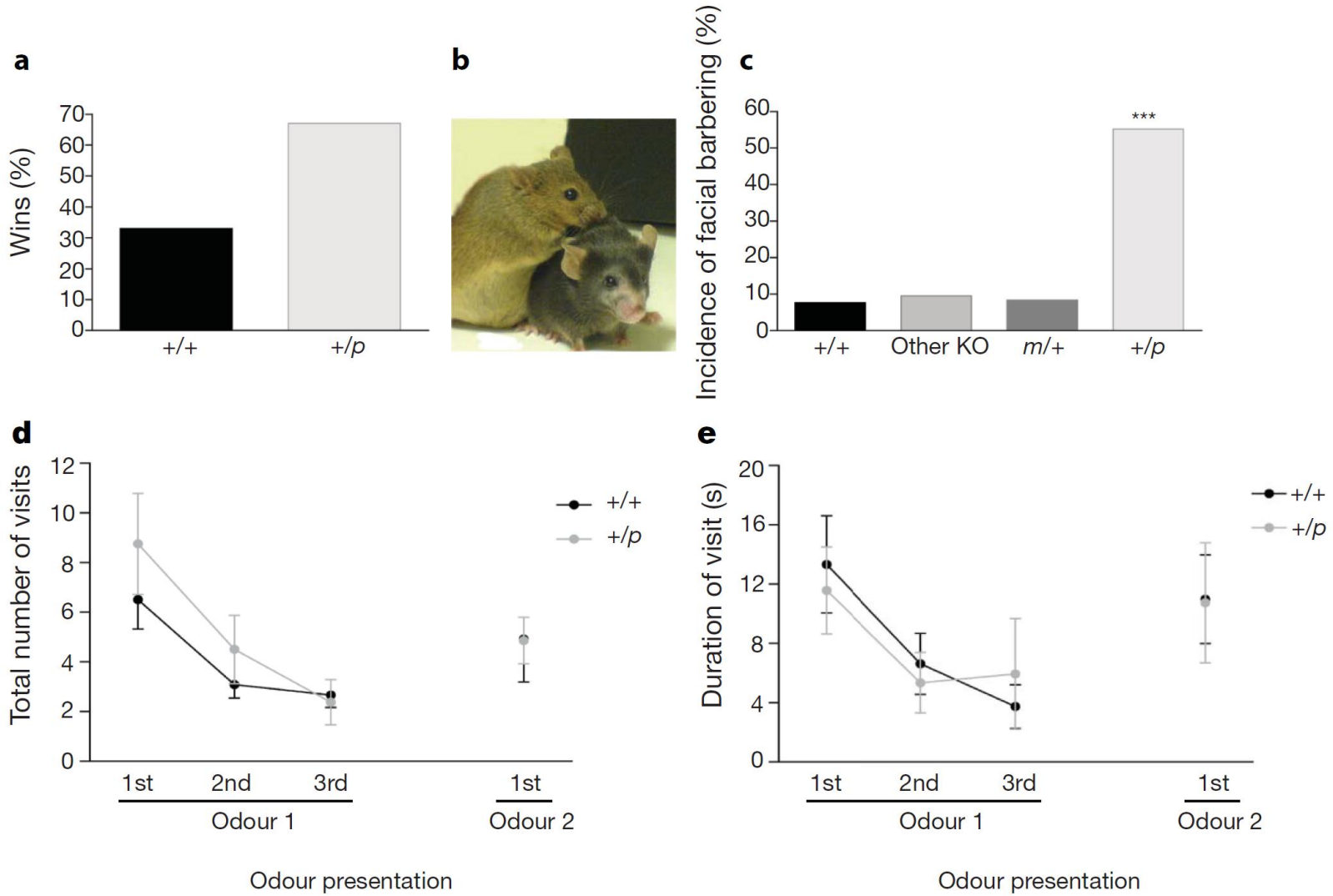
LETTER

doi:10.1038/nature09651

Distinct physiological and behavioural functions for parental alleles of imprinted *Grb10*

Alastair S. Garfield[1,2]†, Michael Cowley[1]†, Florentia M. Smith[1], Kim Moorwood[1], Joanne E. Stewart-Cox[1], Kerry Gilroy[2], Sian Baker[2], Jing Xia[3], Jeffrey W. Dalley[3,4], Laurence D. Hurst[1], Lawrence S. Wilkinson[2], Anthony R. Isles[2] & Andrew Ward[1]

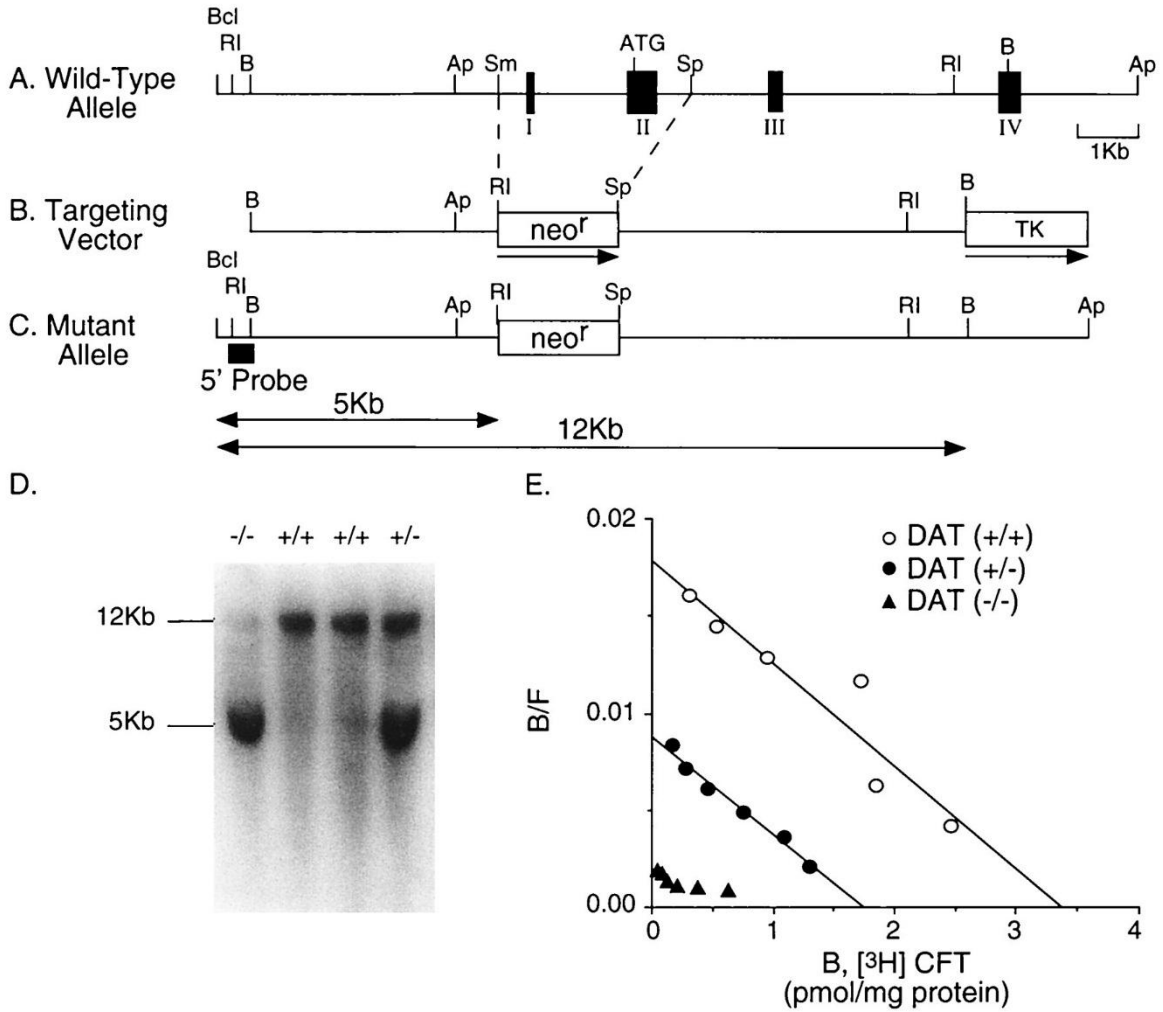

a
1A
1c
1B1
1B2
2
3
4
5
6
7
8–12
13–17
18
Maternal transcripts
Paternal brain-specific transcripts
Probe A
SA
β-geo
pA
b
+/+
m/+
+/p
Grb10
Gapdh
c
+/+
m/+
+/p
Grb10
Gapdh
d
m/+
e
+/p
E11.5
f
m/+
g
+/p
E14.5
h
Body weight (g)
2.0
1.5
1.0
0.5
0.0
***
+/+
m/+
+/p
i
Percentage of total body weight
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
**
***
**
+/+
m/+
+/p
Brain
Liver
Kidney
Lung
Heart
j
Percentage of total body weight
4.0
3.0
2.0
1.0
0
+/+
+/p


a
Wins (%)
70
60
50
40
30
20
10
0
+/+
+/p
b
c
Incidence of facial barbering (%)
60
50
40
30
20
10
0
***
+/+
Other KO
m/+
+/p
d
Total number of visits
12
10
8
6
4
2
0
+/+
+/p
1st
2nd
3rd
1st
Odour 1
Odour 2
Odour presentation
e
Duration of visit (s)
20
16
12
8
4
0
+/+
+/p
1st
2nd
3rd
1st
Odour 1
Odour 2
Odour presentation

可卡因成瘾之谜

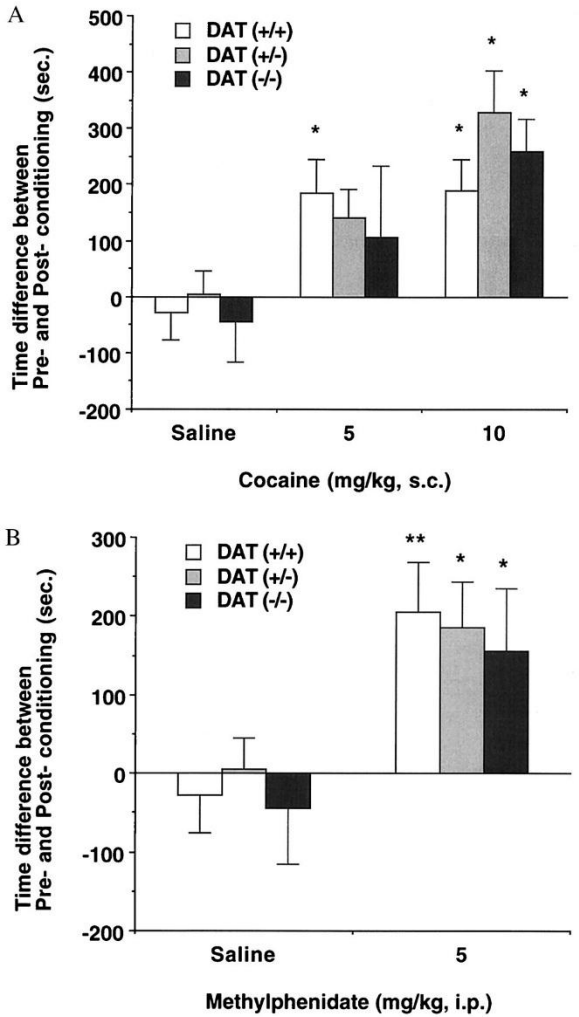
从否定到否定之否定

Disruption of the DAT gene



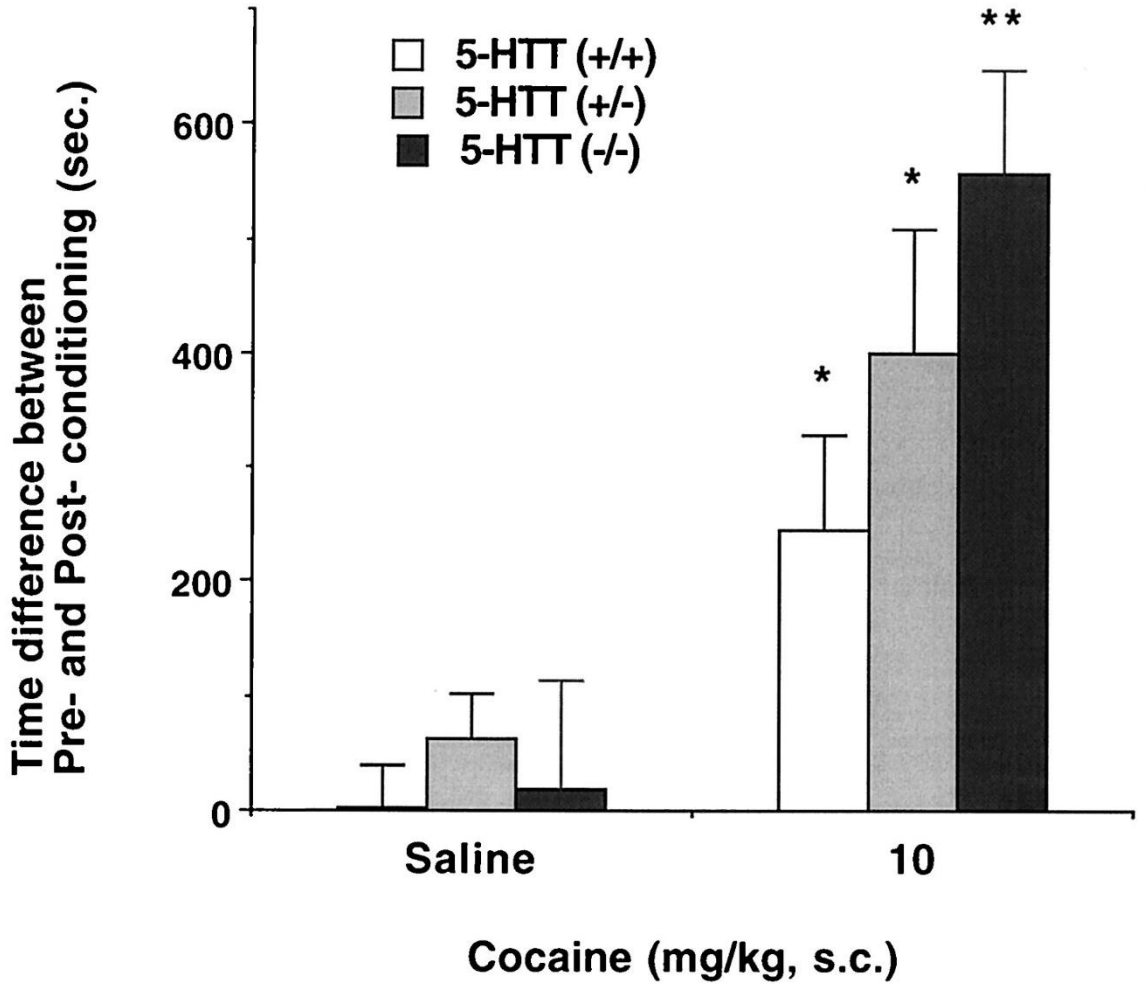
Sora I et al. PNAS 1998;95:7699-7704

Cocaine- and methylphenidate-conditioned place preferences in DAT knockout mice



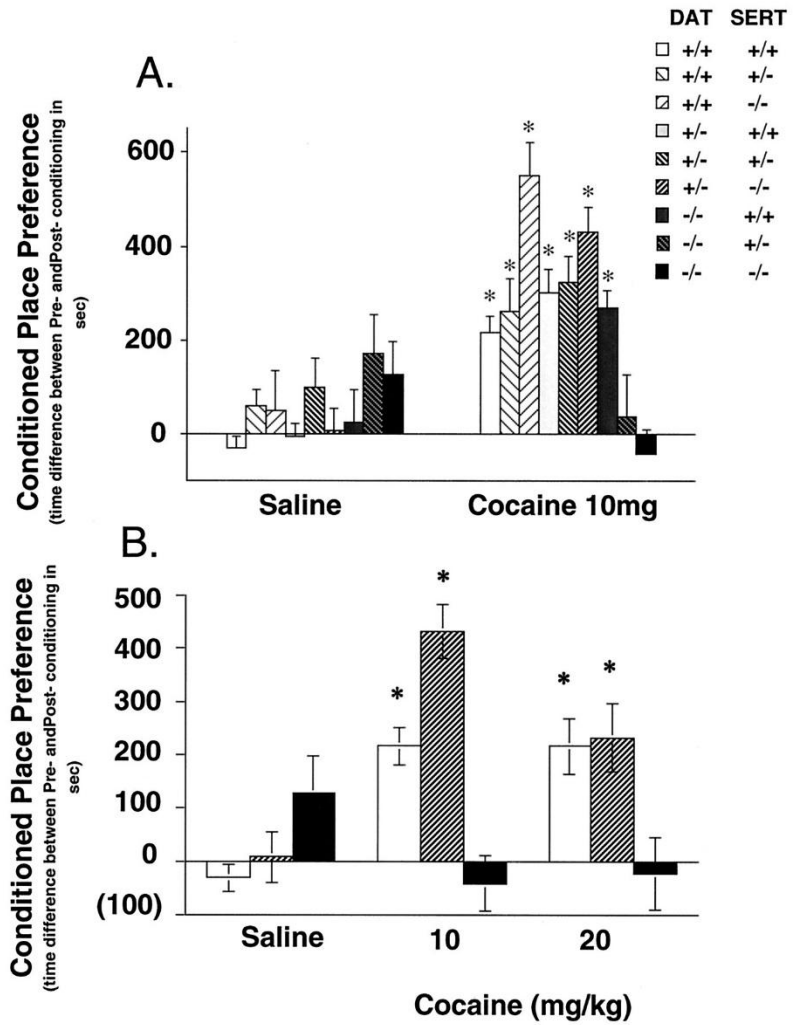
Sora I et al. PNAS 1998;95:7699-7704

Cocaine-conditioned place preferences in 5-HTT knockout mice



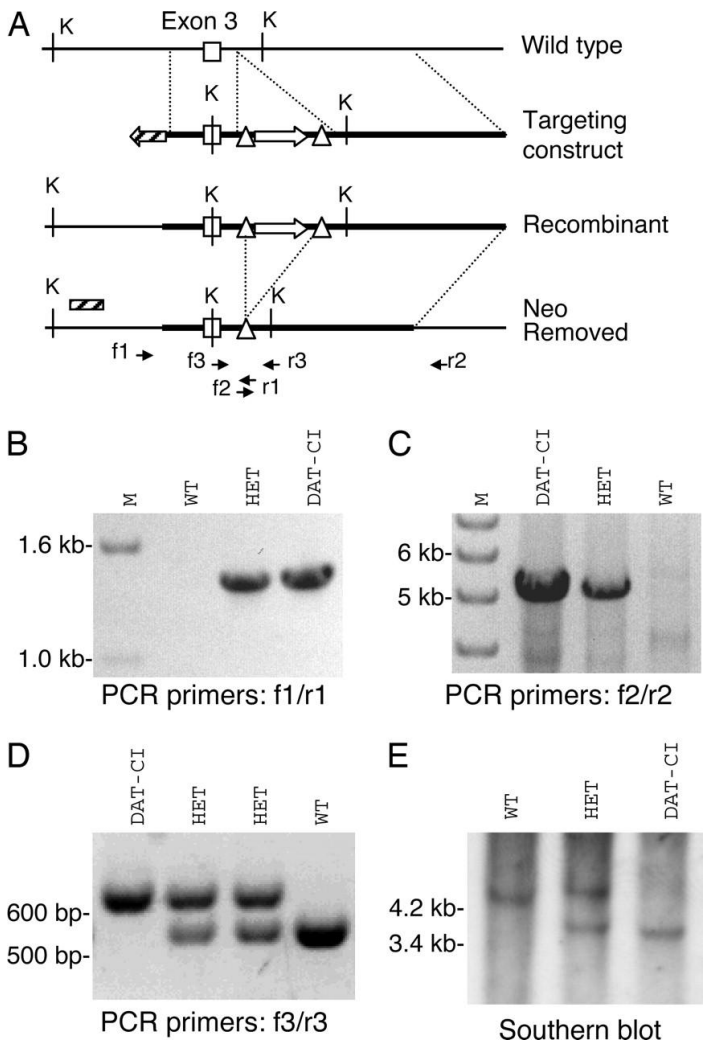
Sora I et al. PNAS 1998;95:7699-7704

Lack of cocaine-conditioned place preference in DAT knockout mice with no or one copy of the SERT gene



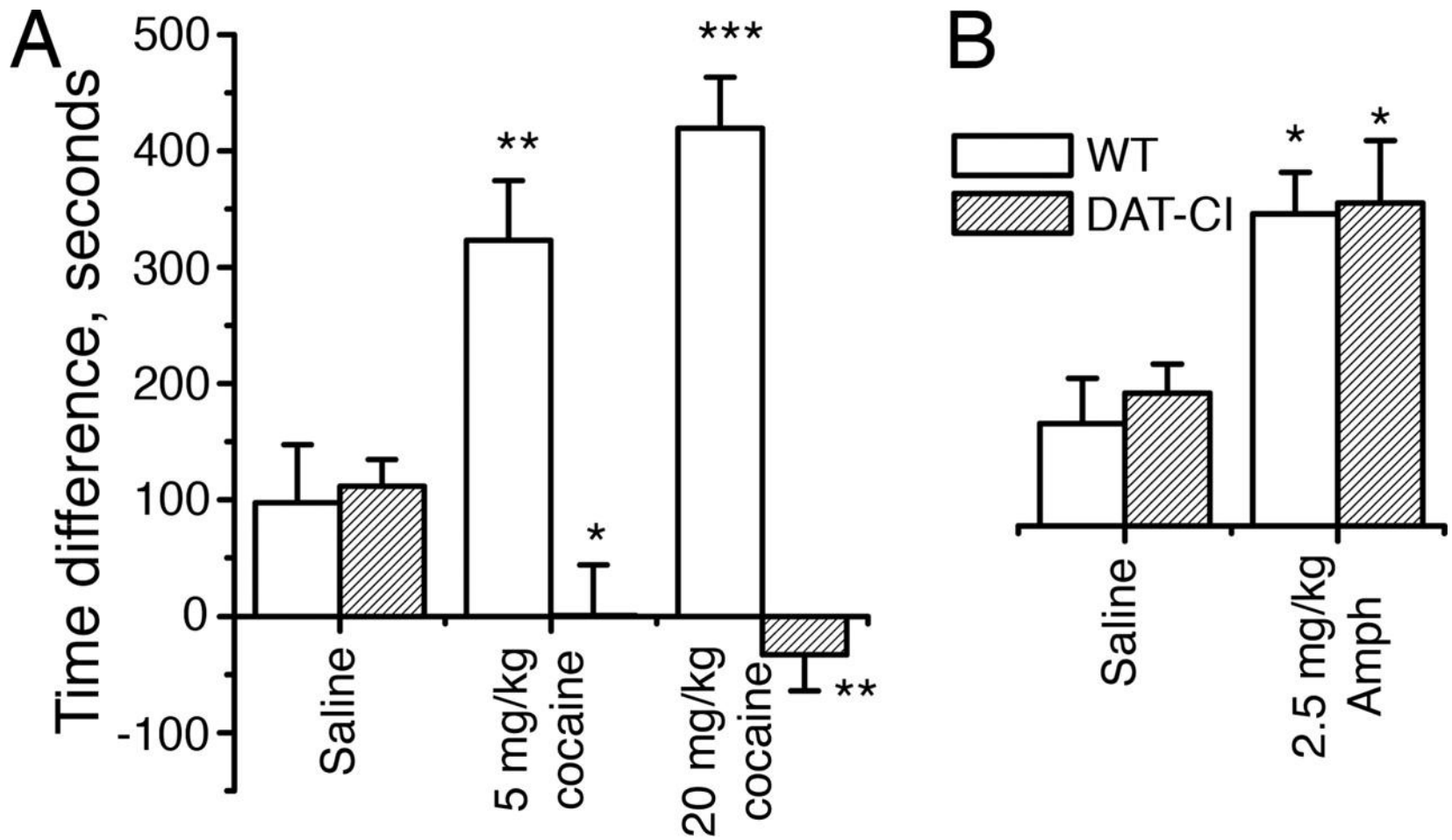
Sora I et al. PNAS 2001;98:5300-5305

Generation of DAT mutant knockin mice(L104V/F105C/A109V)



Chen R et al. PNAS 2006;103:9333-9338

Drug-induced CPP in WT and DAT-CI mice



Chen R et al. PNAS 2006;103:9333-9338

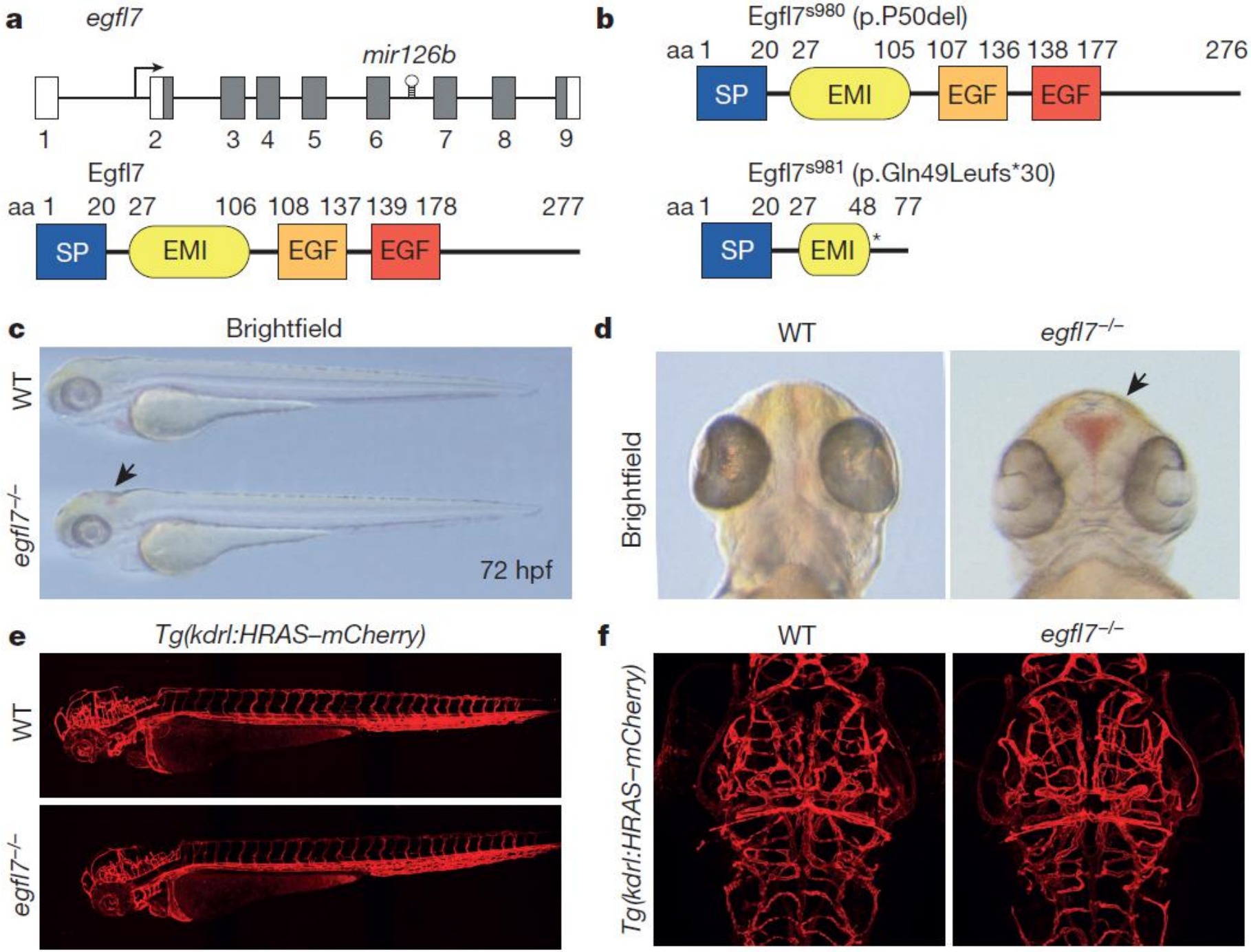
LETTER

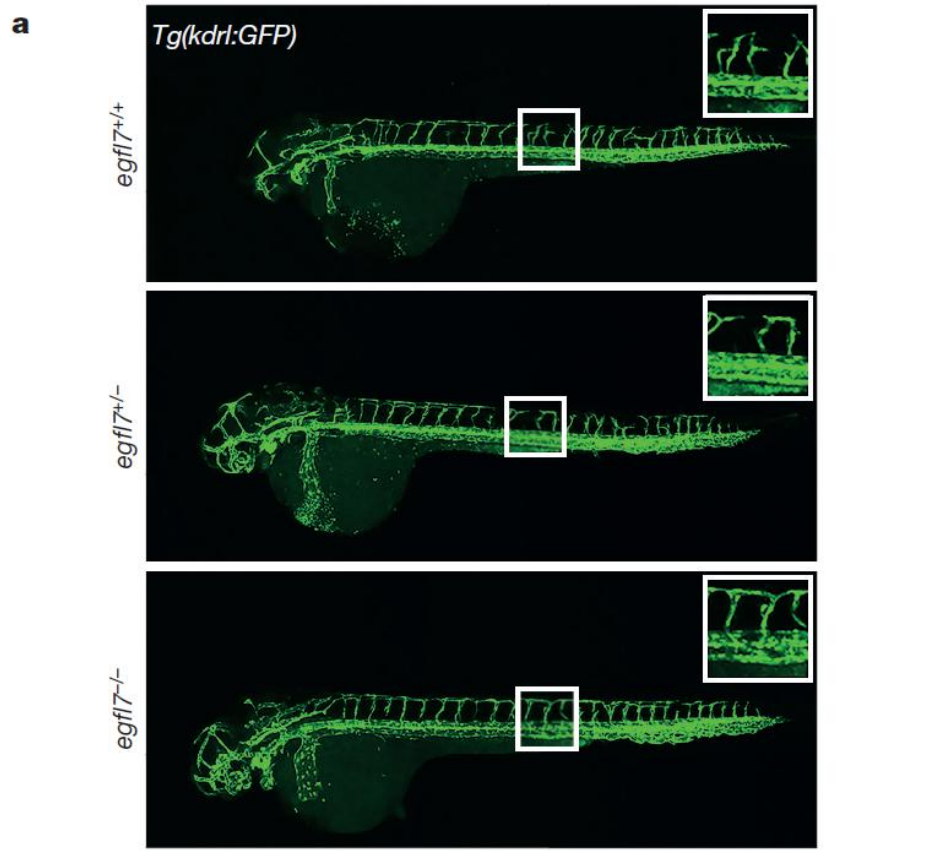
doi:10.1038/nature14580

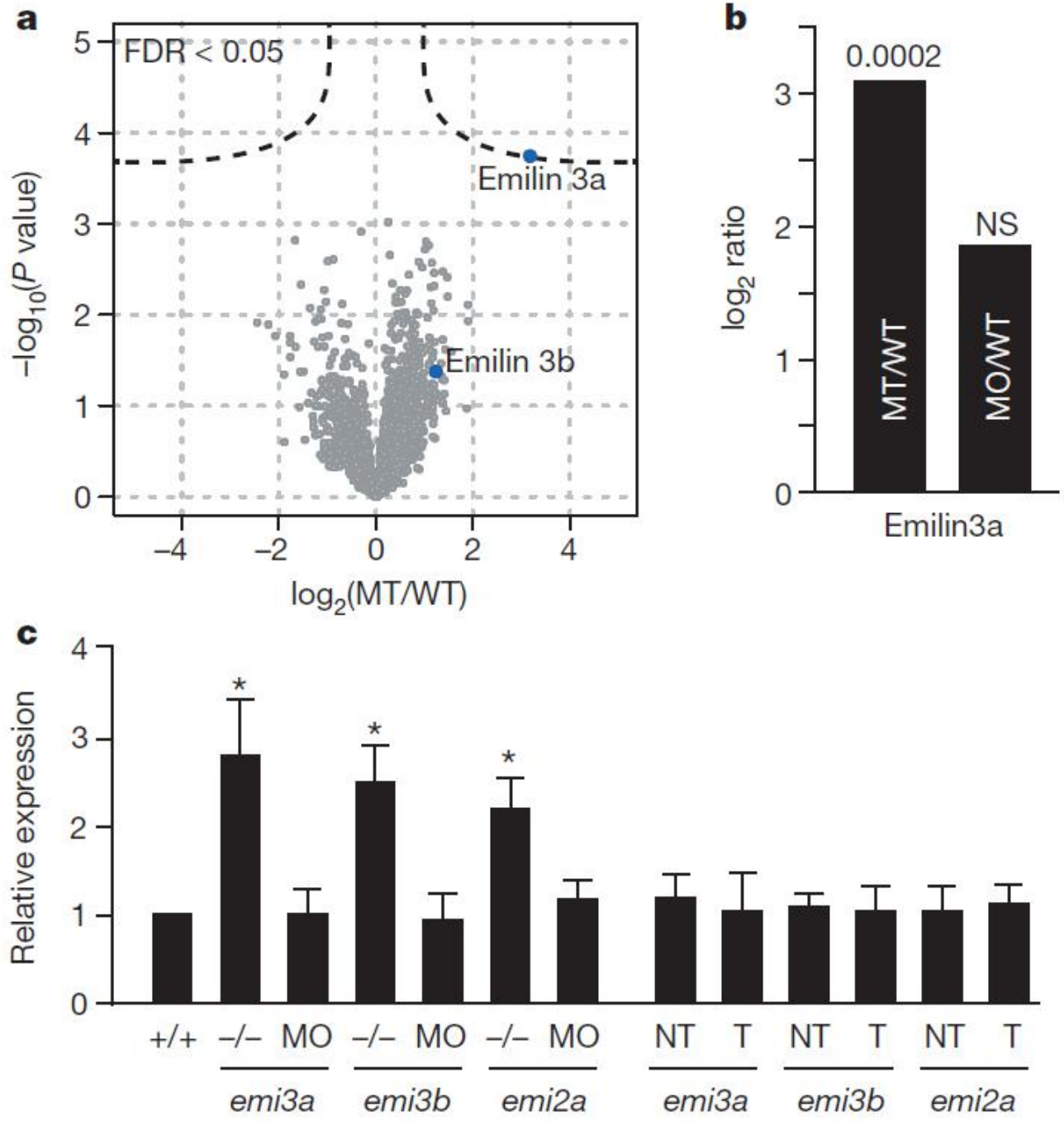
Genetic compensation induced by deleterious mutations but not gene knockdowns

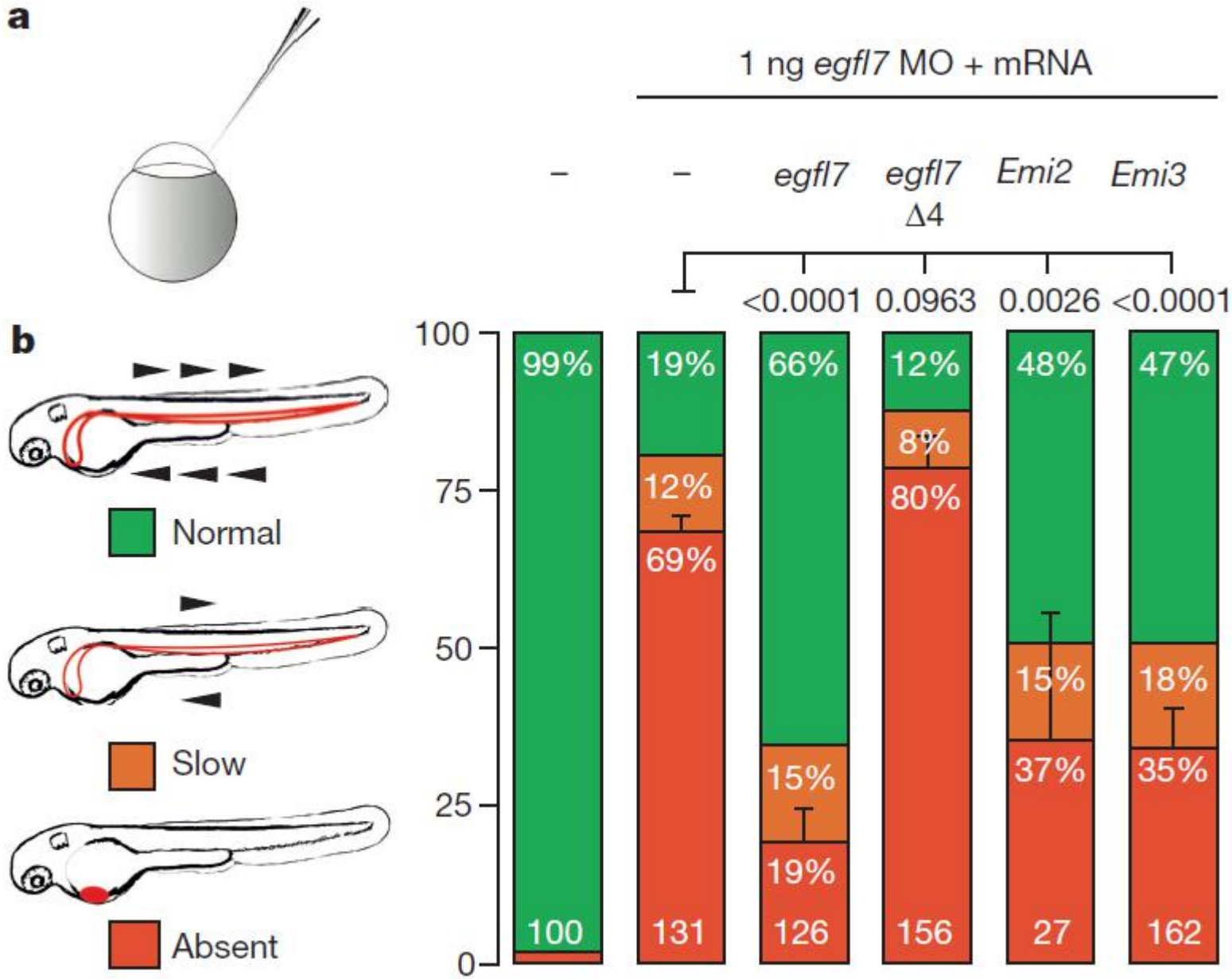
Andrea Rossi[1]*, Zacharias Kontarakis[1]*, Claudia Gerri[1], Hendrik Nolte[1]†, Soraya Hölper[1], Marcus Krüger[1]† & Didier Y. R. Stainier[1]

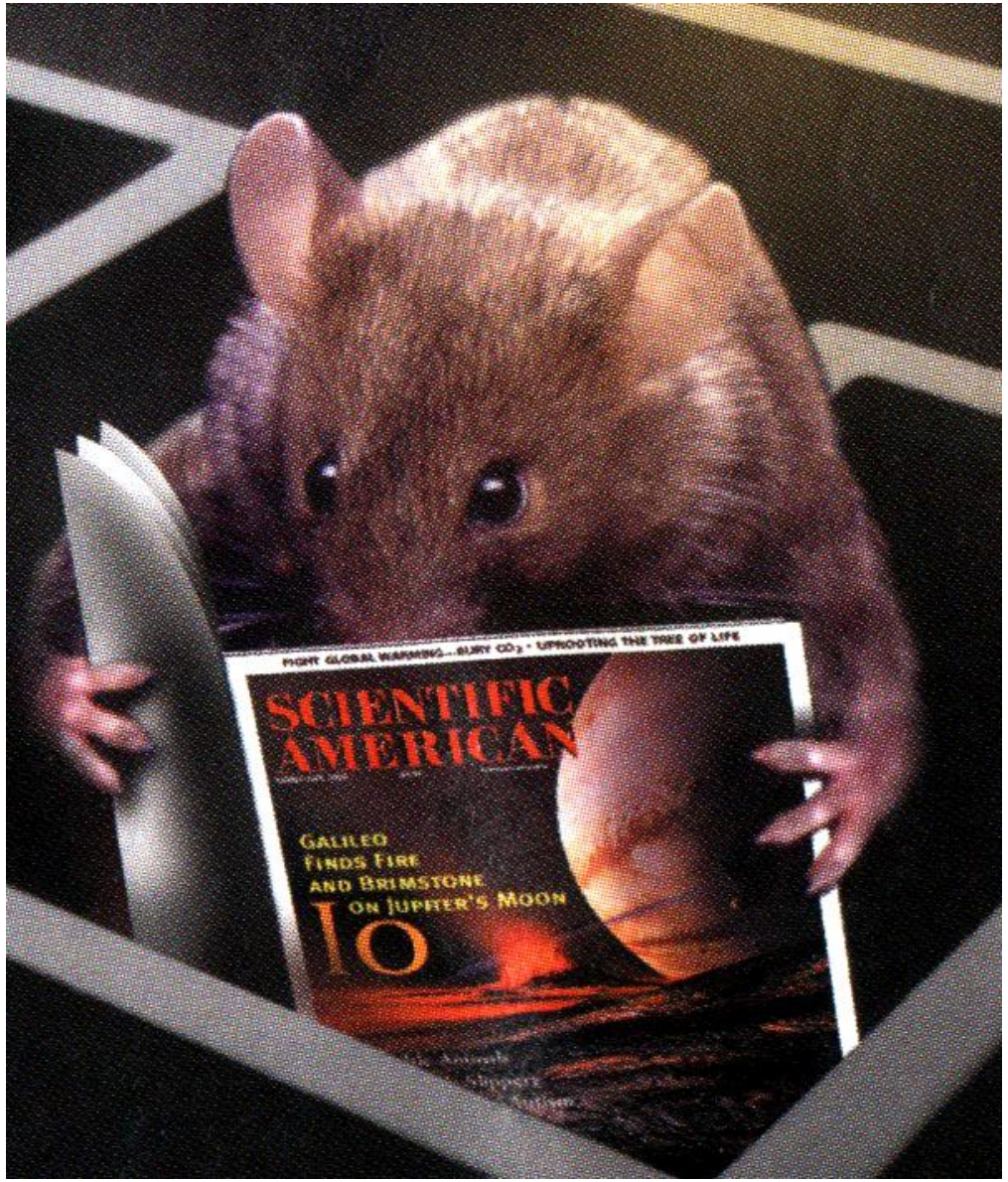
[1]Max Planck Institute for Heart and Lung Research, 61231 Bad Nauheim, Germany. †Present address: Institute for Genetics and CECAD, University of Cologne, 50931 Cologne, Germany.
*These authors contributed equally to this work.


a
egfl7
mir126b
1
2
3
4
5
6
7
8
9
Egfl7
aa 1
20
27
106
108
137
139
178
277
SP
EMI
EGF
EGF
b
Egfl7s980 (p.P50del)
aa 1
20
27
105
107
136
138
177
276
SP
EMI
EGF
EGF
Egfl7s981 (p.Gln49Leufs*30)
aa 1
20
27
48
77
SP
EMI
*
c
Brightfield
WT
egfl7−/−
72 hpf
d
WT
egfl7−/−
Brightfield
e
Tg(kdrl:HRAS–mCherry)
WT
egfl7−/−
f
WT
egfl7−/−
Tg(kdrl:HRAS–mCherry)


a
Tg(kdrl:GFP)
egfl7+/+
egfl7+/–
egfl7–/–
b
egfl7+/+
egfl7+/–
egfl7–/–
3
12
17
Derivative fluorescence
0.12
0.10
0.08
0.06
0.04
0.02
0.00
72
74
76
78
80
Temperature (°C)
c
0.0008
0.0041
0.0087
0.9105
100
75
50
25
0
Percentage
9%
38%
53%
13%
37%
50%
9%
44%
47%
25%
47%
28%
Exp 1
Exp 2
Exp 3
Ctrl


a
FDR < 0.05
Emilin 3a
Emilin 3b
−log10(P value)
log2(MT/WT)
b
0.0002
NS
MT/WT
MO/WT
log2 ratio
Emilin3a
c
Relative expression
+/+
−/− MO
−/− MO
−/− MO
NT T
NT T
NT T
emi3a
emi3b
emi2a
emi3a
emi3b
emi2a


a
1 ng egfl7 MO + mRNA
–
–
egfl7
egfl7 Δ4
Emi2
Emi3
<0.0001
0.0963
0.0026
<0.0001
b
Normal
Slow
Absent
100
75
50
25
0
99%
100
19%
12%
69%
131
66%
15%
19%
126
12%
8%
80%
156
48%
15%
37%
27
47%
18%
35%
162


SCIENTIFIC AMERICAN
GALILEO FINDS FIRE AND BRIMSTONE ON JUPITER'S MOON
IO

谢谢！