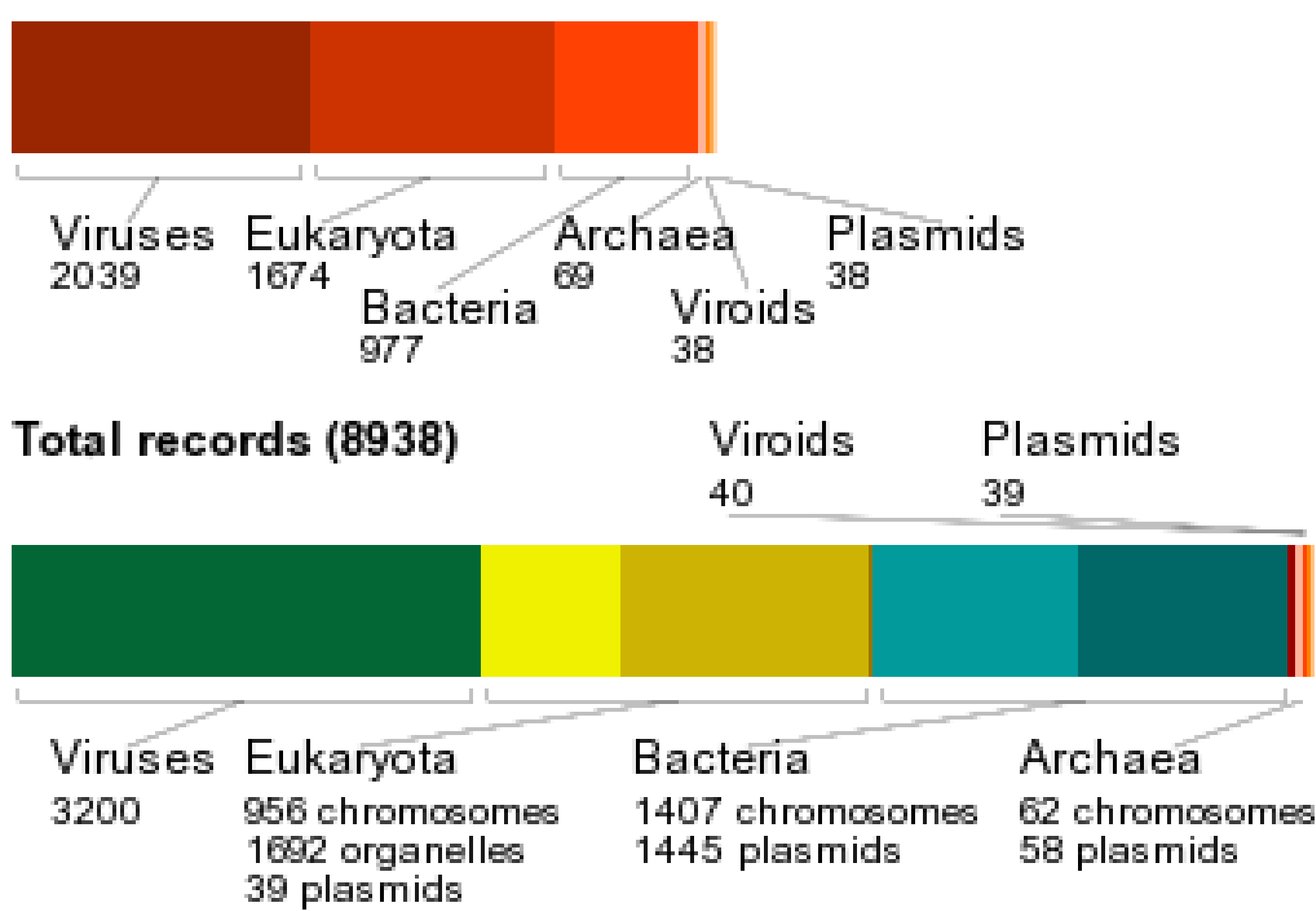
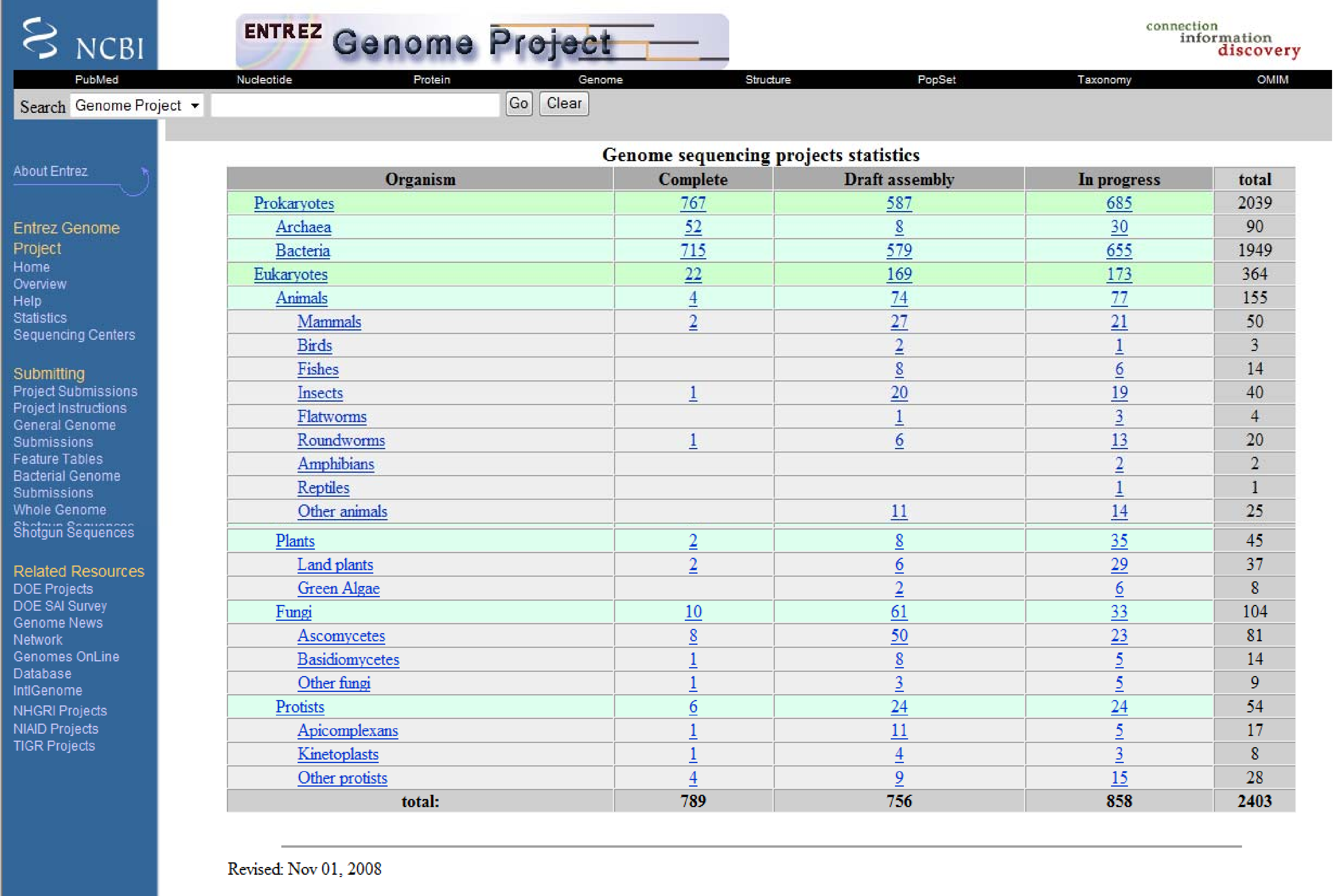
# Genómica Funcional

LABORATORIO DE BIOINFORMATICA Y EXPRESION GENICA-INTA

# Proyectos genoma





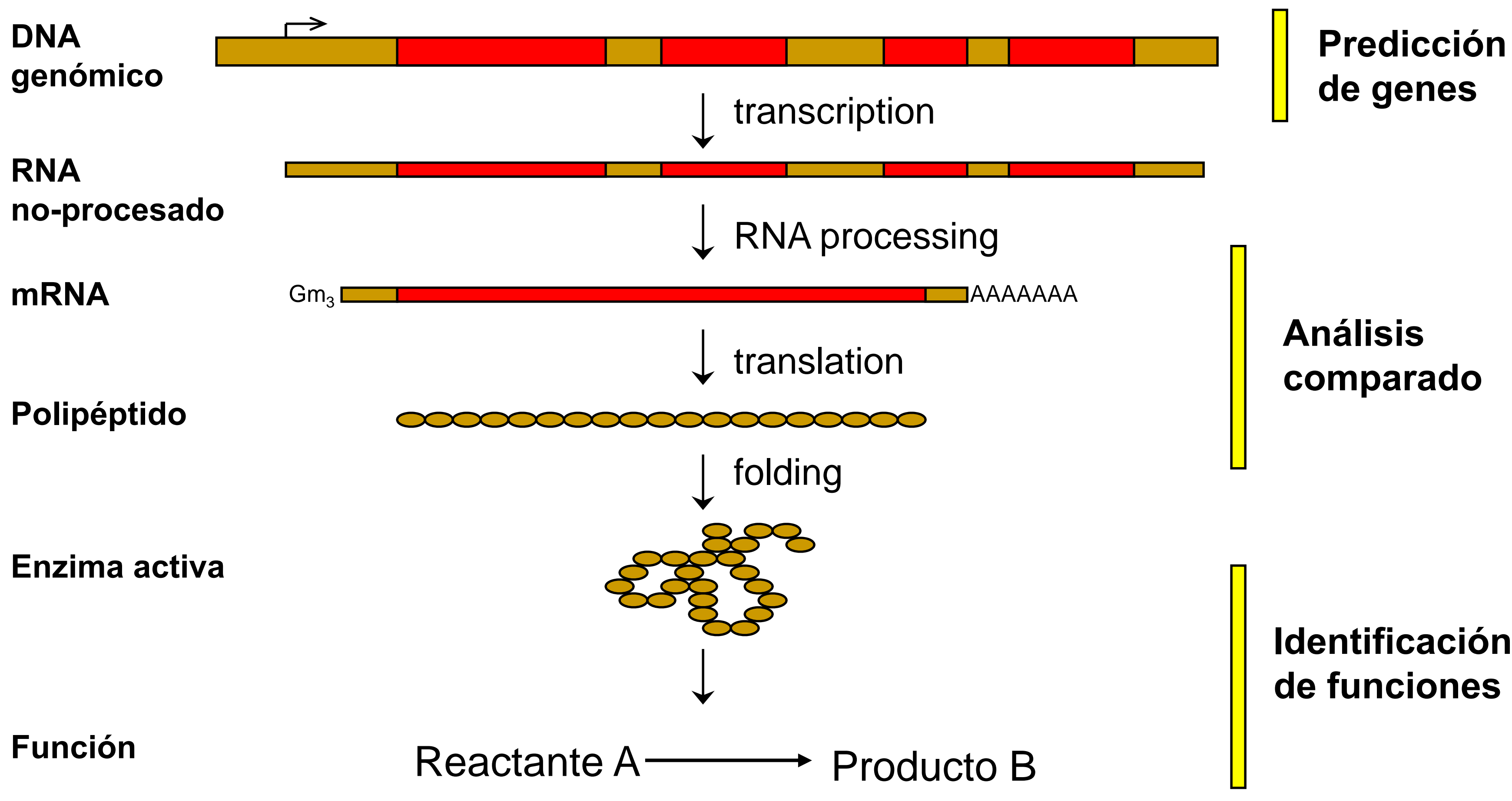

**Genome sequencing projects statistics**

| Organism | Complete | Draft assembly | In progress | total |
|---|---|---|---|---|
| Prokaryotes | 767 | 587 | 685 | 2039 |
| Archaea | 52 | 8 | 30 | 90 |
| Bacteria | 715 | 579 | 655 | 1949 |
| Eukaryotes | 22 | 169 | 173 | 364 |
| Animals | 4 | 74 | 77 | 155 |
| Mammals | 2 | 27 | 21 | 50 |
| Birds | | 2 | 1 | 3 |
| Fishes | | 8 | 6 | 14 |
| Insects | 1 | 20 | 19 | 40 |
| Flatworms | | 1 | 3 | 4 |
| Roundworms | 1 | 6 | 13 | 20 |
| Amphibians | | | 2 | 2 |
| Reptiles | | | 1 | 1 |
| Other animals | | 11 | 14 | 25 |
| Plants | 2 | 8 | 35 | 45 |
| Land plants | 2 | 6 | 29 | 37 |
| Green Algae | | 2 | 6 | 8 |
| Fungi | 10 | 61 | 33 | 104 |
| Ascomycetes | 8 | 50 | 23 | 81 |
| Basidiomycetes | 1 | 8 | 5 | 14 |
| Other fungi | 1 | 3 | 5 | 9 |
| Protists | 6 | 24 | 24 | 54 |
| Apicomplexans | 1 | 11 | 5 | 17 |
| Kinetoplasts | 1 | 4 | 3 | 8 |
| Other protists | 4 | 9 | 15 | 28 |
| total: | 789 | 756 | 858 | 2403 |

# Proyectos genoma

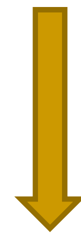
| Organism | Genome Size (bases) | Estimated Genes |
|---|---|---|
| Human (*Homo sapiens*) | 3 billion | 30,000 |
| Laboratory mouse (*M. musculus*) | 2.6 billion | 30,000 |
| Mustard weed (*A. thaliana*) | 100 million | 25,000 |
| Roundworm (*C. elegans*) | 97 million | 19,000 |
| Fruit fly (*D. melanogaster*) | 137 million | 13,000 |
| Yeast (*S. cerevisiae*) | 12.1 million | 6,000 |
| Bacterium (*E. coli*) | 4.6 million | 3,200 |
| Human immunodeficiency virus (HIV) | 9700 | 9 |

# Anotación de genomas eucariontes



| Organismo | # genes | % genes con función inferida | Año de termino de la secuencia genómica |
|---|---|---|---|
| *E. coli* | 4.288 | 60 | 1997 |
| yeast | 6.600 | 40 | 1996 |
| *C. elegans* | 19.000 | 40 | 1998 |
| *Drosophila* | 13.000 | 25 | 1999 |
| *Arabidopsis* | 25.000 | 40 | 2000 |
| mouse | 30.000 | 10-20 | 2002 |
| human | 30.000 | 10-20 | 2000 |

**Muchos genes, pocas funciones**



**Genómica funcional**

# Genómica funcional

- Desarrollo y aplicación de aproximaciones experimentales para examinar la función de genes utilizando la información proporcionada por la genómica estructural.

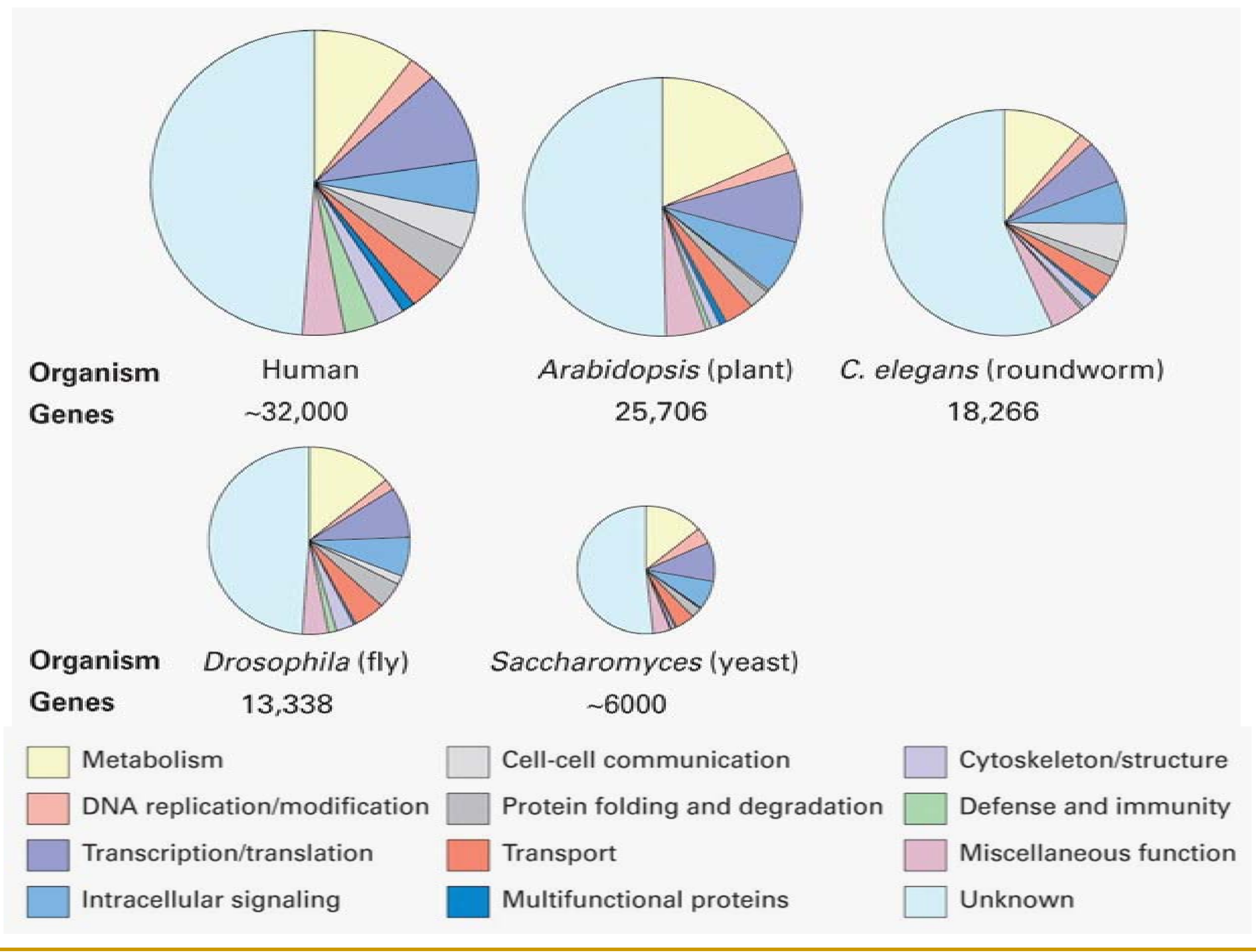
- Investigaciones a gran escala de la función de genes.

- Por ejemplo la forma en la que una célula responde a una señal ambiental puede ser monitoreada analizando simultáneamente el patrón de expresión de todos sus genes.

- **Transcriptoma:** Colección de todos transcritos (RNAs) de una célula.

- **Proteoma:** colección de todos los polypeptidos/proteínas expresados en una célula.

# ¿Por qué necesitamos genómica funcional?



# Métodos de la genómica funcional

**Expresión diferencial de genes**

- **Serial analysis of gene expression (SAGE)**
- **Micro/Macroarrays**

**Función de genes**

- **RNA interference (RNAi)**

# Análisis del transcriptoma

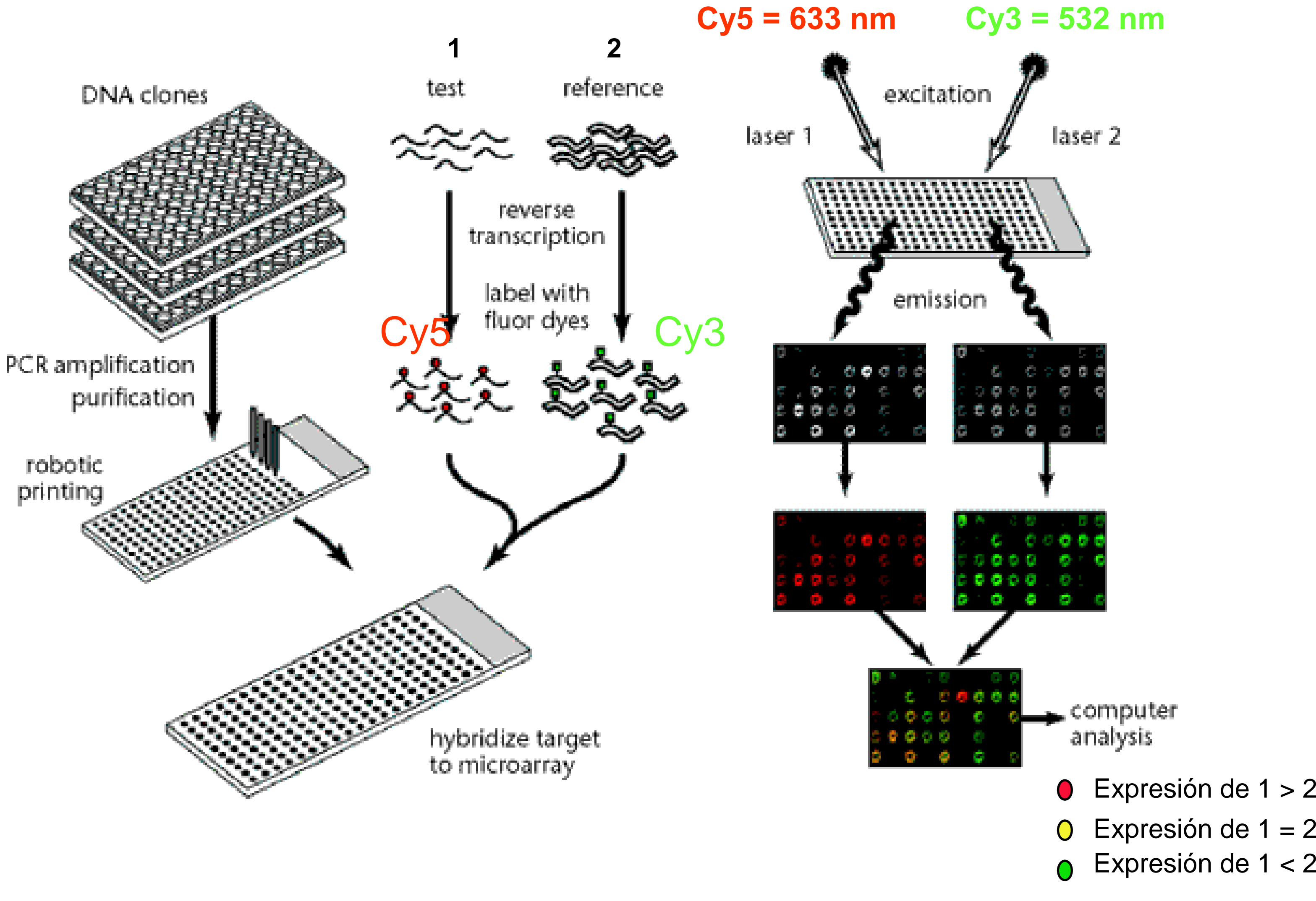
- **Sistema cerrado:**

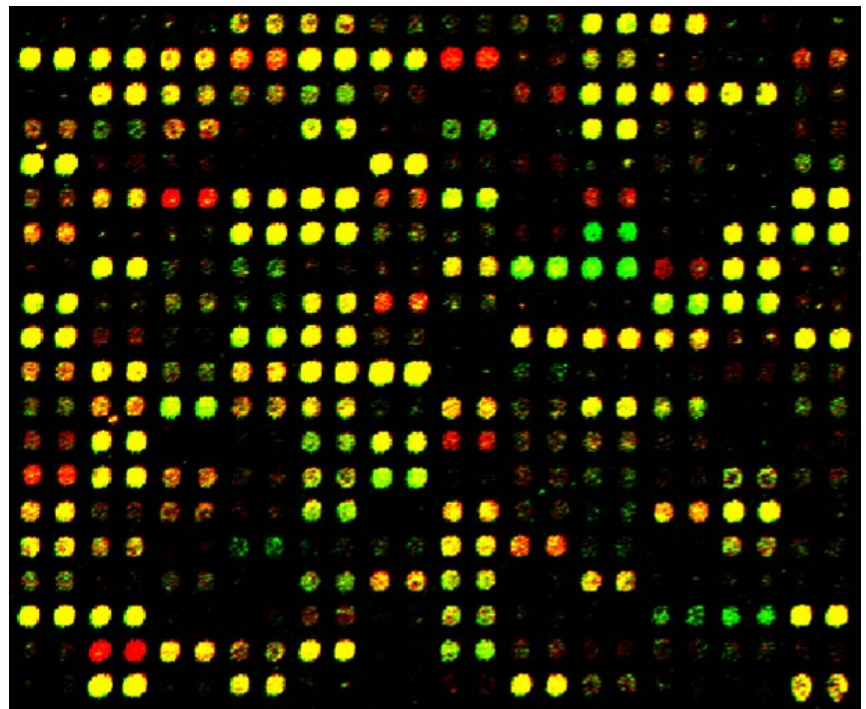
  **Hibridación en Microarray**

- **Sistema abierto:**

  **Serial analysis of gene expression (SAGE)**

# Hibridación en microarrays





Amarillo: Experimental = Control

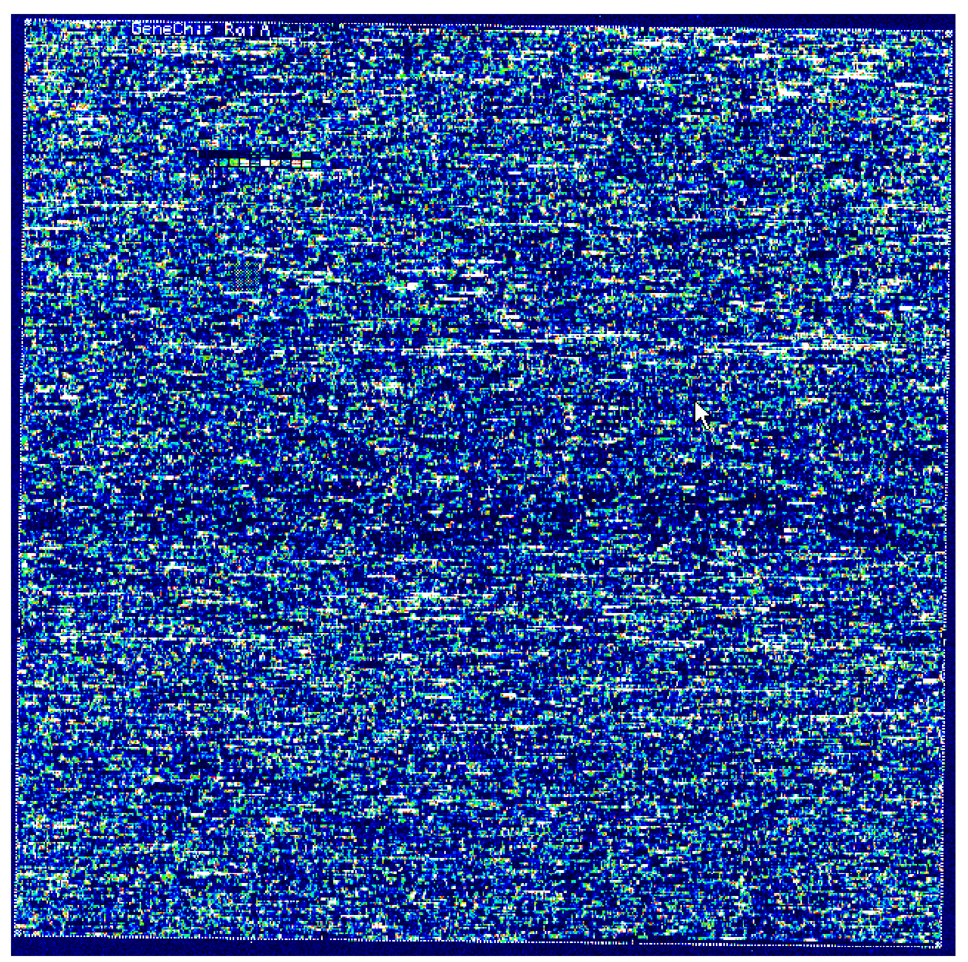
Rojo: Experimental > Control

Verde: Experimental > Control

Límite de detección: 1/30.000 transcritos

Abundancia: ~ 20 transcritos/célula

# Affymetrix GeneChip®



Límite: 1/100,000 transcritos

Abundancia: ~ 5 transcripts/cell

- **Superabundant**
  - **15-90% of mRNA mass**
  - **<10 structural gene transcripts**
  - **>5000 molecules per cell per sequence**
- **Abundant**
  - **50-75% of mRNA mass**
  - **~200-1000 structural gene transcripts (5% of diversity)**
  - **500-2500 molecules per cell per sequence**
- **Rare/complex**
  - **<25% of mRNA mass; individual seqs <0.01%**
  - **<u>95% of mRNA diversity</u>**
  - **1-10 molecules per cell per sequence**

# Affymetrix

| Gene Expression Arrays | Transcripts/Genes |
|---|---|
| *Arabidopsis* Genome | 24,000 |
| *C. elegans* Genome | 22,500 |
| *Drosophila* Genome | 18, 500 |
| *E. coli* Genome | 20, 366 |
| Human Genome U133 Plus | 47,000 |
| Mouse Genome | 39, 000 |
| Yeast Genome | 5, 841 (*S. cerevisiae*) & 5, 031 (*S. pombe*) |
| Rat Genome | 30, 000 |
| Zebrafish | 14, 900 |
| *Plasmodium/Anopheles* | 4,300 (*P. falciparum*) & 14,900 (*A. gambiae*) |

Barley (25,500), Soybean (37,500 + 23,300 pathogen), Grape (15,700)

Canine (21,700), Bovine (23,000)

*B.subtilis* (5,000), *S. aureus* (3,300 ORFS), *Xenopus* (14, 400)

# Microarray y GeneChip

**Ventajas**

- **Rápido**
- **Metodología y análisis de datos bien descritos**
- **Robusto**
- **Conveniente en estudios dirigidos y enfocados**

**Desventajas**

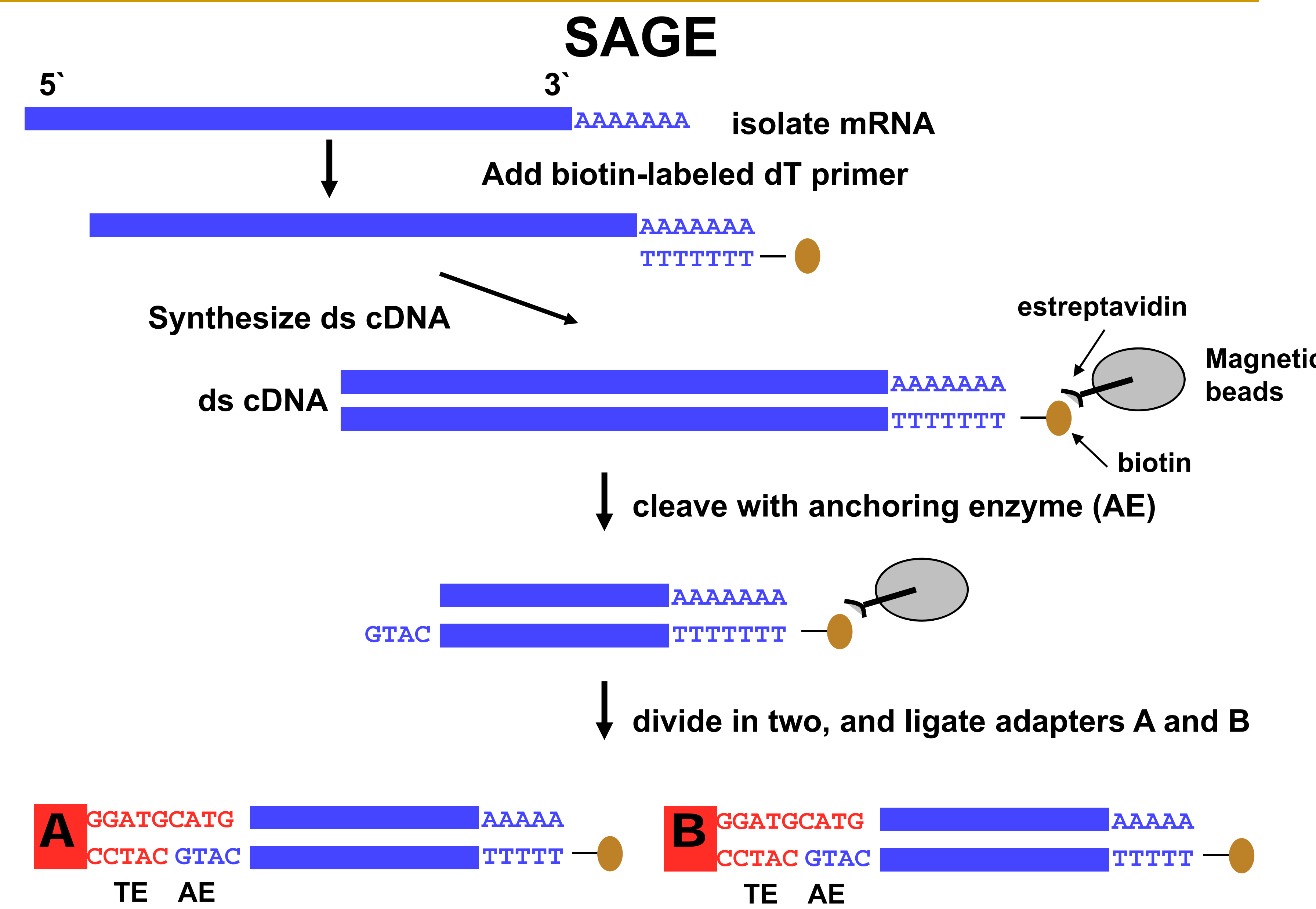
- **Sistema cerrado, sólo revelan la expresión de los genes sembrados en el array.**
- **Dificultad para correlacionar con el numero absoluto de transcritos**
- **Sensible a ambigüedades por procesamiento alternativo de transcritos**

# Serial Analysis of Gene Expression (SAGE)

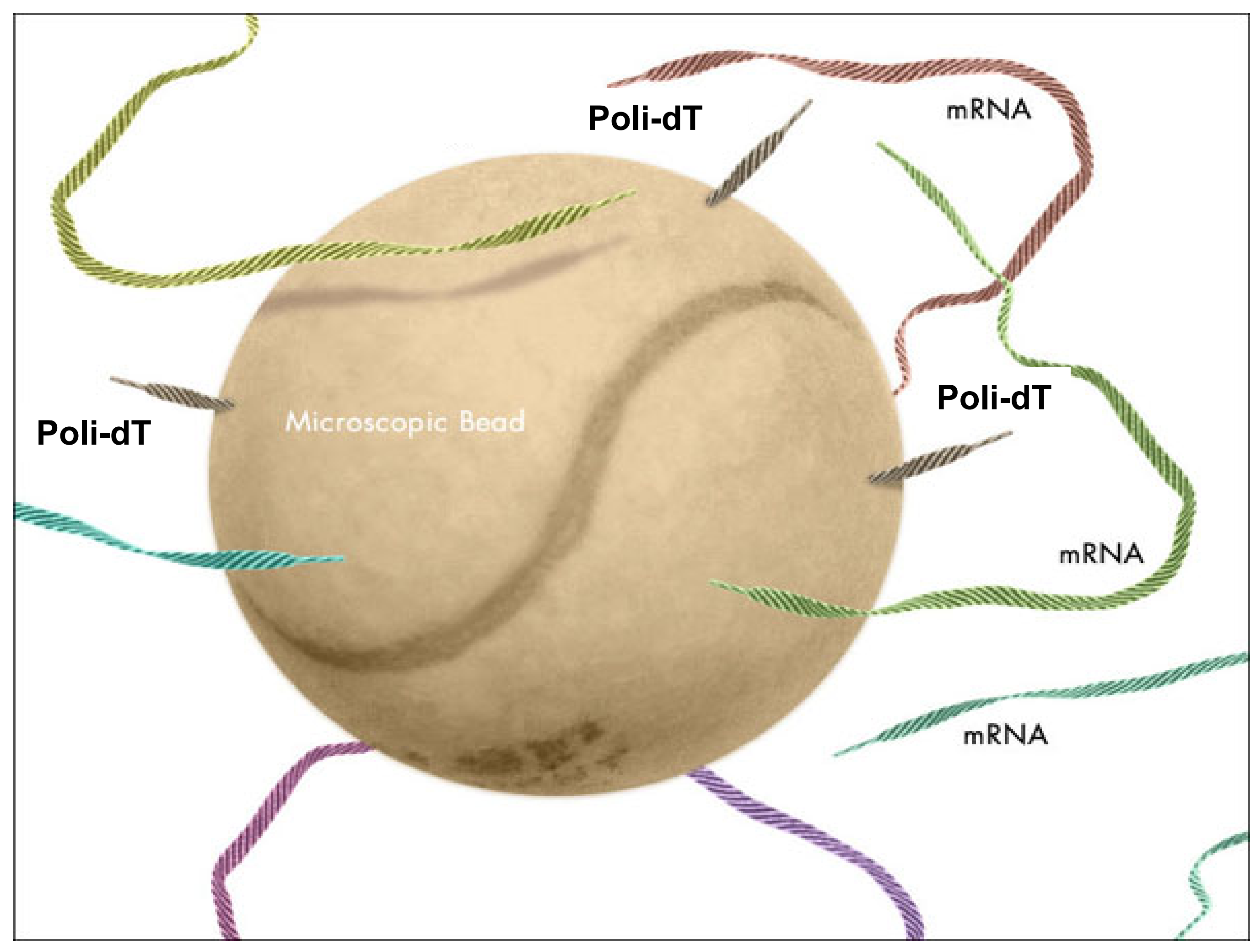
- **Principio: Una secuencia de nucleótidos corta (*tag*) de 9 o 10 pares de bases contiene suficiente información para identificar un transcrito.**
- **Sistema abierto. Puede revelar sin sesgo los niveles de expresión de cientos de miles de genes.**
- **Método para cuantificar niveles de expresión génica en muestras de células.**

Velculescu et al., Science 1995; 270:484-487

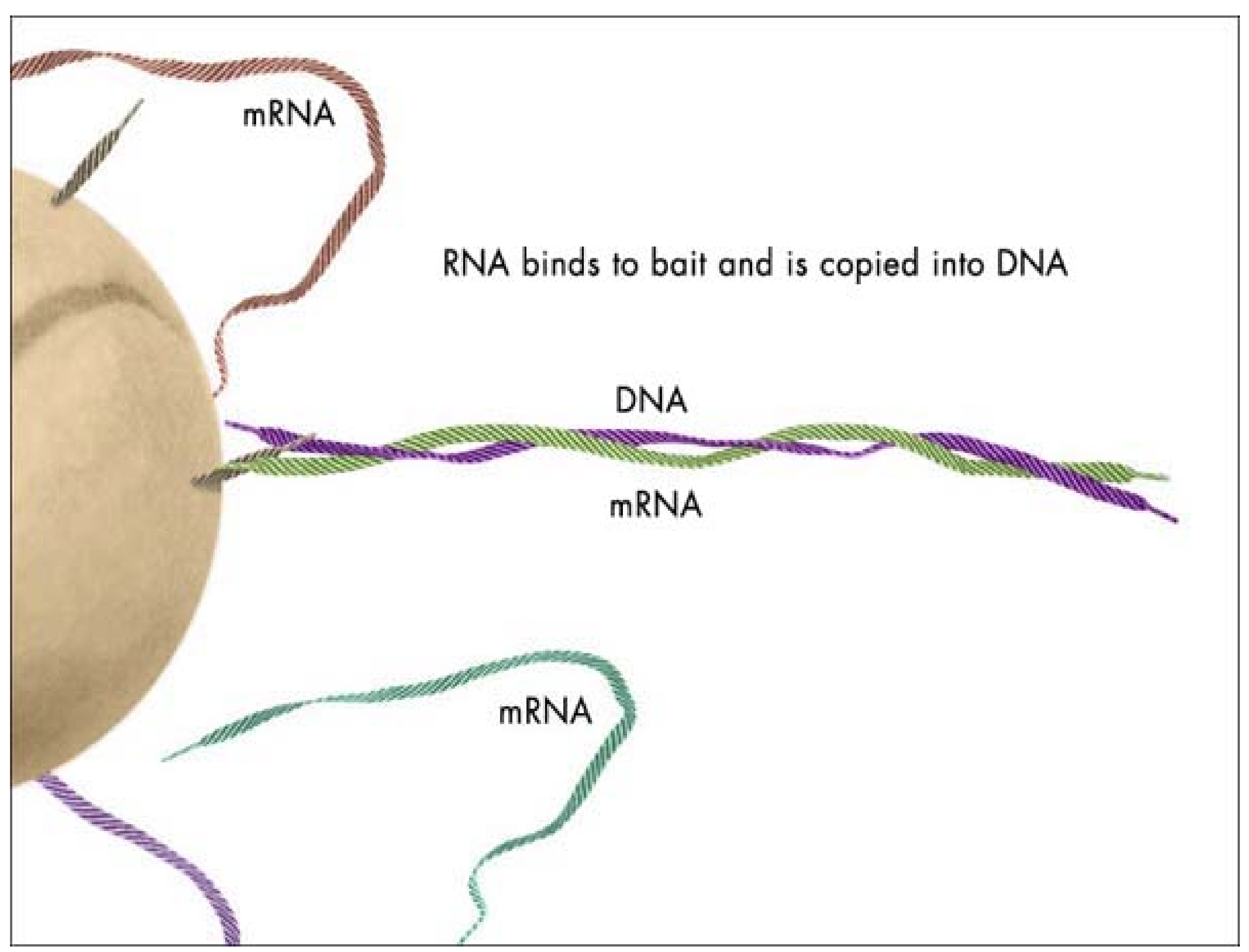
# SAGE



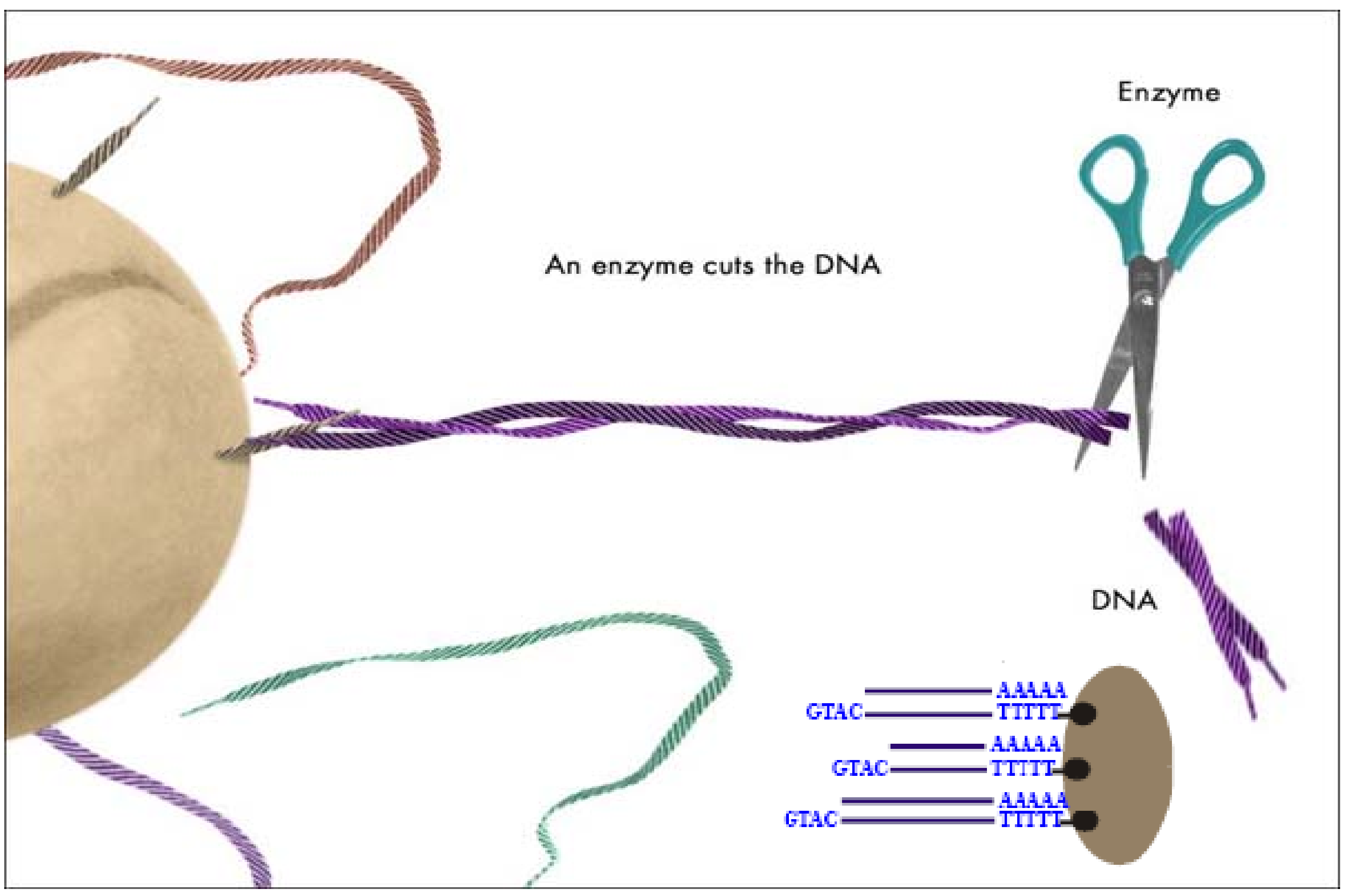
# SAGE



# SAGE

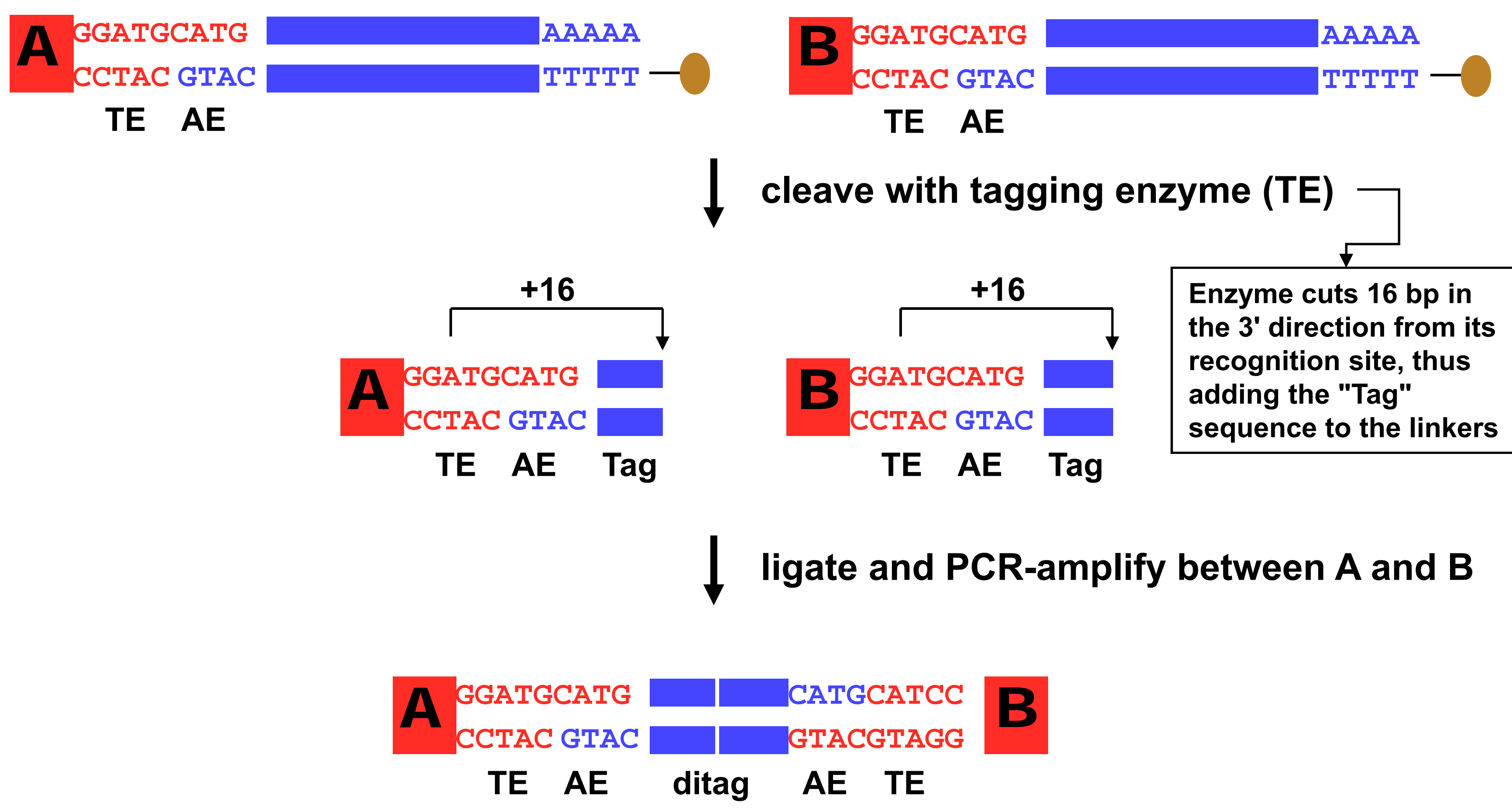


# SAGE

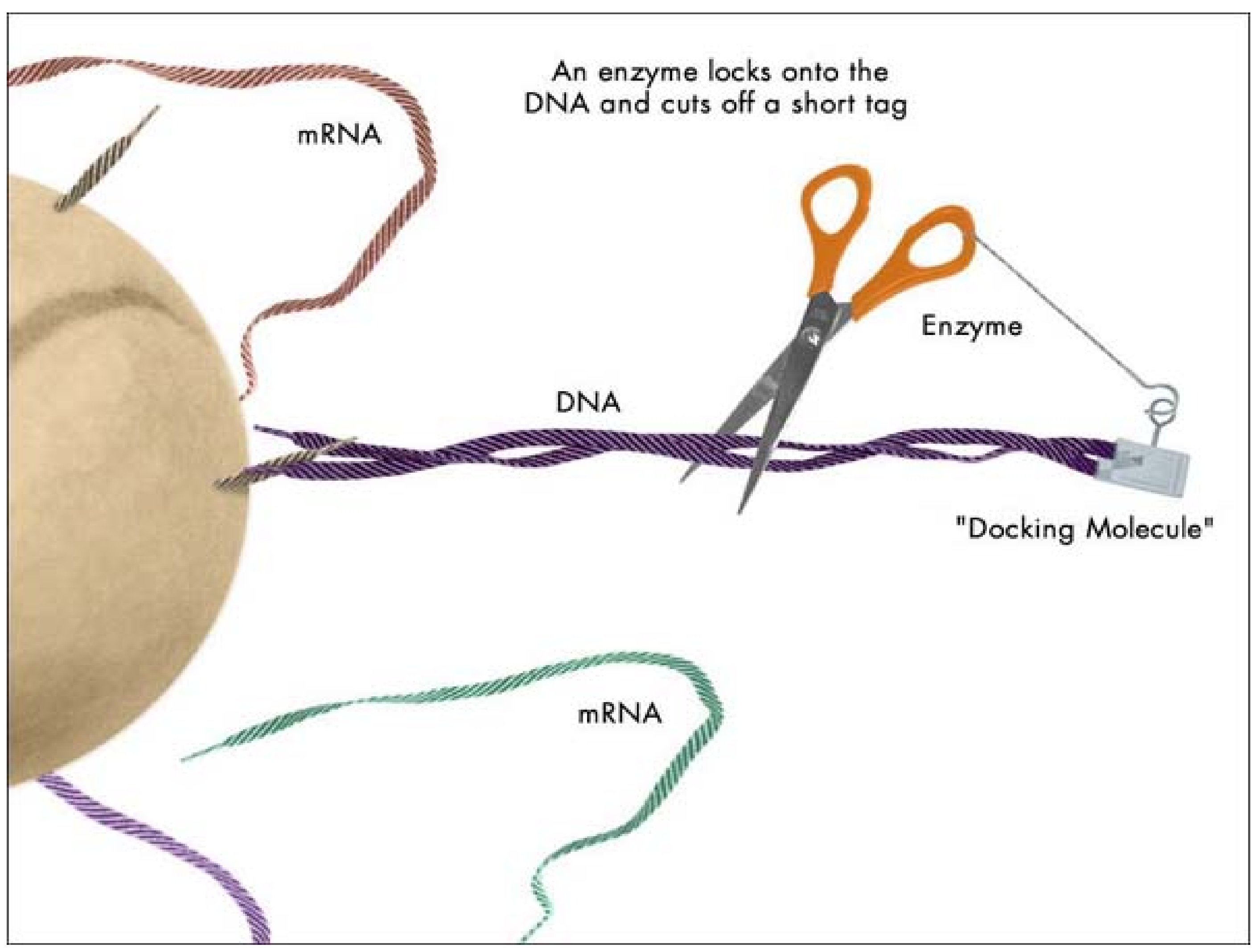



The biotinylated 3’ cDNA are affinity purified using strepatavidin coated magnetic beads.

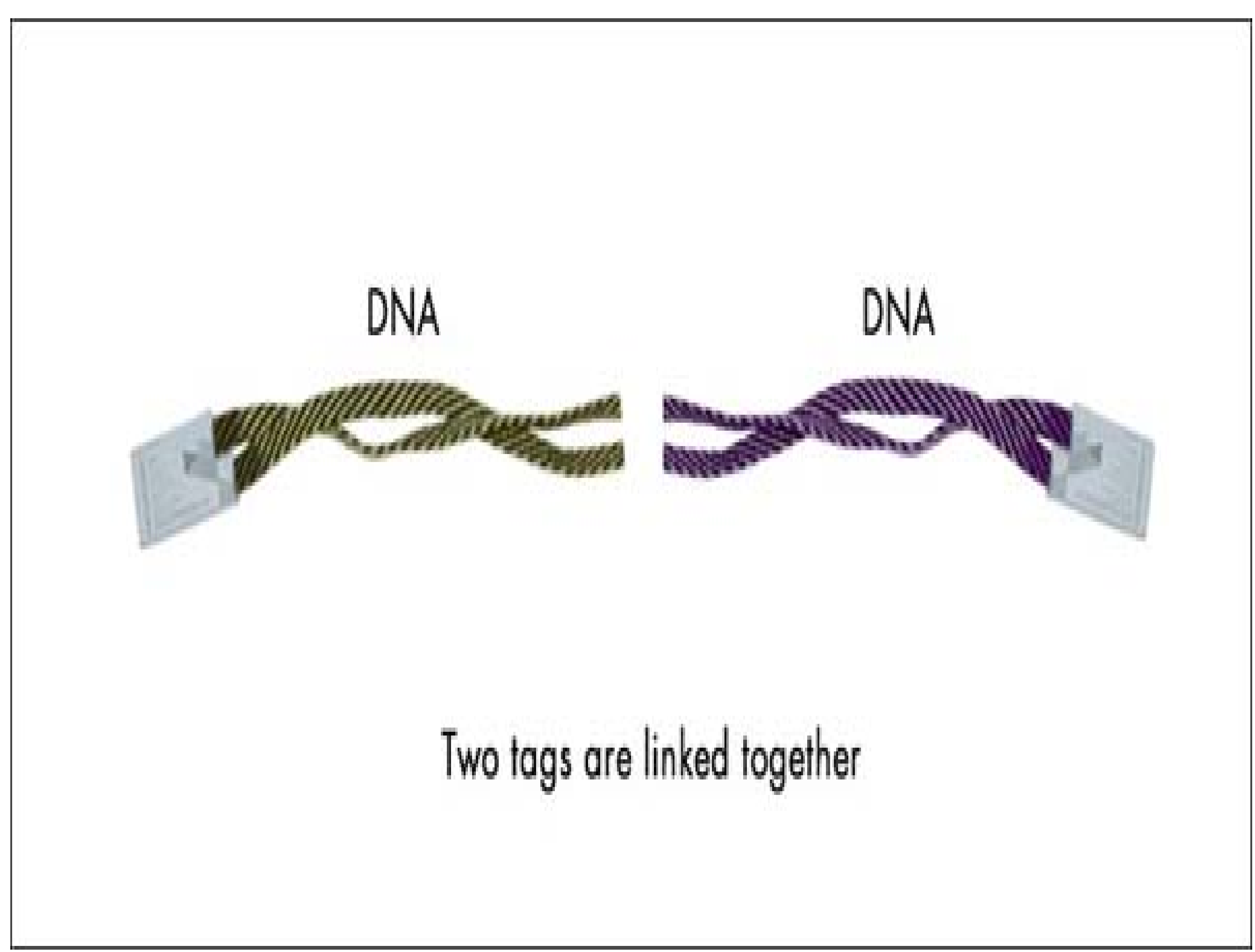
# SAGE



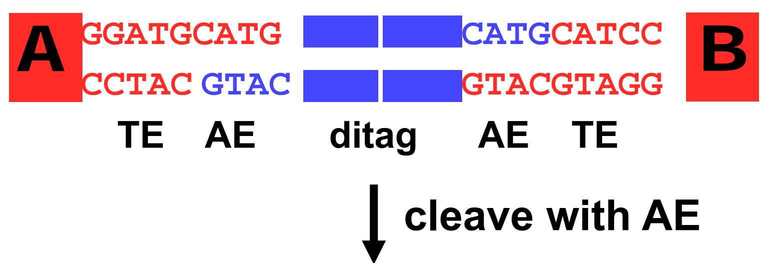
# SAGE



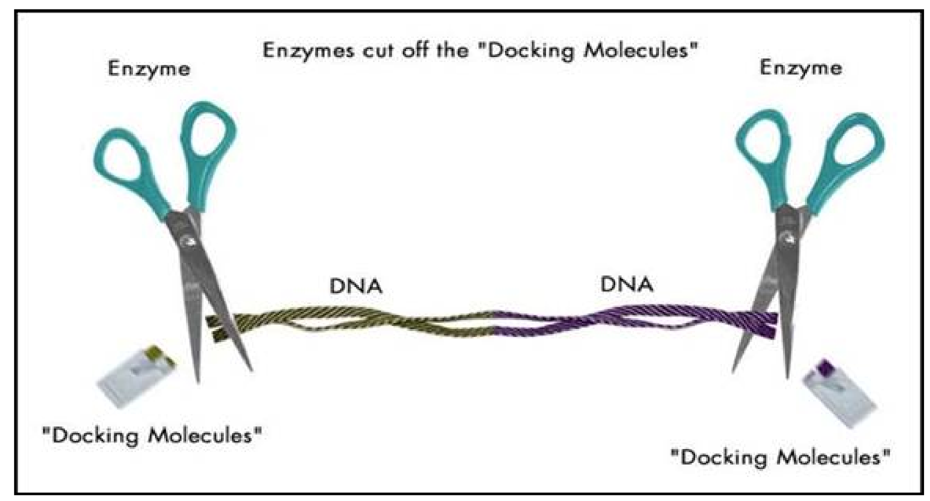
# SAGE



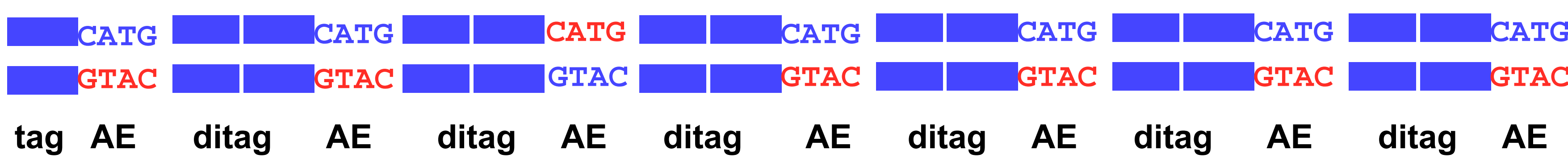
# SAGE



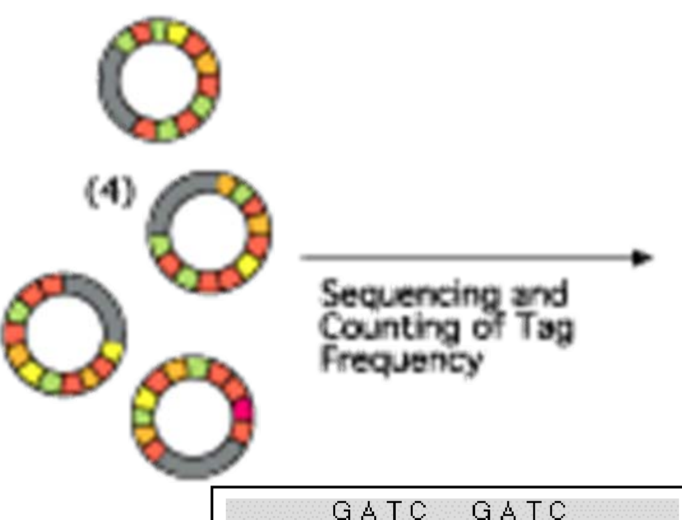

cleave with AE

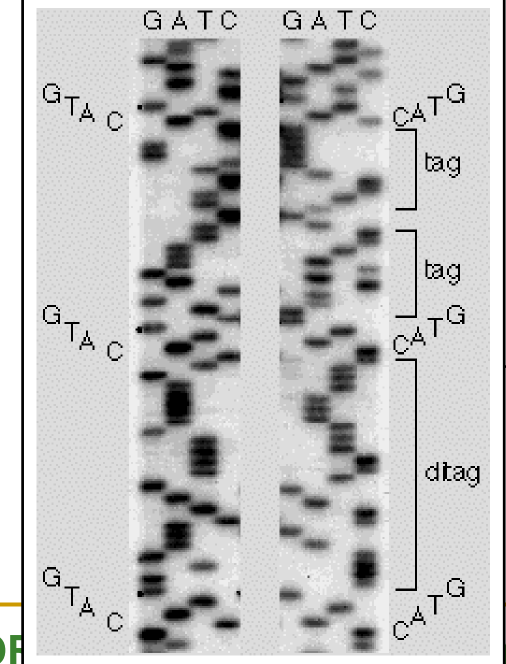



concatenate, clone, sequence, BLAST



# SAGE






**Análisis de datos:**

**- Frecuencia de cada tag**

**- Alineamiento con secuencias en bases de datos**

- Localizar la puntuación “CATG”
- Extraer ditags de 20-26 pb entre los sitios CATG
- Descartar ditags duplicados – probablemente artefactos del PCR
- Contar las veces que aparece cada tag

| TAG | COUNT | | TAG | COUNT | | TAG |
|---|---|---|---|---|---|---|
| CCCATCGTCC | 1286 | | CACTACTCAC | 245 | | TTCACTGTGA |
| CCTCCAGCTA | 715 | | ACTAACACCC | 229 | | ACGCAGGGAG |
| CTAAGACTTC | 559 | | AGCCCTACAA | 222 | | TGCTCCTACC |
| GCCCAGGTCA | 519 | | ACTTTTTCAA | 217 | | CAAACCATCC |
| CACCTAATTG | 469 | | GCCGGGTGGG | 207 | | CCCCCTGGAT |
| CCTGTAATCC | 448 | | GACATCAAGT | 198 | | ATTGGAGTGC |
| TTCATACACC | 400 | | ATCGTGGCGG | 193 | | GCAGGGCCTC |
| ACATTGGGTG | 377 | | GACCCAAGAT | 190 | | CCGCTGCACT |
| GTGAAACCCC | 359 | | GTGAAACCCT | 188 | | GGAAAACAGA |
| CCACTGCACT | 359 | | CTGGCCCTCG | 186 | | TCACCGGTCA |
| TGATTTCACT | 358 | | GCTTTATTTG | 185 | | GTGCACTGAG |
| ACCCTTGGCC | 344 | | CTAGCCTCAC | 172 | | CCTCAGGATA |
| ATTTGAGAAG | 320 | | GCGAAACCCT | 167 | | CTCATAAGGA |
| GTGACCACGG | 294 | | AAAACATTCT | 161 | | ATCATGGGGA |

- Cada tag debe ser alineado con la secuencia del gen que produjo el mRNA, utilizando las secuencias depositadas en bases de datos (UniGene) y algoritmos de alineamiento.
- Esta es la etapa mas larga, dependiendo de la cantidad de mRNAs que se estén analizando puede durar semanas o meses.

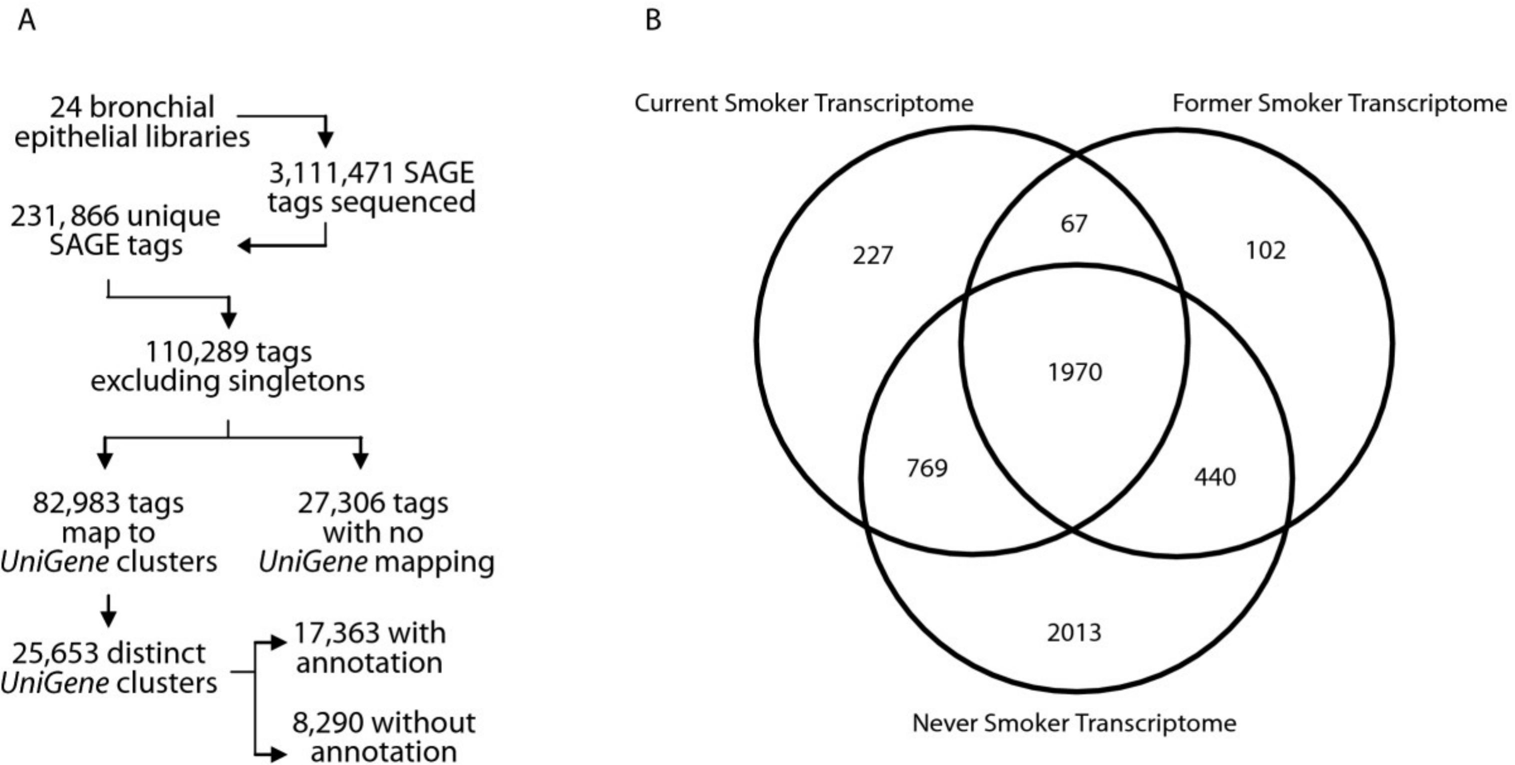
| Tag_Sequence | Count | Gene Name |
|---|---|---|
| ATATTGTCAA | 5 | translation elongation factor 1 gamma |
| AAATCGGAAT | 2 | T-complex protein 1, z-subunit |
| ACCGCCTTCG | 1 | no match |
| GCCTTGTTTA | 81 | rpa1 mRNA fragment for r ribosomal protein |
| GTTAACCATC | 45 | ubiquitin 52-AA extension protein |
| CCGCCGTGGG | 9 | SF1 protein (SF1 gene) |
| TTTTTGTTAA | 99 | NADH dehydrogenase 3 (ND3) gene |
| GCAAAACCGG | 63 | rpL21 |
| GGAGCCCGCC | 45 | ribosomal protein L18a |
| GCCCGCAACA | 34 | ribosomal protein S31 |
| GCCGAAGTTG | 50 | ribosomal protein S5 homolog (M(1)15D) |
| TAACGACCGC | 4 | BcDNA.GM12270 |

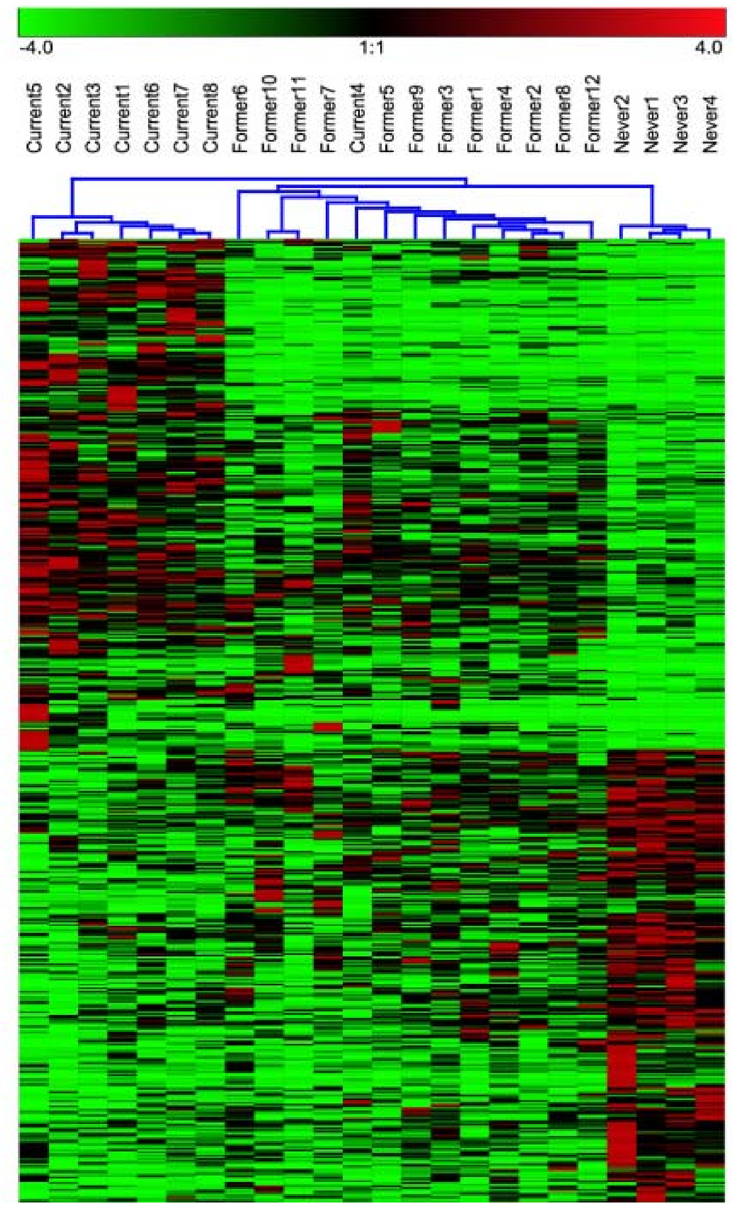
- **Situación ideal**
  - **un gen = un tag**

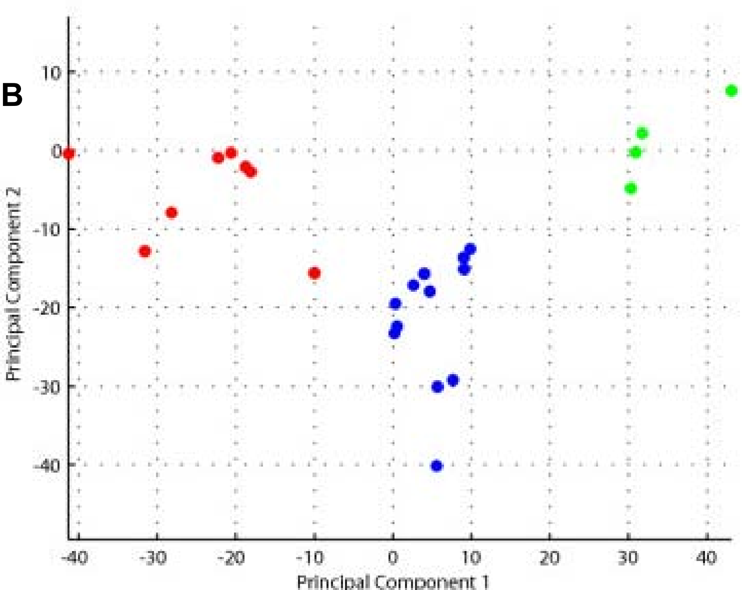
- **Situación real**
  - **un gen = muchos tags (procesamiento alternativo, sitios alternativos de poliadenilación)**
  - **un tag = muchos genes (regiones 3' conservadas)**
  - **Ausencia de sitio de restricción: 0 tags/gen**
  - **Errores en la secuencia (0.7% por base)**

**Effect of active smoking on the human bronchial epithelium Transcriptome.** Chari *et al.* **BMC Genomics, 8**:297, 2007.

- ***Background:*** **In this study, using serial analysis of gene expression (SAGE), we comprehensively examined the effect of active smoking by comparing the transcriptomes of clinical specimens obtained from current, former and never smokers, and identified genes showing both reversible and irreversible expression changes upon smoking cessation.**






**(A) Cluster analysis of current, former and never smokers: using 609 SAGE tags representing tags differentially expressed between current and never smokers. Green rectangles represent samples with lower expression for the particular gene amongst the samples, and red rectangles represent samples where the gene is highly expressed relative to other samples. (B) Principal component analysis of current, former and never smokers. Current smokers are represented in red, former smokers are represented in blue and never smokers are represented in green.**

## Conclusion

- This study represents the largest human SAGE study reported to date. Over three million SAGE tags were sequenced, representing over 110 thousand potentially unique transcripts expressed within the bronchial epithelium relative to cigarette smoke exposure.
- Based on the gene expression profiles of 24 current, former and never smokers, we identified both reversible and irreversible gene expression changes upon smoking cessation.
- Amongst those genes reversibly expressed, three main functions were identified: xenobiotic metabolism, nucleotide metabolism, and mucus secretion.
- Amongst those genes irreversibly expressed, three main functions were identified: cell cycle process and DNA repair.
- Those genes and functions which do not revert to normal levels upon smoking cessation may also provide insight into why former smokers still maintain a risk of developing lung cancer.

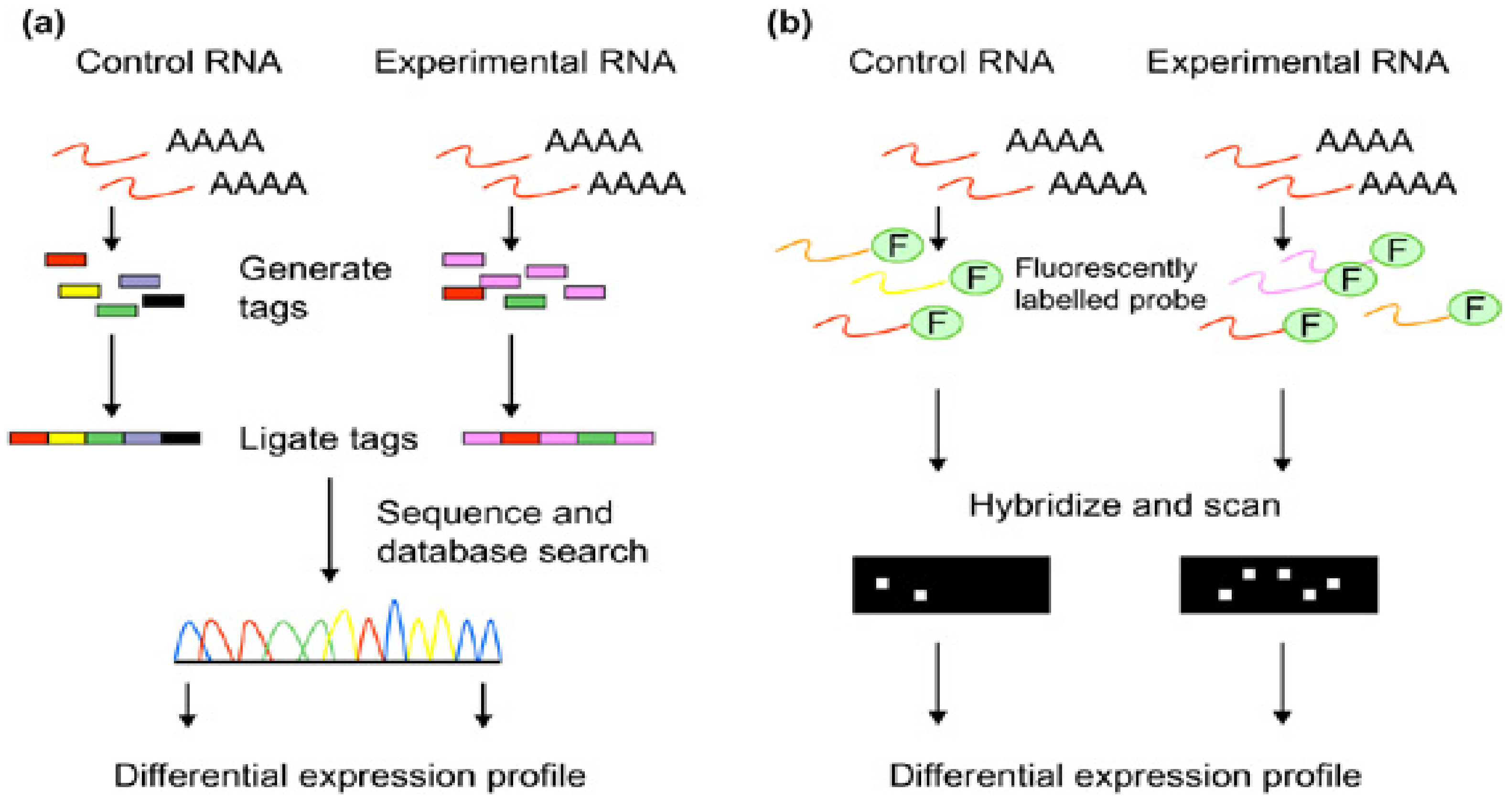
# Serial analysis of gene expression

## Ventajas

- Identificación simultánea de múltiples genes y perfiles de expresión.
- No requiere del conocimiento previo de la secuencia.
- Utiliza herramientas comunes de la biología molecular.
- Cuantitativo

# SAGE v/s MICROARRAY

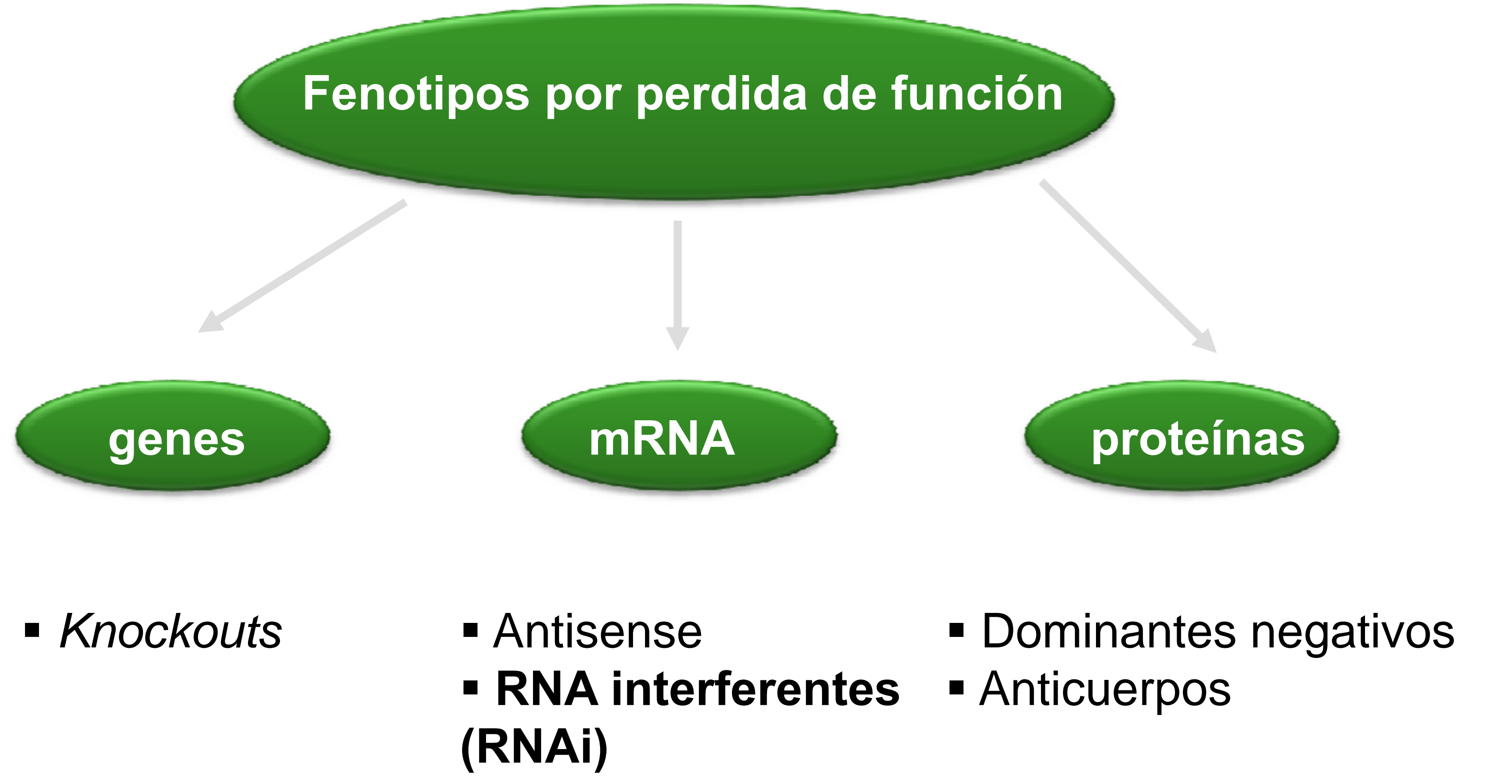
- **SAGE – Sistema abierto detecta genes conocidos y desconocidos.**



# SAGE v/s MICROARRAY

| *Features* | *SAGE* | *Microarray* |
| --- | --- | --- |
| ***Detects unknown transcripts*** | Yes | No |
| ***Quantification*** | Absolute measure | Relative measure |
| ***Sensitivity*** | High | Moderate |
| ***Specificity*** | Moderate | High |
| ***Reproducibility*** | Good for higher abundance transcripts | Good for data from intra-platform comparison |
| ***Direct cost*** | 5-10X higher than arrays. | 5-10 X lower than SAGE |

# Análisis funcionales




- *Knockouts*

- Antisense
- **RNA interferentes (RNAi)**

- Dominantes negativos
- Anticuerpos

- **Permiten demostrar una relación causal entre la función de un gen y la mantención o modulación de un fenotipo.**
- **Se consigue a través de una disminución (*knockdown*) o una eliminación (*knockout*) de la función génica.**

# RNA interference (RNAi)

# RNA interference (RNAi)

❖ Inhibición de la expresión de genes específicos mediada por RNAs de doble hebra (dsRNAs).

❖ Este mecanismo reconoce dsRNAs como señales para gatillar la degradación de su mRNA homólogo.

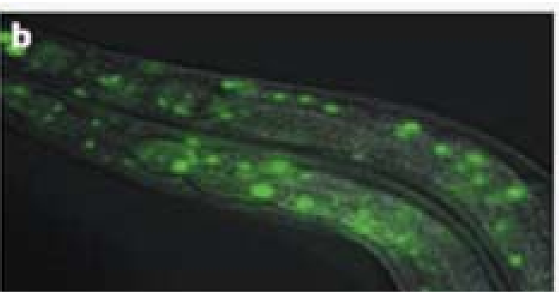
❖ Evolutivamente conservado entre los eucariontes.

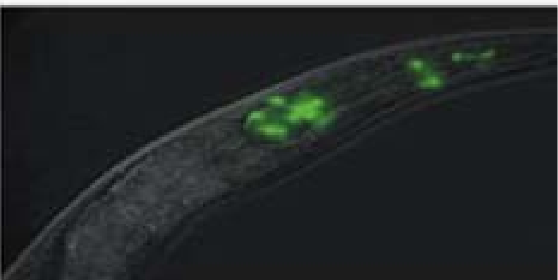
❖ Probablemente este mecanismo ha evolucionado para inmovilizar elementos de transposición e inhibir RNAs exógenos (virus).

# Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

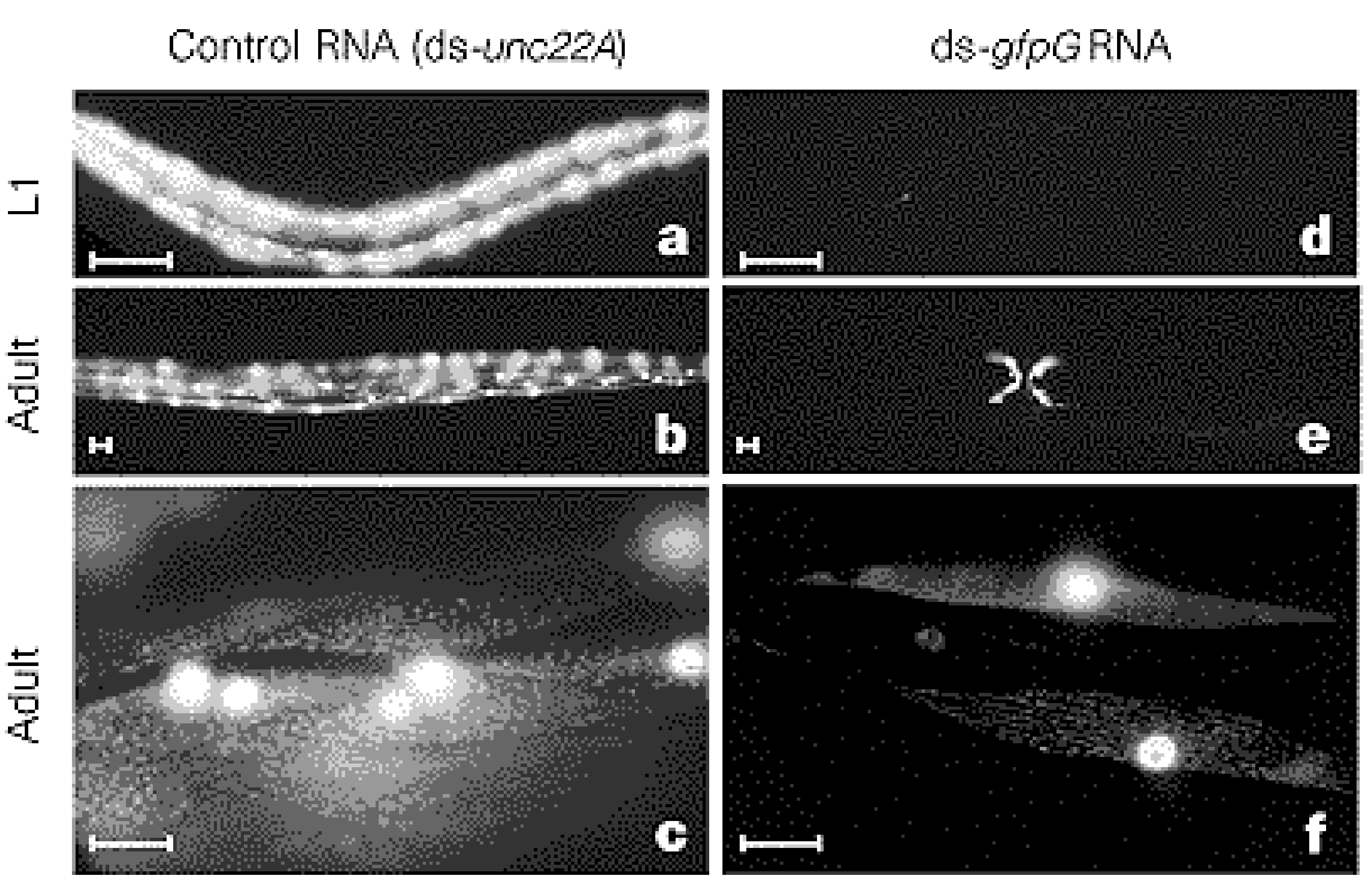
**Andrew Fire, SiQun Xu, Mary K. Montgomery, Steven A. Kostas, Samuel E. Driver & Craig C. Mello**

**Nature 391:806-811, 1998**

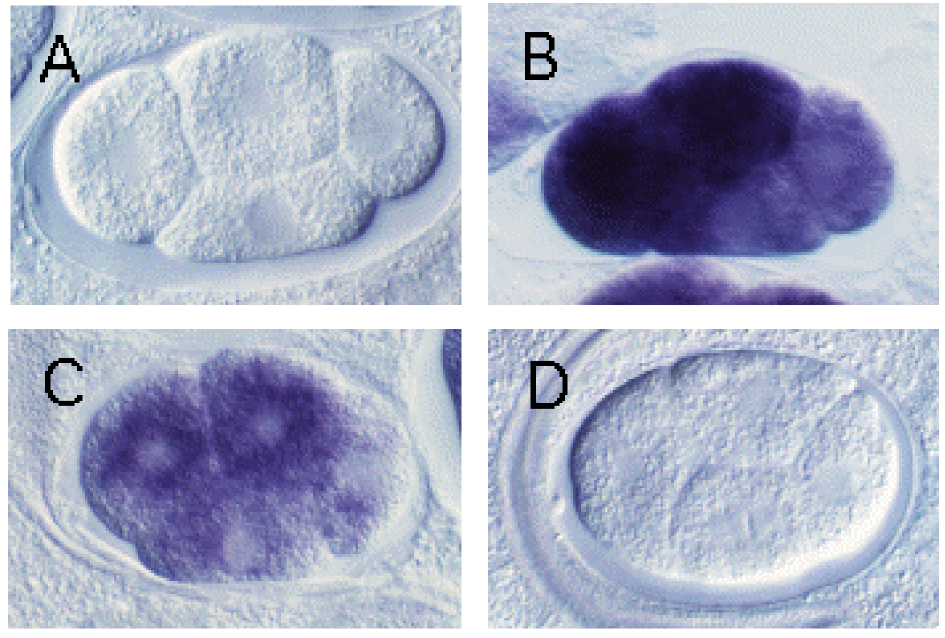


## RNAi en una cepa de *C. elegans* que expresa el reportero GFP



## Efecto del RNAi sobre los niveles del mRNA endógeno de *mex-3B*




a) Control negativo, sin tinción
b) Wild type, hibridación *in situ*
c) Wild type + anti-sense mex-3B RNA
d) Wild type + dsRNA mex-3B.

- dsRNA causa una interferencia potente y específica
- dsRNA es significativamente más efectivo que el antisense

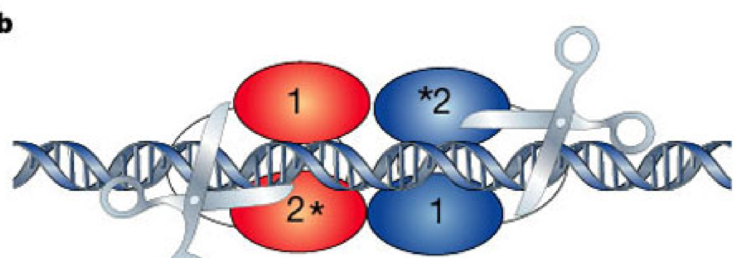
# RNA interferente (RNAi)
# Etapas

**Iniciación**

- **dsRNA es digerido para formar 21-23 nt small interfering RNAs (siRNAs) con la ayuda de una endonucleasa (Dicer).**

**Activación**

- **siRNAs son incorporados en un complejo proteico, *RNA-induced silencing complex* (RISC).**
- **siRNA sirve de guía a RISC para el reconocimiento y le ruptura del mRNA complementario.**




# INICIACION

- La endonucleasa <u>Dicer</u> rompe el dsRNA para generar fragmentos de ~22 nt. Miembro de la familia Rnase III.

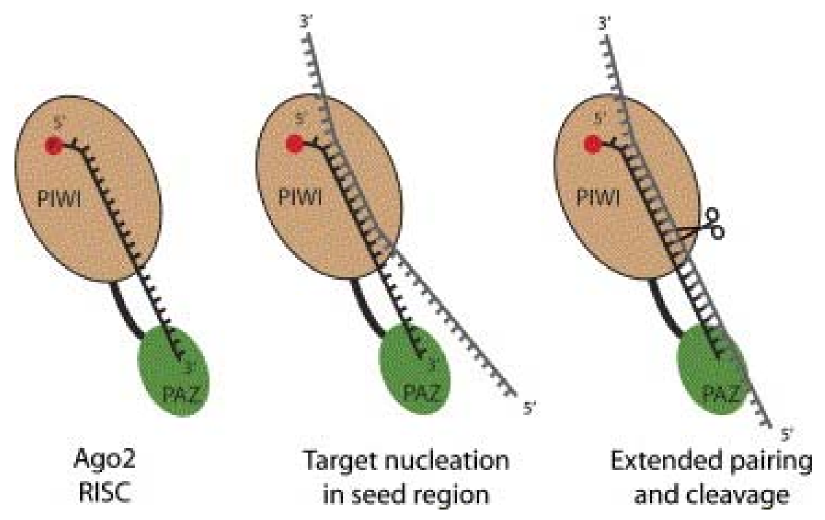
- Los siRNAs son incorporados en el complejo <u>RISC y</u> desenrollados.

- Esto activa a <u>RISC*</u>, el cual utiliza a los siRNAs como guías para la selección del sustrato.

# RNA-induced silencing complex (RISC)

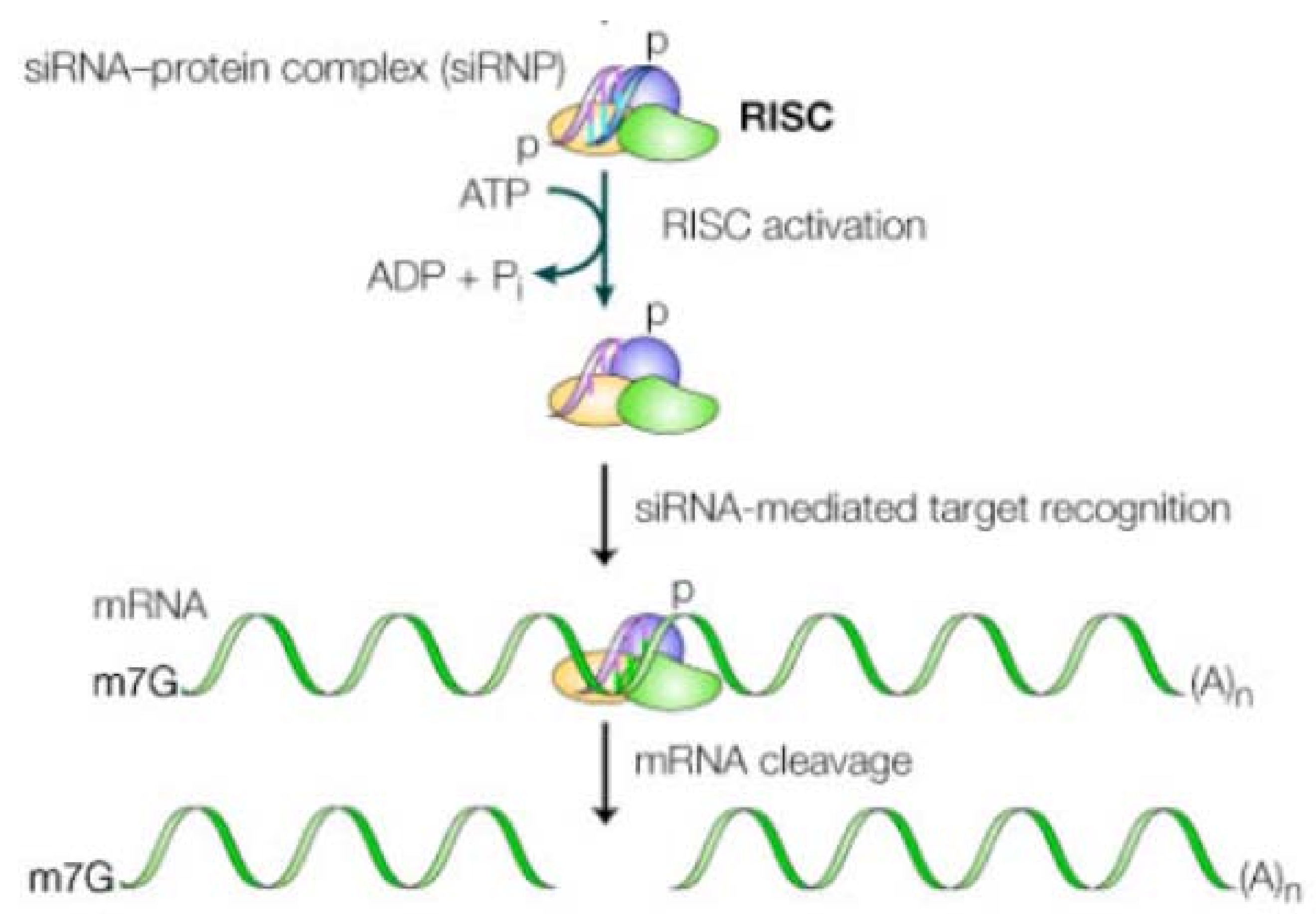
**Reconoce y destruye los mRNAs blanco**
**Compuesto de:**

- **siRNA: Identifica los sustratos mediante apareamiento de bases.**
- **<u>Slicer</u>: *Argonaute family protein* presenta dos dominios estructurales PAZ (une extremo 3` del siRNA) y Piwi (une extremo 5`). Piwi posee actividad RNaseH.**
- **Otras proteínas con dominios de unión a RNA.**

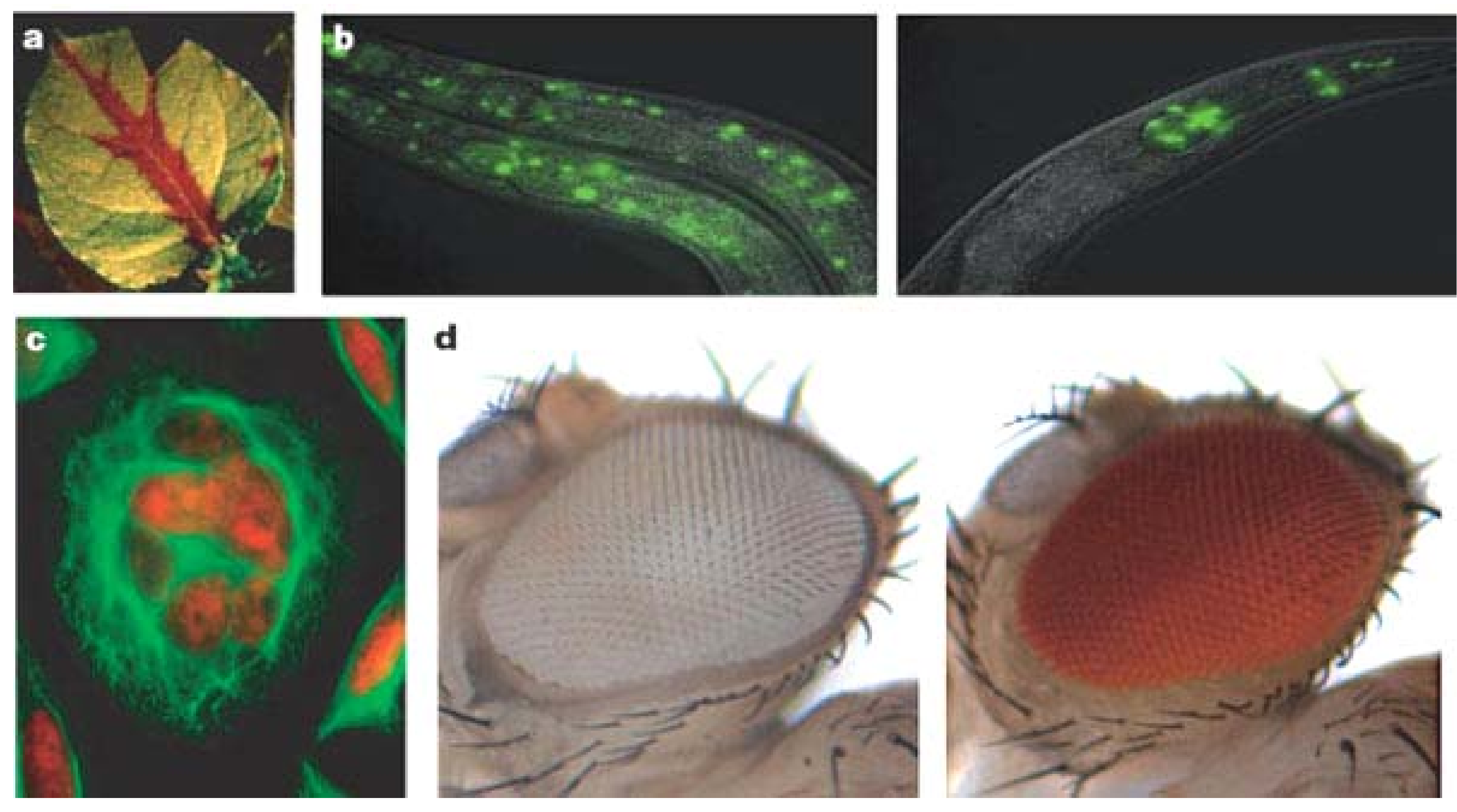


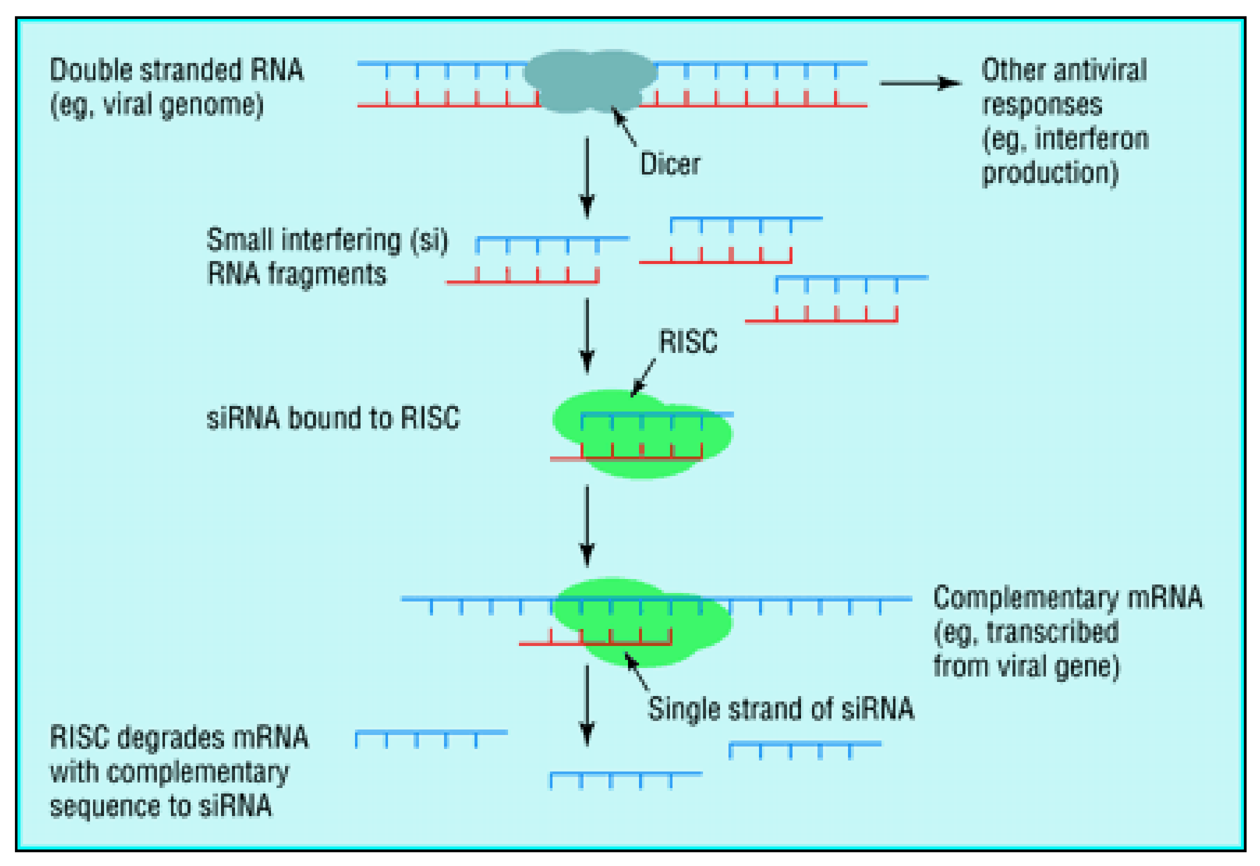

**Model for Slicer catalysis. The siRNA guide strand is bound at the 5′ end by the PIWI domain and at the 3′ end by the PAZ domain. The RNaseH fold hydrolyzes the target in a cation dependent manner.**

# RNA-induced silencing complex (RISC)



# Ejemplos de RNAi






**Natural mechanism of RNA interference.** The appearance of double stranded (ds) RNA within a cell—for example, as a result of viral infection—triggers an RNA interference response. The cellular enzyme dicer binds to the dsRNA and cuts it into short pieces of 20 or so nucleotide pairs in length known as small interfering RNAs or siRNAs. These bind to a cellular enzyme complex RISC (RNA induced silencing complex) that uses one strand of the siRNA to bind to single stranded RNA molecules such as mRNA of complementary sequence. RISC then degrades the mRNA, thus silencing expression of the viral gene. In mammals, other antiviral responses to dsRNA also exist

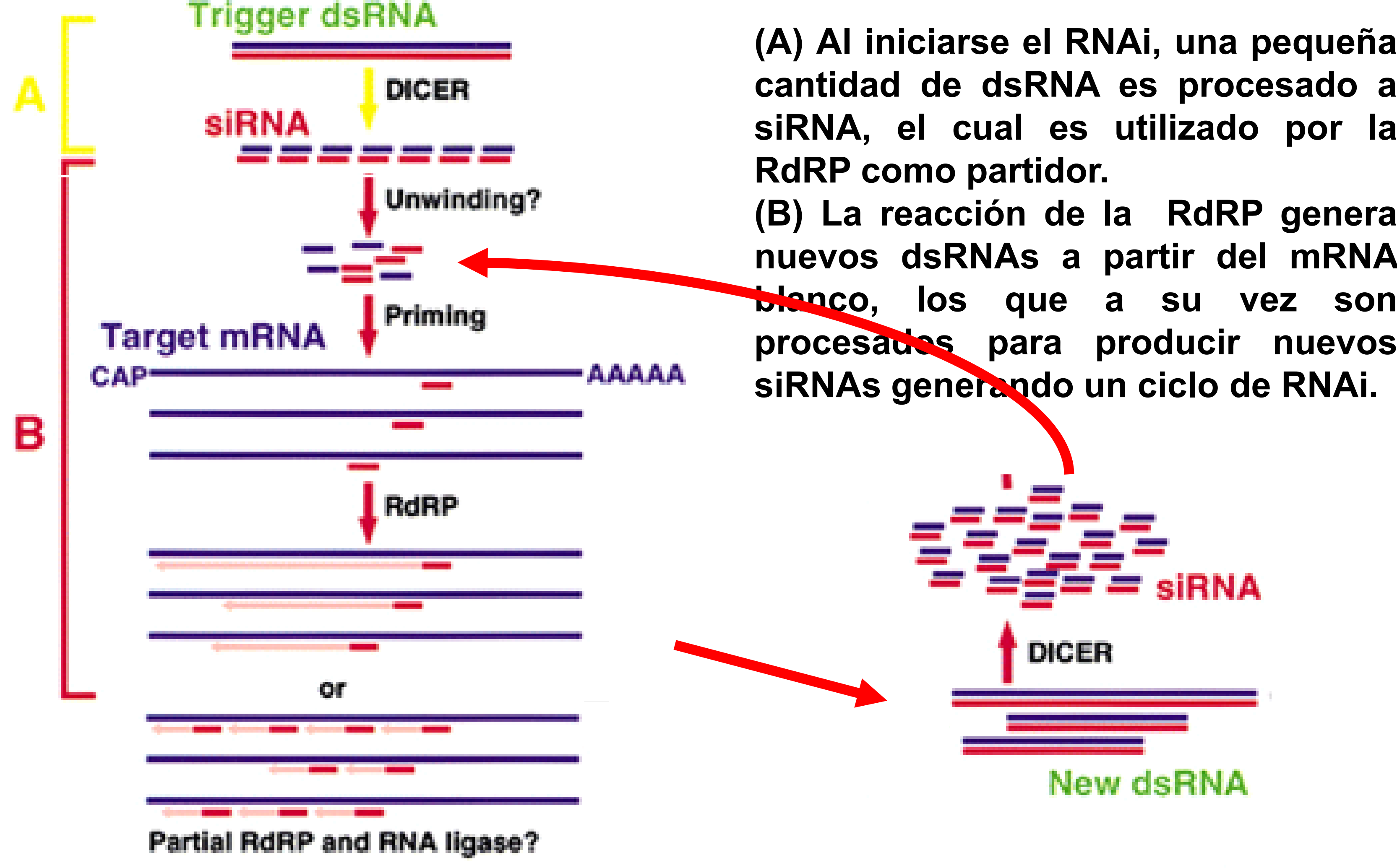
# RNA interferente (RNAi)
# Propiedades

## Amplificación: RNA-directed RNA polymerase (RdRP)

**RdRP se encuentra presente en:**

| | |
|---|---|
| **Tomate** | **RdRP** |
| ***Arabidopsis*** | **SDE1/SGS2** |
| ***Neurospora*** | **QDE-1** |
| ***C.elegans*** | **línea germinal EGO-1** |
| | **soma – RRF-1/RDE-9** |
| ***Drosophila*** | **RdRP** |

# Amplificación: RNA-directed RNA polymerase (RdRP)




**(A) Al iniciarse el RNAi, una pequeña cantidad de dsRNA es procesado a siRNA, el cual es utilizado por la RdRP como partidor.**

**(B) La reacción de la RdRP genera nuevos dsRNAs a partir del mRNA blanco, los que a su vez son procesados para producir nuevos siRNAs generando un ciclo de RNAi.**

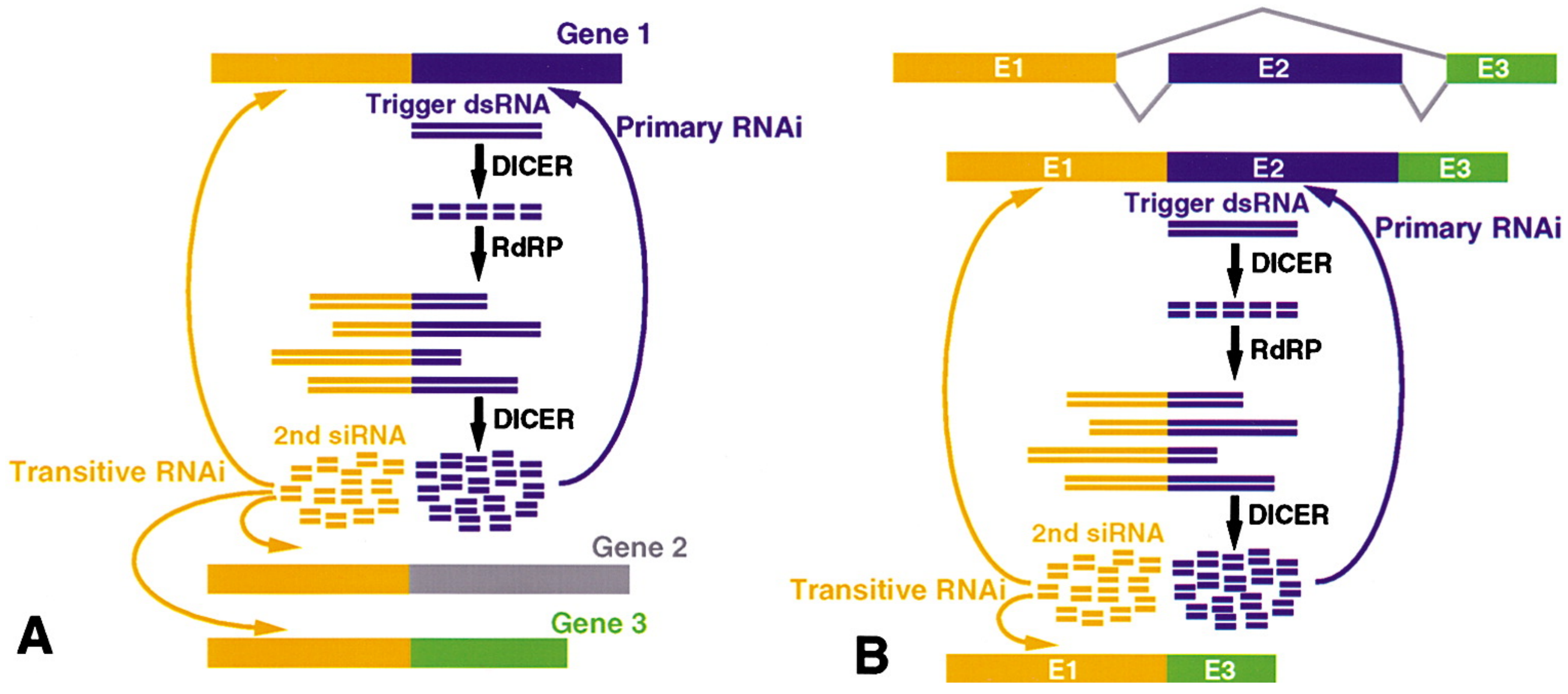
# RNA interferente (RNAi)
# Propiedades

## Transitividad

**La polaridad determinada por la reacción de amplificación de la RdRP predice que:**

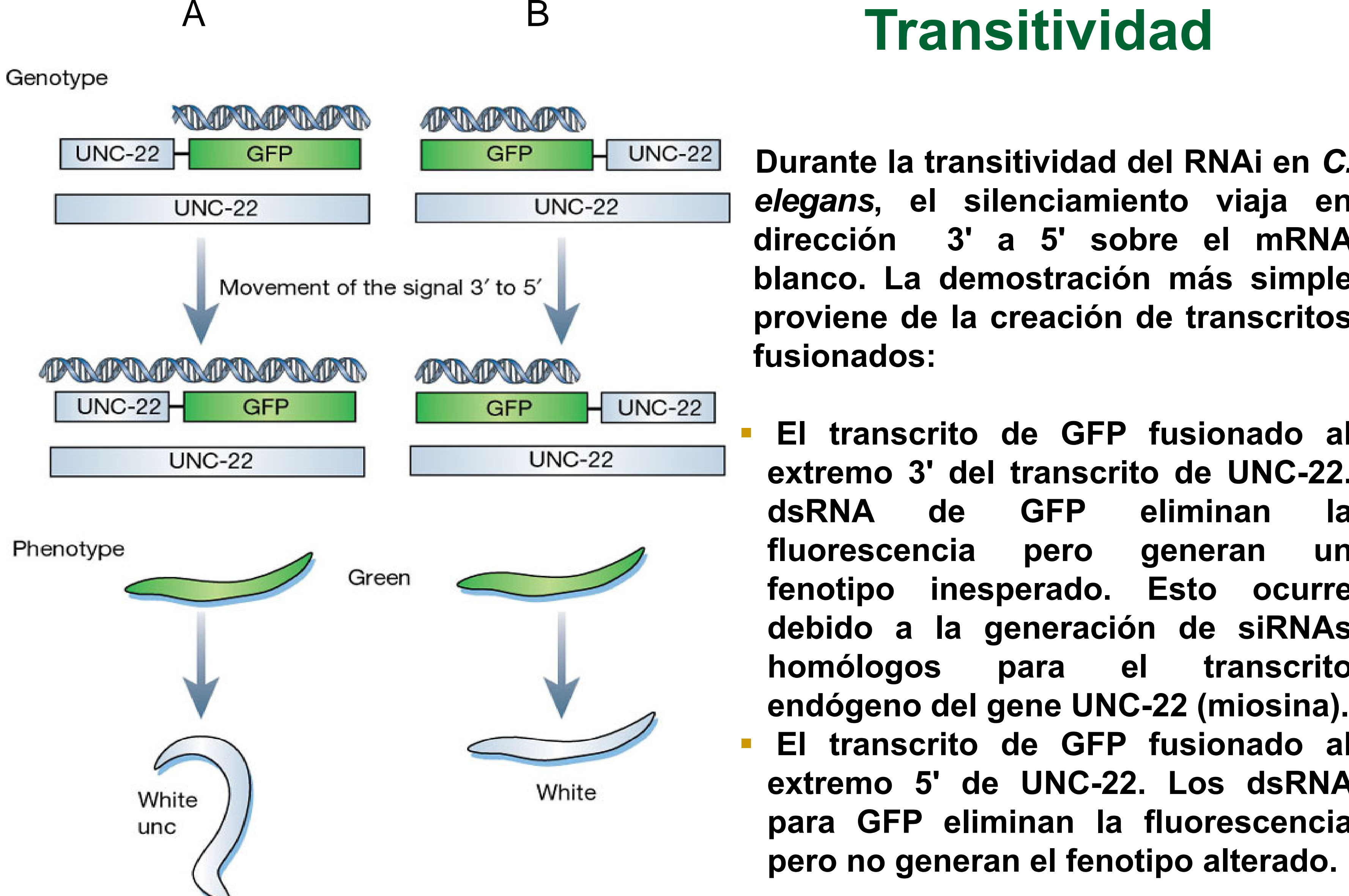
- **El dsRNA sintetizado puede extenderse más allá de la secuencia complementaria del dsRNA inicial, inhibiendo regiones 5` del mRNA blanco.**
- **Una nueva población de dsRNAs secundarios puede generarse a partir de la amplificación del dsRNA.**

# Transitividad



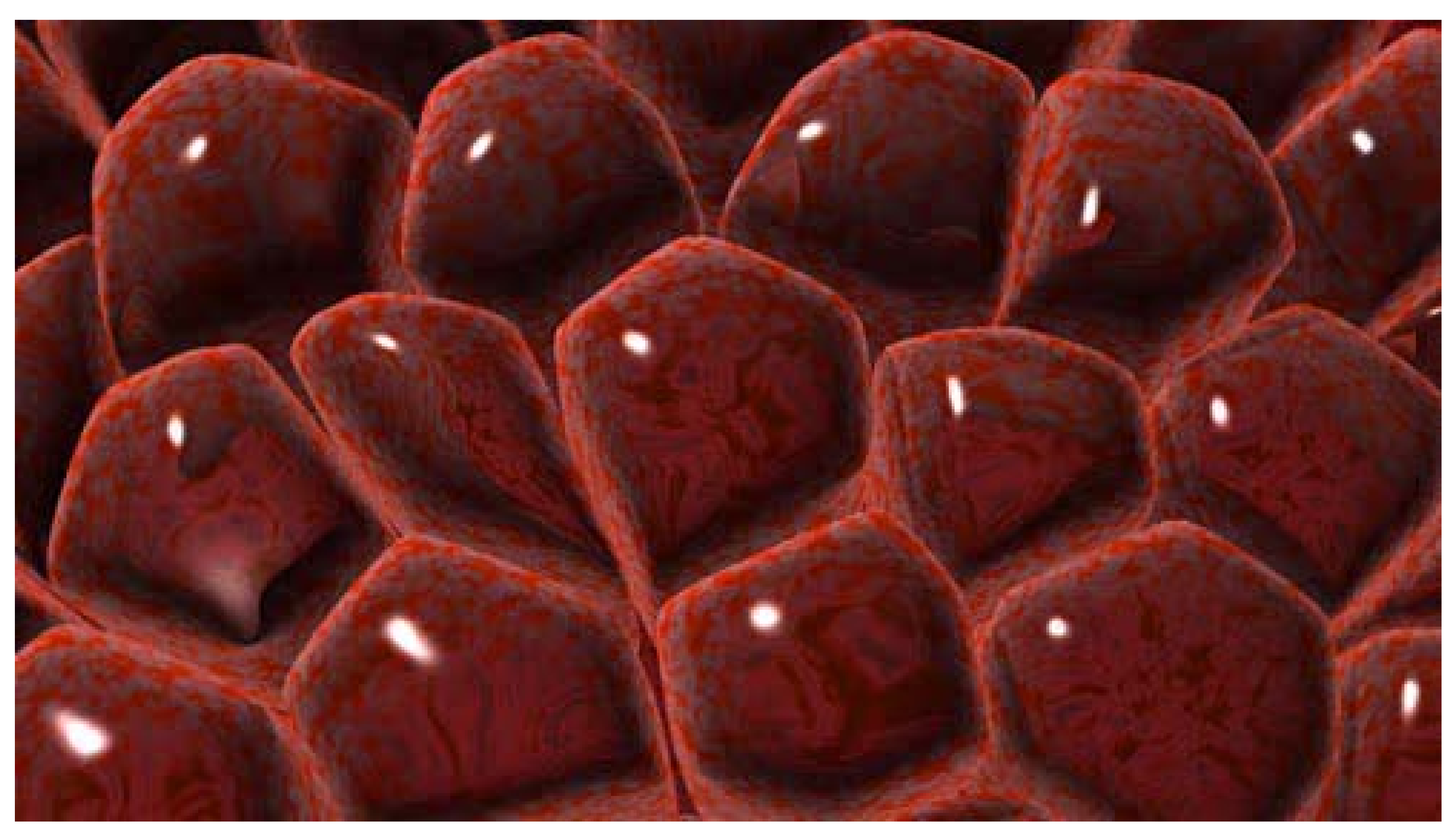

**siRNAs secundarios, generados a partir de la amplificación del dsRNA (naranja) y la extensión hacia la región 5`del blanco primario (azul) mediada por la RdRP, pueden promover la transitividad de la interferencia afectando secuencias homólogas (A) o mensajeros generados por procesamiento alternativo (B).**

# Transitividad




**Durante la transitividad del RNAi en *C. elegans*, el silenciamiento viaja en dirección 3' a 5' sobre el mRNA blanco. La demostración más simple proviene de la creación de transcritos fusionados:**

- **El transcrito de GFP fusionado al extremo 3' del transcrito de UNC-22. dsRNA de GFP eliminan la fluorescencia pero generan un fenotipo inesperado. Esto ocurre debido a la generación de siRNAs homólogos para el transcrito endógeno del gene UNC-22 (miosina).**
- **El transcrito de GFP fusionado al extremo 5' de UNC-22. Los dsRNA para GFP eliminan la fluorescencia pero no generan el fenotipo alterado.**



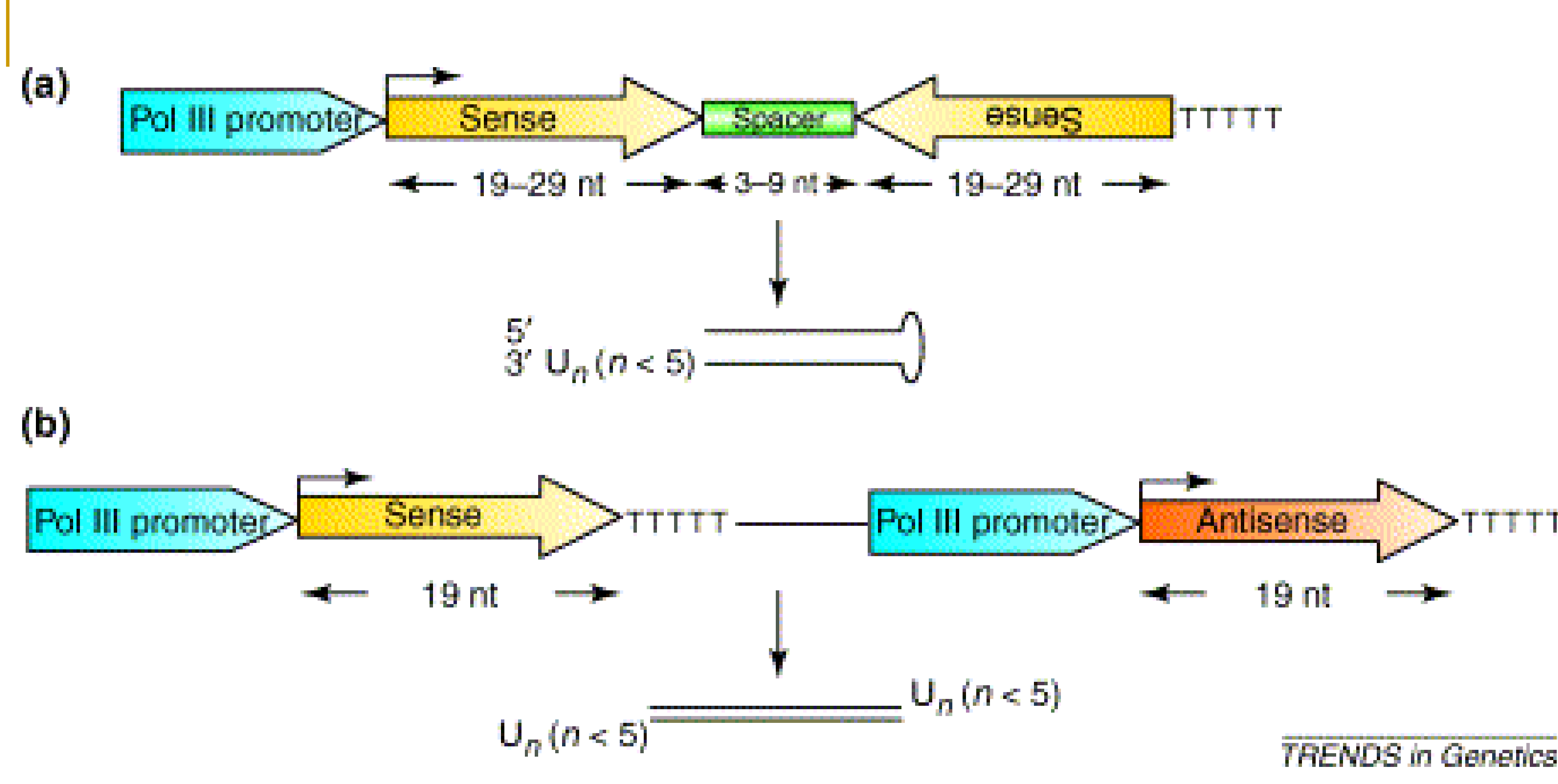
# RNA interference (RNAi)

## En células de mamíferos

## A System for Stable Expression of Short Interfering RNAs in Mammalian Cells

**Thijn R. Brummelkamp, Rene Bernards, Reuven Agami**

**Science 296:550-553, 2002**




**The DNA vector-based RNA interference (RNAi) technology.**

A Stable clones after 8 weeks

**Co-transfectadas con pSuper-dsRNA-p53 + pBabe-puro**

pSUPER pSUPER-p53

**Verde: p53** **Rojo: actina**

C **Northern blot**

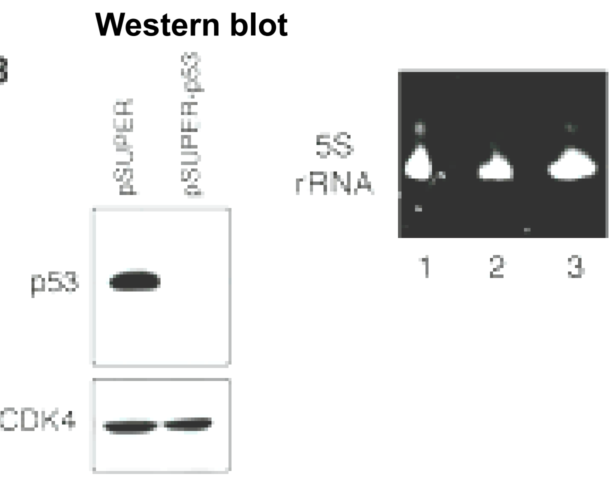
Stable clones

pSUPER-p53 pSUPER pSUPER-p53

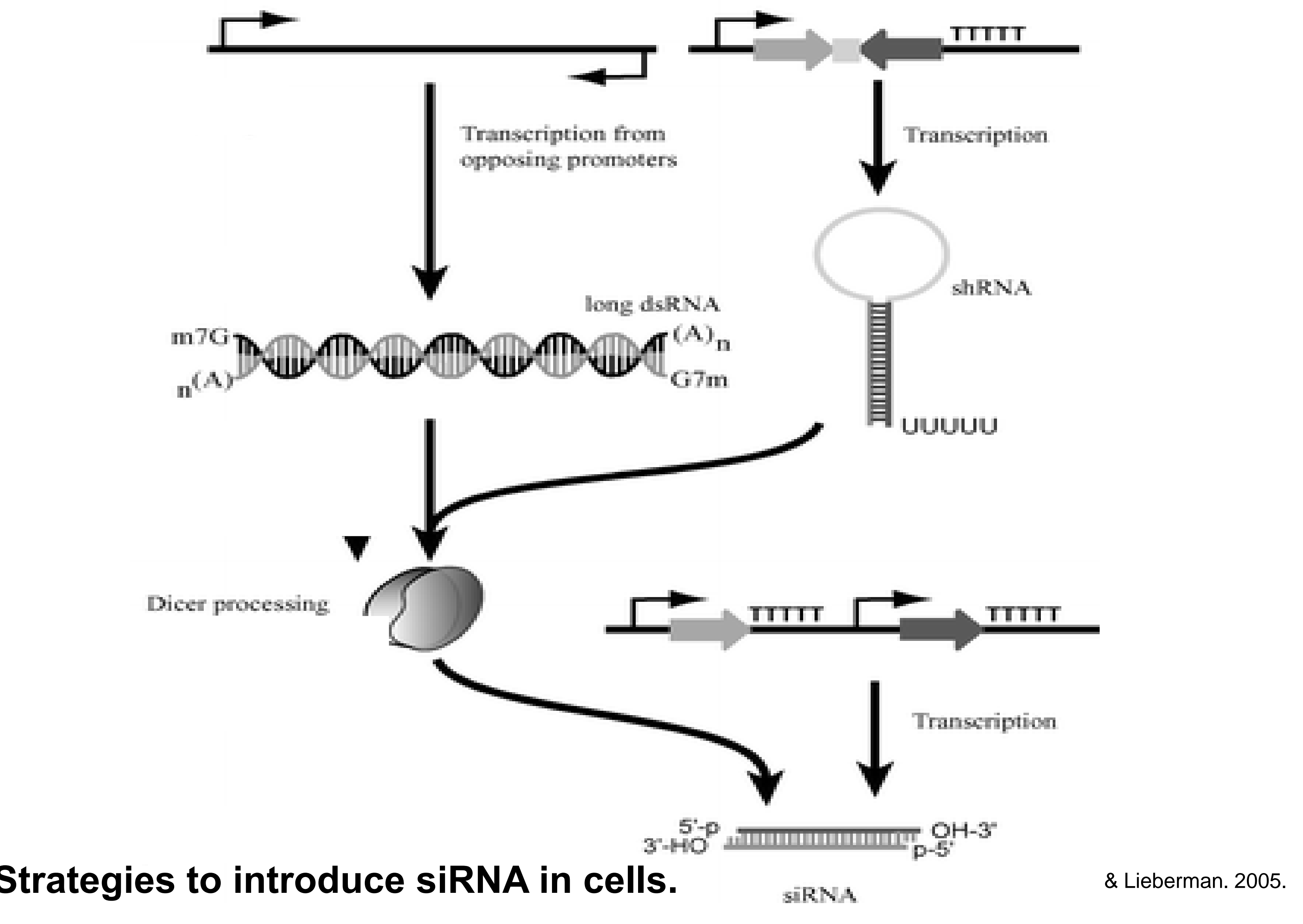
1 2 3

p53 siRNAs

19 nt —

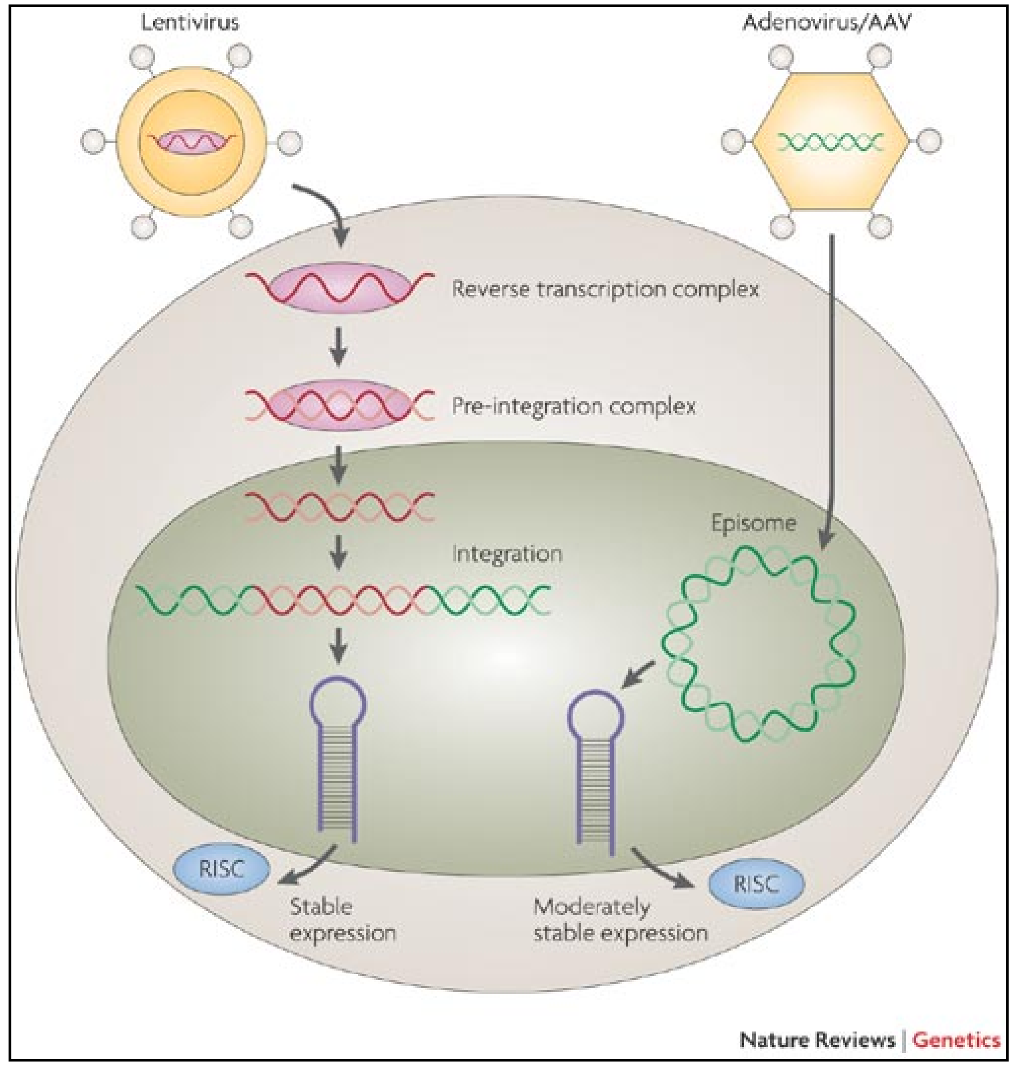



**(A) Immunofluorescence using antibodies against p53 (green) and against actin, as a control (red). (B) Immunoblot analysis for p53 and control (CDK4). (C) Stable clones for pSUPER and pSUPER-p53 after 2 months in culture (lanes 2 and 3) and transiently transfected cells with 1 µg pSUPER-p53 after 48 hours (lane 1) were analyzed for p53-specific siRNAs expression. Blots were probed with a $^{32}$P-labeled sense p53 19-nt probe corresponding to the targeting sequence.**

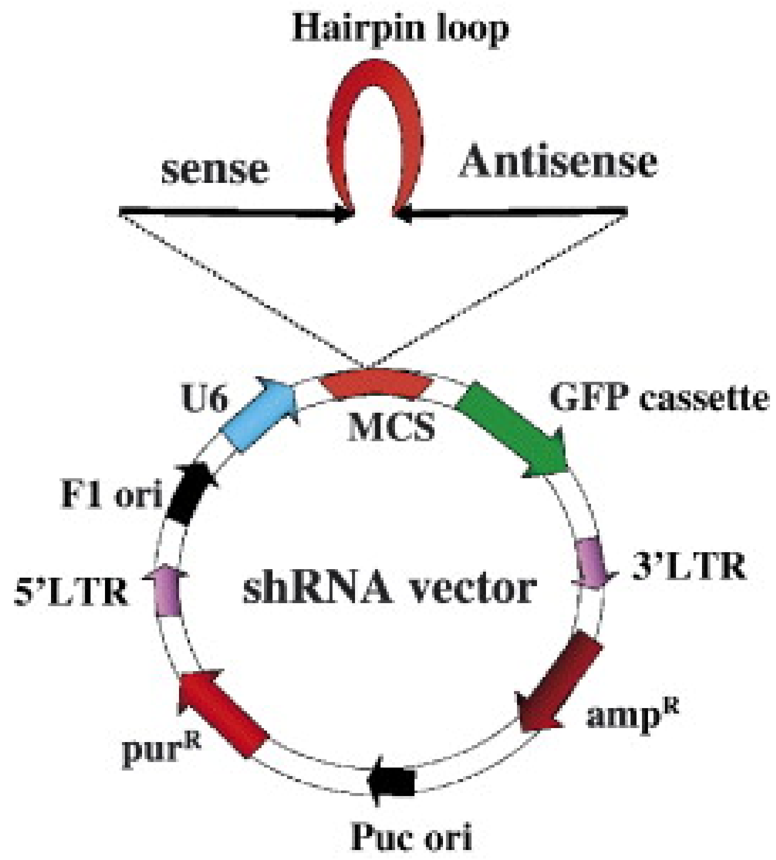



**Strategies to introduce siRNA in cells.**

& Lieberman. 2005.




**Lentiviral vectors are used to deliver therapeutic, short hairpin RNA (shRNA)-expressing transgenes that integrate into the genome for stable shRNA expression.**




**Representation of a typical shRNA viral based vector system. Shown here are two expression cassettes, one with a U6 promoter for the expression of the shRNA and a GFP cassette to mediate reporter gene expression. puc ori-origin of replication ; F1 ori — origin of replication; ampR — ampicillin resistance gene for bacterial selection ; purR — puromycin resistance for mammalian selection 3′LTR — left terminal repeats; 5′LTR — left terminal repeats.**

El RNAi es una poderosa herramienta que puede aplicarse con fines terapéuticos, debido principalmente a dos hechos:

- Todas las células contienen la maquinaria necesaria para poner en marcha los procesos mediados por el RNAi.
- Todos los genes son potenciales blancos.

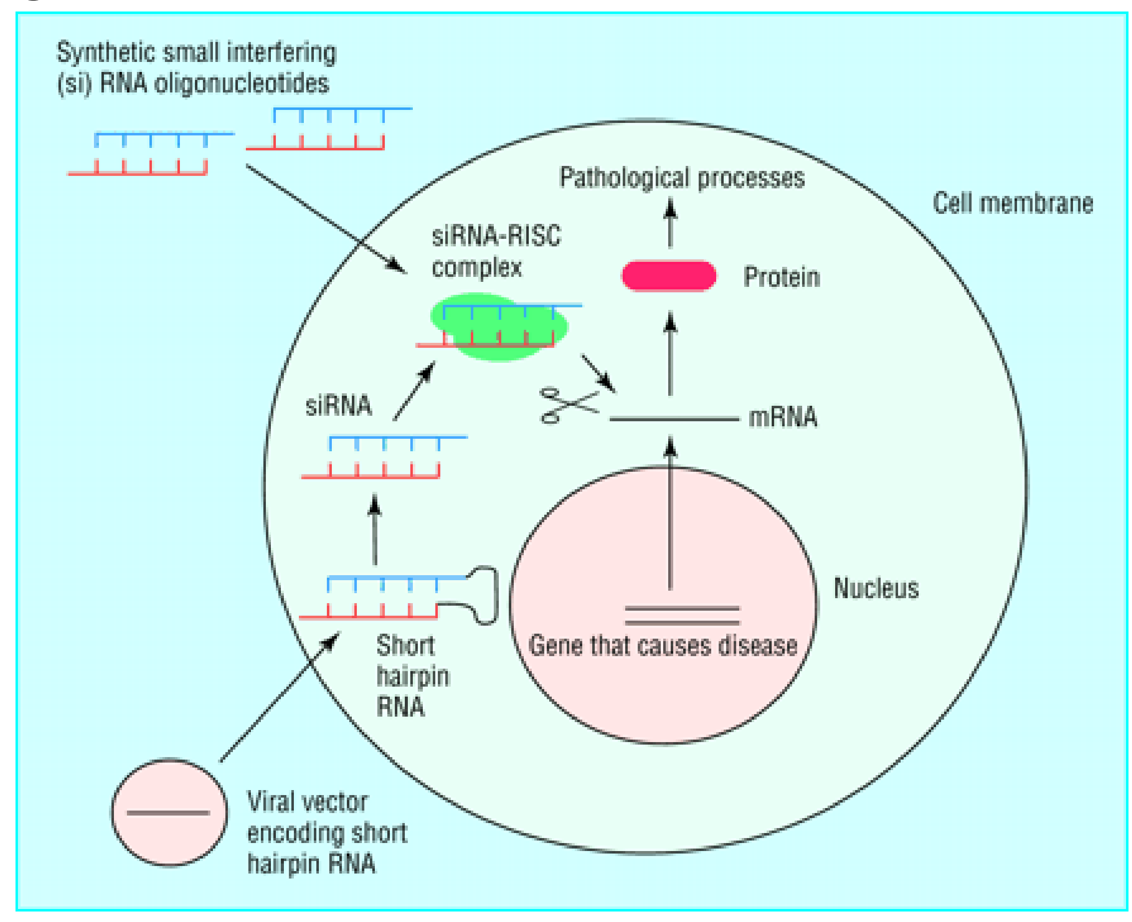


Table 3
Therapeutic targets of RNAi tested in vivo

| Field | Disease | Target | Route | Vehicle | References |
|---|---|---|---|---|---|
| Neurological | Amyotrophic lateral sclerosis | SOD1 | i.m. | Lentivirus | [126] |
| | | SOD1 | Intraspinal | Lentivirus | [127] |
| | Spinocerebellar ataxia | Ataxin1 | i.c. | AAV | [40] |
| | Huntington's disease | Huntingtin | i.c. | AAV | [128] |
| | Neuropathic pain | P2X3 cation channel | Intrathecal | None | [39] |
| Ocular | Inflammation in eye | TGFβ RII | Local | None | [129] |
| | AMD | VEGF | Local | Transit TKO | [130] |
| | Herpetic stromal keratitis | VEGF/R | i.v. | Ligand directed | [131] |
| Hearing | Autosomal dominant | Gap junction β2 | Local | Liposome | [132] |
| Inflammation | Rheumatoid arthritis | TNFα | Local | None | [133] |
| | Sepsis | TNFα | i.p. | None | [44] |
| Apoptosis | Acute liver failure | Fas | hd | None | [134] |
| | | Caspase 8 | hd/p.v. | None | [135] |
| | Liver ischemia/reperfusion | Caspase 8/3 | hd | 10%lipiodol | [136] |
| | Renal ischemia/reperfusion | Fas | hd | None | [137] |
| | Lung ischemia/reperfusion | Heme oxygenase1 | i.n. | None | [37] |
| Metabolism | Obesity | AGRP | i.c | None | [38] |
| | Cholestrol | ApoB | i.v. | Modified | [41] |

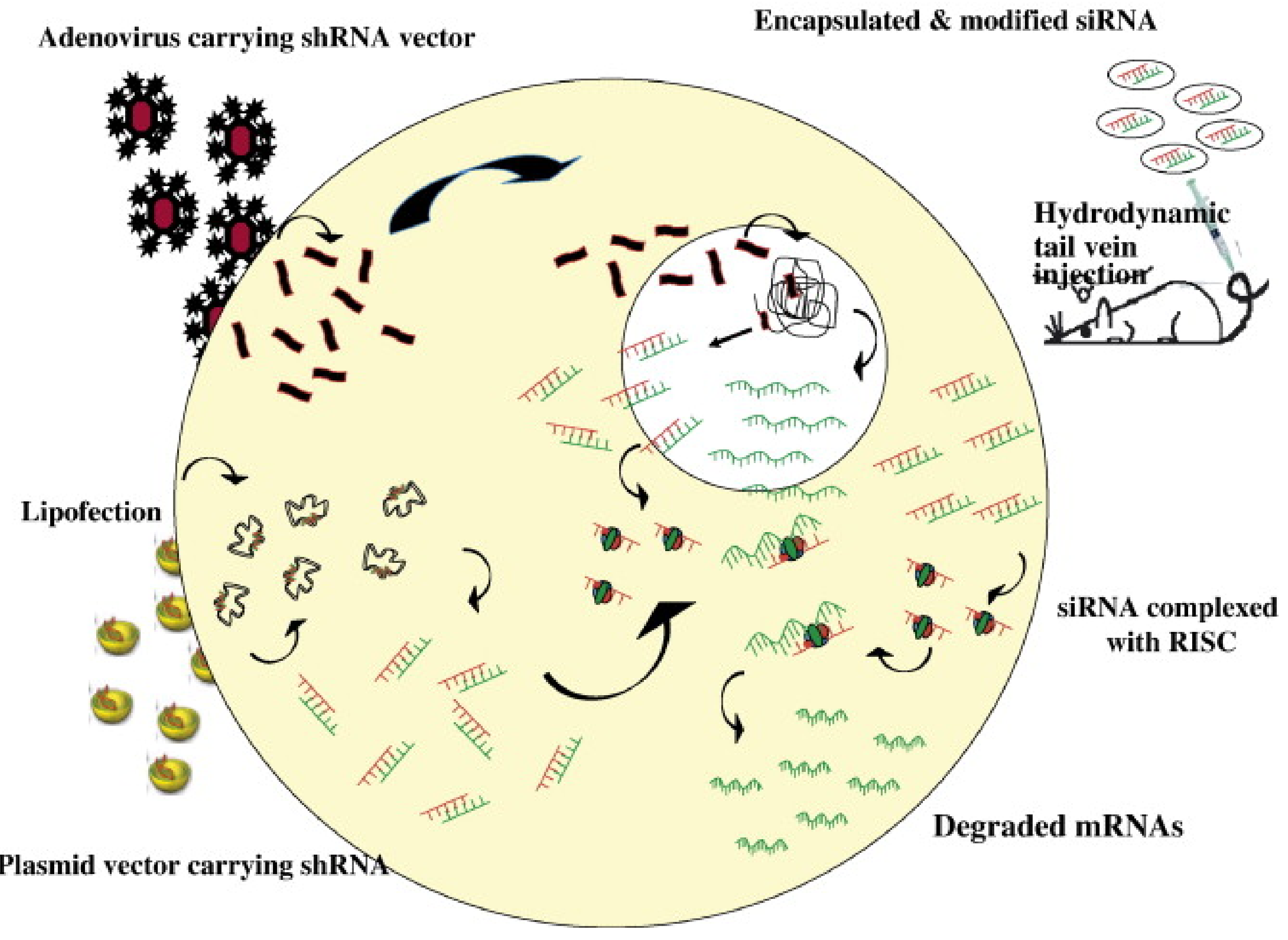
hd, hydrodynamic injection; i.v., intravenous; i.n., intranasal; i.c., intracranial; i.m., intramuscular; AAV, adeno-associated virus; AMD, age-related macular degeneration; AGRP, agouti related peptide.

Uprichard, SL. 2005. The therapeutic potential of RNA interference. FEBS Letters 579:5996-6007.

Table 4
Anti-cancer RNAi targets tested in vivo

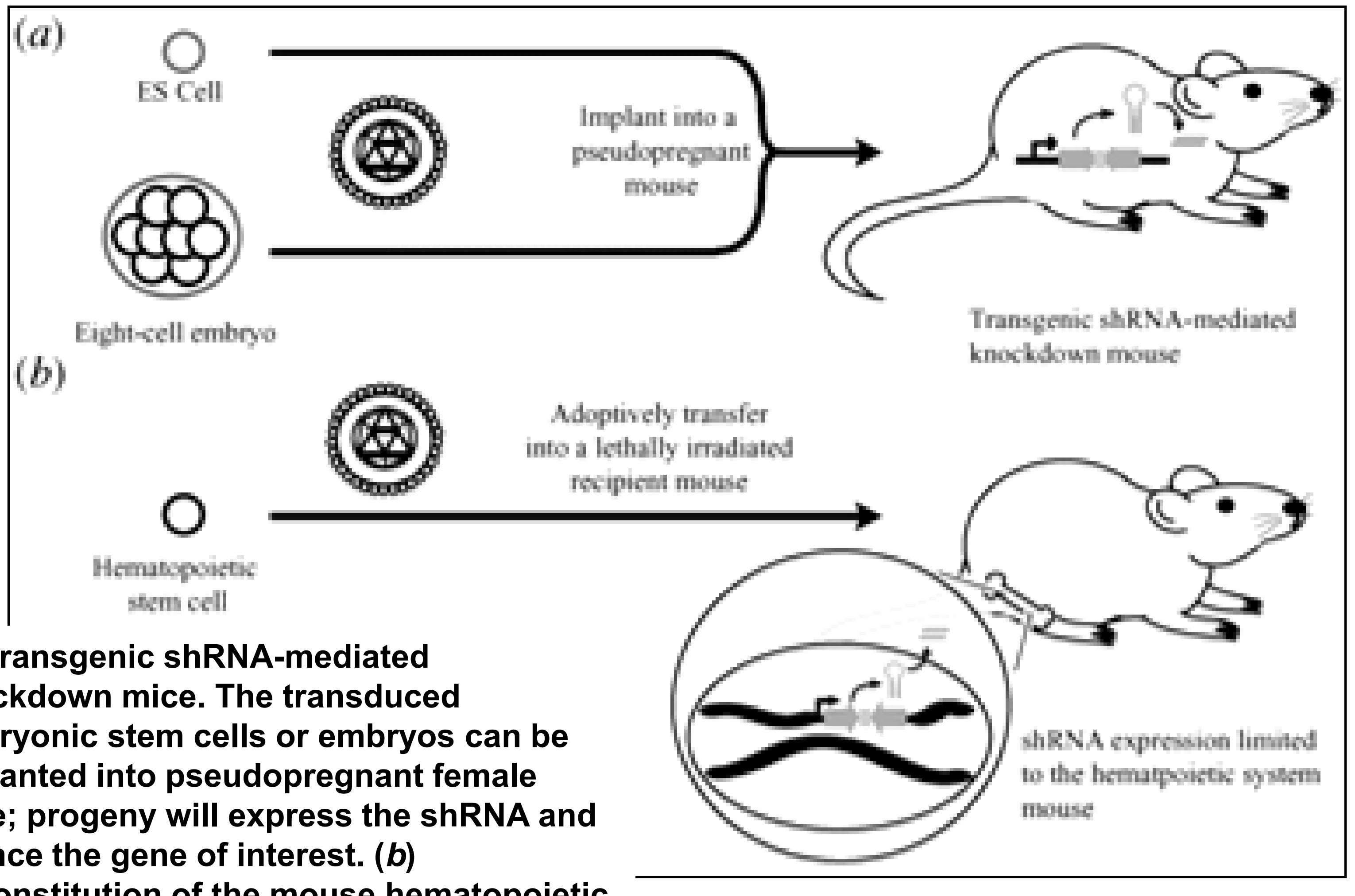
| Target | Route | Vehicle | References |
|---|---|---|---|
| Bcl-2 | i.v. | Liposome | [145] |
| Cxcr4 | i.v. | None | [146] |
| Focal adhesion kinase | i.v. | None | [147] |
| EphA2 | i.v. | None | [148] |
| Polo-like kinase 1 | i.v. | ATA-treated | [149] |
| Colony-stimulating factor | i.t. | None | [150] |
| survivin | hd | DNA | [151] |
| CEACAM6 | i.v. | None | [152] |
| EGFR | i.v. | Ligand-targeted | [49] |
| Erbb2/neu (HER2)R | i.p. | PEI-complex | [47] |
| Skp-2 | i.t. | Adenovirus | [153] |
| Spingosine-1 phosphate-R | i.t. | Liposome | [154] |
| RhoA | i.t. | None | [155] |
| VEGF-R | i.v. | Ligand-targeted | [46] |
| VEGF | i.t./i.v. | Atelocollagen | [156] |
| FGF4 | i.t. | Atelocollagen | [157] |

i.v., intravenous; i.t., intratumoral; hd, hydrodynamic injection; ATA, aurintricarboxylic acid; CEACAM6, carcinoembryonic antigen-related adhesion molecule 6.




**A schematic summary of several different methods of siRNA delivery *in vivo* leading to silencing of the target gene within the cell.**

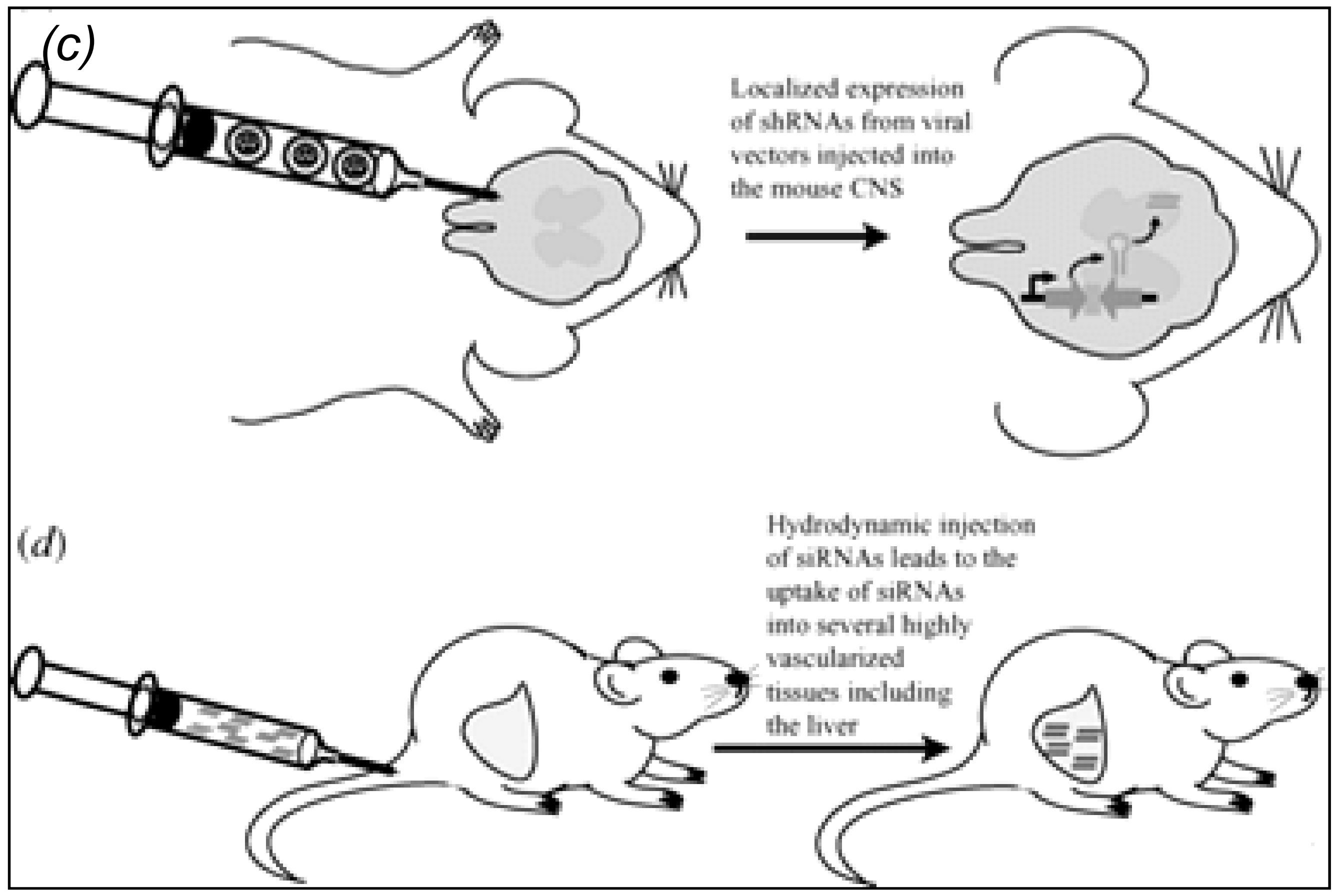
# RNAi *in vivo*




**(*a*) Transgenic shRNA-mediated knockdown mice. The transduced embryonic stem cells or embryos can be implanted into pseudopregnant female mice; progeny will express the shRNA and silence the gene of interest. (*b*) Reconstitution of the mouse hematopoietic system with shRNA-expressing stem cells.**

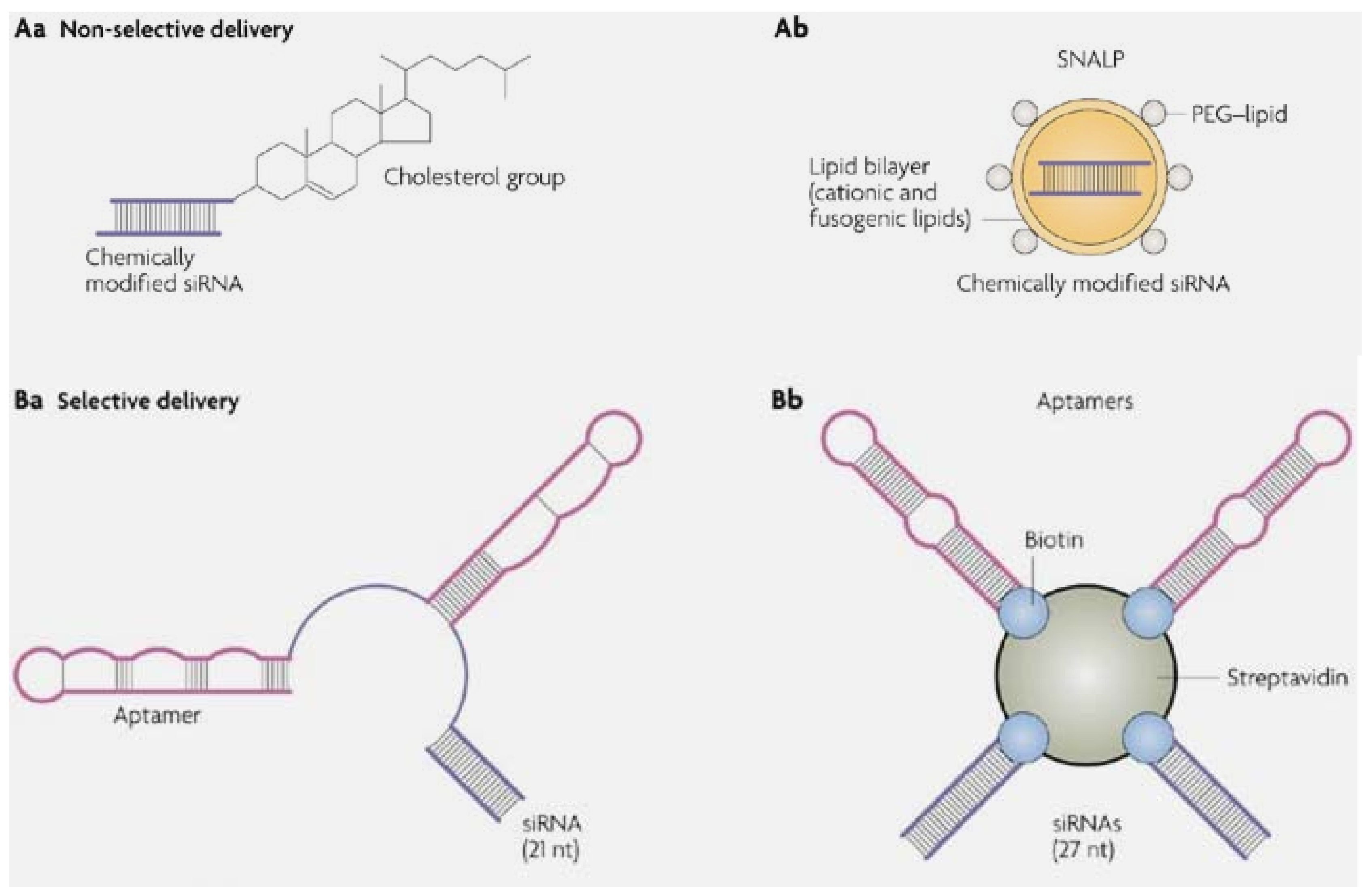
Dykxhoorn & Lieberman. 2005.

# RNAi *in vivo*

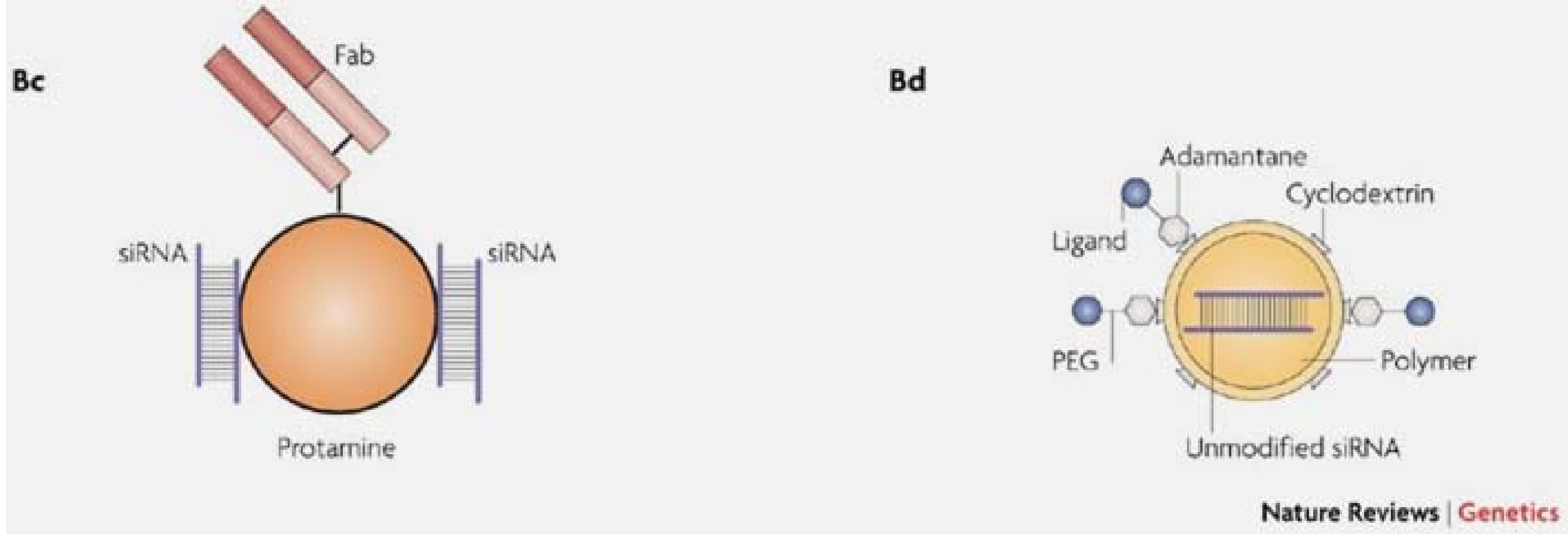



**(*c*) The injection of viral constructs into the central nervous system of mice.**
**(*d*) The hydrodynamic (high-pressure, high-volume, rapid) injection of siRNAs into the tail vein of mice leads to the uptake ("hydroporation") of siRNAs into a variety of tissues including the liver, pancreas, lung, and spleen.**

# Delivery of small interfering RNAs.



# Delivery of small interfering RNAs.



| Disease | Stage | RNAi reagent | Delivery | Company/institution |
|---|---|---|---|---|
| *Ocular diseases* | | | | |
| AMD | Preclinical stage | siRNA | Direct intravitreal injection | Quark Biotech |
| | Clinical trial phase I | siRNA | Direct intravitreal injection | Sirna |
| | Clinical trial phase II | siRNA | Direct intravitreal injection | Acuity |
| *Viral infections* | | | | |
| Hepatitis B and C | Preclinical stage | shRNA | Liganded nanoparticle | Nucleonics/Intradigm |
| RSV | Clinical trial phase I | siRNA | Aerosol | Alnylam |
| HIV | Clinical trial phase I (scheduled for 2007) | shRNA | Lentivirus | Benitec/City of Hope |
| *Cancer* | | | | |
| Hepatic cancer | Preclinical stage | siRNA | Liganded nanoparticle | Calando |
| Solid tumour cancers | Preclinical stage | siRNA | Liganded nanoparticle | Intradigm |
| *Other disease types* | | | | |
| ALS | Preclinical stage | siRNA | N/A | CytRx |
| Inflammatory diseases | Preclinical stage | siRNA | Peptide | Nastech |

ALS, amyotrophic lateral sclerosis; AMD, age-related macular degeneration; RNAi, RNA interference; RSV, respiratory syncytial virus; shRNA, short hairpin RNA; siRNA, small interfering RNA.

Kim and Rossi *Nature Reviews Genetics* **8**, 173–184, March 2007

**Table 1 RNAi biotechnology companies**

| Company | Founded | Founders and advisors | Technology focus | Business focus |
|---|---|---|---|---|
| Acuity Pharmaceuticals (Philadelphia, PA, USA) | 2002 | Michael Tolentino and Samuel Reich (University of Pennsylvania) | Use of RNAi against vascular endothelial growth factor in ophthalmic diseases | Therapeutics against macular degeneration and diabetic retinopathy |
| Alnylam Holding Company (Cambridge, MA, USA) 2003 merger between Alnylam and Ribopharm AG | 2002 | Phil Sharp (MIT), David Bartel (The Whitehead), Paul Schimmel (Scripps Institute), Tom Tushl (Rockefeller University), and Phillip Zamore (U. Mass Medical School), Roland Kreutzer and Stefan Limmer (founders of Ribopharma) | Therapeutic use of delivered RNA in cells and adult mammals | Therapeutics against viral, cancer, metabolic, central nervous system (CNS), and autoimmune diseases. |
| Atugen (Berlin, Germany) | 1998 | Spin-off from Ribozyme Pharmaceuticals (now Sirna Therapeutics) | Exclusive licensee of Sirna's RNAi target discovery and validation technologies | Cancer therapeutics, pathway analysis and target validation |
| Avocel (Sunnyvale, CA, USA) | 2003 | Mark Kay (Stanford University) | Exclusive license for expressed RNAi in non-embryonic mammals (Stanford University) and co-exclusive license to deliver RNAi to non-embryonic mammals | Therapeutics against chronic hepatitis B and C |
| Benitec (Queensland, Australia) | 1997 | Queensland Department of Primary Industries | DNA-directed RNAi (ddRNAi) | Therapeutics against cancer, autoimmune, HIV/AIDS and chronic viral disease |
| Cenix BioScience (Dresden, Germany) | 1999 | Christophe Echeverri, Pierre Gonczy, Anthony Hyman (European Molecular Biology, Heidelberg, Germany; Max Planck Laboratory, Dresden, Germany) | Genome-scale application of RNAi | Custom design of large-scale RNAi libraries (offered by Ambion), target discovery and validation |

**Table 1 RNAi biotechnology companies**

| Company | Founded | Founders and advisors | Technology focus | Business focus |
|---|---|---|---|---|
| CytRx (Los Angeles, CA, USA) | 2002 | Merger with Global Genomics, changed company focus to RNAi | Nonexclusive licensee of U Mass Medical School patents covering gene silencing of specific diseases using RNAi | Therapeutics against obesity, type 2 diabetes and amyotrophic lateral sclerosis |
| Devgen (Ghent, Belgium) | 1997 | Thierry Bogaert (MRC, Cambridge, UK), Michael Hengartner (University of Zurich) | Genome-wide *Caenorhabditis. elegans* RNAi feeding library | Therapeutics against metabolic and CNS disorders |
| Intradigm (Rockville, MD, USA) | 2001 | Martin Woodle (Novartis, Cambridge, MA, USA) | Gene delivery and gene therapy vectors developed at Genetic Therapy for use with RNAi (subsidiary of Novartis) | Therapeutics against cancer |
| Nucleonics (Malvern, PA, USA) | 2001 | C. Satishchandran and Catherine Pachuk (Thomas Jefferson University, Philadelphia, PA, USA) | Expressed long interfering RNA (eiRNA) | Therapeutics from expressed interfering RNA |
| Polgen (Cambridge, UK), a division of Cyclacel (Dundee, UK) | 2000 | David Glover (University of Cambridge, Cambridge, UK) | Identifies cell cycle targets from whole genome screens using RNAi in Drosophila cell lines | Cancer targets and pathways. Phenotypic characterization after genetic knock down and small molecule inhibitors |
| Sequitur (Natick, MA, USA) (The company was acquired in November by life sciences product and services company Invitrogen (Carlsbad, CA, USA).) | 1996 | Tod Woolf, Craig Mello (U. Mass Medical School), and Richard Wagner (Phylos, Lexington, MA, USA) | Proprietary 'stealth' RNAi technology | Therapeutics against hepatic insufficiency, respiratory syncitial virus, asthma and breast cancer |
| Sirna Therapeutics (formerly Ribozyme Pharmaceuticals) (Boulder, CO, USA) | 1992 | Ralph 'Chris' Christoffersen (Morgenthaler Ventures, Boulder, CO, USA) | Therapeutic use of RNAi and expression of siRNA in cells. (Max Planck, MIT, U Mass Medical school, Whitehead). Chemically modified siRNA and RNA. RNA synthesis and manufacturing | Therapeutics against hepatitis C, macular degeneration (VEGF pathway), oncology, inflammation, metabolic diseases and CNS |

**Summary**

➢RNA interference is an ancient natural antiviral mechanism that directs silencing of gene expression in a sequence specific manner

➢RNA interference can be exploited artificially to inhibit the expression of any gene of interest

➢The principal systems for achieving RNA interference are short synthetic double stranded RNA molecules and gene expression vectors that direct their production in the cell

➢ RNA interference systems could be used clinically to suppress gene expression as a therapeutic strategy in many diseases characterised by elevated gene function