Cosa sono le piante GM: basi scientifiche e storia

Alessandro Vitale
Istituto di Biologia e Biotecnologia Agraria, Milano
CNR
vitale@ibba.cnr.it

1. genomi, geni, DNA, DNA ricombinante
2. Le piante GM
3. Genome editing, o tecnologie di evoluzione assistita (TEA)
4. La situazione delle coltivazioni

The Nobel Prize in Physiology or Medicine 1962
Francis Crick, James Watson, Maurice Wilkins

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The Nobel Prize in Physiology or Medicine 1962

Francis Harry Compton Crick
Prize share: 1/3

James Dewey Watson
Prize share: 1/3

Maurice Hugh Frederick Wilkins
Prize share: 1/3

«Per le loro scoperte riguardo la struttura molecolare degli acidi nucleici e il suo significato per il trasferimento di informazioni nella materia vivente»

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

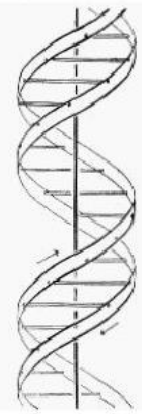
We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons:

(1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining ß-D-deoxy-ribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain, does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain, is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in time following, communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereo-chemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

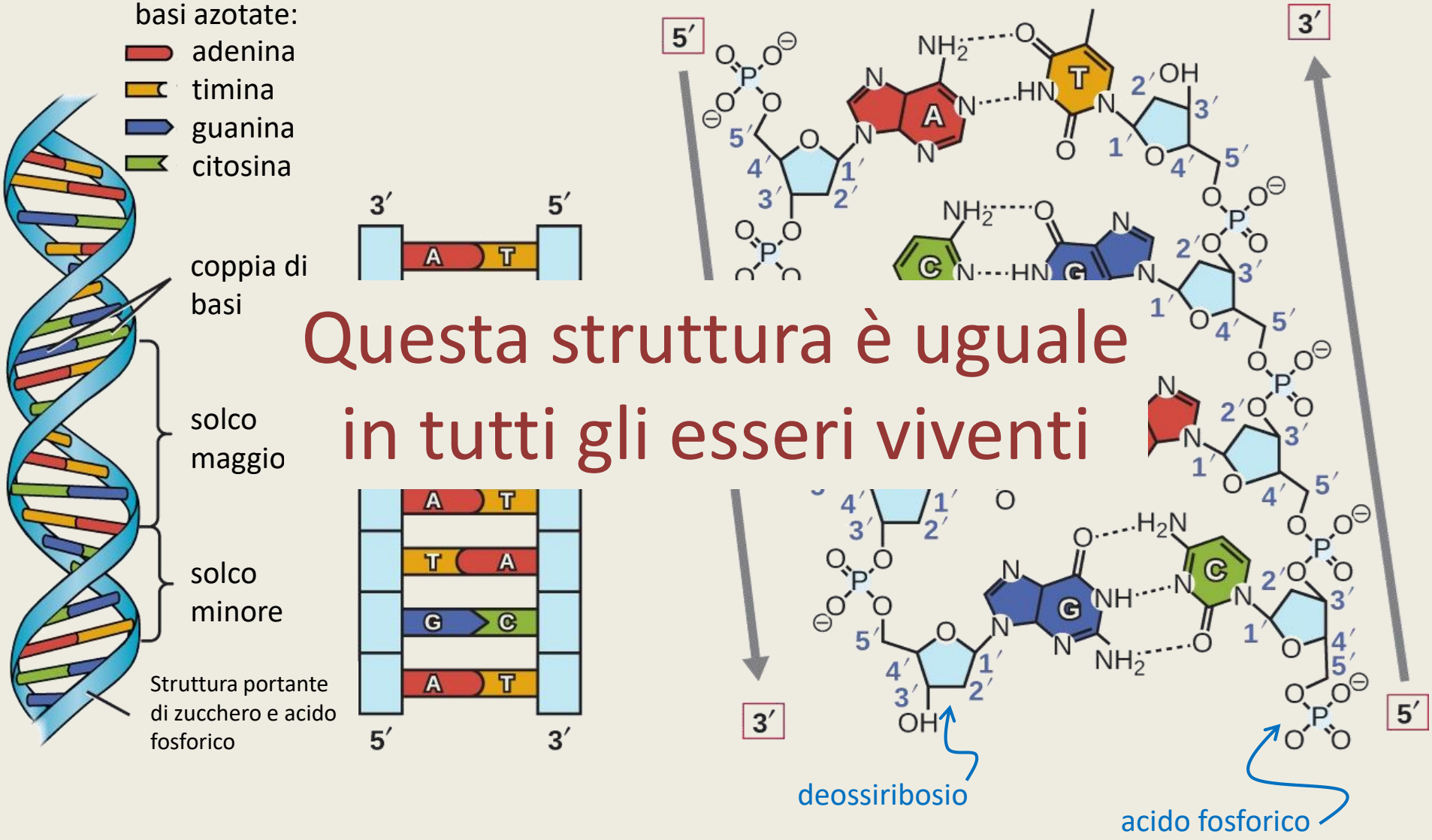
We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. *M.* H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J.D.W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J.D. WATSON
F.H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

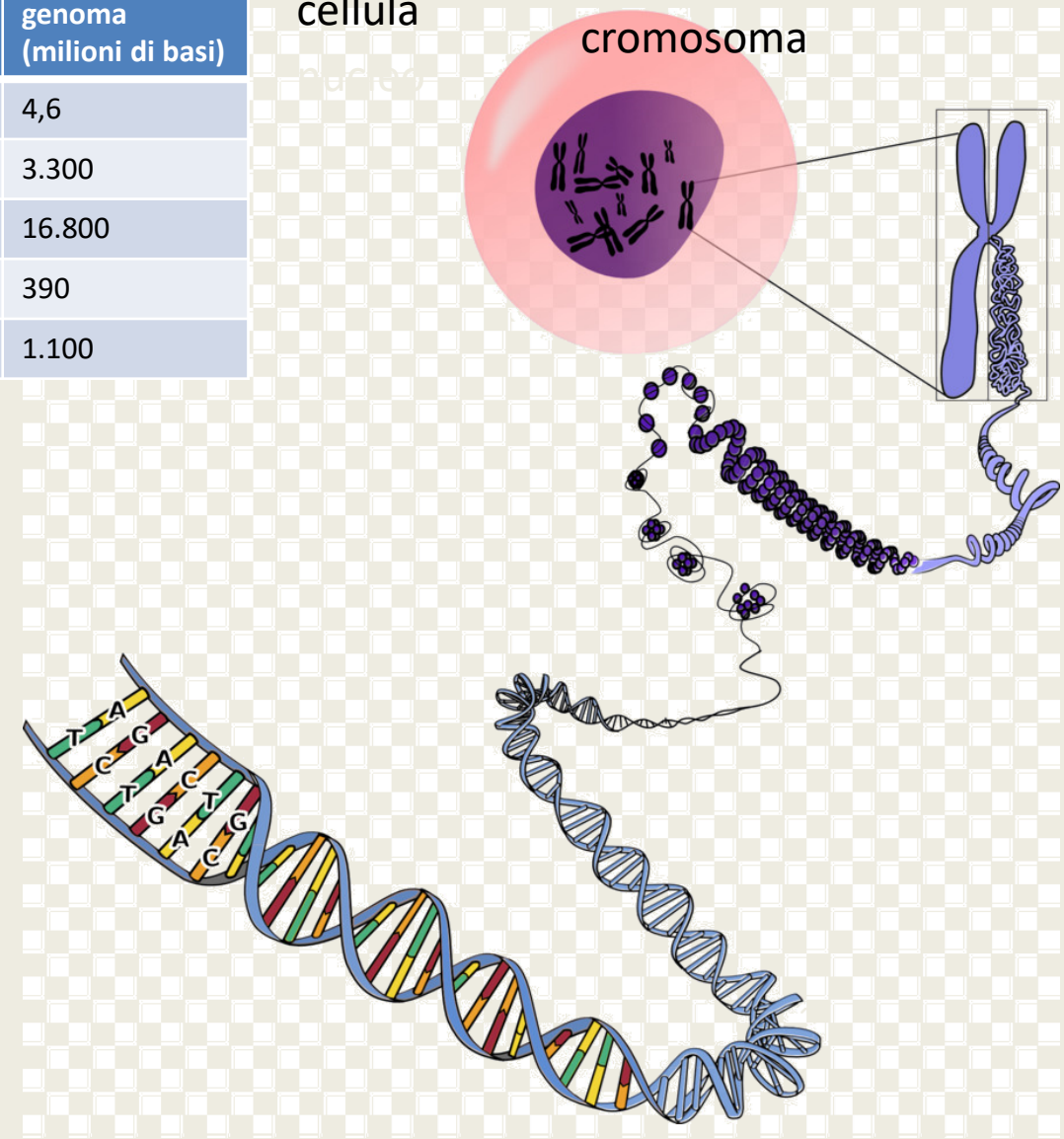
[1] Pauling, L., and Corey, R. B. nature, 171, 346 (1953); Proc. U.S. Nat Acad. Sci., 39, 84 (1953).
[2] Furberg, S., Acta Chem. Scand., 6, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., Biochim. et Biophys. Acta, 9 402 (1952).
[4] Wyatt, G.R. J. Gen. Physiol., 36 201 (1952).
[5] Astbury, W.T., Symp. Soc. Exp. Biol. I, Nucleic Acid, 66 (Camb. Univ. Press, 1947)
[6] Wilkins, M. H. F. and Randall, J. T. Biochim. et. Biophys. Acta, 10, 102 (1953).

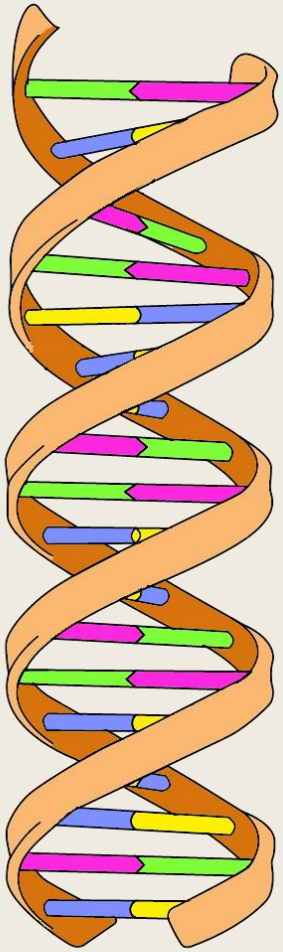
Struttura dell'acido deossiribonucleico (DNA)



Questa struttura è uguale in tutti gli esseri viventi

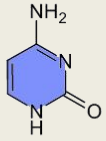
specie	Numero di cromosomi	Dimensione del genoma (milioni di basi)
E. Coli (batterio)	1	4,6
umani	23 (x2)	3.300
frumento	7 (x6)	16.800
riso	12 (x2)	390
tacchino	40 (x2)	1.100



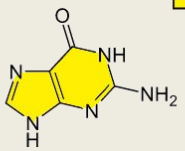


DNA
Acido Desossiribonucleico

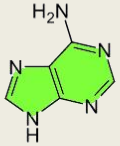
Citosina **C**



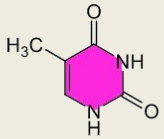
Guanina **G**



Adenina **A**

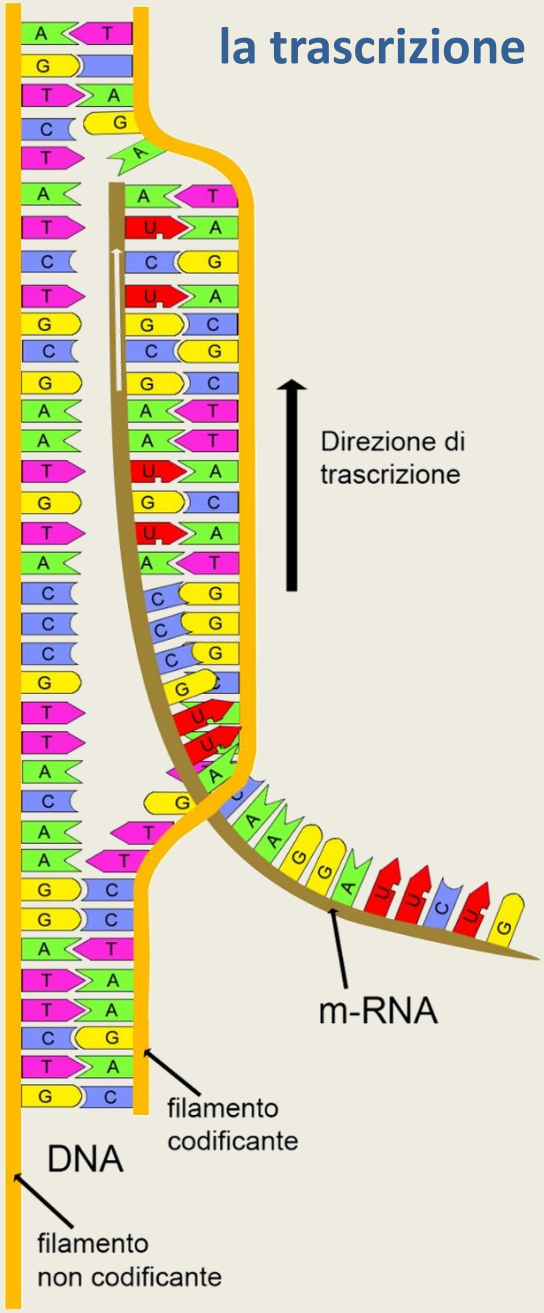


Timina **T**

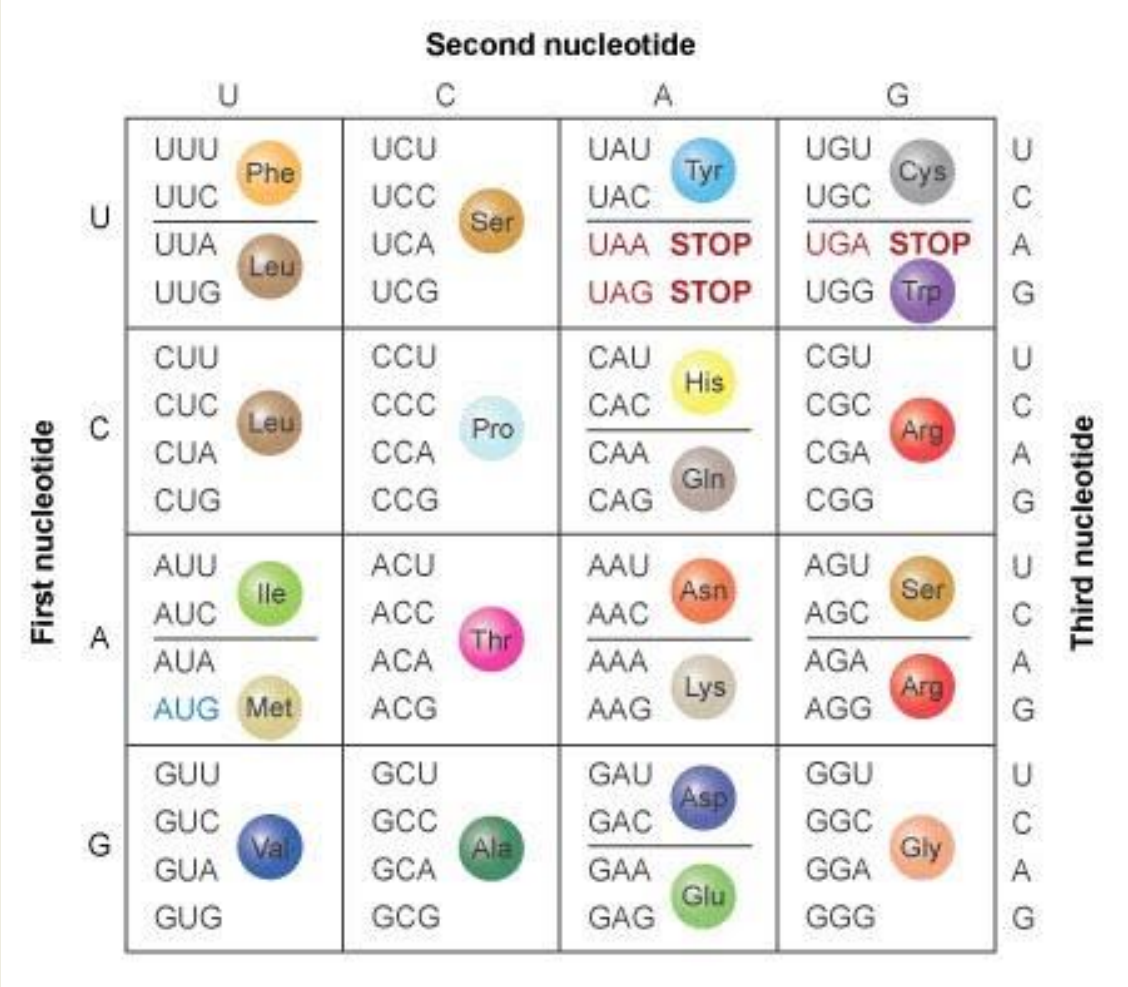


Basi azotate

la trascrizione

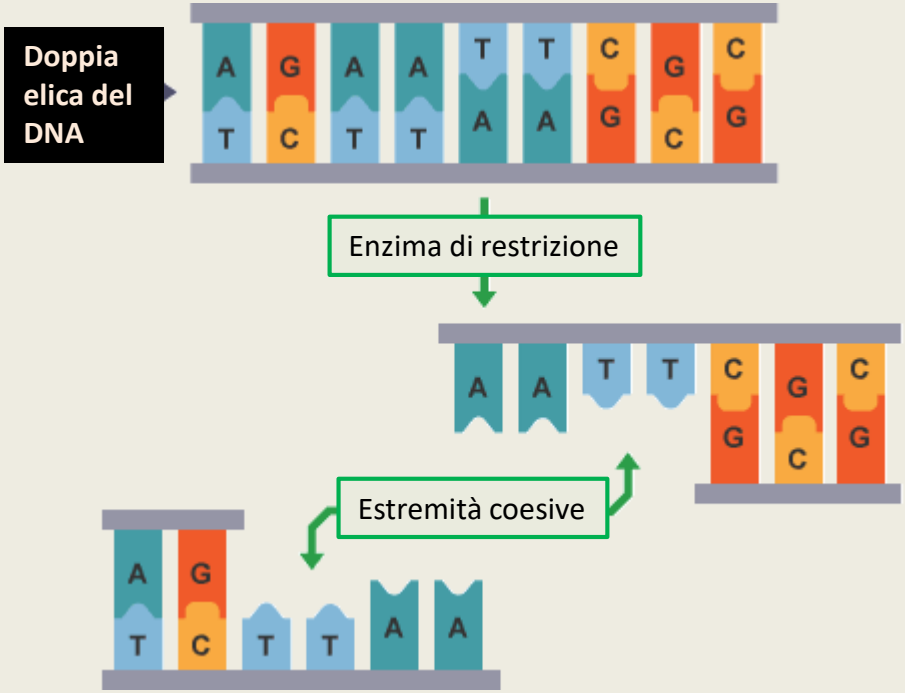


Il codice genetico e la traduzione



ACU CCC AUU CUA GGC CAA UGU GUC GAA UUC

Thr Pro Ile Leu Gly Gln Cys Val Glu Phe

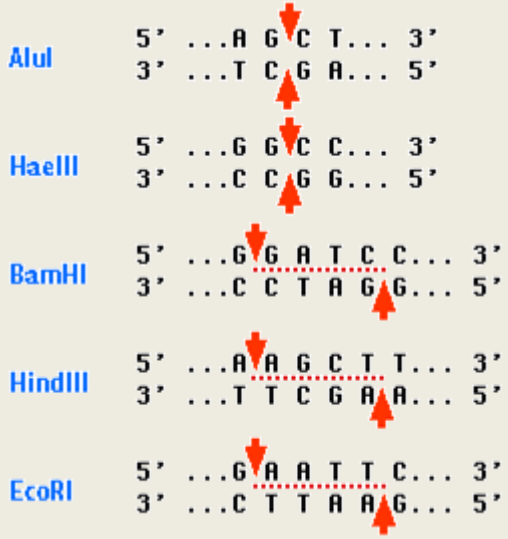


The Nobel Prize in Physiology or Medicine 1978

Werner Arber
Prize share: 1/3

Daniel Nathans
Prize share: 1/3

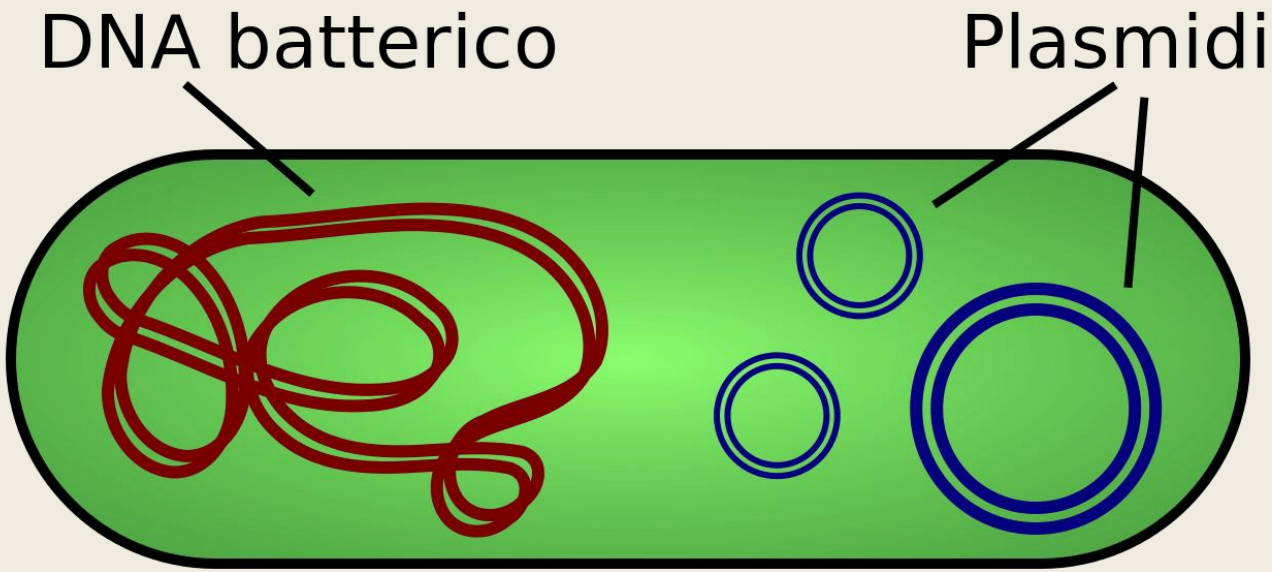
Hamilton O. Smith
Prize share: 1/3

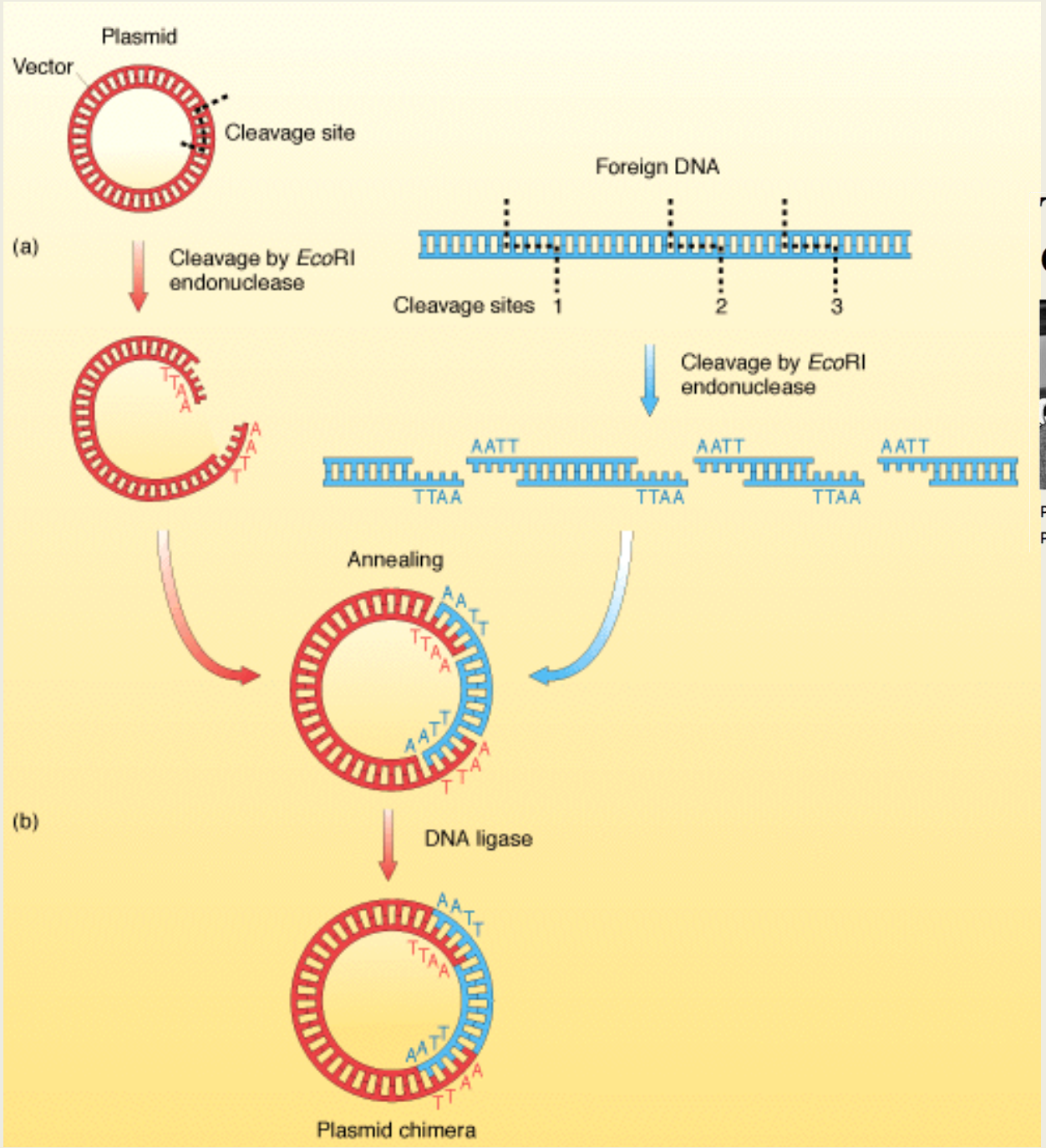


«Per la scoperta degli enzimi di restrizione e la loro applicazione nei problemi di genetica molecolare»

I plasmidi

- **DNA circolari che si possono trovare nei batteri**
- **Contengono geni che non sono necessari per funzioni fondamentali ma conferiscono particolari proprietà**
  - ✓**es.: resistenza a un antibiotico**
- **Si possono trasferire da una batterio a un altro**





The Nobel Prize in Chemistry 1980

Paul Berg
Prize share: 1/2

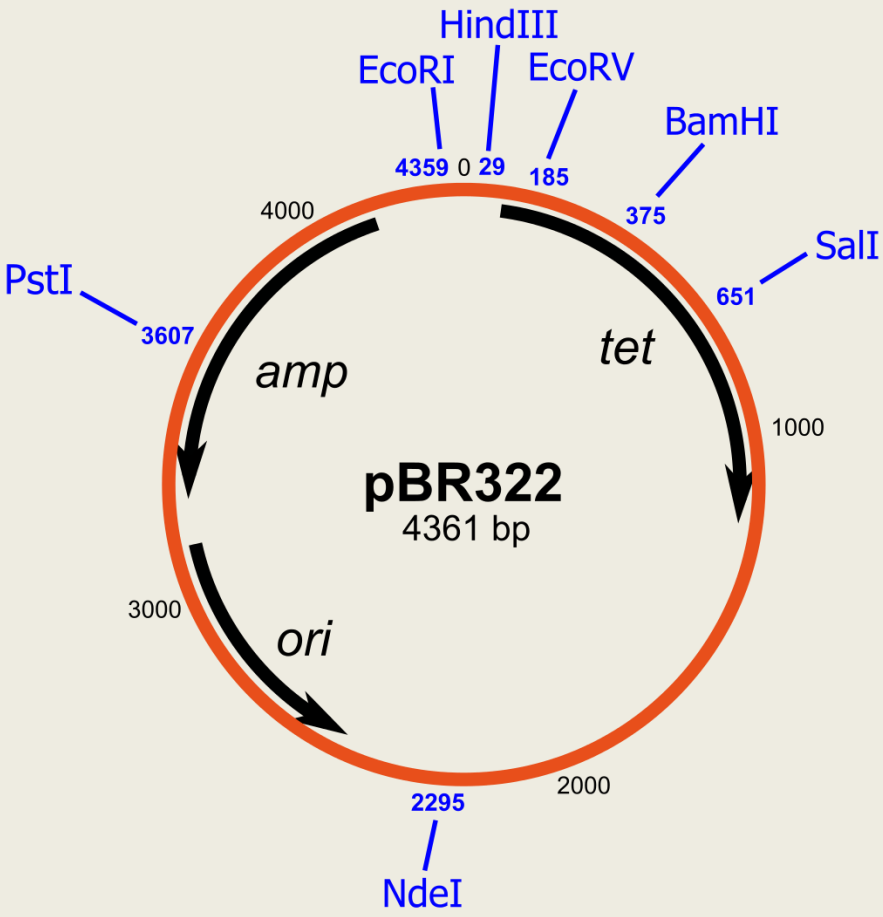
Walter Gilbert
Prize share: 1/4

Frederick Sanger
Prize share: 1/4

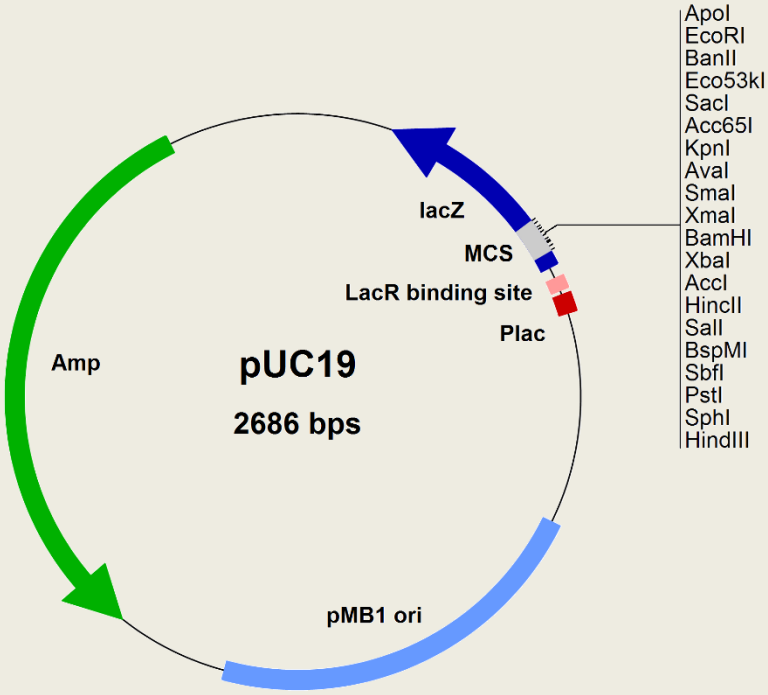
A Paul Berg *«per i suoi studi fondamentali sulla biochimica degli acidi nucleici, in particolare riguardo il* ***DNA ricombinante****»* A Walter Gilbert e Frederick Sanger *«per i loro contributi nel determinare le sequenze delle basi negli acidi nucleici»*

Esempi di plasmidi prodotti per clonare geni

1977



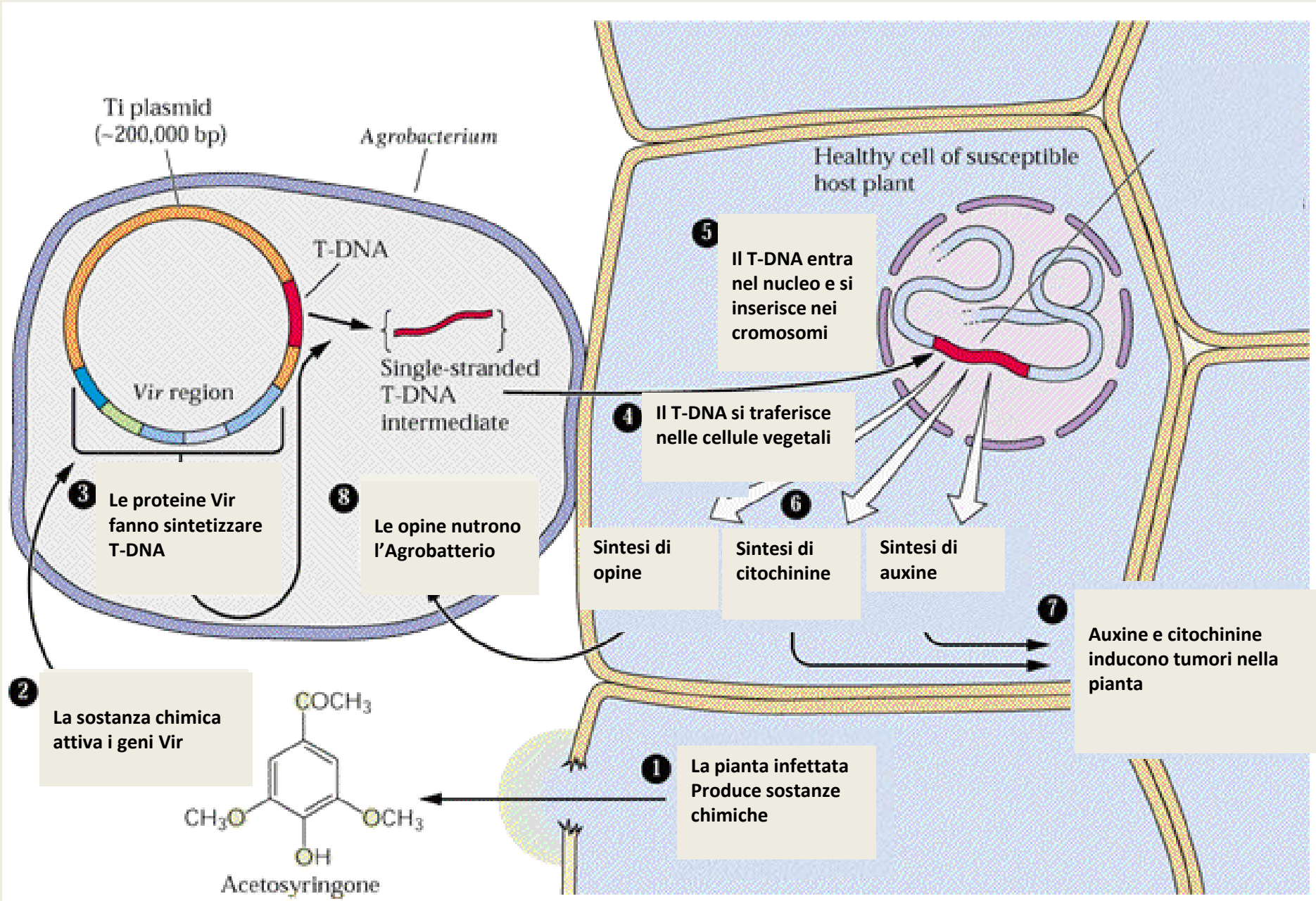
1985



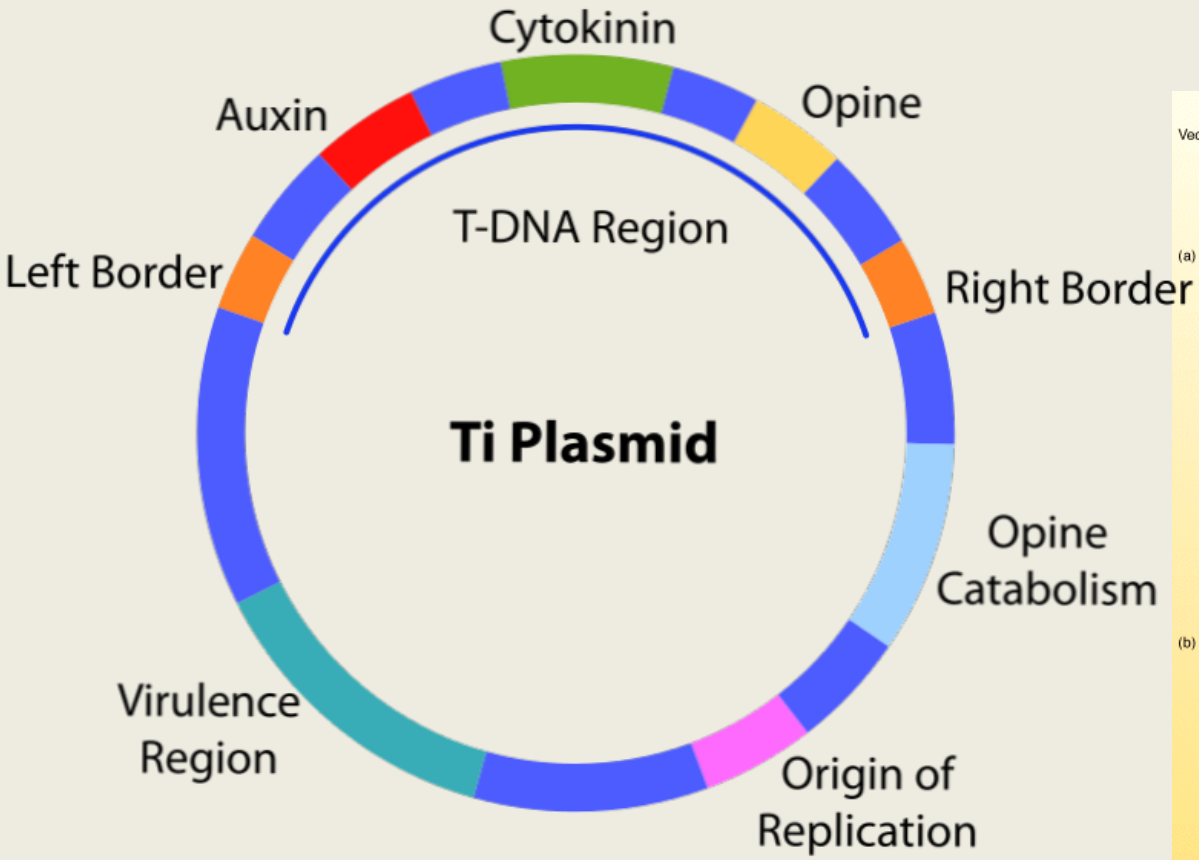
1. genomi, geni, DNA, DNA ricombinante
2. **Le piante GM**
3. Genome editing, o tecnologie di evoluzione assistita (TEA)
4. La situazione delle coltivazioni

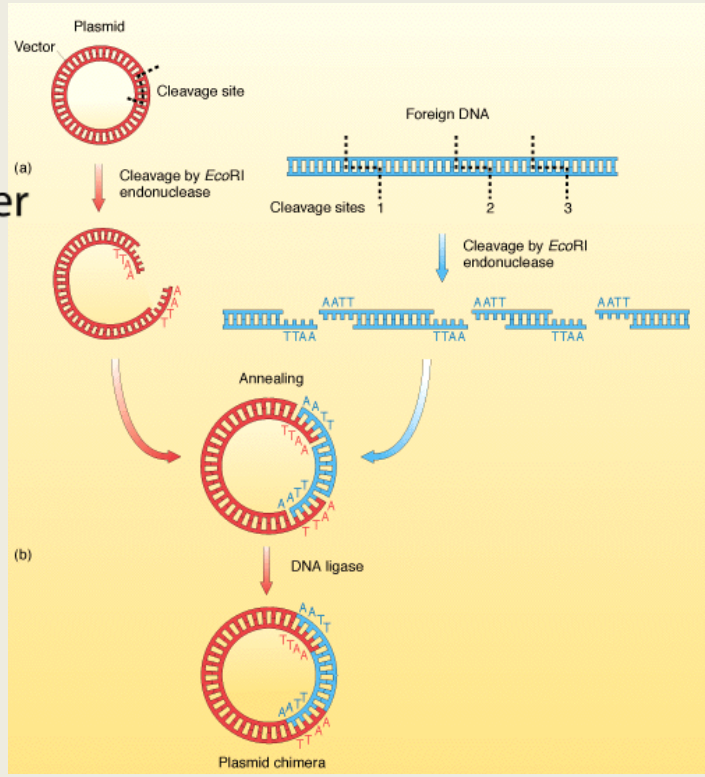
Il tumore batterico del colletto, causato dal batterio *Agrobacterium tumefaciens*

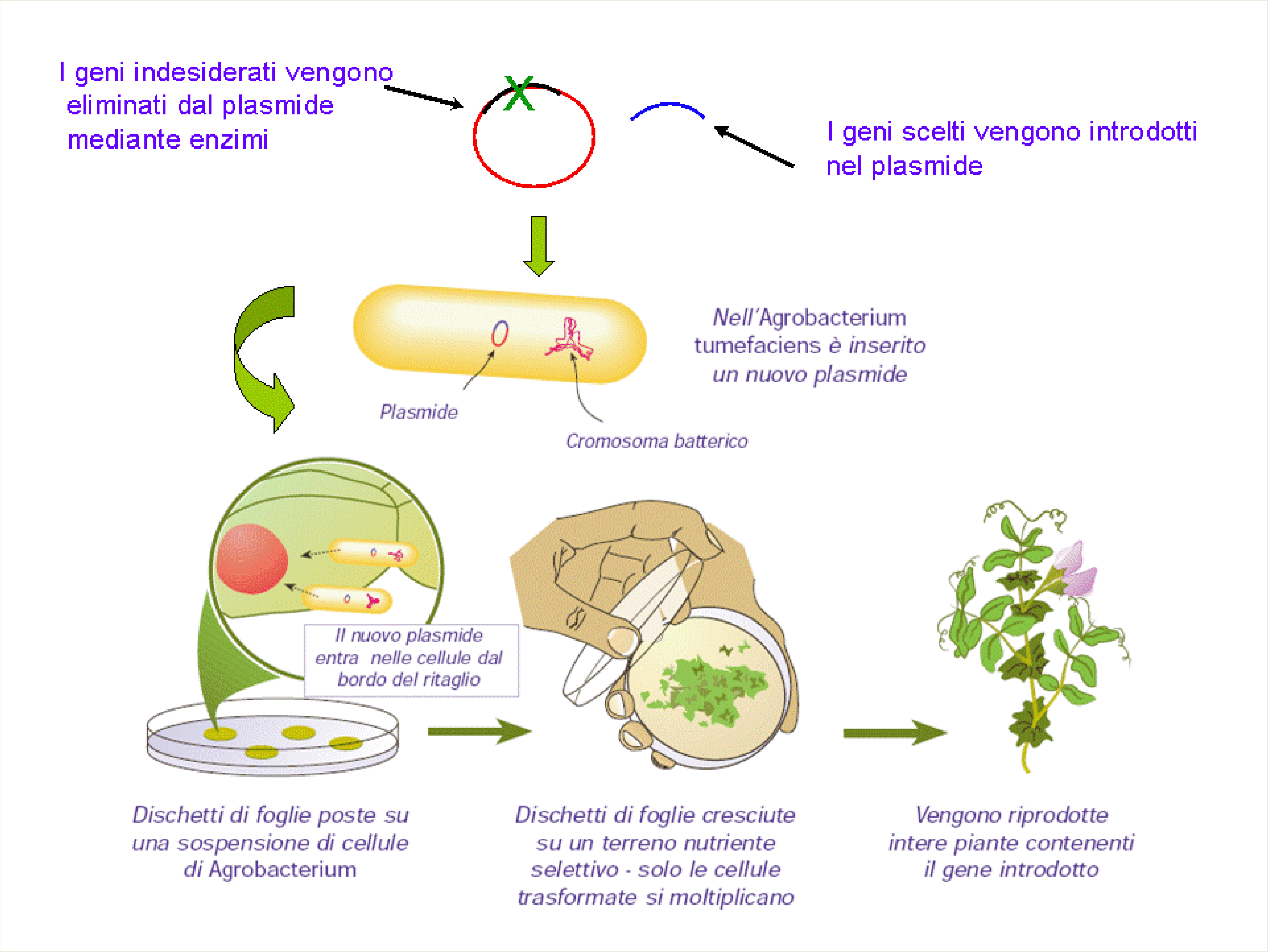
Il batterio *Agrobacterium tumefaciens*



Il plasmide Ti, contenuto naturalmente in *Agrobacterium tumefaciens*






I geni indesiderati vengono eliminati dal plasmide mediante enzimi
I geni scelti vengono introdotti nel plasmide
Nell'Agrobacterium tumefaciens è inserito un nuovo plasmide
Plasmide
Cromosoma batterico
Il nuovo plasmide entra nelle cellule dal bordo del ritaglio
Dischetti di foglie poste su una sospensione di cellule di Agrobacterium
Dischetti di foglie cresciute su un terreno nutriente selettivo - solo le cellule trasformate si moltiplicano
Vengono riprodotte intere piante contenenti il gene introdotto

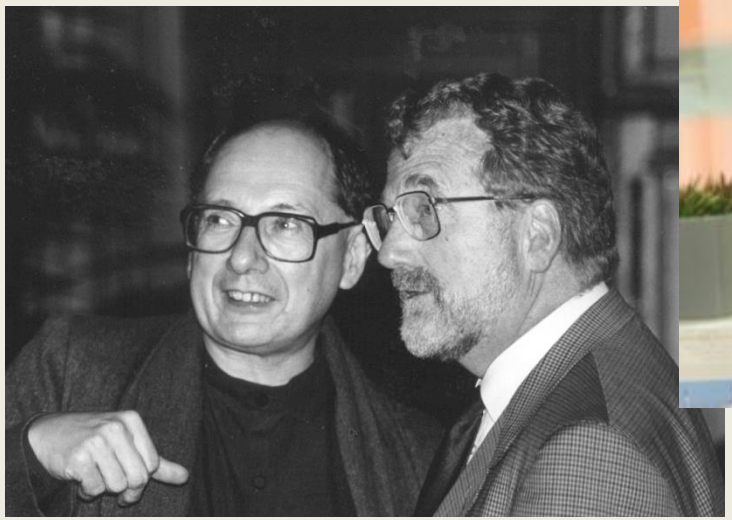
Maggio 1983, le prime piante transgeniche

NATURE VOL. 303 19 MAY 1983 ARTICLES 209

Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector

Luis Herrera-Estrella*, Ann Depicker*, Marc Van Montagu* & Jeff Schell*†

* Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium
† Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG

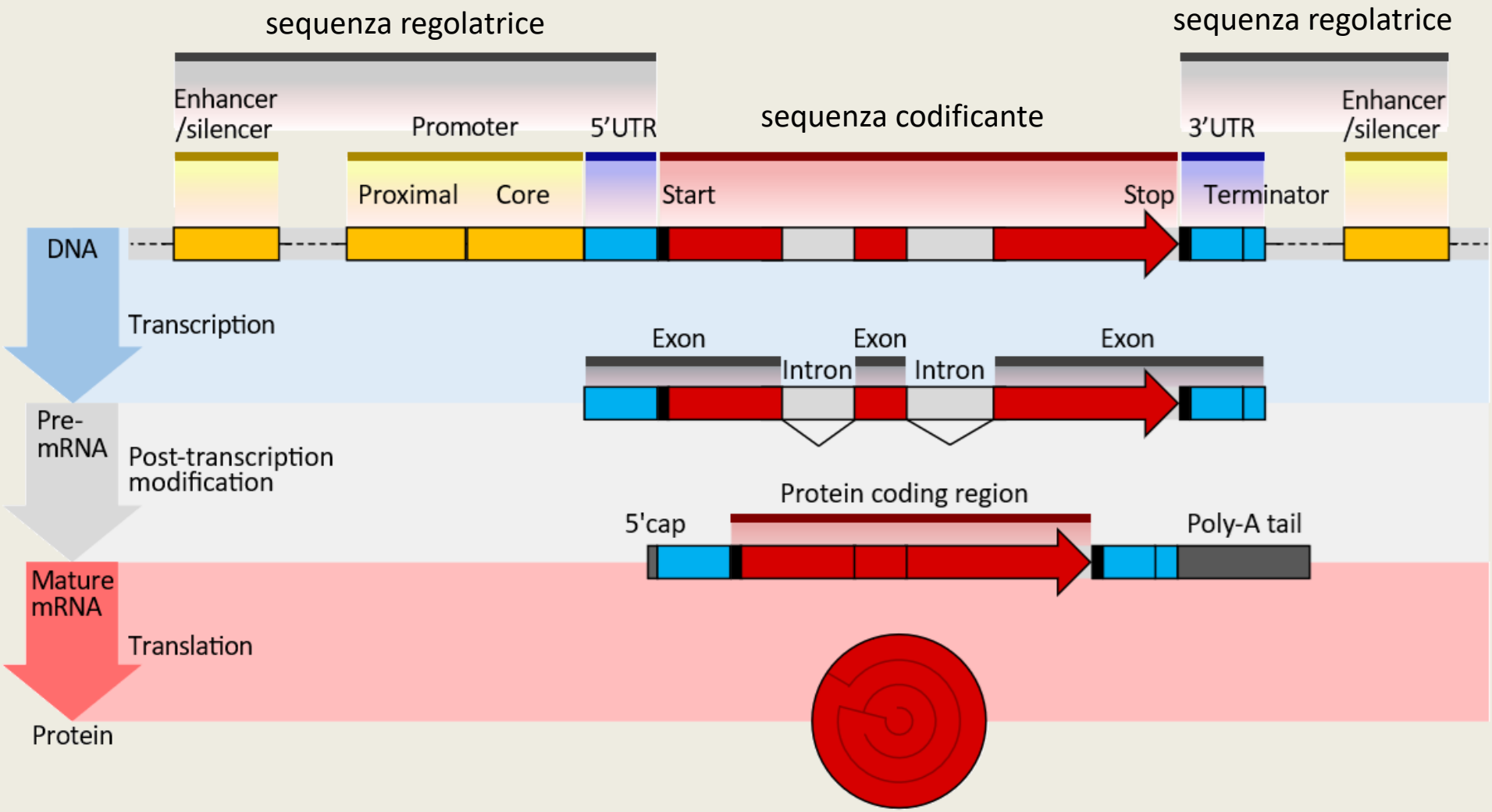


Definizioni riconosciute dall'EFSA

Transgenica
Cisgenica
Intragenica

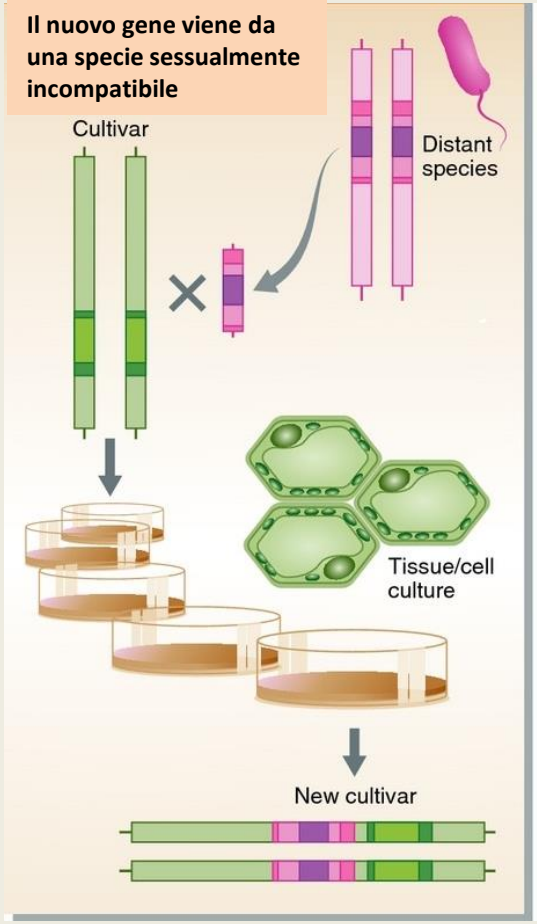
Cos'è precisamente un gene?

Cos'è un gene


sequenza regolatrice
sequenza regolatrice
Enhancer /silencer
Promoter
5'UTR
sequenza codificante
3'UTR
Enhancer /silencer
Proximal
Core
Start
Stop
Terminator
DNA
Transcription
Exon
Exon
Exon
Intron
Intron
Pre-mRNA
Post-transcription modification
Protein coding region
5'cap
Poly-A tail
Mature mRNA
Translation
Protein

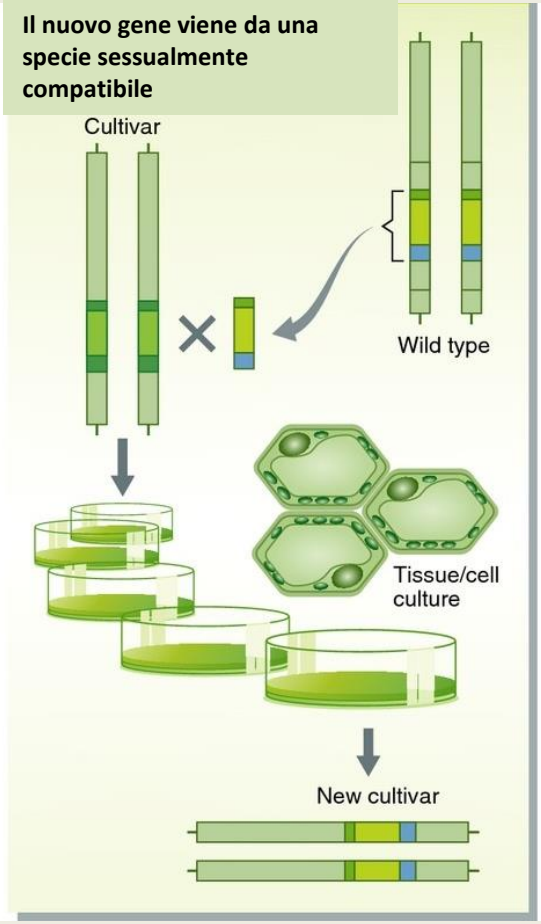
Transgenica

Il nuovo gene viene da una specie sessualmente incompatibile



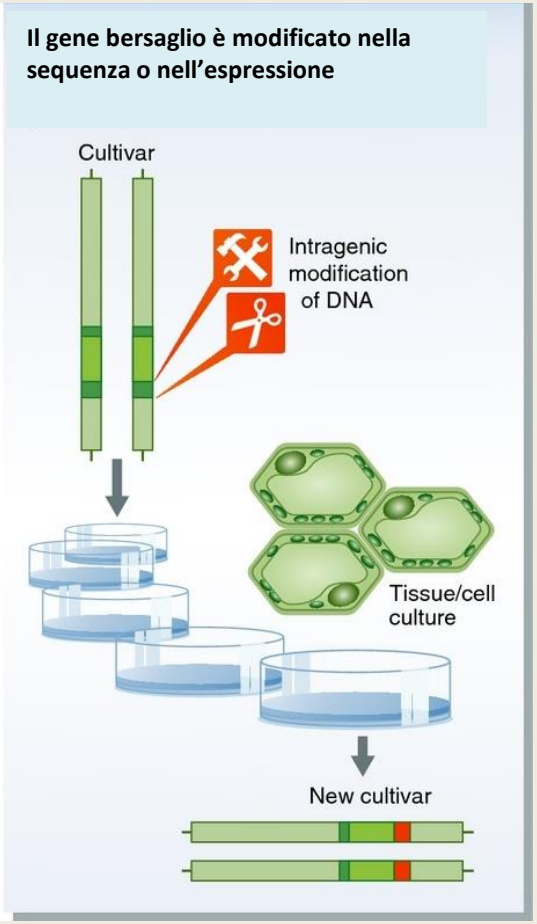
Cisgenica

Il nuovo gene viene da una specie sessualmente compatibile

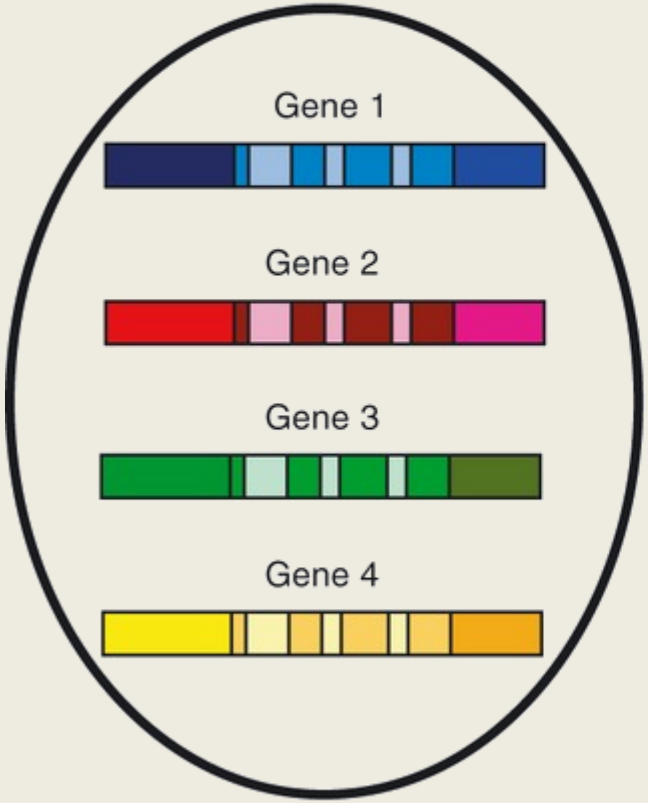


Intragenica

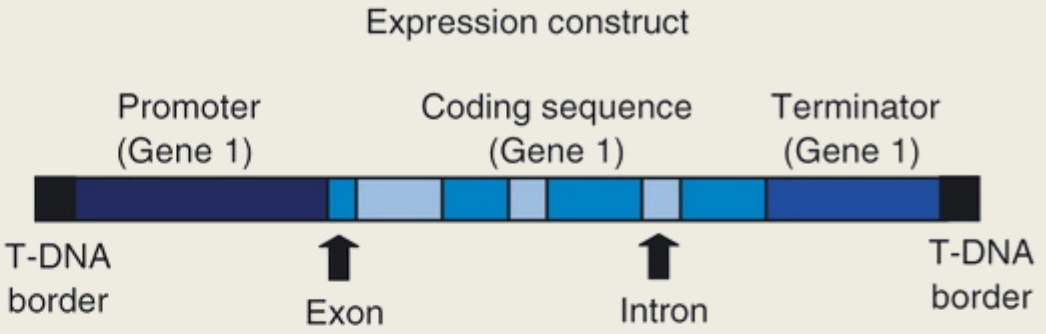
Il gene bersaglio è modificato nella sequenza o nell'espressione



Alcuni geni naturali di una pianta

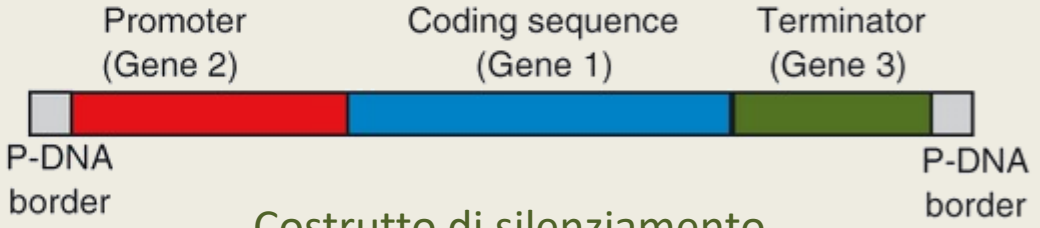


Pianta cisgenica

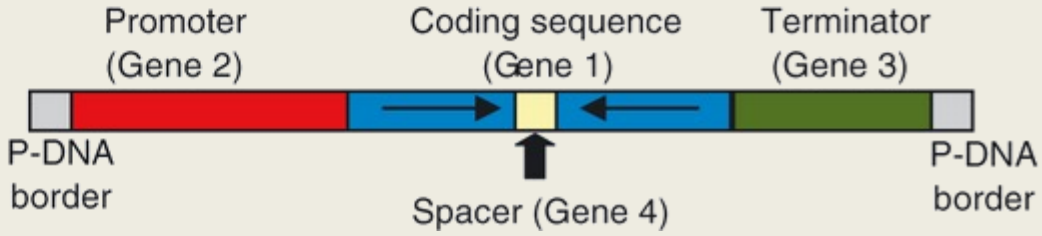


Pianta intragenica

Costrutto di espressione



Costrutto di silenziamento



1. genomi, geni, DNA, DNA ricombinante
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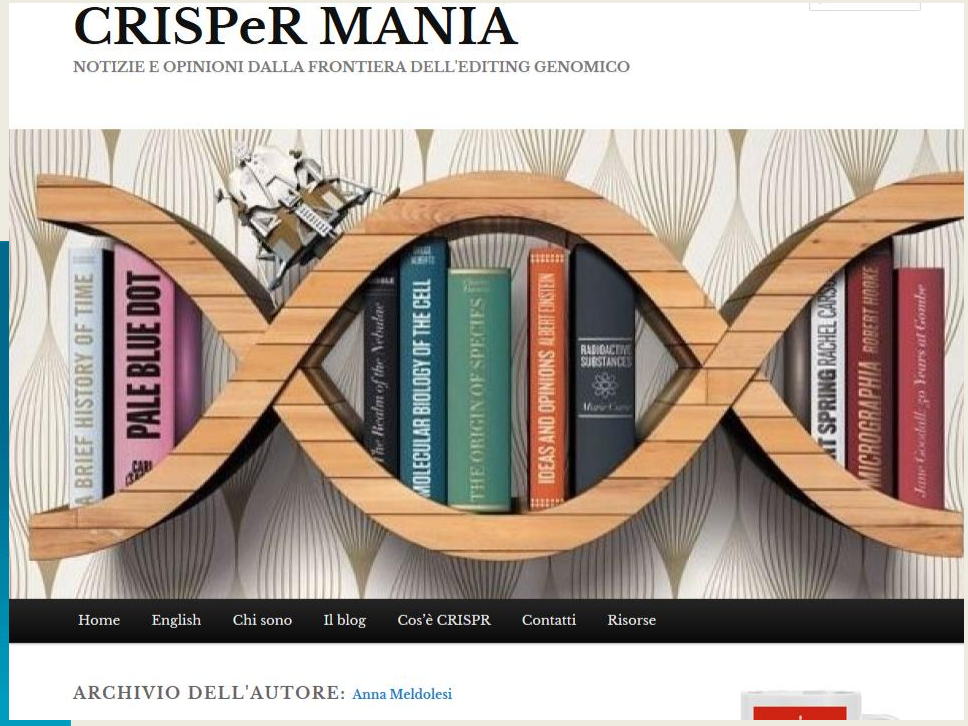
La grande novità

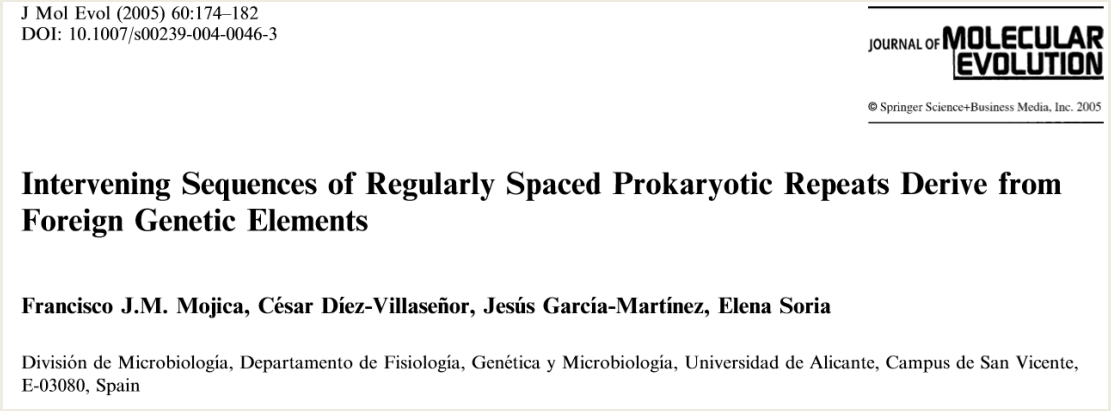


1997 I biologi che sequenziano i genomi dei batteri notano che sono piuttosto comuni brevi sequenze di DNA con queste caratteristiche:

- una sequenza di DNA
- una sequenza quasi identica ma orientata al contrario
  Esempio: **GTTTCAATACTTCCTTAGAGGGTATGGAAAC**
- circa 30 basi «spaziatrici» apparentemente casuali
- una ripetizione delle stesse due sequenze iniziali seguita da una diversa sequenza spaziatrice.

Un singolo batterio può avere svariate sequenze di questo tipo, ognuna con diverse sequenze ripetute e spaziatrici. Viene coniato il nome CRISPR (clustered regularly interspaced short palindromic repeats)

2005 I bioinformatici notano che le sequenze spaziatrici sono spesso identiche a sequenze che si trovano nei fagi. Questo suggerisce che CRISPR abbia un ruolo di difesa immunitaria nei batteri.



J Mol Evol (2005) 60:174–182
DOI: 10.1007/s00239-004-0046-3

JOURNAL OF MOLECULAR EVOLUTION

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Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements

Francisco J.M. Mojica, César Díez-Villaseñor, Jesús García-Martínez, Elena Soria

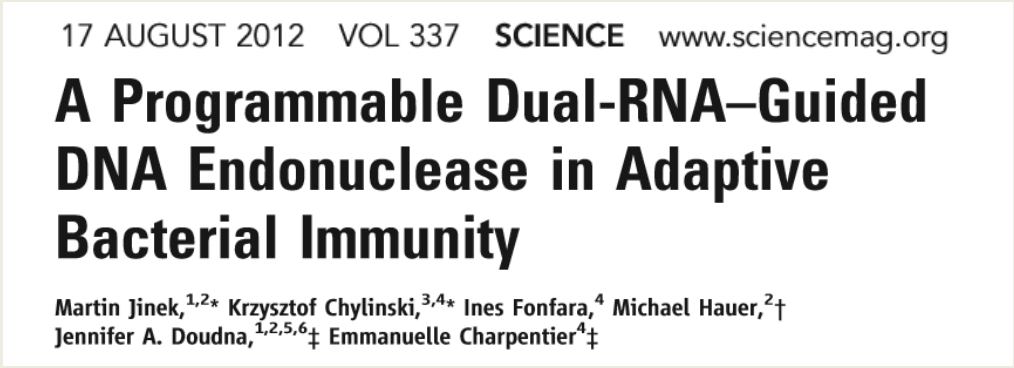
División de Microbiología, Departamento de Fisiología, Genética y Microbiología, Universidad de Alicante, Campus de San Vicente, E-03080, Spain

"Sebbene le nostre conoscenze siano al momento limitate, diverse osservazioni suggeriscono che le sequenze CRISPR possano essere coinvolte nel conferire ai batteri immunotà contro DNA estraneo"

2012 Due scienziate che lavorano in Germania e negli USA alla ricerca di proteine che permettono a CRISPR a funzionare uniscono le loro forze e scoprono un sistema CRISPR molto semplice che funziona grazie a una sola proteina, Cas9 **(C**RISPR **as**sociated protein **9**).

- quando un fago invade il batterio, CRISPR è trascritto
- si forma una lunga molecola di RNA, che è tagliata in corti RNA (crRNA) derivati dalle sequenze spaziatrici
- un altro RNA (tracrRNA) lavora con Cas9 e crRNA per riconoscere il DNA del fago
- a questo punto Cas9, che è una nucleasi, taglia il DNA del fago nella zona riconosciuta

Le ricercatrici dimostrano che se si uniscono crRNA e tracrRNA in un'unica molecola il meccanismo funziona ugualmente


17 AUGUST 2012 VOL 337 SCIENCE www.sciencemag.org

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]†
Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

"Il nostro studio rivela una famiglia di endonuclease che usano un sistema di due RNA per tagliare il DNA in punti precisi, e mostra la possibilità di sfruttare tale sistema per correggere i genomi (editing genomes) in maniera programmata"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Cas9 endonuclease
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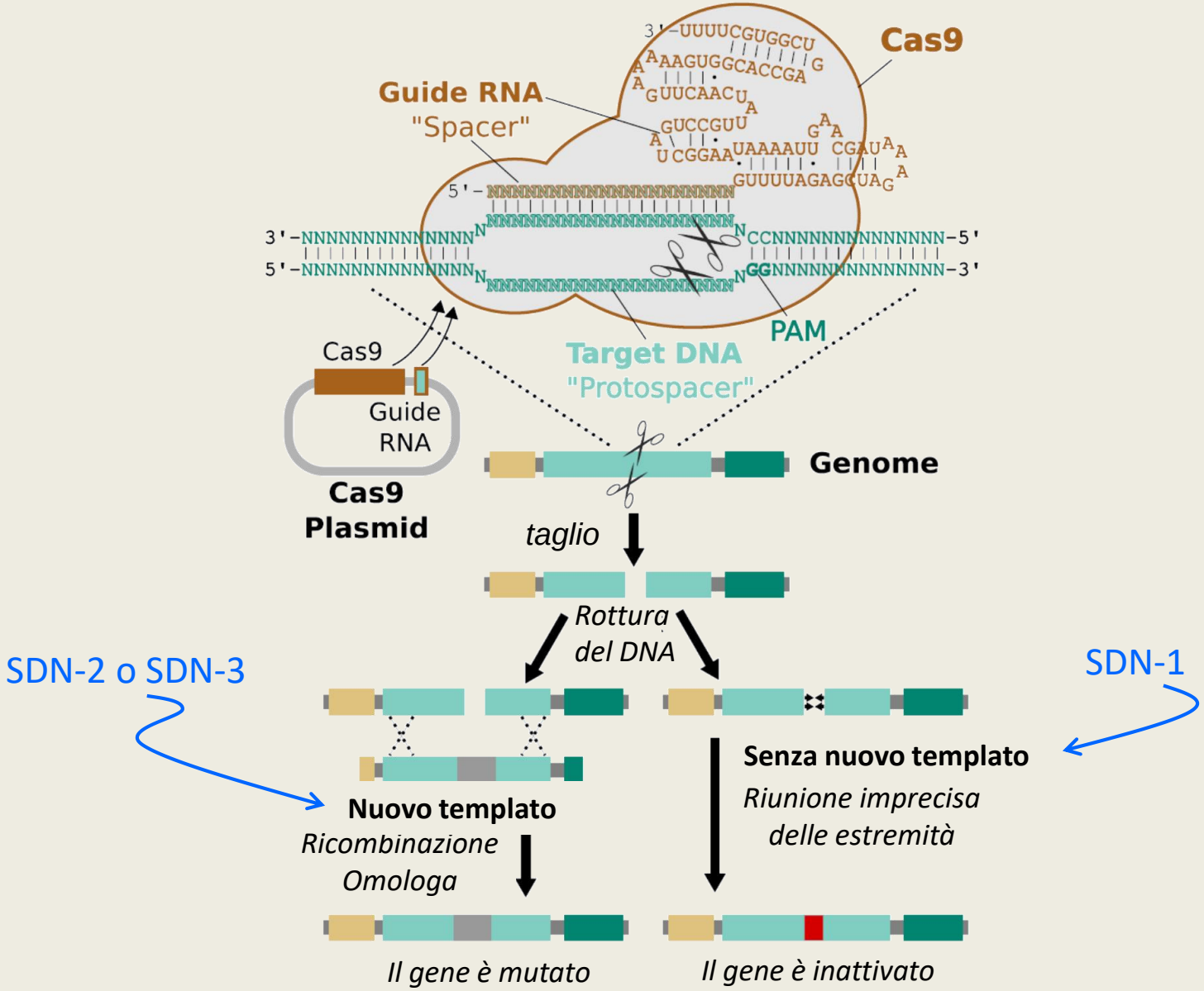
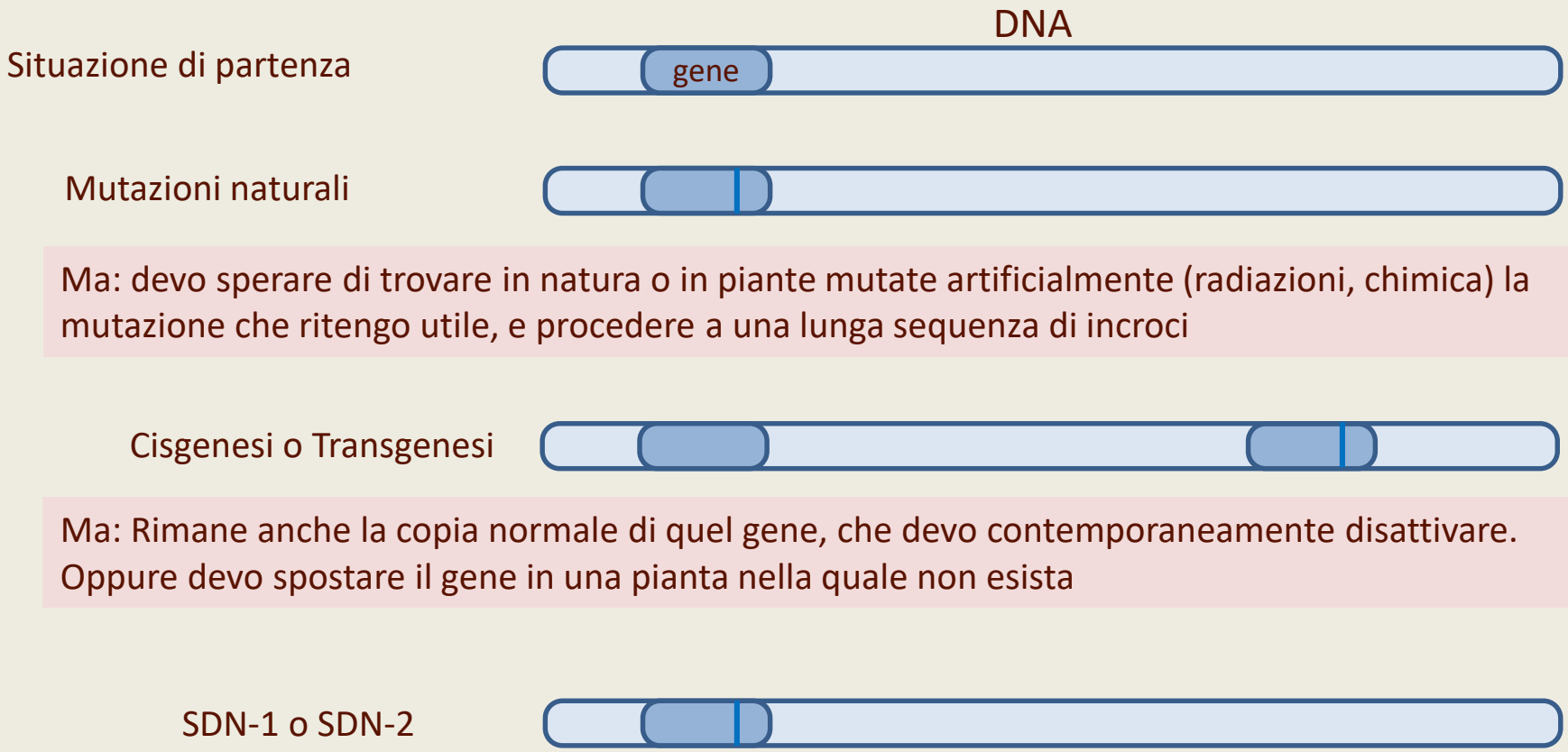
Emmaneulle Charpentier
Jennifer Doudna

Cas9 è una nucleasi sito-diretta (SDN)



La sostanziale novità

Modificare a piacimento un gene senza spostarlo dalla sua posizione naturale nel genoma e senza aggiungere nuove copie (normali o mutate) dello stesso gene.



Ma: devo sperare di trovare in natura o in piante mutate artificialmente (radiazioni, chimica) la mutazione che ritengo utile, e procedere a una lunga sequenza di incroci

Ma: Rimane anche la copia normale di quel gene, che devo contemporaneamente disattivare. Oppure devo spostare il gene in una pianta nella quale non esista

Risolvo i problemi dell'utilizzo di mutazioni naturali e transgenesi

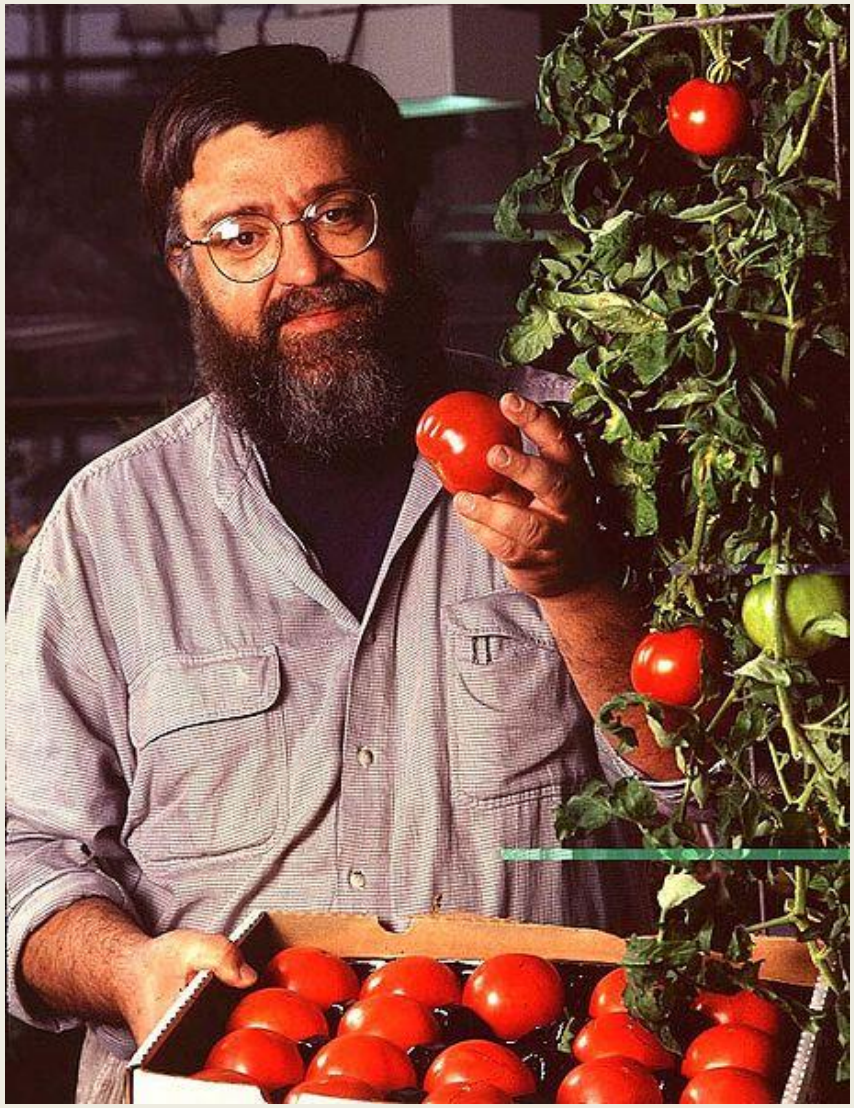
1. genomi, geni, DNA, DNA ricombinante
2. Le piante GM
3. Genome editing, o tecnologie di evoluzione assistita (TEA)
4. **La situazione delle coltivazioni**

Il pomodoro FlavrSavr

E’ una pianta intragenica
Non ha una proteina in più, ne ha una in meno

prodotto da Calgene (California)
entra in commercio nel 1994
la produzione cessa nel 1997

FlvrSavr è un insuccesso commerciale (il frutto invecchia più lentamente ma non ha maggiore consistenza)



Papaya transgenica, coltivata dal 1998

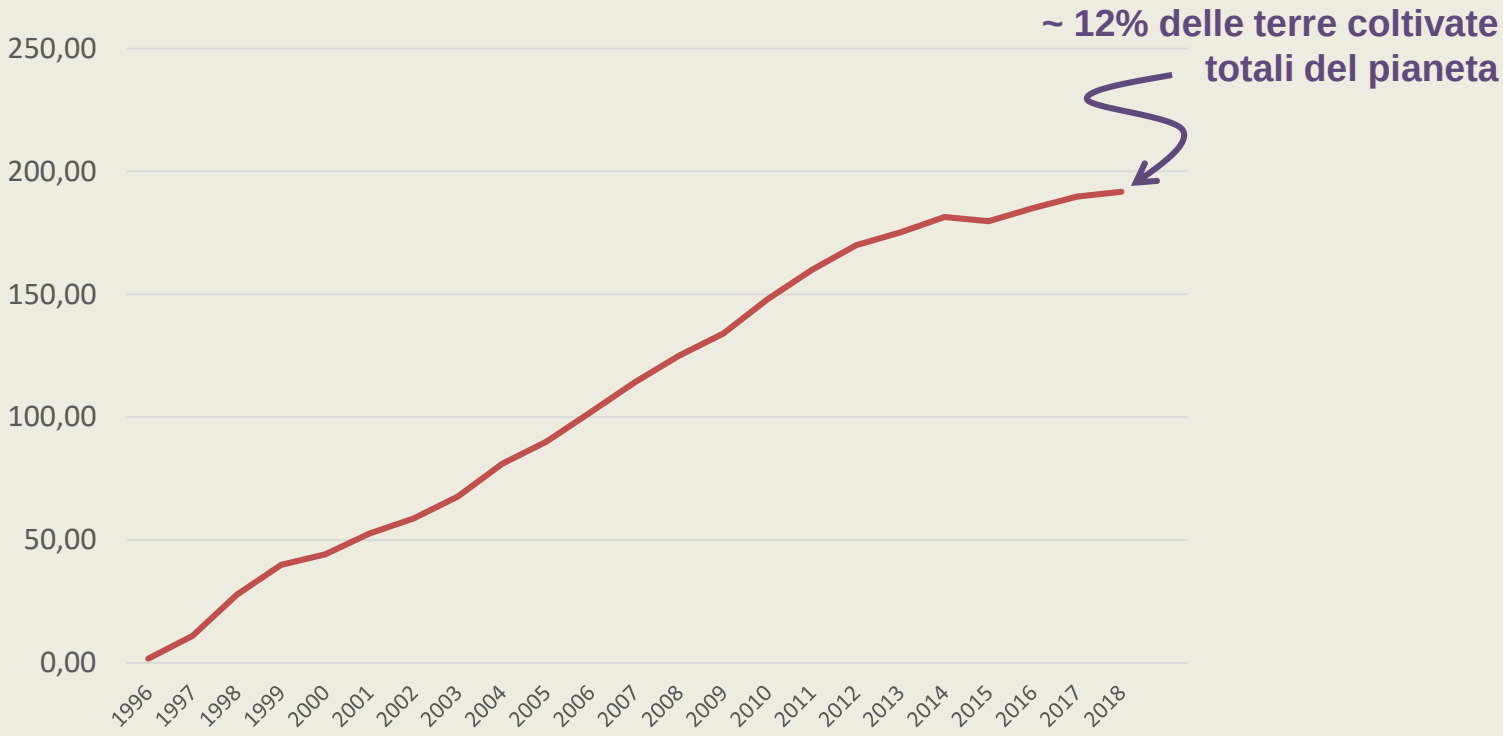
- **Prodotta dalla University of Hawaii e dalla Cornell University**


Puna, Hawaii: 1992


1994
Un’epidemia di Papaya ringspot virus devasta le coltivazioni


1996
Papaya transgenica che resiste al virus
Papaya convenzionale

Area coltivata GM globale (milioni di ettari)



Domande per la coltivazione approvate globalmente

Situazione nel 2020	
Tipi di coltivazioni (specie vegetale con specifico evento di trasformazione) approvate dal 1992	827
Eventi di trasformazione	387
Specie di piante	27
Nazioni	70

Le maggiori coltivazioni GM

pianta	% del totale coltivato nel mondo	
	2014	2018
Soia	82	78
Mais	30	30
Cotone	68	76
colza	25	39

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Classifica	Nazione	Area coltivata (milioni di ettari)	Raccolto
1	USA	75.0	mais, soia , cotone, colza, barbabietola, erba medica, papaya, zucca, patata, melo
2	Brazil	51.3	soia, mais, cotone, canna da zucchero
3	Argentina	23.9	soia, mais, cotone
4	Canada	12.7	colza, mais, soia, barbabietola, erba medica, papata
5	India	11.6	cotone
6	Paraguay	3.8	soia, mais, cotone
7	China	2.9	cotone, papaya
8	Pakistan	2.8	cotone
9	South Africa	2.7	mais, soia, cotone
10	Uruguay	1.3	soia, mais
11	Bolivia	1.3	soia
12	Australia	0.8	cotone, colza
13	Philippines	0.6	mais
14	Myanmar	0.3	cotone
15	Sudan	0.2	cotone
16	Mexico	0.2	cotone
17	Spain	0.1	mais
18	Colombia	0.1	cotone, mais
19	Vietnam	<0.1	mais
20	Honduras	<0.1	mais
21	Chile	<0.1	mais, soia, colza
22	Portugal	<0.1	mais
23	Bangladesh	<0.1	barbabietola
24	Costa Rica	<0.1	cotone, soia
25	Indonesia	<0.1	canna da zucchero
26	eSwatini	<0.1	cotone
	Totale	**191,7**	

Approvate nell'Unione Europea

Evento di trasformazione	Industria e nazione	Nome commerciale
Garofano - *Dianthus caryophyllus* : 7 eventi		
Sulfonylurea herbicide tolerance , Modified flower color	Florigene Pty Ltd. (Australia)	Moonshadow™
Sulfonylurea herbicide tolerance , Modified flower color	Florigene Pty Ltd. (Australia)	Moonshade™
Sulfonylurea herbicide tolerance , Modified flower color	Florigene Pty Ltd. (Australia)	Moonlite™
Sulfonylurea herbicide tolerance , Modified flower color	Suntory Limited (Giappone)	Moonberry™
Sulfonylurea herbicide tolerance , Modified flower color	Suntory Limited (Japan)	Moonvelvet™
Sulfonylurea herbicide tolerance , Modified flower color	Florigene Pty Ltd. (Australia)	Moonshade™
Sulfonylurea herbicide tolerance , Modified flower color	Florigene Pty Ltd. (Australia)	Moonshade™
Cotone - *Gossypium hirsutum* : 1 evento		
Glyphosate herbicide tolerance , Isoxaflutole herbicide tolerance	BASF (Germania)	Non disponibile
Mais - *Zea mays* : 2 eventi		
Glyphosate herbicide tolerance , Lepidopteran insect resistance , Antibiotic resistance	Monsanto (USA, Germania)	YieldGard™, MaizeGard™
Glufosinate herbicide tolerance , Antibiotic resistance	Bayer CropScience (Germania)	Liberty Link™ Maize
Patata - *Solanum tuberosum* : 1 evento		
Modified starch/carbohydrate , Antibiotic resistance	BASF (Germania)	Amflora™