Genómica Funcional

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Genome sequencing projects statistics

Organism	Complete	Draft assembly	In progress	total
Prokaryotes	595	400	476	1471
Archaea	47	4	31	82
Bacteria	548	396	445	1389
Eukaryotes	23	129	186	338
Animals	4	53	90	147
Mammals	2	21	26	49
Birds		1	2	3
Fishes		3	6	9
Insects	1	19	20	40
Flatworms		1	3	4
Roundworms	1	3	13	17
Amphibians			2	2
Reptiles			2	2
Other animals		6	19	25
Plants	3	3	34	40
Land plants	2	2	27	31
Green Algae	1	1	7	9
Fungi	10	52	31	93
Ascomycetes	8	46	21	75
Basidiomycetes	1	4	6	11
Other fungi	1	2	4	7
Protists	6	19	27	52
Apicomplexans	1	10	6	17
Kinetoplasts	1	2	6	9
Other protists	4	7	14	25
total:	**618**	**529**	**662**	**1809**

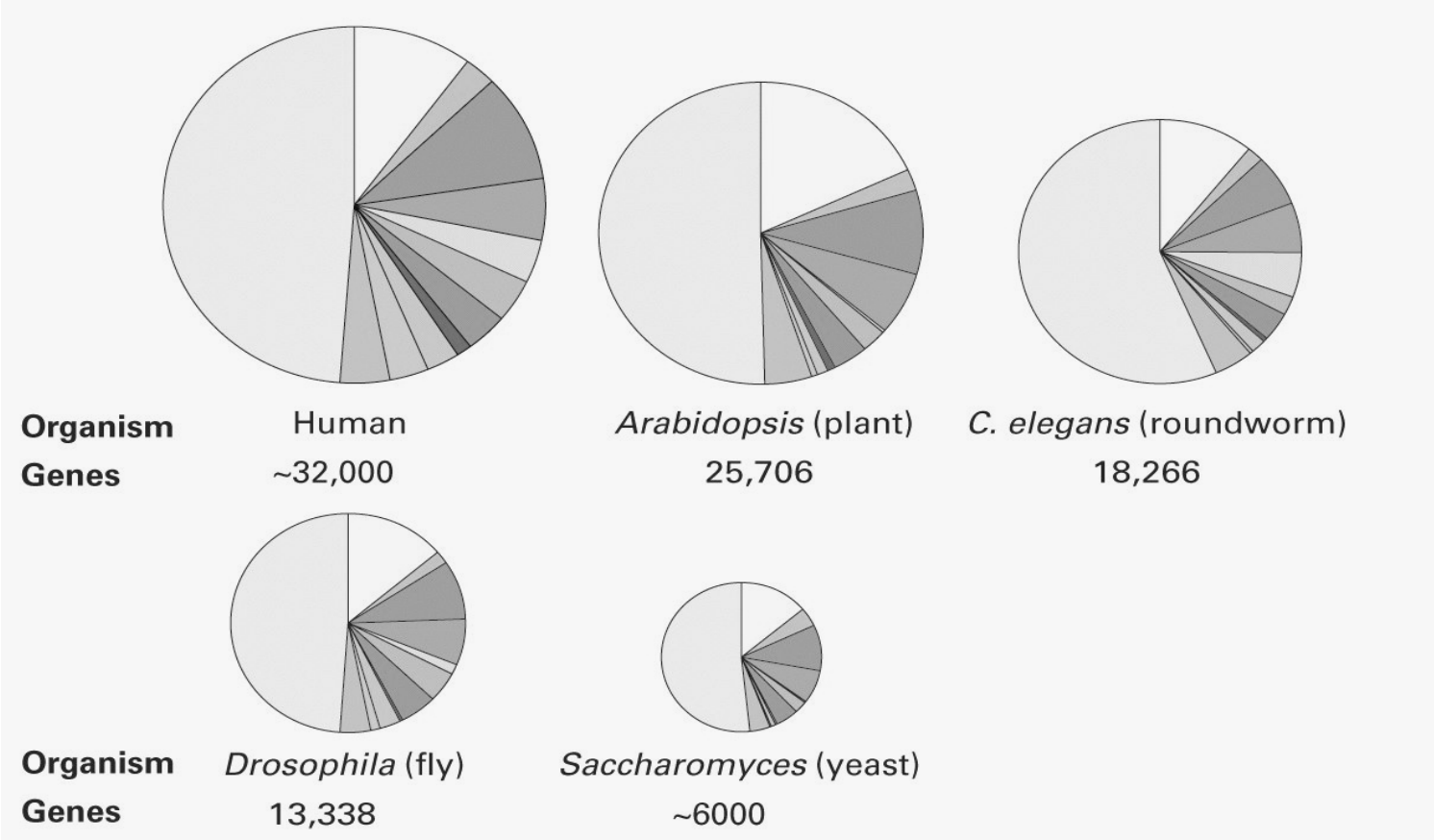
Muchos genes, pocas funciones

Genómica funcional

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

"estudio de todos los genes expresados por una célula o grupo de ellas y los cambios en su expresión bajo diferentes condiciones".

¿Por qué necesitamos genómica funcional?



¿Por qué necesitamos genómica funcional?

Organismo	# genes	% genes con función inferida	Año de termino de la secuencia genómica
E. coli	4288	60	1997
yeast	6,600	40	1996
C. elegans	19,000	40	1998
Drosophila	12-14K	25	1999
Arabadopsis	25,000	40	2000
mouse	~30,000?	10-20	2002
human	~30,000?	10-20	2000

Métodos de la genómica funcional

Expresión diferencial de genes

- Serial analysis of gene expression (SAGE)
- Suppression subtractive hybridization (SSH)
- Micro/Macroarrays

Función de genes

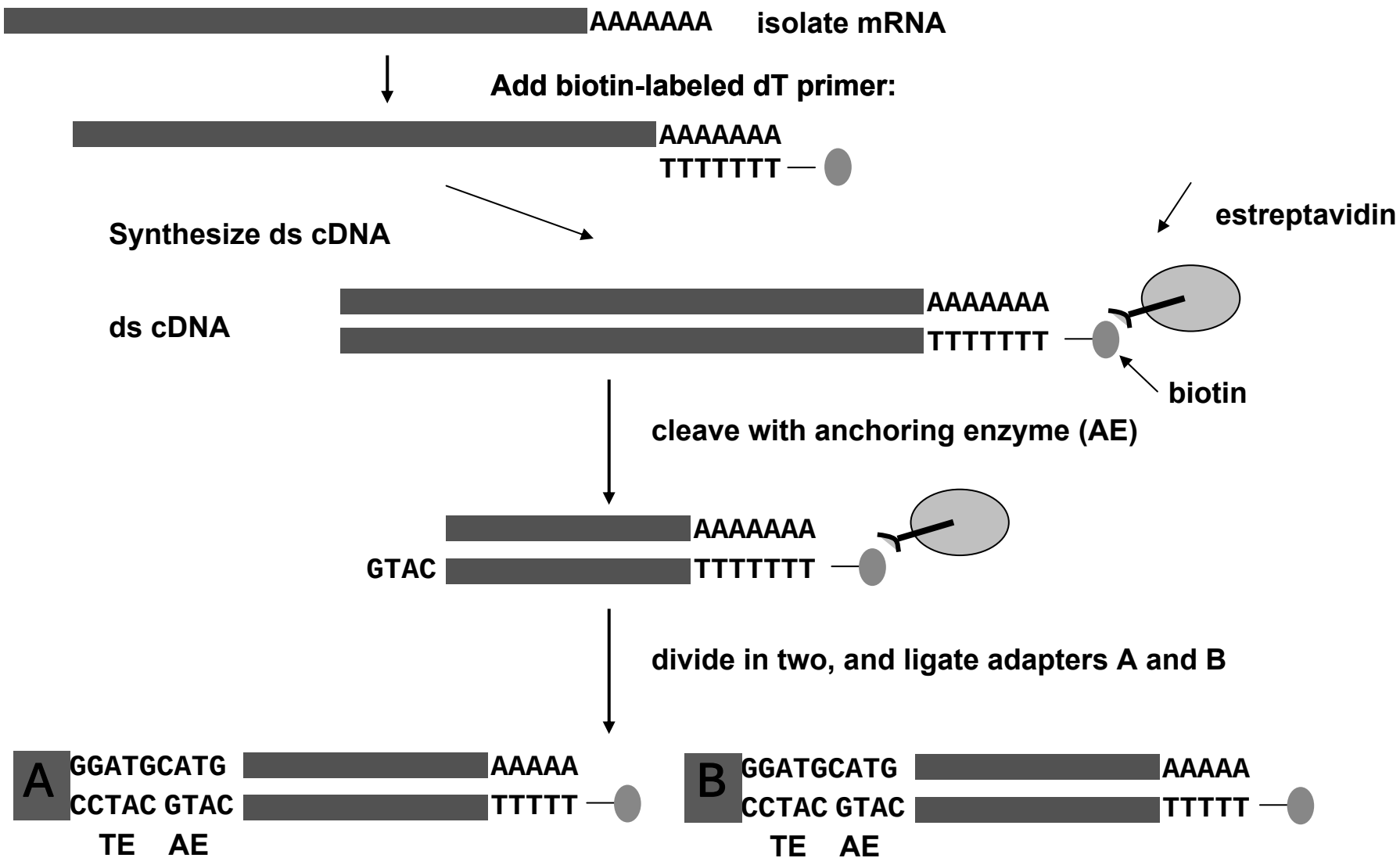
- RNA interference (RNAi)

Serial Analysis of Gene Expression (SAGE)

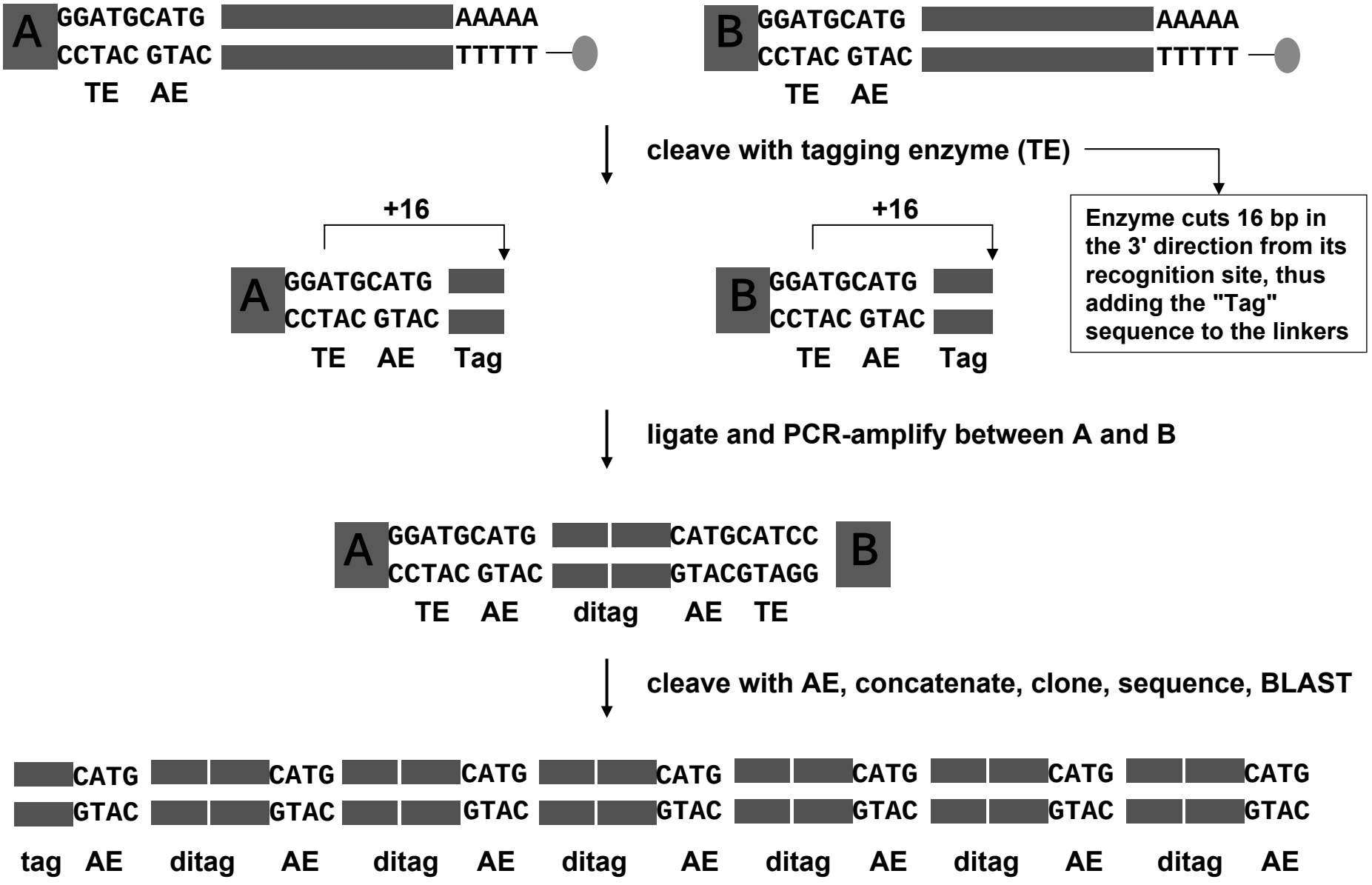
- Principio: Una secuencia de nucleótidos corta (tag) de 9 ó 10 pares de bases contiene suficiente información para identificar un transcrito
- Método para cuantificar niveles de expresión génica en muestras de células.
- Puede revelar sin sesgo los niveles de expresión de cientos de miles de genes. Sistema abierto.
- Los microarrays constituyen un sistema cerrado, sólo revelan la expresión de los genes sembrados en el array.

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

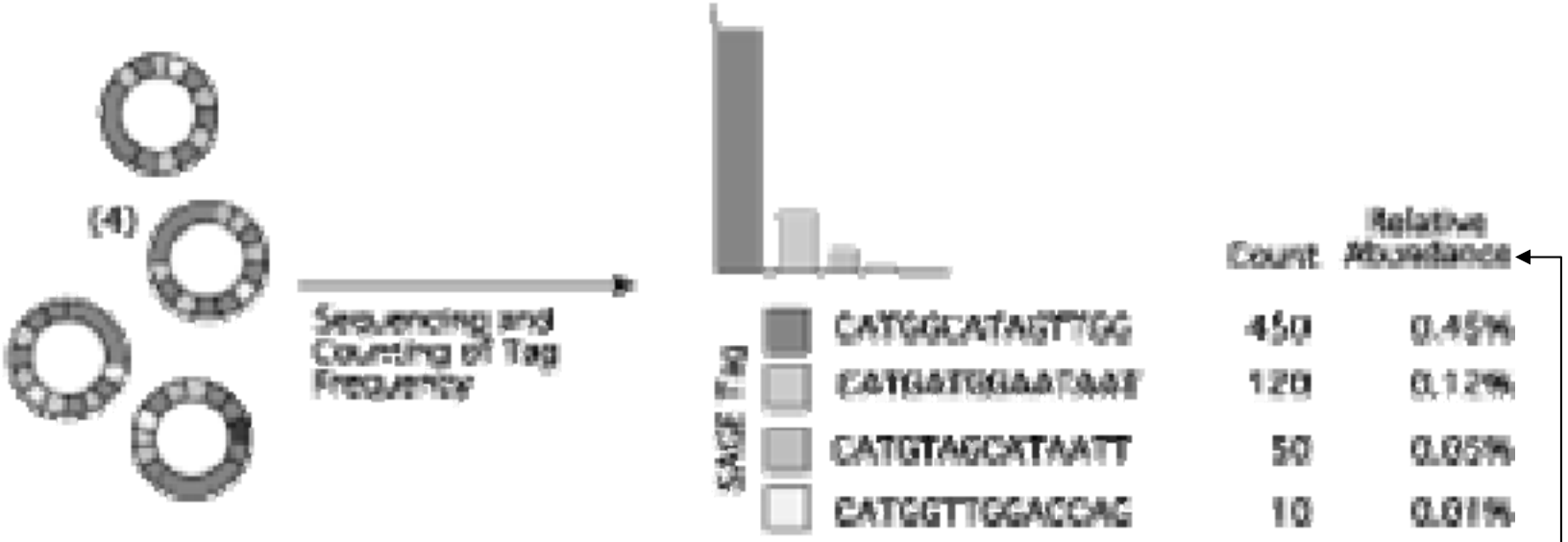
SAGE

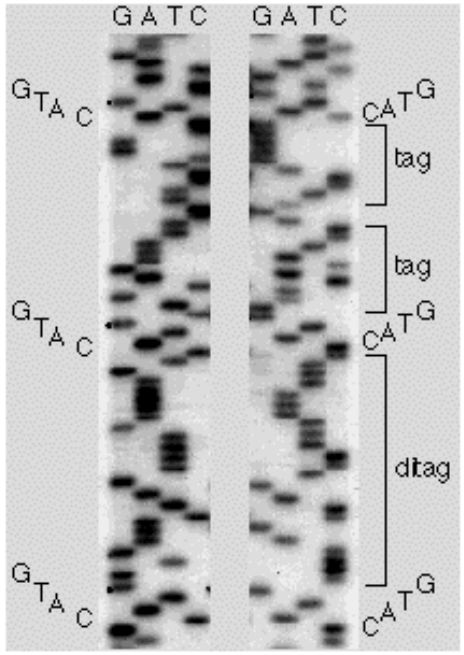

AAAAAAA
isolate mRNA
Add biotin-labeled dT primer:
AAAAAAA
TTTTTTT
estreptavidin
Synthesize ds cDNA
ds cDNA
AAAAAAA
TTTTTTT
biotin
cleave with anchoring enzyme (AE)
AAAAAAA
GTAC
TTTTTTT
divide in two, and ligate adapters A and B
A
GGATGCATG
AAAAA
CCTAC GTAC
TTTTT
TE AE
B
GGATGCATG
AAAAA
CCTAC GTAC
TTTTT
TE AE

SAGE


A
GGATGCATG
AAAAA
CCTAC GTAC
TTTTT
TE AE
B
GGATGCATG
AAAAA
CCTAC GTAC
TTTTT
TE AE
cleave with tagging enzyme (TE)
+16
A
GGATGCATG
CCTAC GTAC
TE AE Tag
+16
B
GGATGCATG
CCTAC GTAC
TE AE Tag
Enzyme cuts 16 bp in the 3' direction from its recognition site, thus adding the "Tag" sequence to the linkers
ligate and PCR-amplify between A and B
A
GGATGCATG
CCTAC GTAC
CATGCATCC
GTACGTAGG
B
TE AE ditag AE TE
cleave with AE, concatenate, clone, sequence, BLAST
CATG
GTAC
tag AE ditag AE ditag AE ditag AE ditag AE ditag AE ditag AE ditag AE

SAGE





Análisis de datos:
- Frecuencia de cada tag
- Alineamiento con secuencias en bases de datos

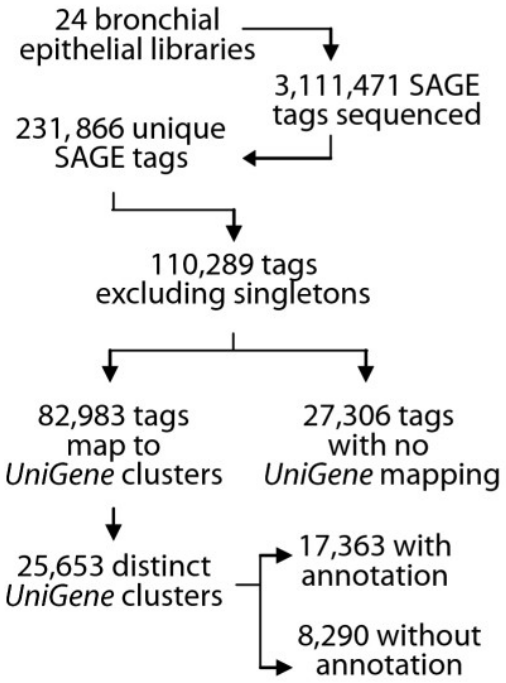
- **Locate the punctuation “CATG”**
- **Extract ditags of length 20-26 between the punctuation**
- **Discard duplicate ditags (including in reverse direction) - probably PCR artifacts**
- **Count occurrences of each tag**

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

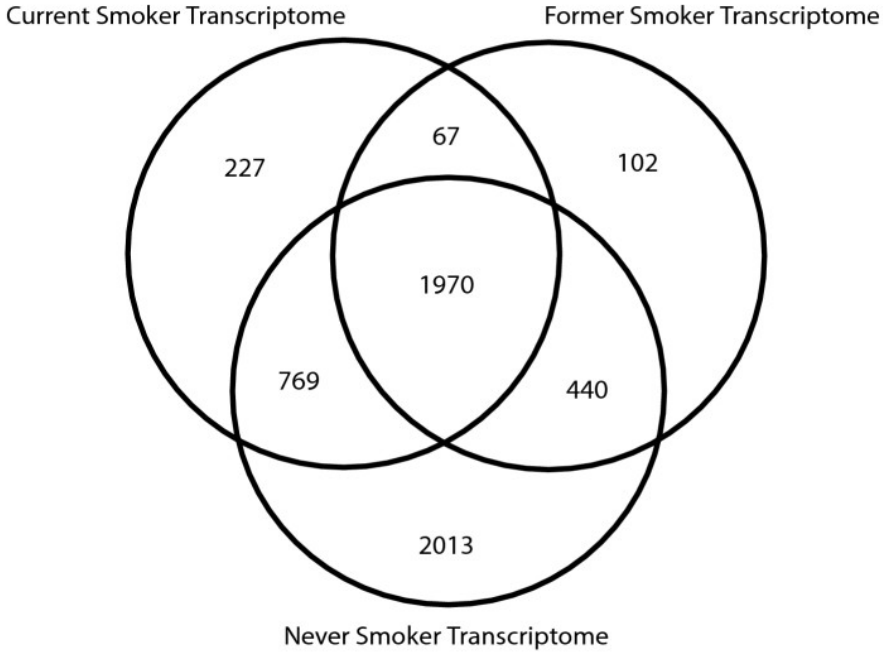
• ***Background***

Lung cancer is the most common cause of cancer-related deaths. Tobacco smoke exposure is the strongest aetiological factor associated with lung cancer. 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.

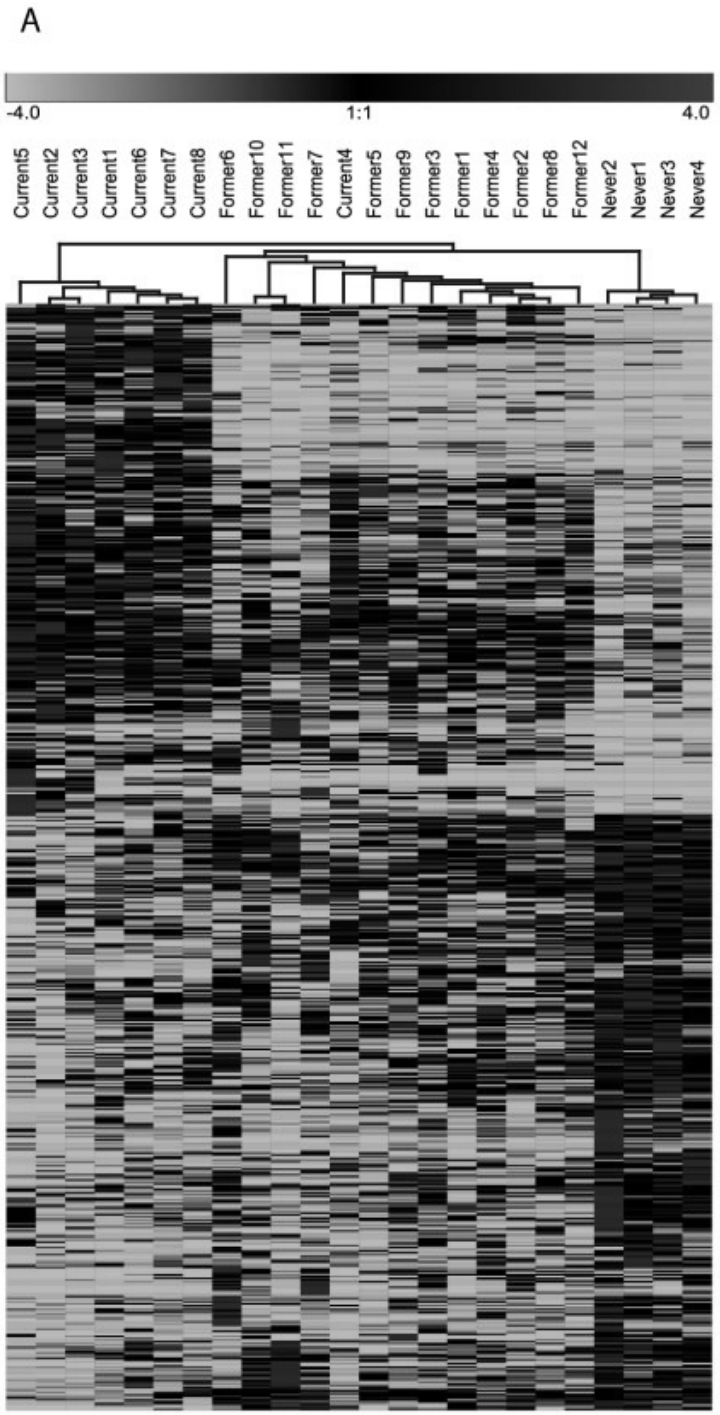
A

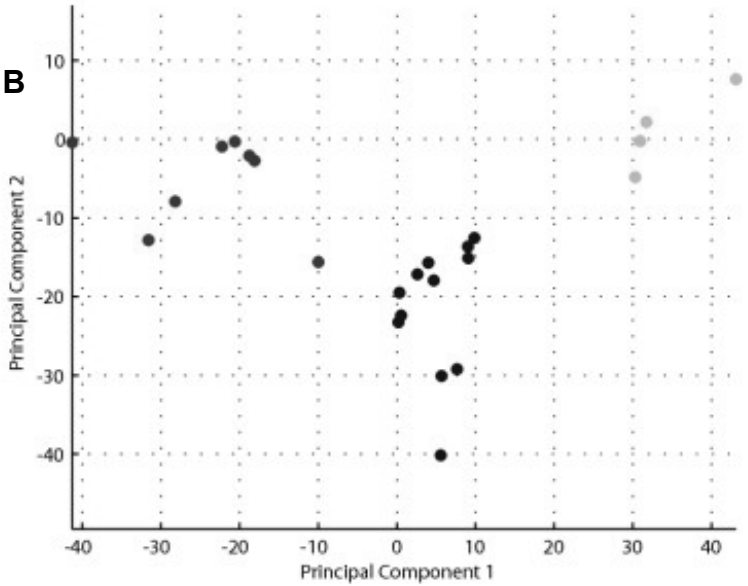


B



SAGE library statistics





(A) Cluster analysis of current, former and never smokers: Single link hierarchical clustering using 609 SAGE tags representing tags differentially expressed between current and never smokers. Distance measure used was a Euclidean distance. 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

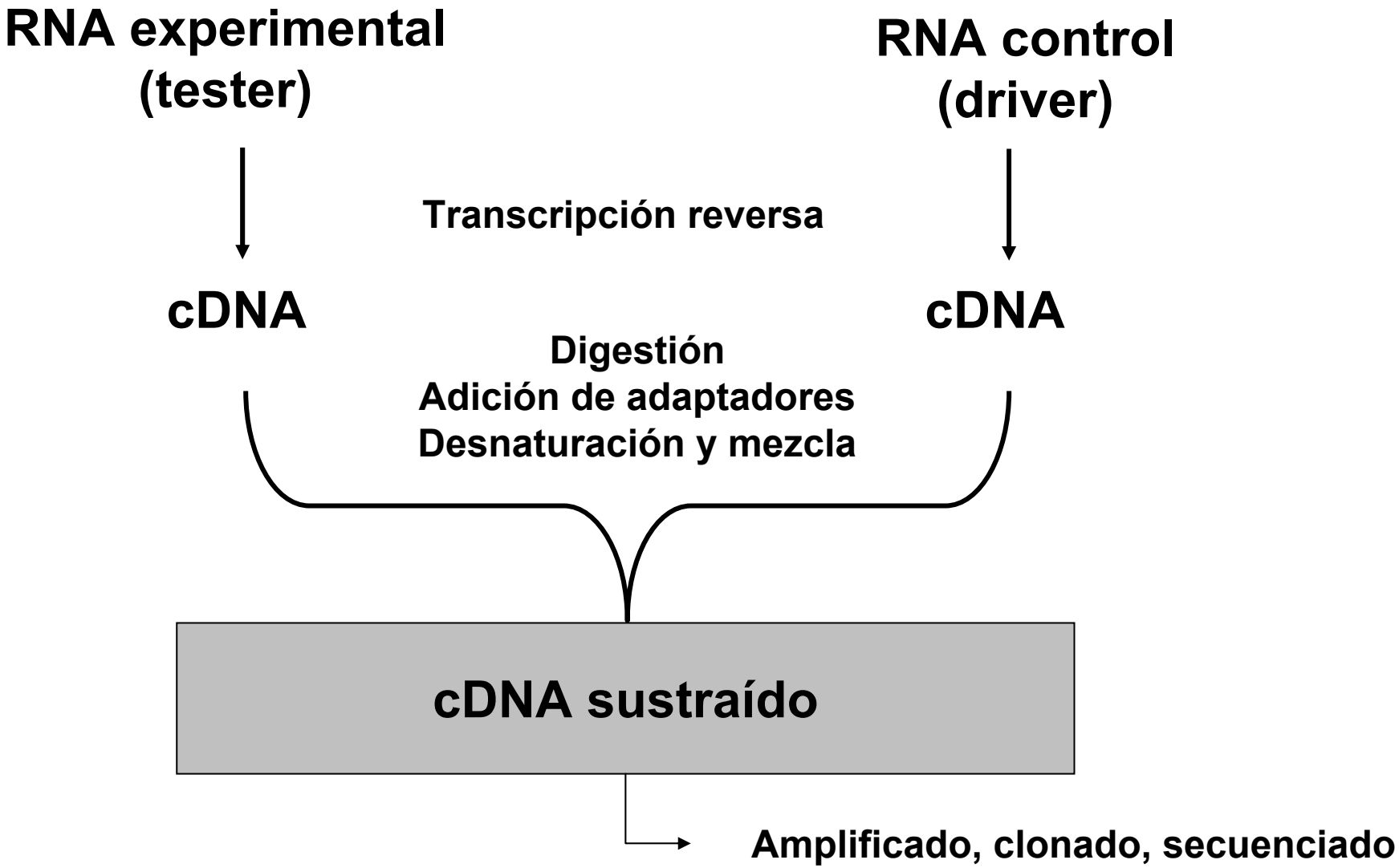
- This study represents the largest human SAGE study reported to date. Over three million SAGE tags were sequenced, representing over 110 thousand potentially unique transcripts expressed within the bronchial epithelium relative to cigarette smoke exposure.
- Based on the gene expression profiles of 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 reversibly expressed, three main functions were identified: cell cycle process and DNA repair.
- By comprehensively identifying gene expression changes that are reversible upon smoking cessation, we have introduced genes which may be investigated for polymorphisms, as those genes which are not sufficiently induced in response to smoking may identify candidate loci of susceptibility.
- Similarly, 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

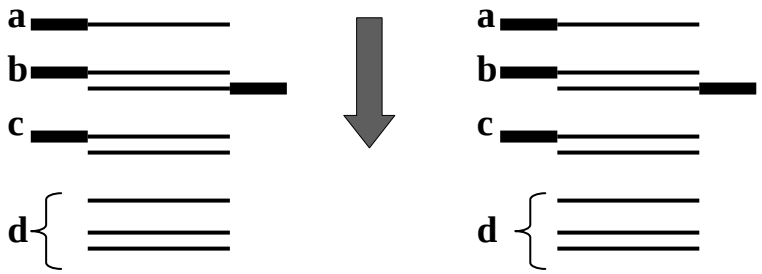
Hibridación Sustractiva por Supresión (SSH)



Hibridación Sustractiva por Supresión

Preparación de cDNAs, digestión con Rsa I, ligación con distintos adaptadores (1, 2R)

Primera hibridación → Con exceso de driver

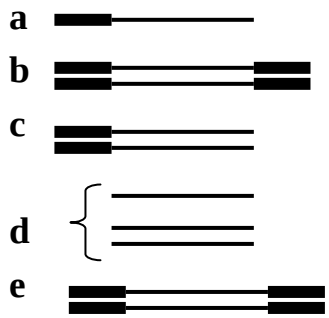


En este paso ocurre la normalización de cada población de tester, gracias a la formación de los respectivos híbridos

Segunda hibridación

(Con exceso de driver, y sin desnaturar)

Completar los extremos (75 ºC)



Amplificación por PCR con primers

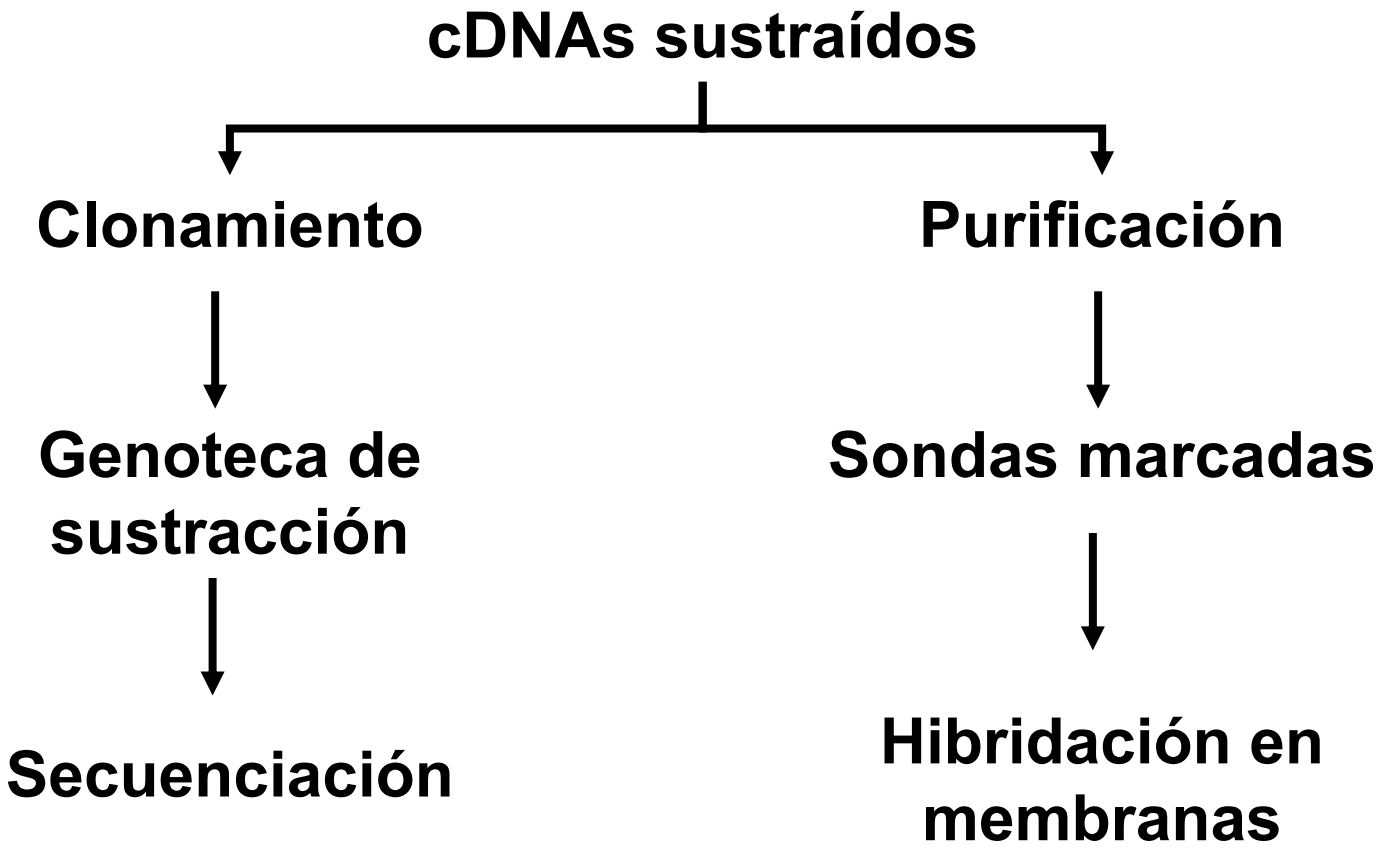
a, d: no amplifican

b: supresión por PCR

c: amplificación lineal

e: amplificación exponencial

Hibridación Sustractiva por Supresión



Hibridación Sustractiva por Supresión

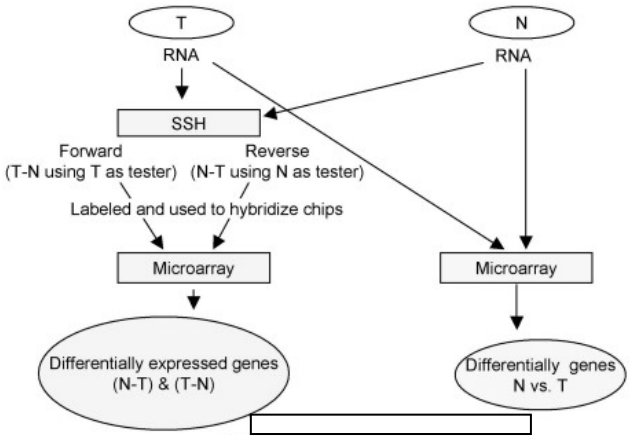
Ventajas

- Permite enriquecer en secuencias poco comunes

Desventajas

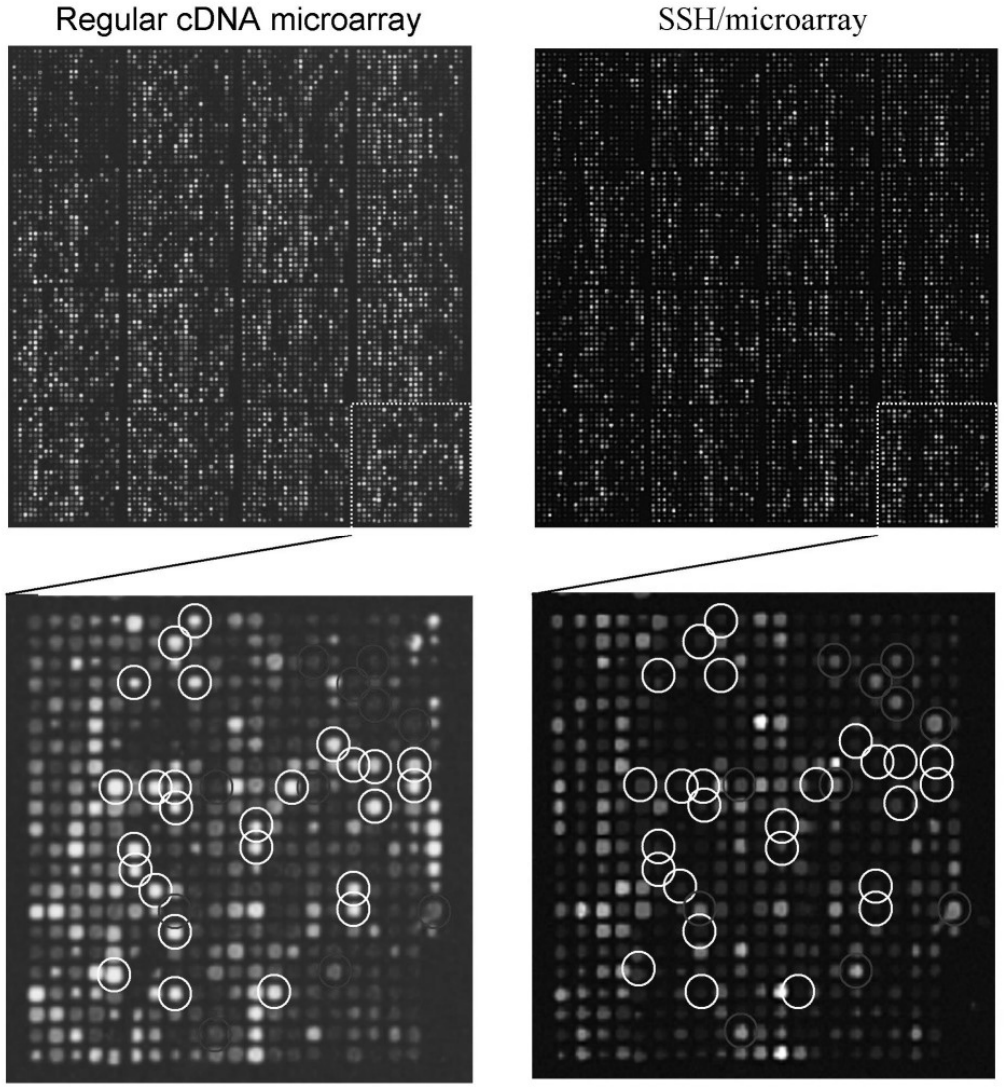
- Requiere 1-2 μg de mRNA
- No genera cDNAs de largo completo

Hibridación Sustractiva por Supresión



mRNA was prepared from hepatoma (T) and non-hepatoma liver tissues (N). Subjected to (1) SSH followed by using the resulted subtracted cDNAs as targets for cDNA microarray analysis and (2) conventional cDNA microarray analysis. SSH was performed in both the forward (T as tester) and reverse (N as tester) direction to enrich up-regulated (T-N amplicon) as well as down-regulated transcriptomes (N-T amplicon). The two subtracted amplicons were labeled with fluorescent cy-dyes as targets for microarray analysis. The results thus obtained were then compared to those obtained from the conventional cDNA microarray assays.

Human 15 K chip, 12,530 distinct genes



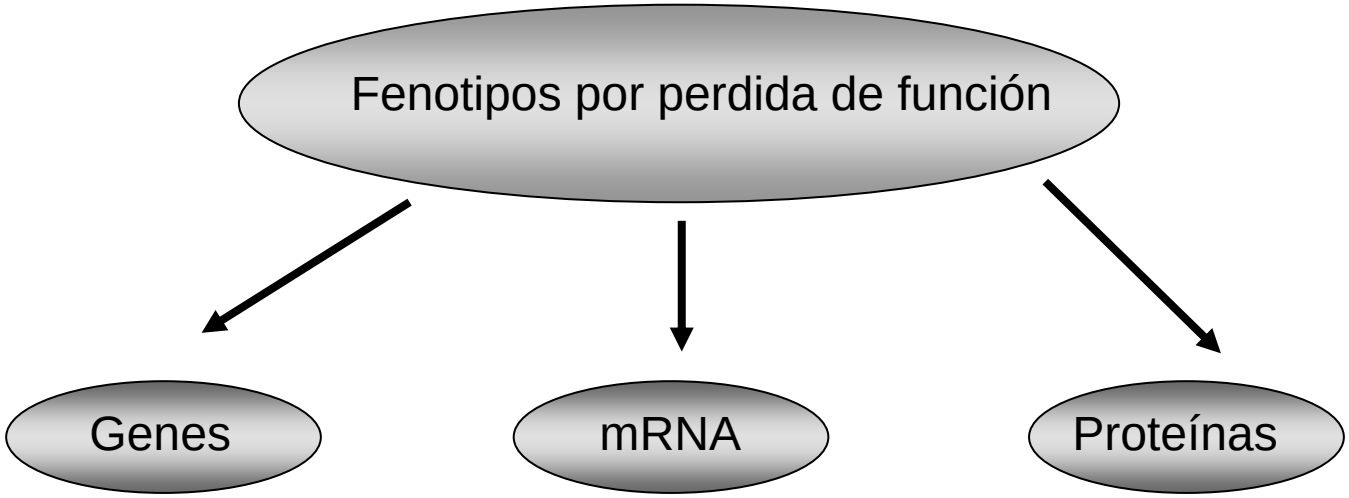
Differentially profiling the low-expression transcriptomes of human hepatoma using a novel SSH/microarray approach. Pan et al. BMC Genomics 7:131, 2006

- 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%
  - 95% of mRNA diversity
  - 1-10 molecules per cell per sequence

Eficiencia de la Hibridación Sustractiva por Supresión

- Diatchenko y cols., 1996; pudieron enriquecer en 1000-5000 veces transcritos poco abundantes en la muestra.
- Un factor crítico es la concentración relativa de un transcrito en las poblaciones de tester y driver
- Un enriquecimiento efectivo ocurre cuando:
  - El transcrito está presente > 0.01%

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.**

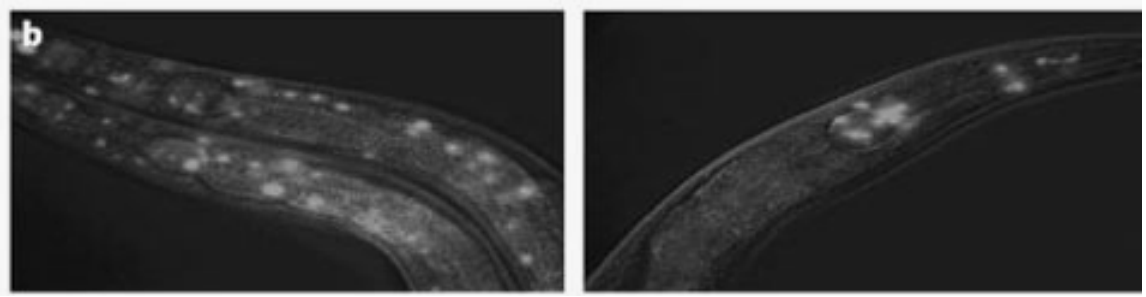
- **Evolutivamente conservado entre los eucariontes.**

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

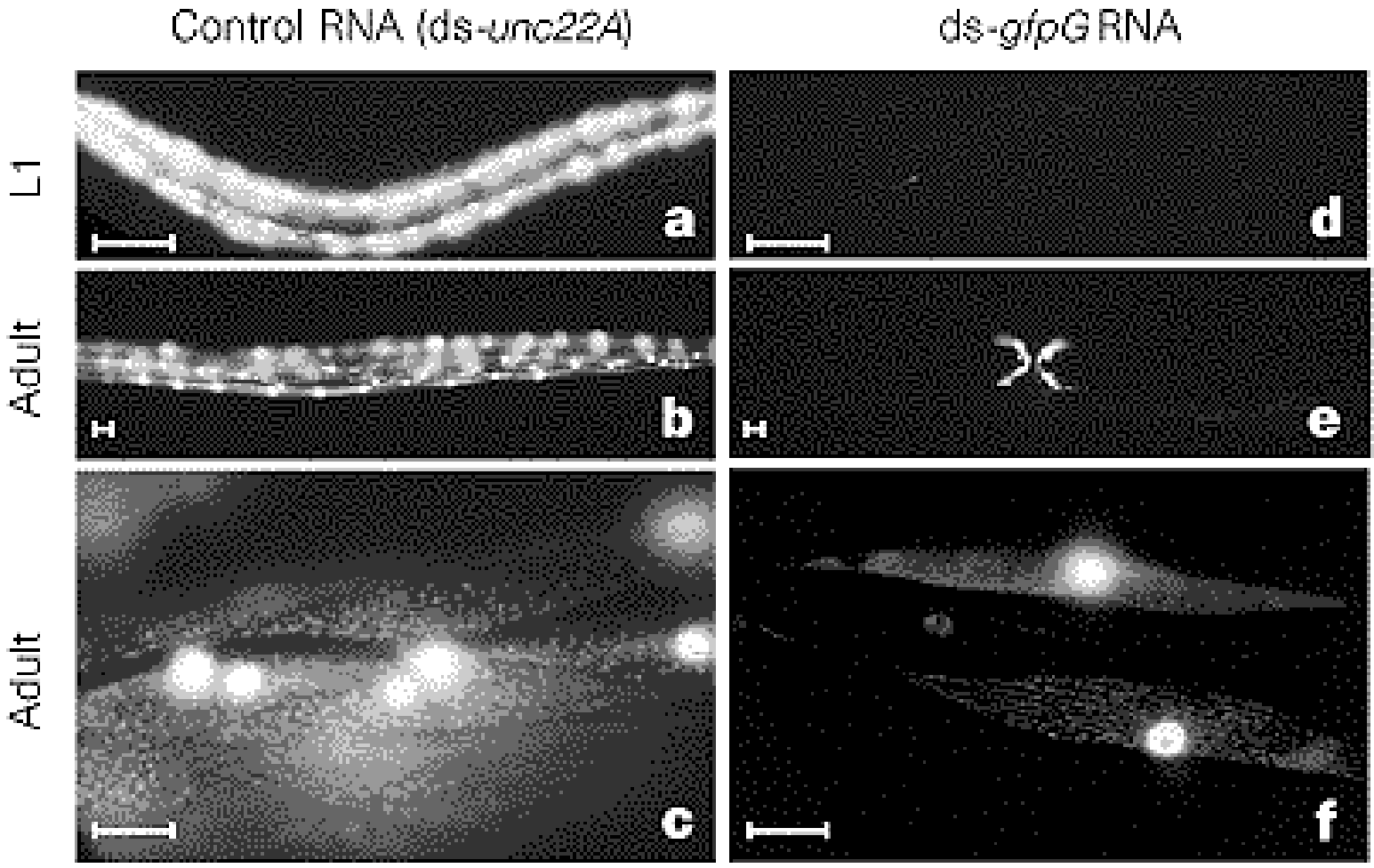
Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

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

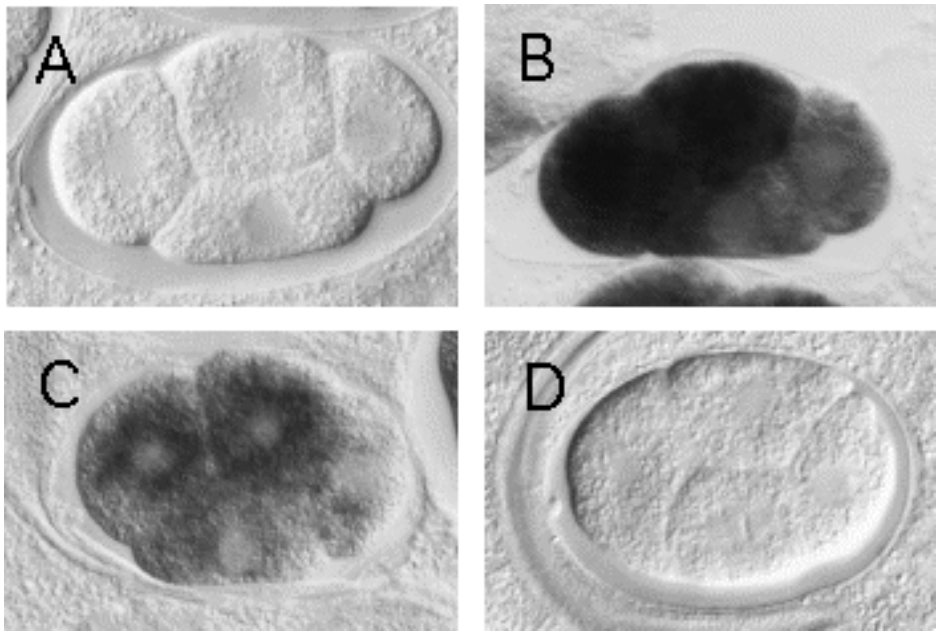
Nature 391:806-811, 1998



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

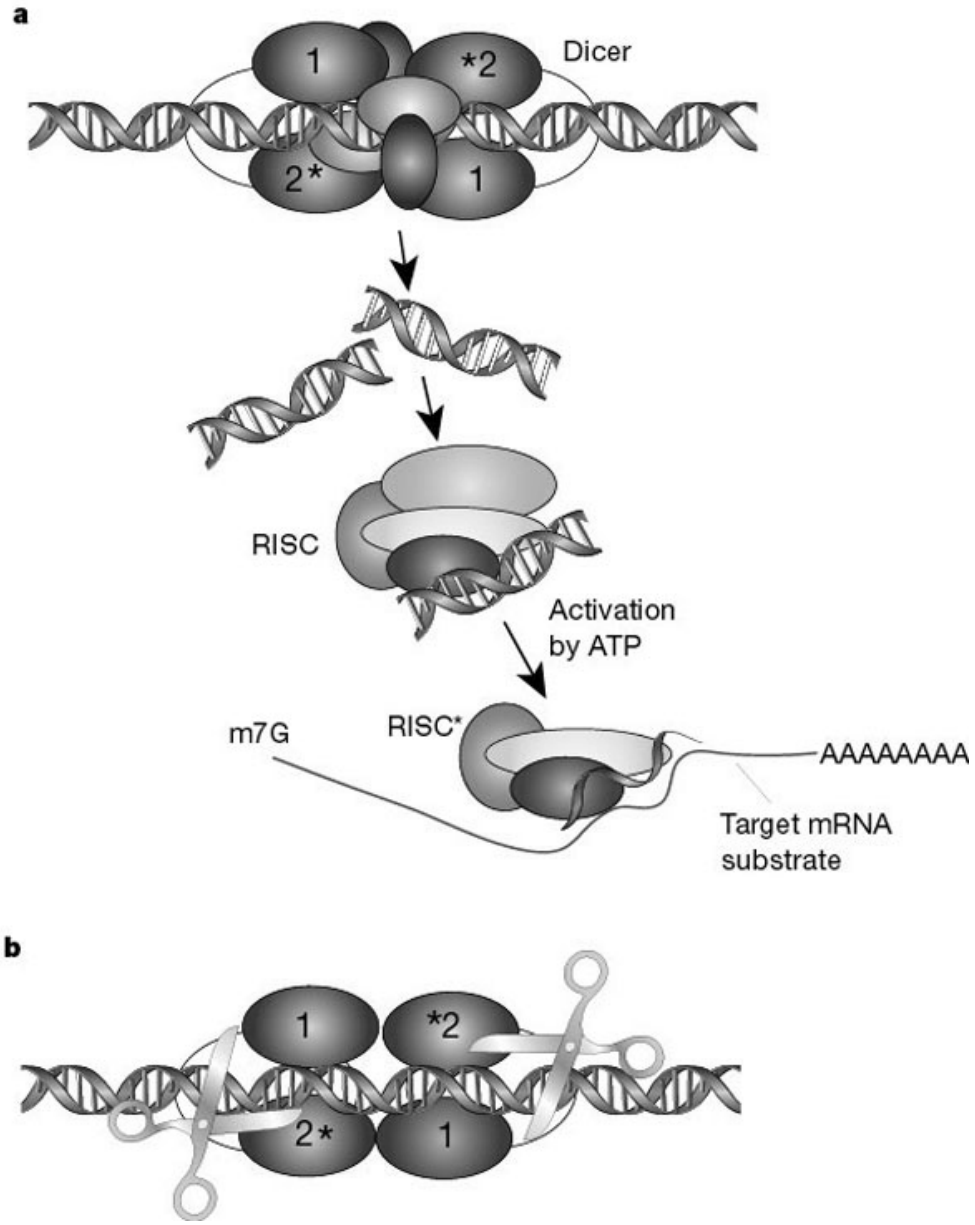
Iniciación

- **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.**

Mecanismo propuesto



La endonucleasa Dicer rompe el dsRNA para generar fragmentos de ~22 nt.
Requiere ATP
Miembro de la familia de Rnase III

Los siRNAs son incorporados en el complejo RISC.
Los siRNAs son desenrollados en una reacción dependiente de ATP.

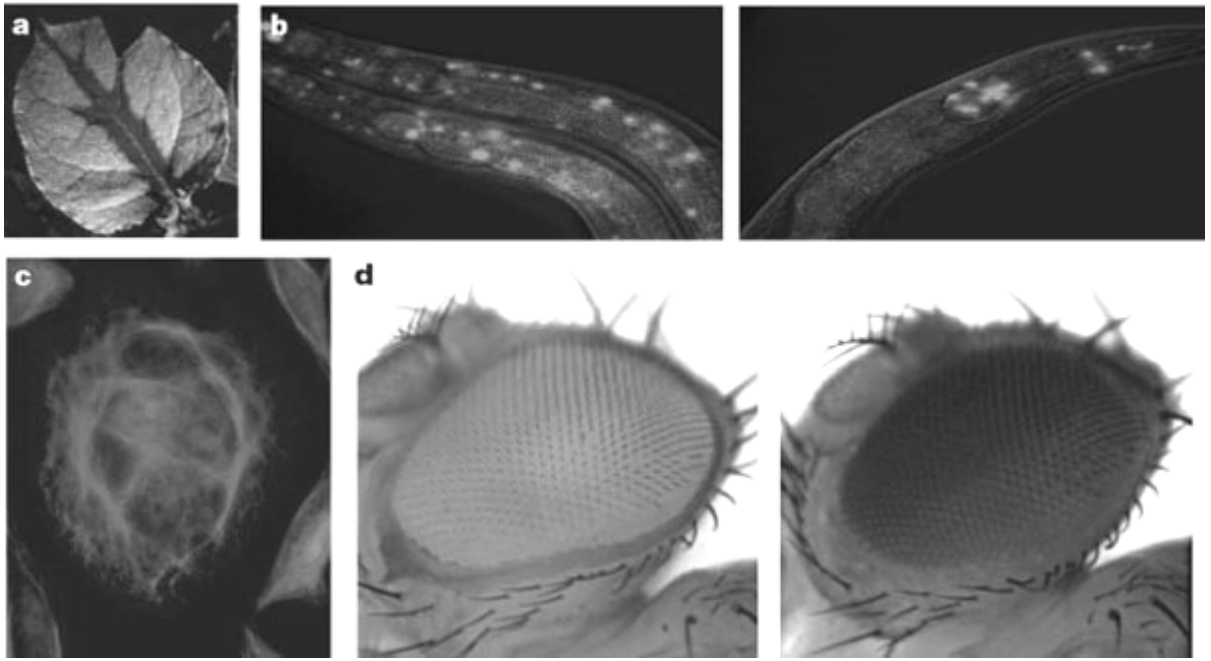
Esto activa a RISC*, el cual utiliza a los siRNAs como guías para la selección del sustrato.

RNA-induced silencing complex (RISC)

Reconoce y destruye los mRNAs blanco

Compuesto de:

- **siRNA: Identifica los sustratos mediante apareamiento de bases.**
- **Endonucleasa: Dicer**
- **Exonucleasa: Slicer**
- **Proteínas *Argonaute*: Muy conservadas presentan dos dominios estructurales PAZ (Piwi/Argonaute/Zwille) y Piwi en el C-terminal. Necesarias para ensamblar el complejo.**



In plants, silencing can be triggered, for example, by engineered RNA viruses or by inverted repeat transgenes. In worms, silencing can be triggered by injection or feeding of dsRNA. In both of these systems, silencing is systemic and spreads throughout the organism. **a**, A silencing signal moves from the veins into leaf tissue. Green is green fluorescent protein (GFP) fluorescence and red is chlorophyll fluorescence that is seen upon silencing of the GFP transgene. **b**, *C. elegans* engineered to express GFP in nuclei. Animals on the right have been treated with a control dsRNA, whereas those on the left have been exposed to GFP dsRNA. Some neuronal nuclei remain florescent, correlating with low expression of a protein required for systemic RNAi. **c**, HeLa cells treated with an ORC6 siRNA and stained for tubulin (green) and DNA (red). Depletion of ORC6 results in accumulation of multinucleated cells. Stable silencing can also be induced by expression of dsRNA as hairpins or snap-back RNAs. **d**, Adult *Drosophila* express a hairpin homologous to the white gene (left), which results in unpigmented eyes compared with wild type (right).

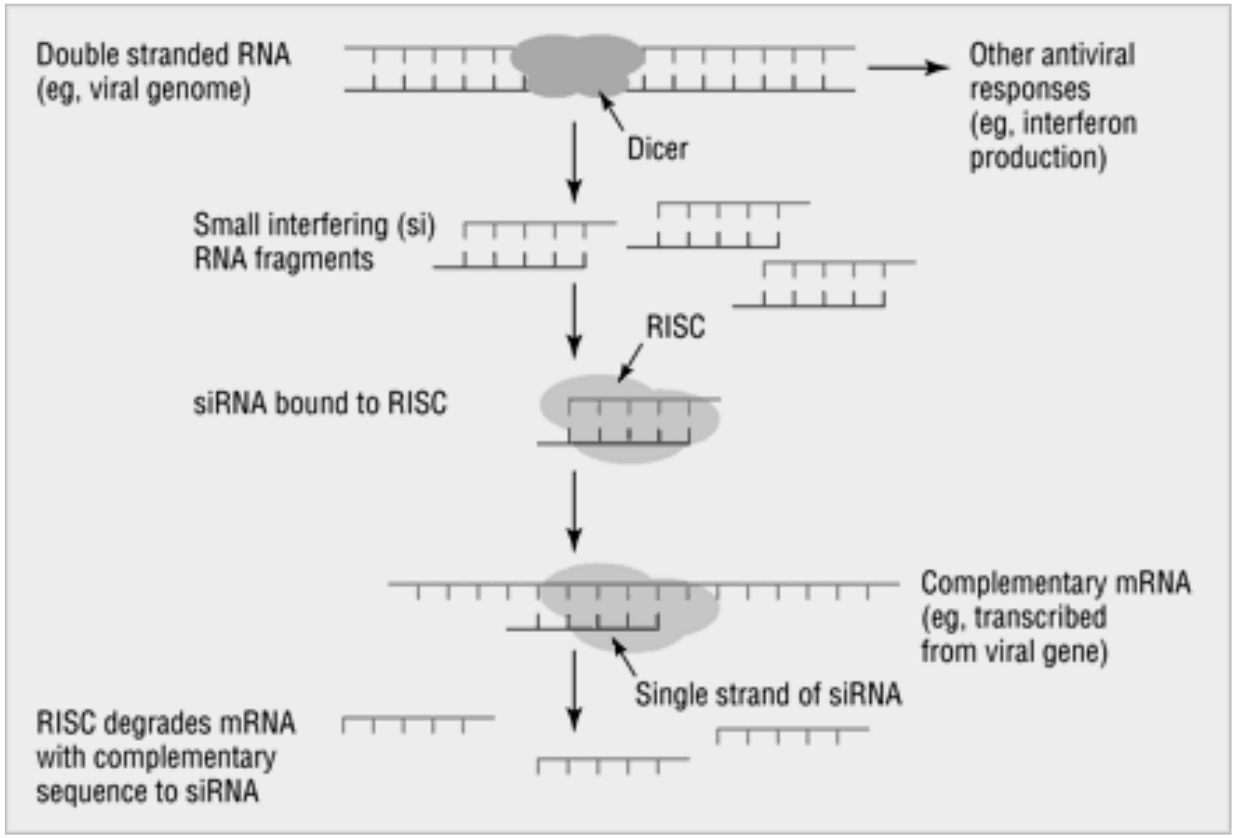


Fig 1 Natural mechanism of RNA interference. The appearance of double stranded (ds) RNA within a cell—for example, as a result of viral infection—triggers an RNA interference response. The cellular enzyme dicer binds to the dsRNA and cuts it into short pieces of 20 or so nucleotide pairs in length known 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

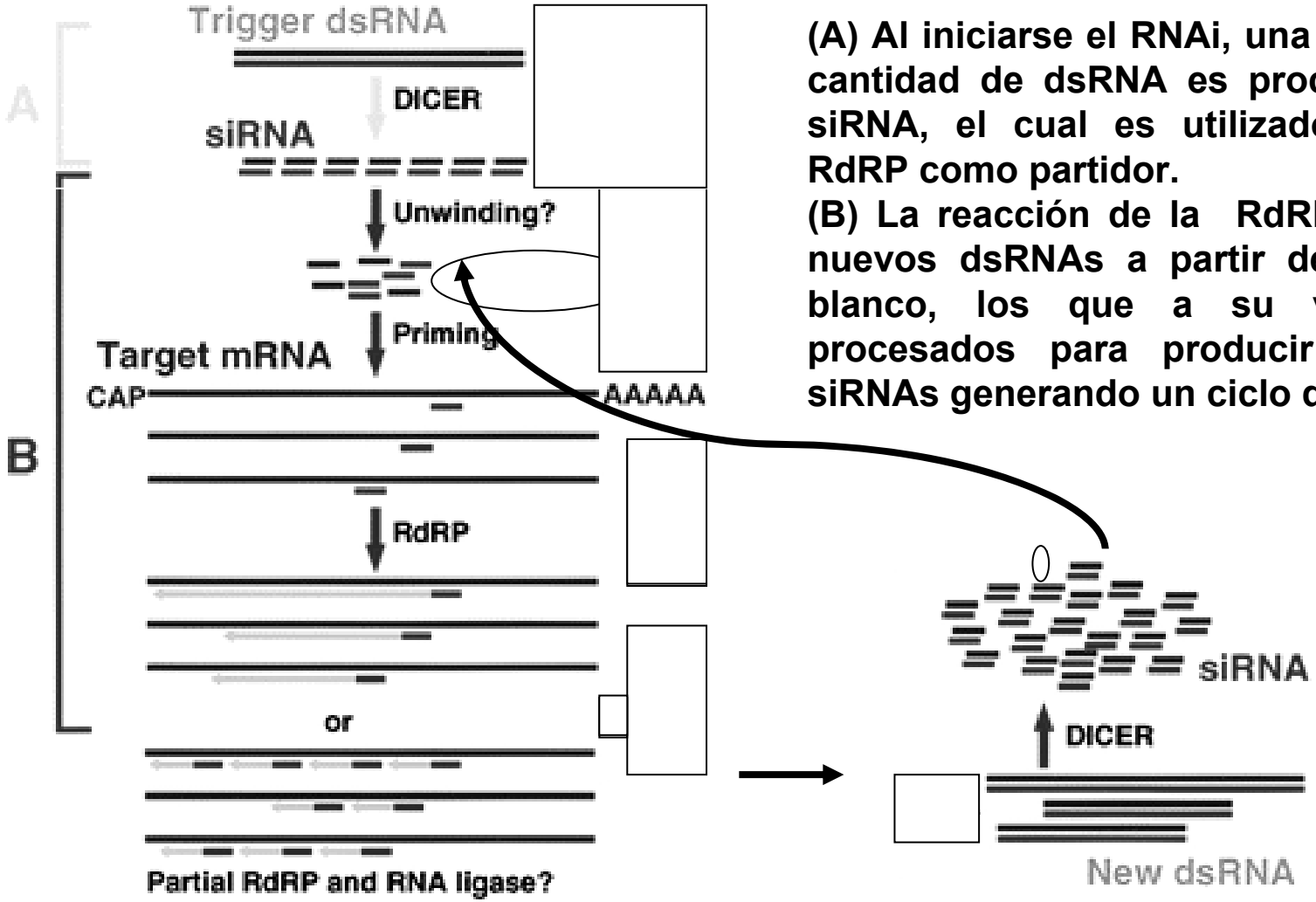
RNA interferente (RNAi)
Propiedades

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.

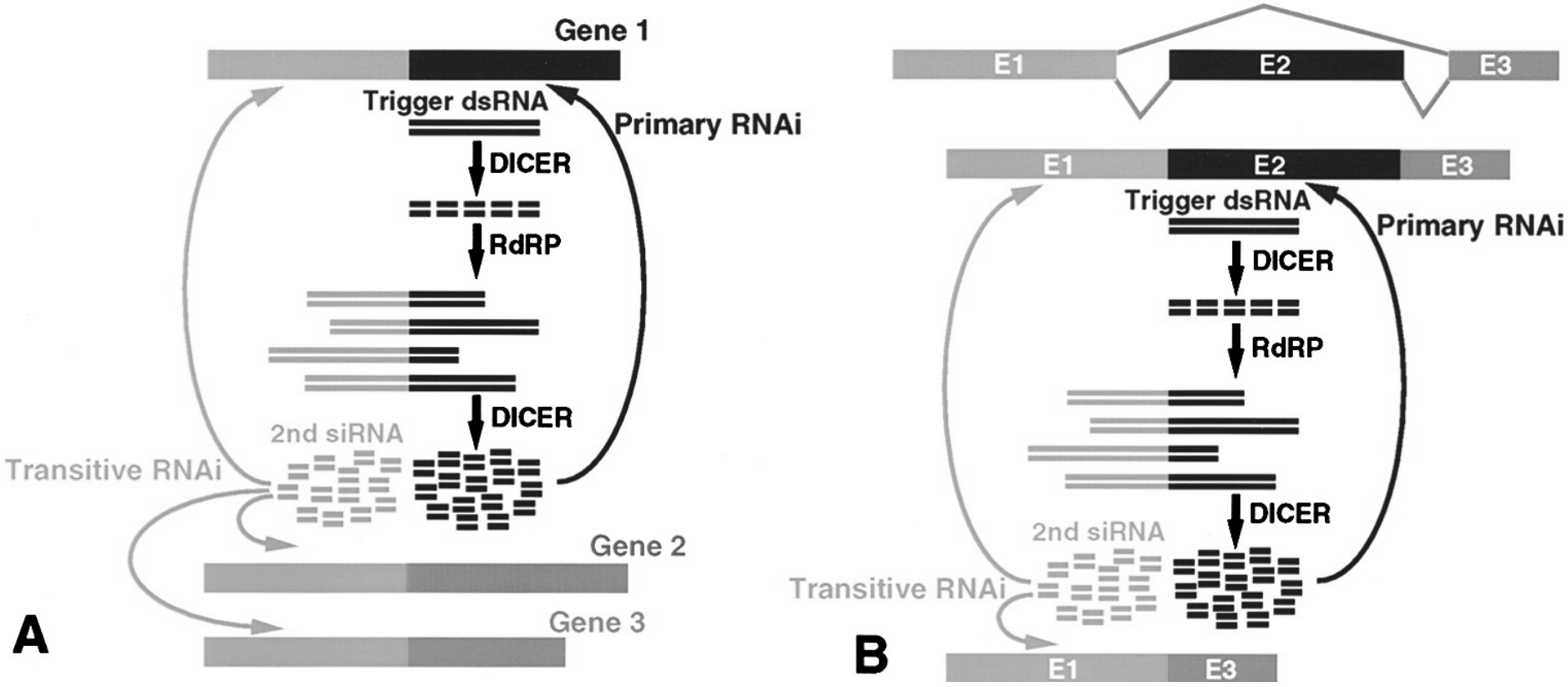
RNA interferente (RNAi)
Propiedades

Transitividad

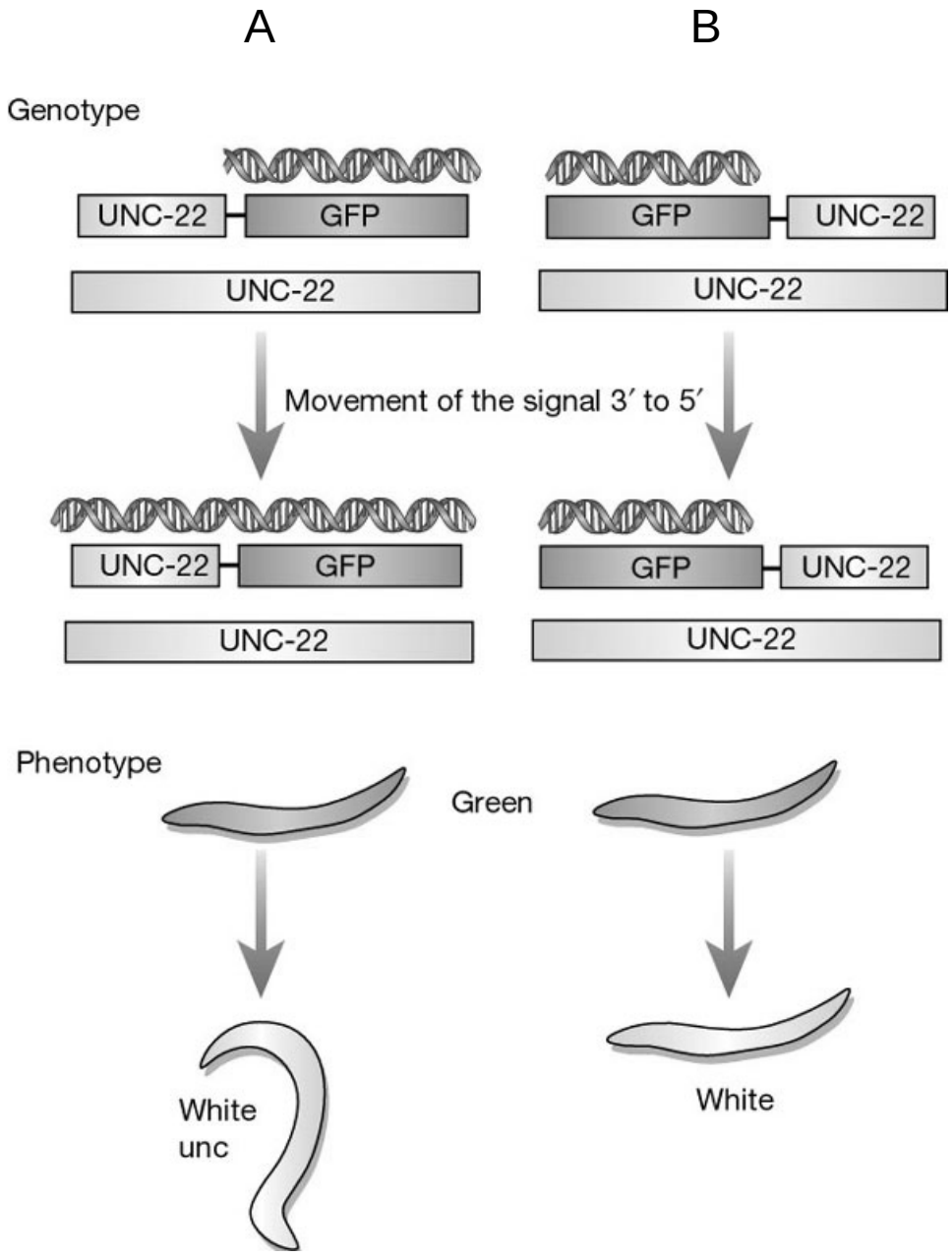
La polaridad determinada por la reacción de amplificación de la RdRP predice que:

- **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:

A) 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).

B) El transcrito de GFP fusionado al extremo 5' de UNC-22. Los dsRNA para GFP eliminan la fluorescencia pero no generan el fenotipo alterado.

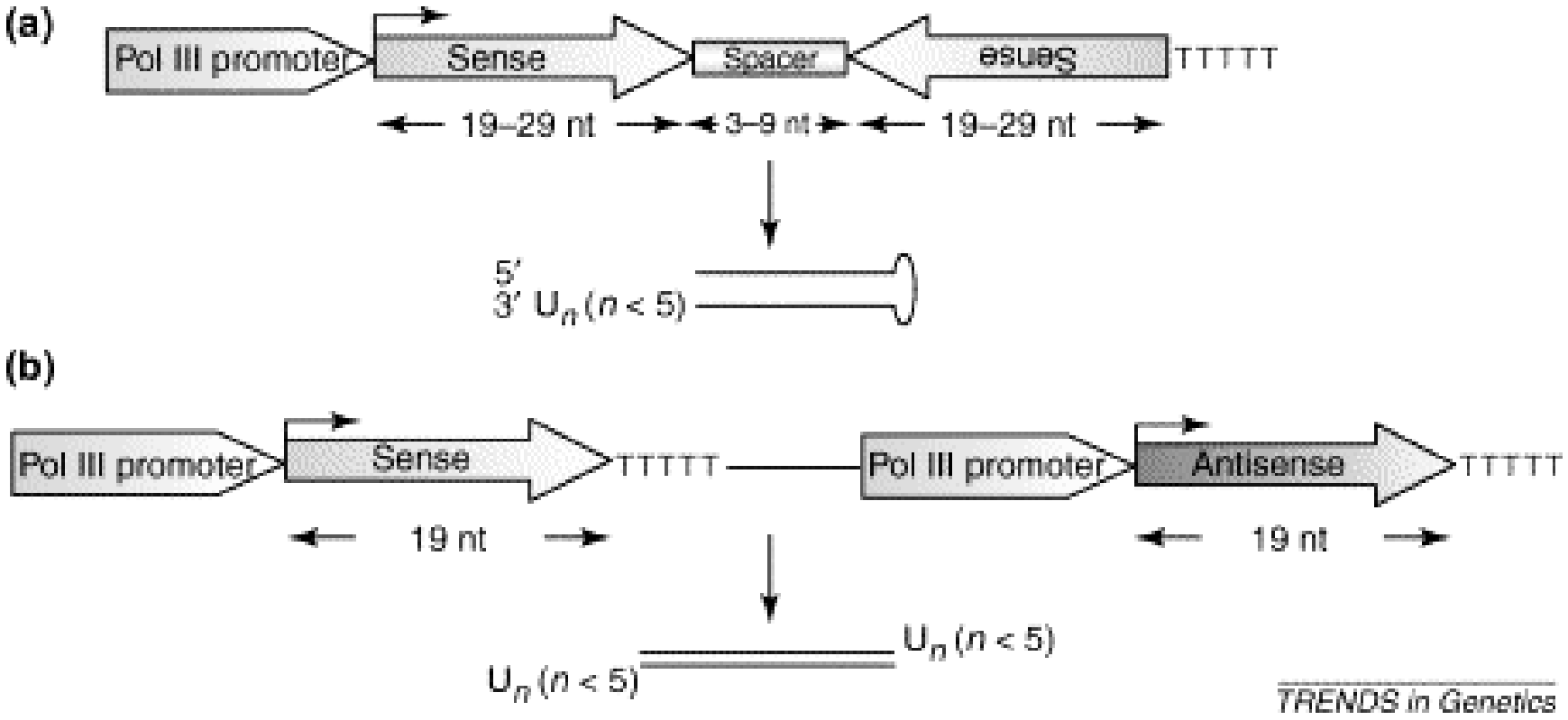
RNA interference (RNAi)

En células de mamíferos

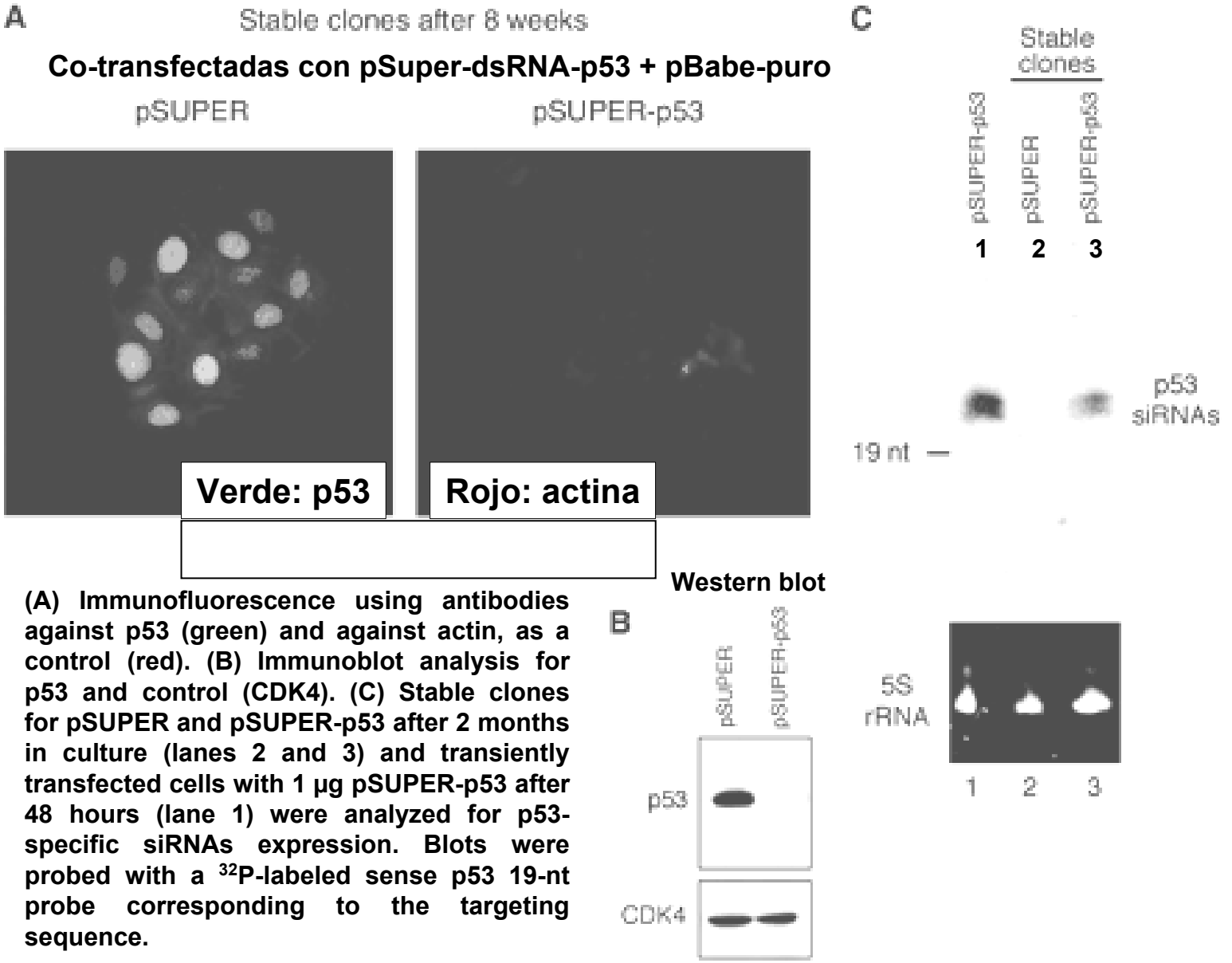
A System for Stable Expression of Short Interfering RNAs in Mammalian Cells

Thijn R. Brummelkamp, Rene Bernards, Reuven Agami

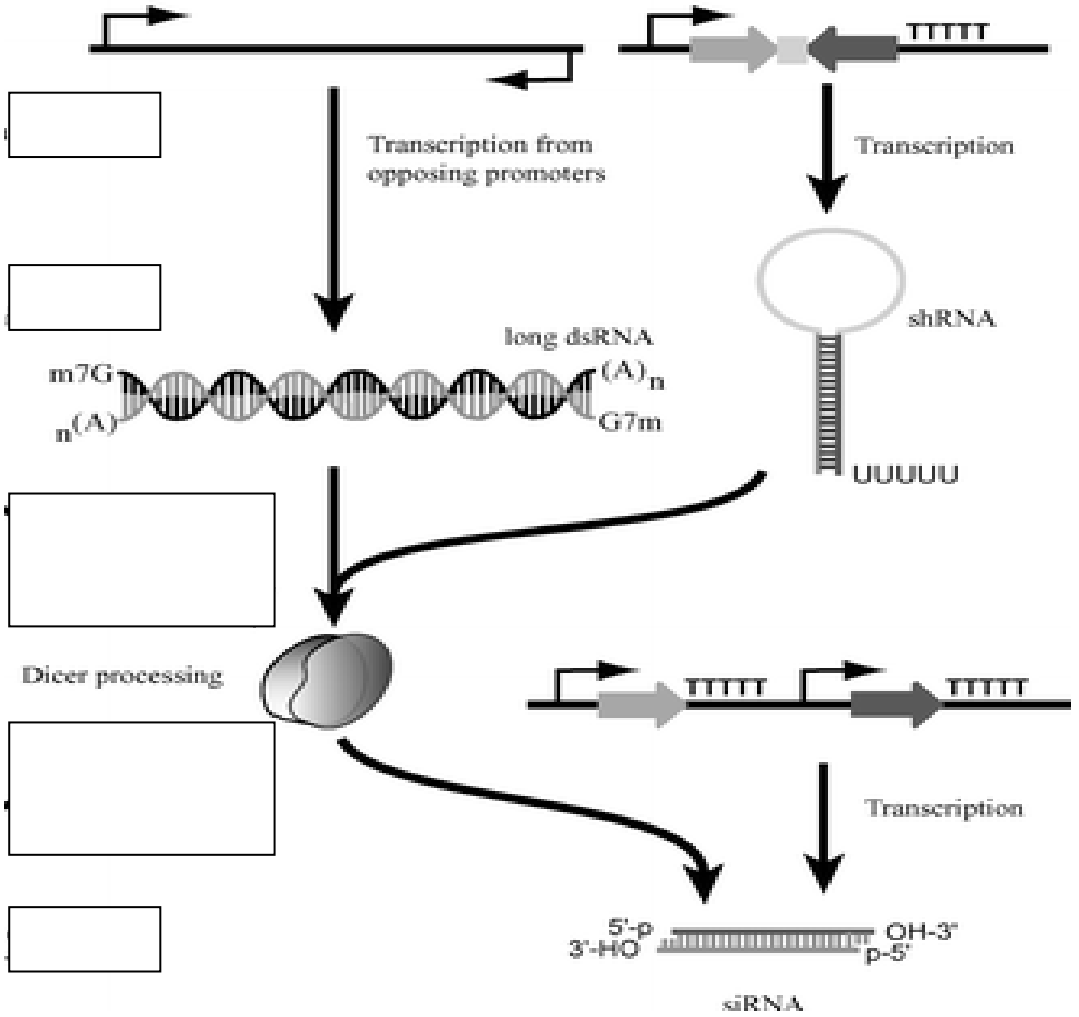
Science 296:550-553, 2002



The DNA vector-based RNA interference (RNAi) technology. (a) Generation of a hairpin siRNA directed by a Pol III promoter. An inverted repeat is inserted at the +1 position of the U6 promoter (-351 to +1). The individual motif is 19–29 nt, corresponding to the coding region of the gene of interest. The two motifs that form the inverted repeat are separated by a spacer of three to nine nt. The transcriptional termination signal of five Ts are added at the 3' end of the inverted repeat. The resulting RNA is predicted to fold back to form a hairpin dsRNA as shown. The resulting siRNA starts with either a G or an A at the 5' end, dependent on the promoter used (U6 or H1) and ends with one to four uridines, forming a 3' overhang that is not complementary to the target sequences. (b) Generation of two complementary siRNA strands synthesized by two U6 promoters. Two U6 promoters either placed in tandem or on two separate plasmids (not shown) direct transcription of a sense and an antisense strand of 19-nt RNAs. The two RNA strands are predicted to form a duplex siRNA in the transfected cells, with 3' overhangs of one to four uridines.

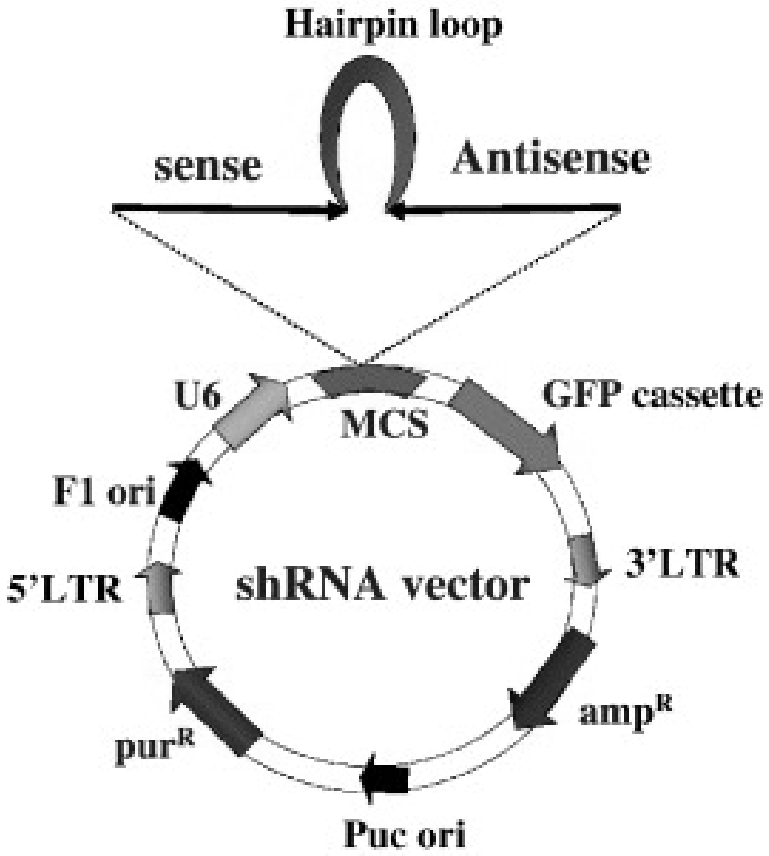


(A) Immunofluorescence using antibodies against p53 (green) and against actin, as a control (red). (B) Immunoblot analysis for p53 and control (CDK4). (C) Stable clones for pSUPER and pSUPER-p53 after 2 months in culture (lanes 2 and 3) and transiently transfected cells with 1 µg pSUPER-p53 after 48 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.

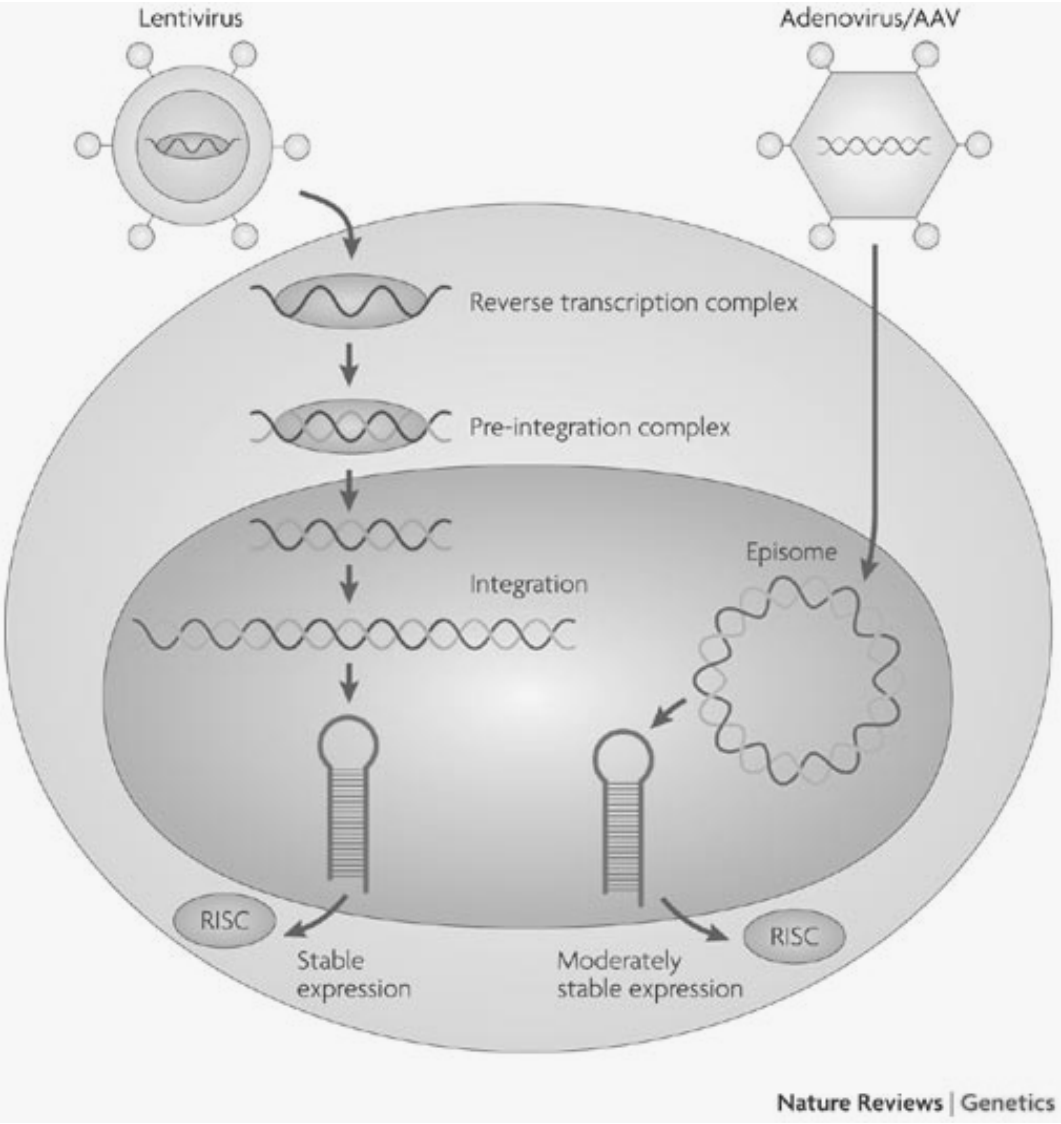


Strategies to introduce siRNA in cells. Dicer can cleave exogenously introduced substrates. Hairpin RNA that can be introduced directly into the cells or can be produced by transcription from plasmid or viral vectors. An RNAi response can also be generated by introducing in vitro synthesized siRNAs or by expressing individual sense and antisense strands of the siRNA from a vector containing tandem promoters

Dykxhoorn & Lieberman. 2005. The silent revolution**:** RNA Interference as Basic Biology, Research Tool, and therapeutic. Annu. Rev. Med. 56:401-423.

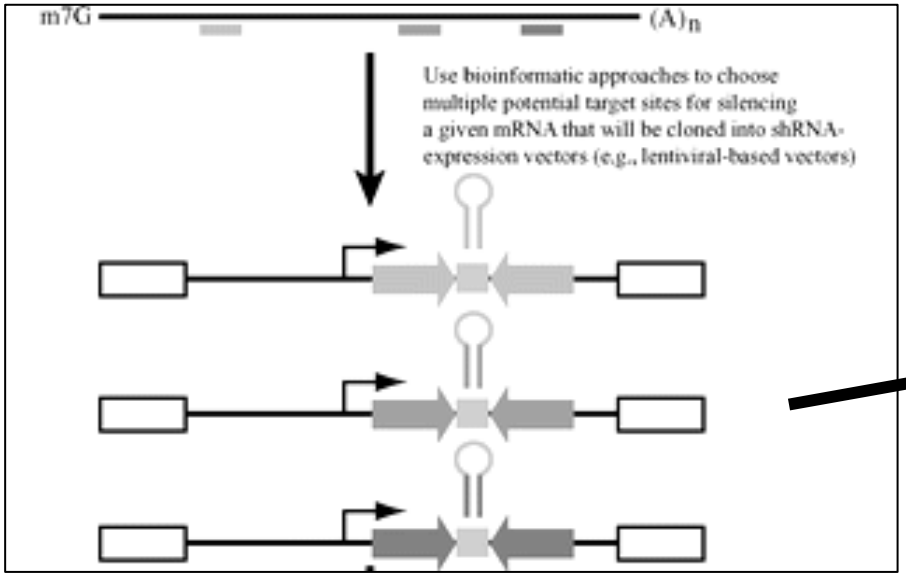


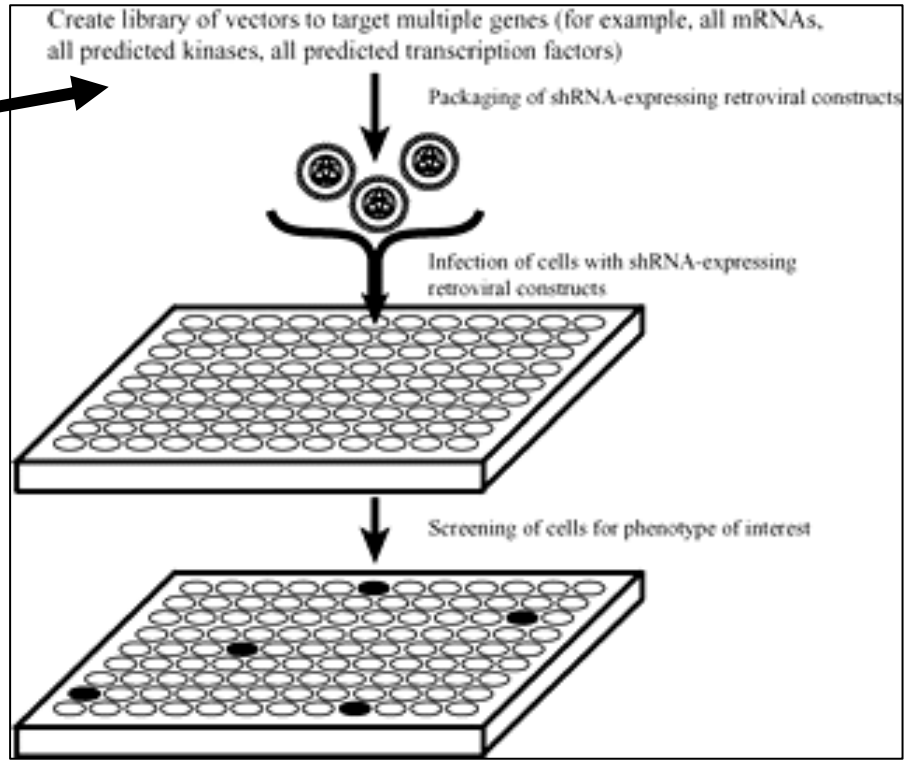
Representation of a typical shRNA viral based vector system. Shown here are two expression cassettes, one with a U6 promoter for the expression of the shRNA and a GFP cassette to mediate reporter gene expression. puc ori-origin of replication ; F1 ori — origin of replication; ampR — ampicillin resistance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



Lentiviral vectors are used to deliver therapeutic, short hairpin RNA (shRNA)-expressing transgenes that integrate into the genome for stable shRNA expression. Adenoviral and adeno-associated virus (AAV) vectors fail to integrate their transgenes into the genome but instead express shRNAs episomally for moderately stable levels of shRNA expression. RISC, RNA-induced silencing complex

Genomic RNAi screens in mammalian cells.



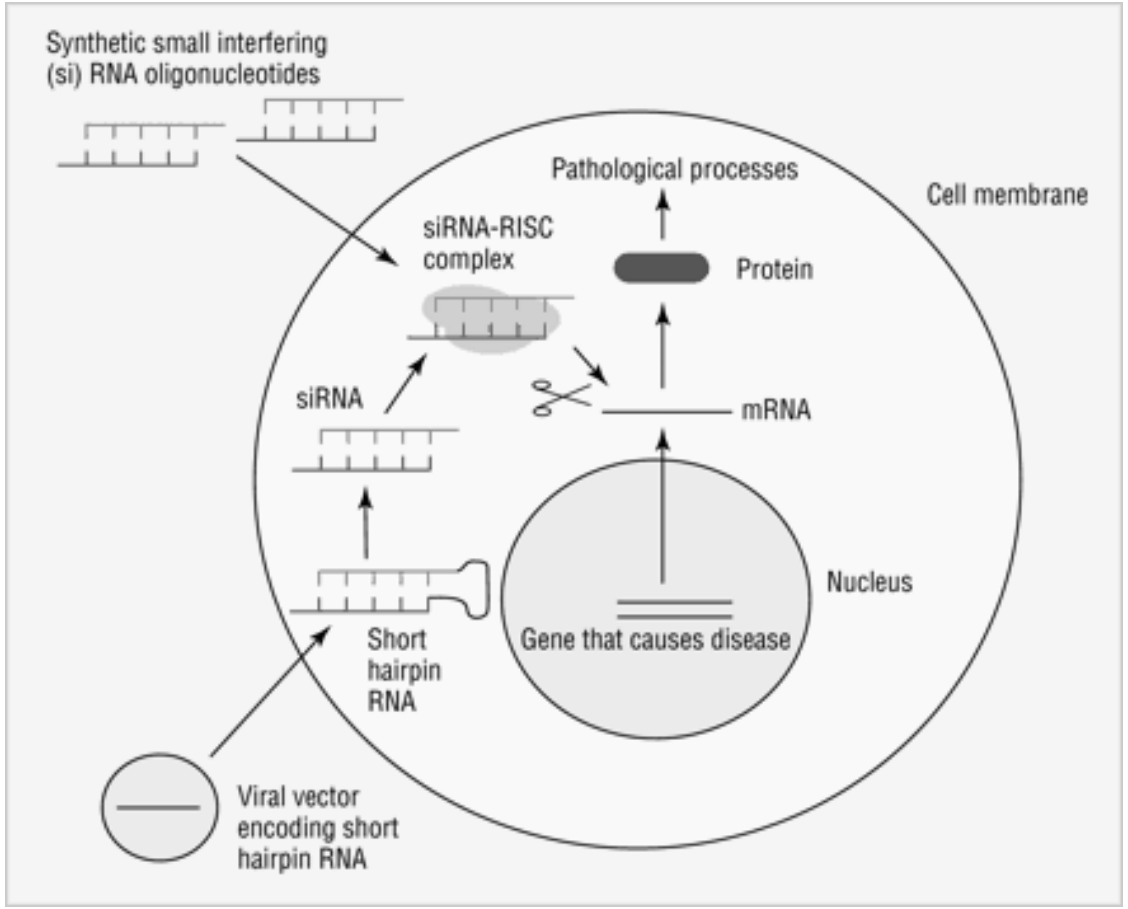


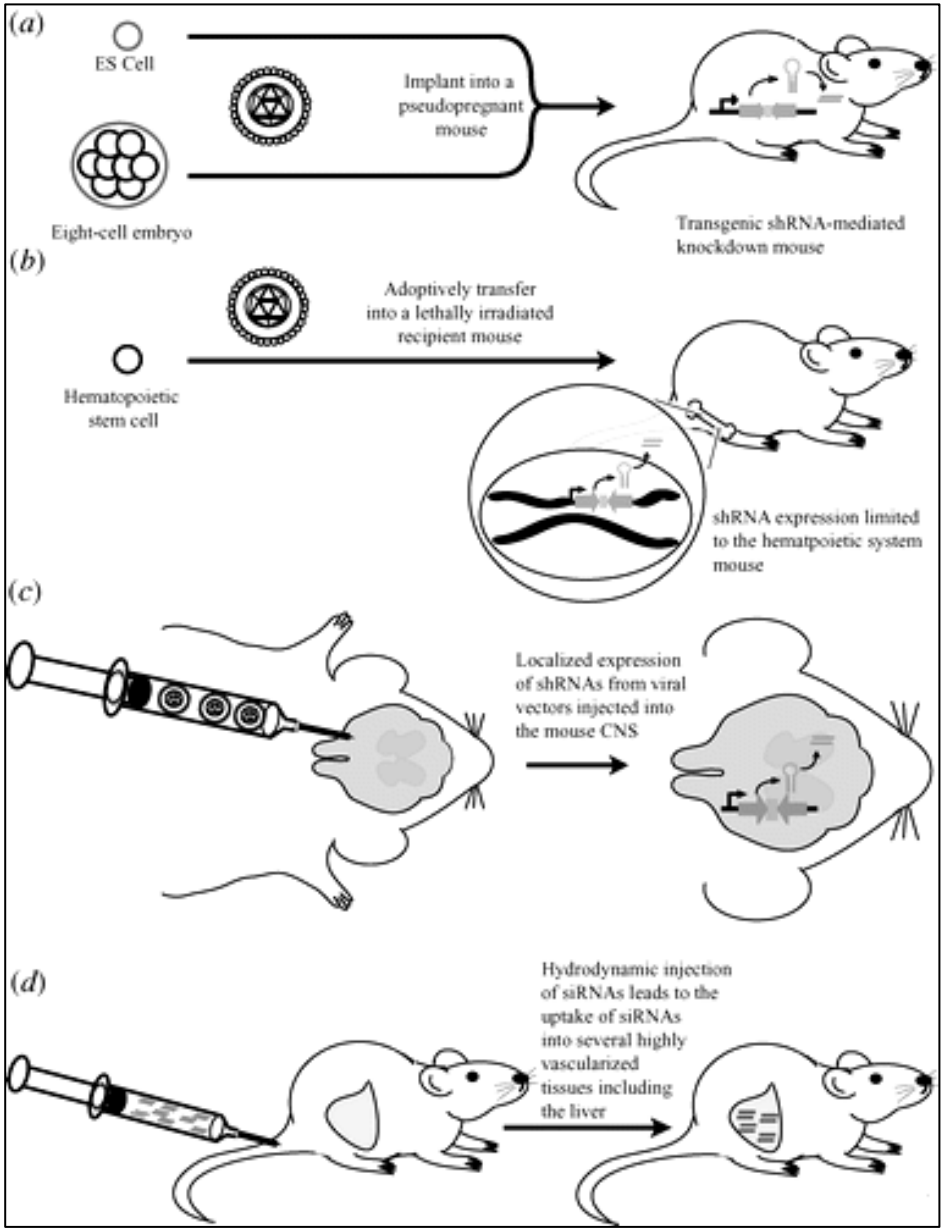
Dykxhoorn & Lieberman. 2005. The silent revolution**:** RNA Interference as Basic Biology, Research Tool, and therapeutic. Annu. Rev. Med. 56:401-423.

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.





Strategies for in vivo RNAi. (*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. (*c*) The injection of viral constructs into the central nervous system of mice can lead to localized gene silencing. For example, the intracerebellar injection of adeno-associated viruses expressing shRNAs against ataxin-1 led to a loss of inclusion body formation and improved motor coordination (112). (*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 (99).

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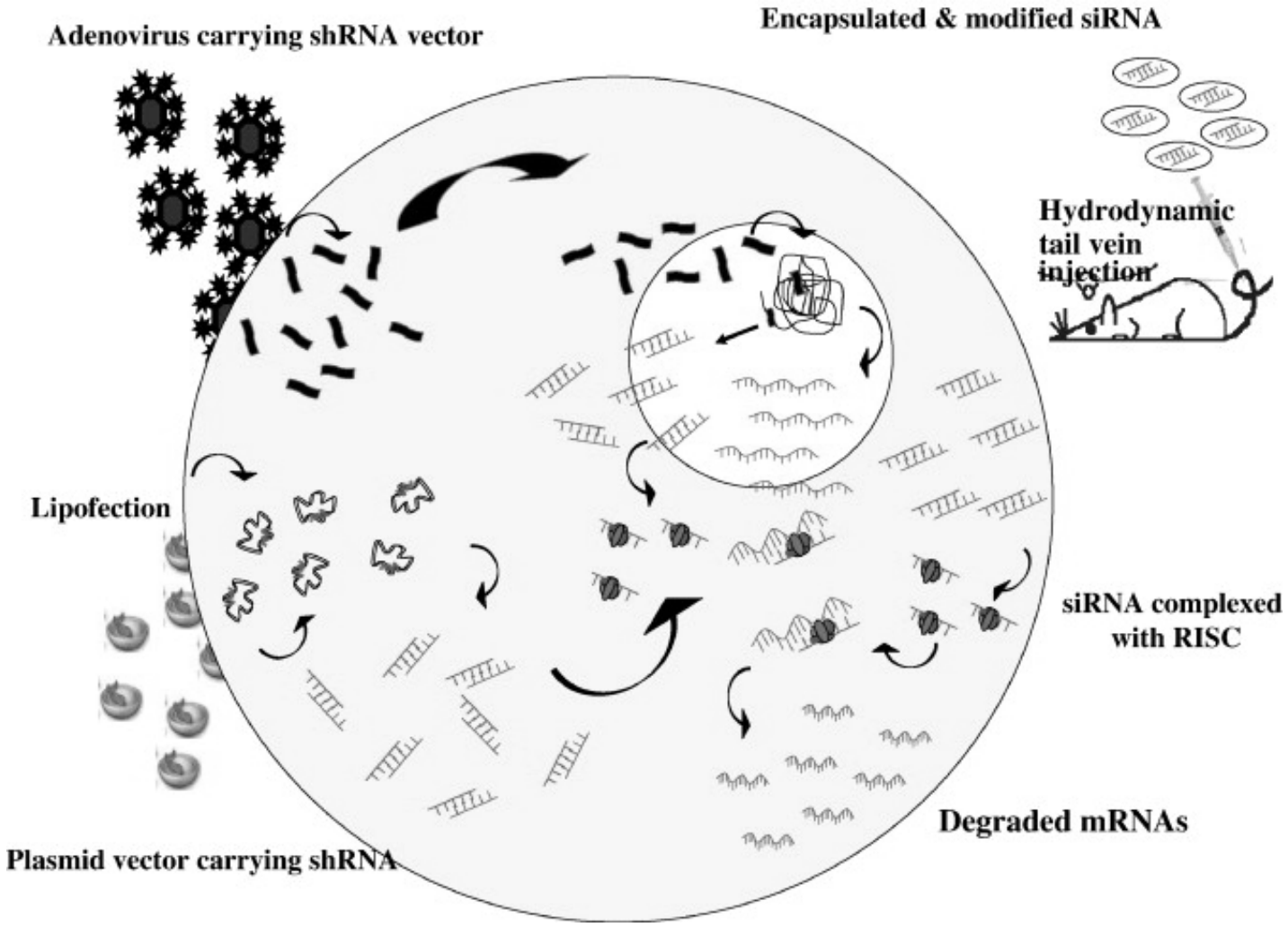
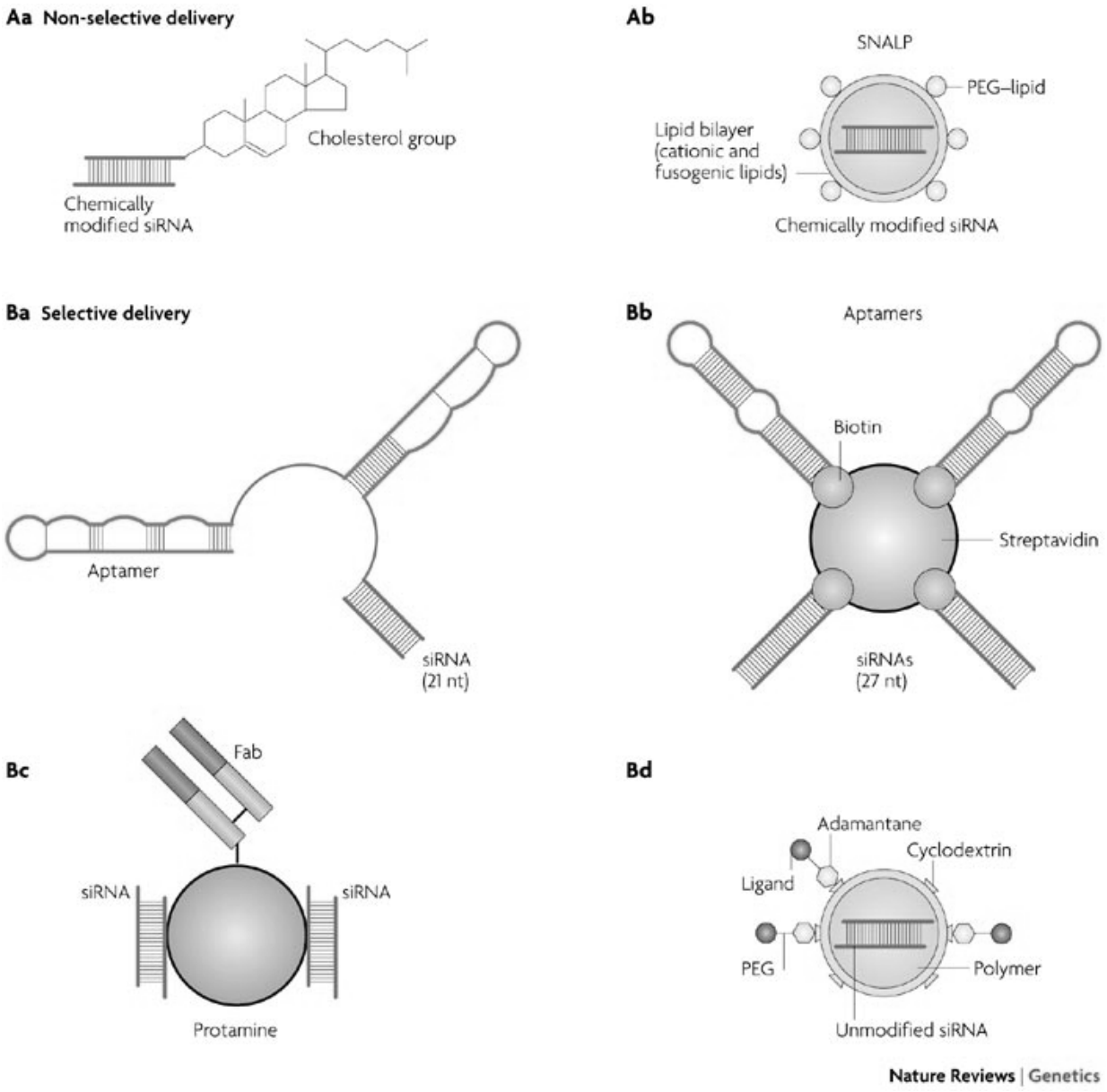


A schematic summary of several different methods of siRNA delivery *in vivo* leading to silencing of the target gene within the cell, These include: Hydrodynamic tail vein injection of mice by which modified siRNAs are delivered; Viral shRNA vectors, with stable integration into the genome and the production of siRNAs; Lipofection of plasmid shRNA vectors which are then maintained as episomal elements for the production of siRNAs. Each of these methods could independently bring about silencing.



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) | doi:10.1038/nrg2006

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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.

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

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 points

- 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

- Libraries of RNA interference molecules have been constructed that allow the analysis of gene function on a genome-wide scale

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