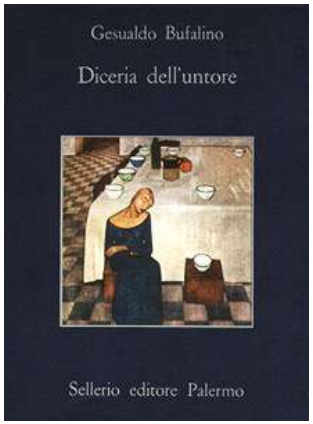
TORINO 1 GIUGNO 2024

IN DOCTRINA ET IN USU

Praticamente...Diabetologia

TERZA SESSIONE | "Diceria dell'untore" G. Bufalino



Benché sapessi già allora che avrei preferito starmene zitto e portarmi lungo gli anni la mia diceria al sicuro sotto la lingua, come un obolo di riserva, con cui pagare il barcaiolo il giorno in cui mi fossi sentito, in séguito ad altra e meno remissibile scelta o chiamata, sulle soglie della notte.

Virus e Diabete: Le "relazioni pericolose" tra esordio e complicanze

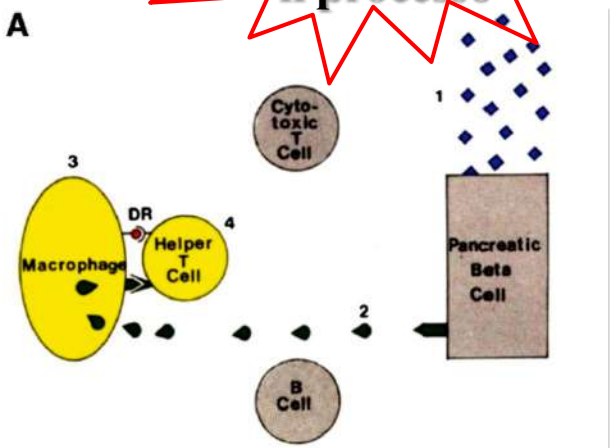
Franco Gregorio Responsabile UOS Diabetologia – Jesi -AN

Death of a Beta Cell: Homicide or Suicide?

Gian Franco Bottazzo

Department of Immunology Middlesex Hospital, Medical School, London W1P 9PG, UK

Anno 1985: il processo

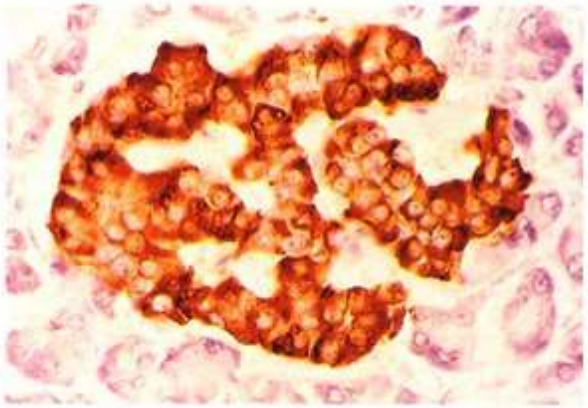


Based on the 1985 R. D. Lawrence Lecture delivered by the author at the Spring Meeting of the Medical and Scientific Section of the British Diabetic Association, Oxford, March 1985, and up-dated for the Diaz-Cristobal Lecture delivered at the XII Congress of the International Diabetes Federation, Madrid, September 1985. The Lecture was delivered in a judicial courtroom style which has been followed in the edited transcript. The reader is invited to join the Jury.

KEY WORDS Diabetes mellitus Beta cell Islet cell antibodies Histocompatibility antigens Chromosomes Viruses Organ specific auto antibodies Genetics

Counsel for the Prosecution

Members of the Jury, today in this Court of Justice we shall debate one of the most intriguing cases in the history of criminal investigation. Nobody will dispute that a death has occurred.[1] This is a healthy normal islet (Exhibit 1(a)), Numerous functioning beta cells occupy most of the space in the islet. In dramatic contrast, the view of an islet from a newly diagnosed diabetic child stained by the same peroxidase method reveals only a few scattered, brown insulin cells (Exhibit 1(b)).[2] Of the original beta cell mass 90% is gone! But, if these cells are dead, how did they die? Was it *Homicide* or *Suicide*? As Counsel for the Prosecution, there is no doubt in my mind that this is a case of *Homicide*. I am confident that I can prove to you that the beta cell has indeed been killed, and indicate where the responsibility for the crime



(a)

Arguments for homicide included, but were not limited to, a theoretic scenario wherein type 1 diabetes was posed to be initiated by an ill-defined environmental attack resulting in the release of B-cell autoantigens.

The suicide model suggested the death of a B-cell was a matter of destiny, imprinted at the time of conception, linked to genetic associations: chromosome 6 (HLA), 11 (insulin), 14 (Gm allotypes), 2 (k light chains) etc.

How Does Type 1 Diabetes Develop?

DIABETES, VOL. 60, MAY 2011

The Notion of Homicide or β-Cell Suicide Revisited

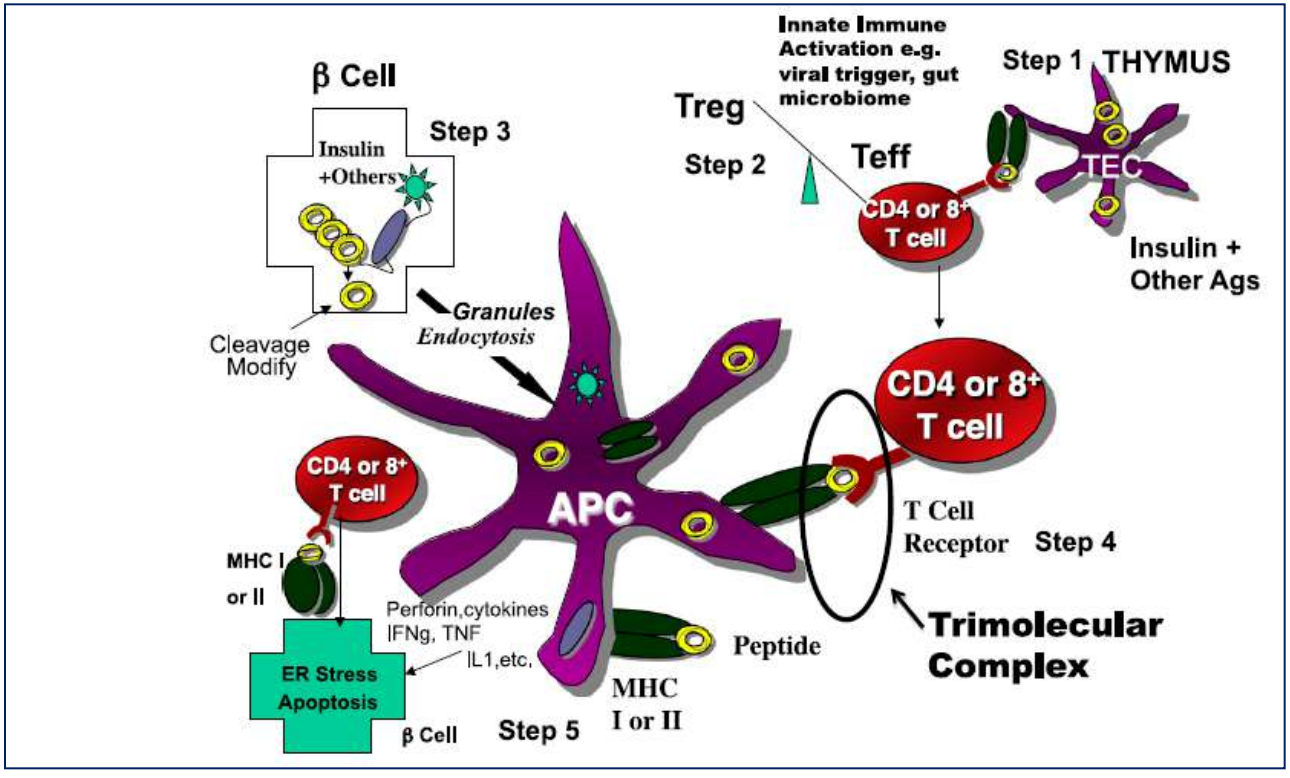


FIG. 2. Simplified model of the immune pathogenesis of type 1 diabetes. Major components include *Step 1*) The thymus where peptides of peripheral antigens are expressed and presented by HLA molecules on the surface of medullary thymic epithelial cells (mTECs cells) to T-cell receptors, leading to deletion of many but not all anti-islet autoreactive T-cells. *Step 2*) Regulatory T-cells (Treg) and effector T-cells are both produced, and their balance is crucial for maintaining tolerance. Innate immune activation can affect the balance in terms of activating autoimmunity. *Step 3*) The β-cell itself not only produces target antigens but also modifies molecules, such as chromogranin, by cleavage at critical sites, thus creating peptides recognized by pathogenic T-cells. There is evidence that processing of molecules such as insulin within the β-cell creates peptides that are then taken up by antigen-presenting cells either as whole, dead β-cells, or specifically, granules of β-cells, for eventual further processing and presentation of islet peptides to effector T-cells. *Step 4*) The trimolecular complex, involving the MHC-presenting molecule/peptide in the appropriate "register"/T-cell receptor recognizing both and, like a lock and key, is the essential recognition unit for adaptive organ-specific autoimmunity. *Step 5*) Finally, CD4 T-cells orchestrate multiple arms of the immune system (e.g., CD8 cytotoxic T-cells, pathogenic cytokine production), resulting in specific destruction of islet β-cells.

Authors	Viruses	Consequences
	Viruses	
Rewers and Ludvigsson, 2016)	Enterovirus (EV)	Development of β-cell autoimmunity, the presence of enterovirus in pancreatic islets of type 1 diabetic patients.
(Esposito, S.; 2014)	Herpesviridae, ParvoviridaeTogaviridae, Paramyxoviridae, Retroviridae, Picornaviridae,	Induce islet autoimmunity and ß-cell damage and reduce insulin production, leading to full-blown T1DM.
(Krogvold et al., 2015)	Enterovirus (EV)	The presence of enterovirus in pancreatic islets of type 1 diabetic patients.
(Vehik et al., 2019)	Enterovirus A, B, mast adenovirus C, Coxsackievirus, adenovirus	Enterovirus B infections may be involved in the development of islet autoimmunity, but not T1DM; in some young children, coxsackie and adenovirus receptor (*CXADR*) genes independently correlated with islet autoimmunity.
(Hayakawa et al., 2019)	Coxsackievirus B1	Fulminant T1DM in pregnancy may be associated with Coxsackievirus B1 infection.
(Butalia et al., 2016)	Mumps, Rubella, Rotavirus, Rnterovirus, Cytomegalovirus	Development of β-cell autoimmunity, molecular mimicry, in vitro, viruses may induce markers of inflammation and alter HLA class I molecule expression.
(Hodik et al., 2016)	Coxsackievirus	Thecoxsackie–adenovirusreceptor (CAR) is expressed in pancreatic islets of patients with T1DM.
(Aarnisalo et al., 2008)	Cytomegalovirus (CMV)	Development of beta-cell autoimmunity.
(Yoneda et al., 2017)	Cytomegalovirus (CMV)	Significantly increased numbers of alpha cells expressing RIG-I and IRF3 development and progression of T1DM.
(Ekman, et al., 2019)	Cytomegalovirus (CMV)	Development and progression of T1DM.
(Al-Hakami, 2016)	Cytomegalovirus (CMV)	No correlation between T1DMandvirus infectivity.
(Nishiumi et al., 2014)	Parvovirus B19	Fulminant type 1 diabetes mellitus associated with parvovirus B 19.
(Selver Eklioglu et al., 2017)	Parvovirus B19	Diabetic ketoacidosis (DKA) and acute fulminan hepatitis.
(O'Bryan et al., 2005)	Parvovirus B19	No association between parvovirus B19 infection and the development of T1DM.
(Honeyman, 2005)	Rotavirus	Molecular mimicry, pancreatic β cell destruction.
(Harrison et al., 2019)	Rotavirus	Molecular mimicry, development of β-cell autoimmunity.
(Vaarala et al., 2017)	Rotavirus	Molecular mimicry, development and progression of T1DM.
(Glanz et al., 2020)	Rotavirus	Molecular mimicry, development of β-cell autoimmunity, rotavirus vaccination does not appear to be associated with T1DM in children.
(Ramondetti et al., 2012)	Rubella virus, Mumps virus	Mumps and rubella viral infections are associated with T1DM.
(Korkmaz and Ermiş, 2019)	Rubella virus	Rubella viral infections are associated with T1DM.
(Gale, 2008)	Rubella virus	Rubella infections predispose to autoimmunity.
(Vuorinen et al., 1992)	Mumps Virus, Coxsackievirus	In vitro model indicated that mumps and coxsackie B3 viruses infect human fetal pancreatic endocrine cells and are able to alter beta-cell function.
(Precechtelova et al., 2014)	Human Cytomegalovirus, Parvovirus, Rotavirus, Coxsackievirus, Human Parechovirus, Enteric Cytopathic Human Orphan viruses, Mumps virus, Rubella virus	Persistent infection, molecular mimicry, autoimmune destruction of pancreatic ≤ β-cells, congenital infection, loss of regulatory T-cells. Infection by rubella virus during pregnancy has been related to increased risk of diabetes in the offspring suffering from congenital rubella syndrome.
(Levet et al., 2017)	Human endogenous retroviruses (HERV)	Pancreatic β cell destruction.
(Parkkonen et al., 1992)	Mumps virus	The infection is associated with an increase in the expression of HLA class I molecules.

Authors	Viruses	Consequences
	Viruses	
(Al-Hakami, 2016)	Viracela, measles	Hemagglutinin peptide and Hsp60 peptide induce the cellular immune response; varicella and measles are risk factors in developing type 1 diabetes.
(Rubino et al., 2020)	SARS-CoV-2	SARS-CoV-2 virus leads to diabetes via binding to its cellular entry—ACE-2 receptors, which are abundant in pancreatic beta cells and adipose tissue, leading to glucose metabolism abnormalities, and pancreatic beta cells destruction.
(Suwanwongse and Shabarek, 2021)	SARS-CoV-2	The aberrant immunity caused by SARS-CoV-2 may induce an auto-immune attack on the pancreatic islet cells mimicking the pathogenesis of insulin-dependent DM.
(Lança et al., 2022)	SARS-CoV-2	Delayed diagnosis, low socioeconomic status, and infection have been associated with diabetic ketoacidosis (DKA) in type 1 diabetes mellitus.

-Molecular Mimicry per la somiglianza fra la proteina 2C del Coxackie e il GAD. Ma cloni di T-cell Autoreattive per GAD non proliferano in presenza dell'epitopo virale nè cross-reagiscono gli Ab anti-GAD con le proteine Coxackie

-Bystander Activation per attivazione di T-cell potenzialmente auto-reattive a seguito della liberazione di antigeni sequestrati da parte dei tessuti infetti

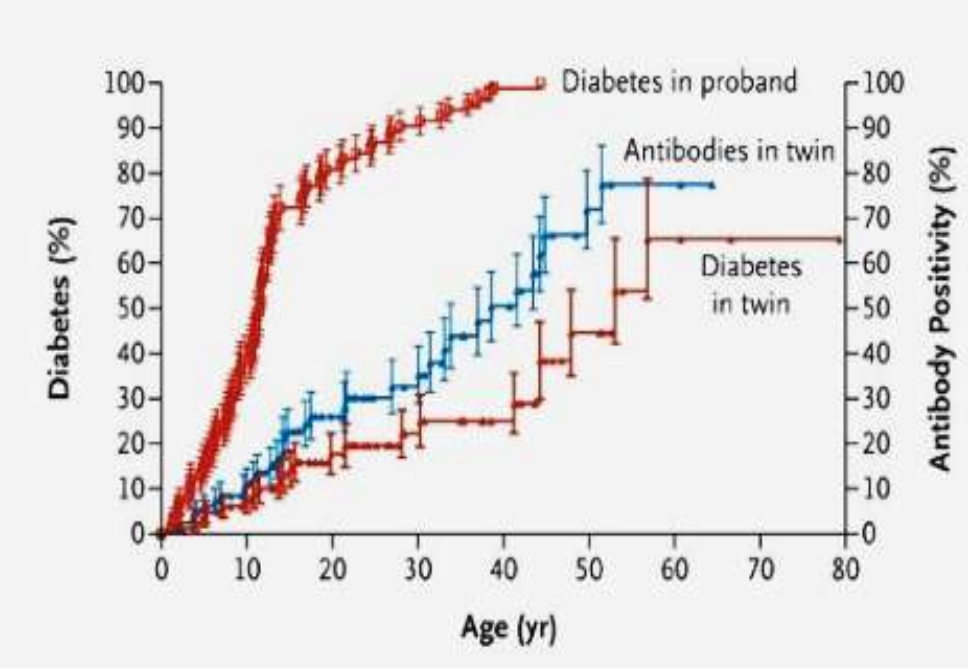
-Fertile Field determinato da una infiammazione persistente con conseguente produzione di INFα che stimola l'espressione anomala di antigeni HLA classe I. Questi "presentando" self-antigeni e/o rilasciando autoantigeni sequestrati attivano i linfociti T (auto)citotossici ($CD8^{+}$)

-Hygiene hypothesis The rate of type 1 diabetes among the children of Pakistanis who migrated to the United Kingdom is the same as the rate among nonimmigrants in the United Kingdom (11.7 per 100,000), about 10 times as high as the incidence in Pakistan (1 per 100,000)

Type1 diabetes familial aggregation

Concordance for Islet Autoimmunity among Monozygotic Twins

N ENGL J MED 359;26 2008

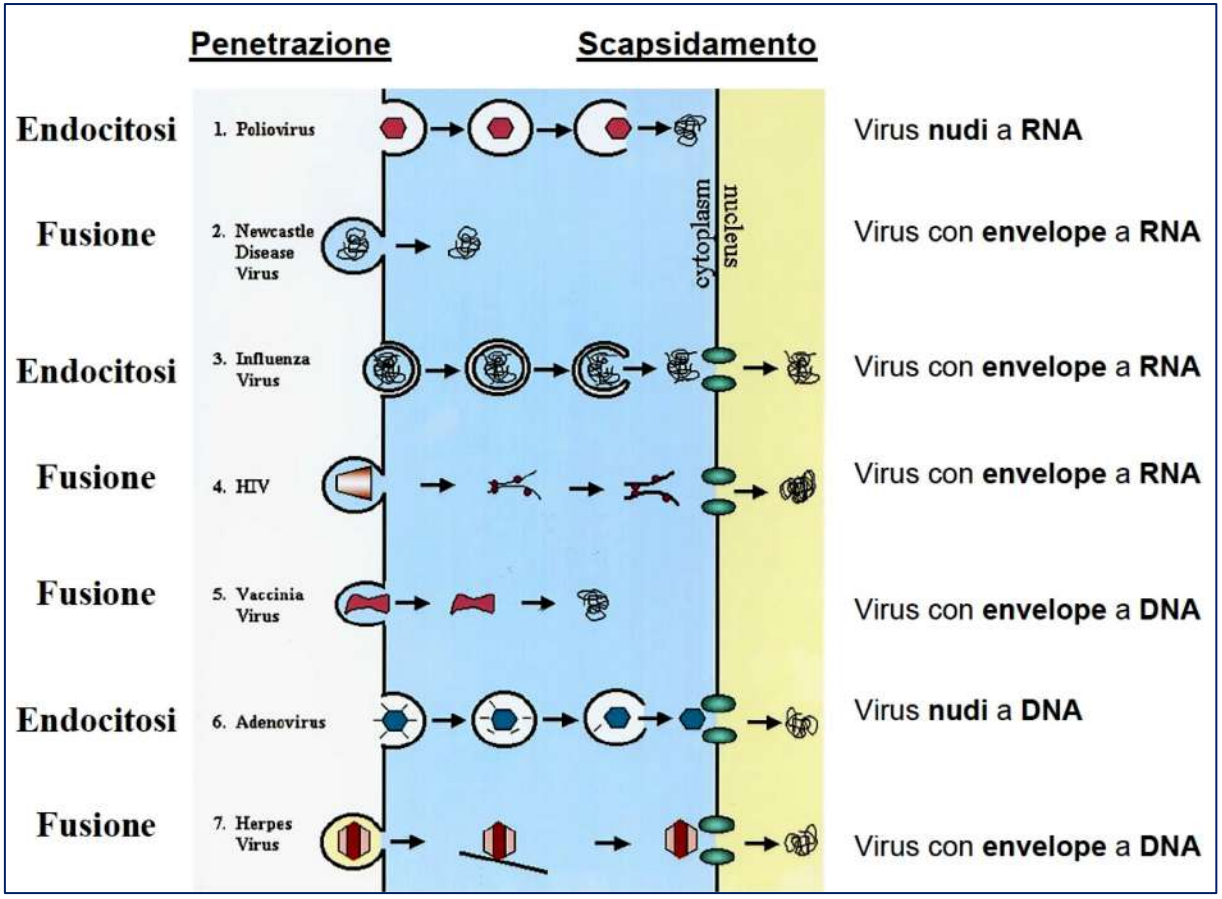


- General population: 0.3%
- Siblings of patients: 5%
- Children of male patient: 6-9%
- Children of female patient: 1.3-4%
- Monozigotic twins: 40%
- Monozygotic twins of a patients: 60%

Anche con un follow-up a lungo termine (>30 anni)
il rischio tra gemelli monozigoti raggiunge appena il 65%

VIROSFERA

si stima che ci siano 10^{31} virus; per paragone si stima che le stelle siano 10^{24}



1. Molti virus con envelope penetrano fondendo il proprio rivestimento con la membrana cellulare.
2. Molti virus a DNA (mono o bicatenario) e a RNA (retrovirus) duplicano il loro materiale genetico nel nucleo cellulare, spesso integrandosi con il DNA della cellula ospite.

Virus is ”simply a piece of bad news wrapped in a protein”

Peter Medawar (Nobel per la medicina nel 1960)

THERAPEUTIC VIRUSES

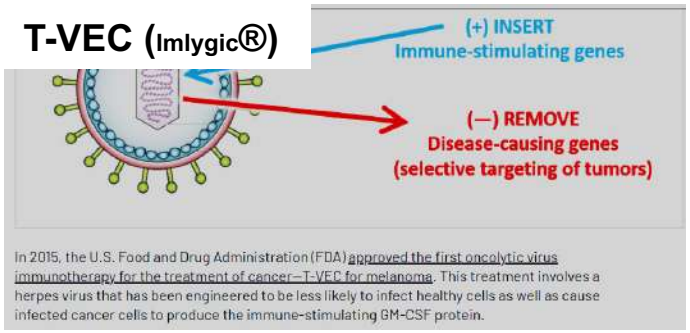
SEZIONI | CERCA — IL SECOLO XIX

Studio italiano rivela: «Una terapia genica pu riparare il cuore dopo l’infarto»

08 Maggio 2019 alle 18:49 | 2 minuti di lettura

Nella ricerca sono state trasferite nel cuore di maiali colpiti da infarto sequenze di micro-Rna (microRNA-199) attraverso un virus ingegnerizzato che ha stimolato la rigenerazione del cuore nel maiale portando al recupero quasi completo della sua funzionalità in un mese.

T-VEC (Imlygic®)



In 2015, the U.S. Food and Drug Administration (FDA) approved the first oncolytic virus immunotherapy for the treatment of cancer—T-VEC for melanoma. This treatment involves a herpes virus that has been engineered to be less likely to infect healthy cells as well as cause infected cancer cells to produce the immune-stimulating GM-CSF protein.

Lentivirus-based Gene Therapy of Hematopoietic Stem Cells in Wiskott-Aldrich Syndrome *Science*. 2013 August 23;

Oncolytic viruses and pancreatic cancer

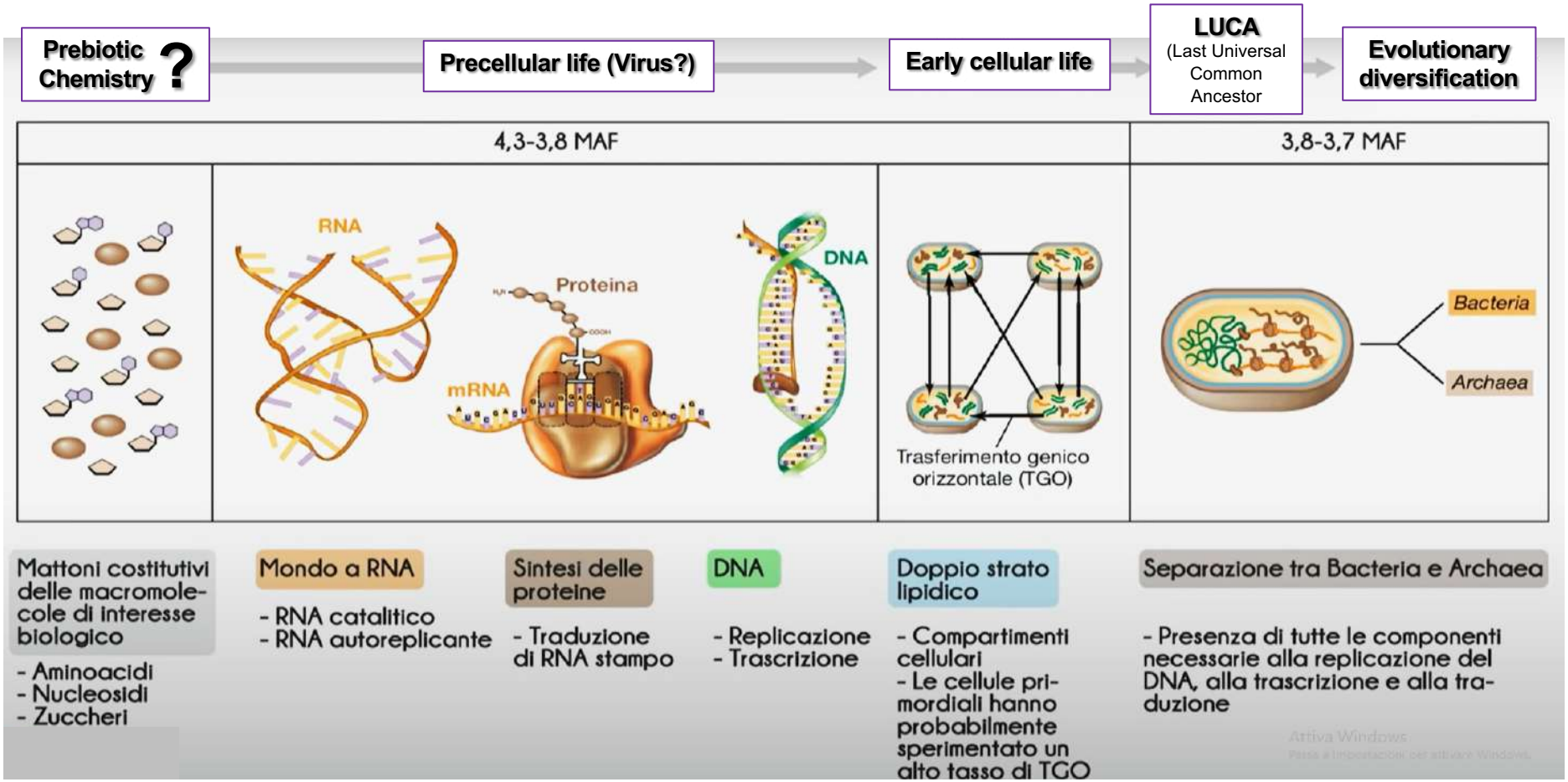
Cancer Treatment and Research Communications 31 (2022)

TERAPIA FAGICA

Poiché la resistenza ai fagi è 10 volte inferiore a quella degli antibiotici si sta testando la terapia fagica in caso di infezioni MDR:

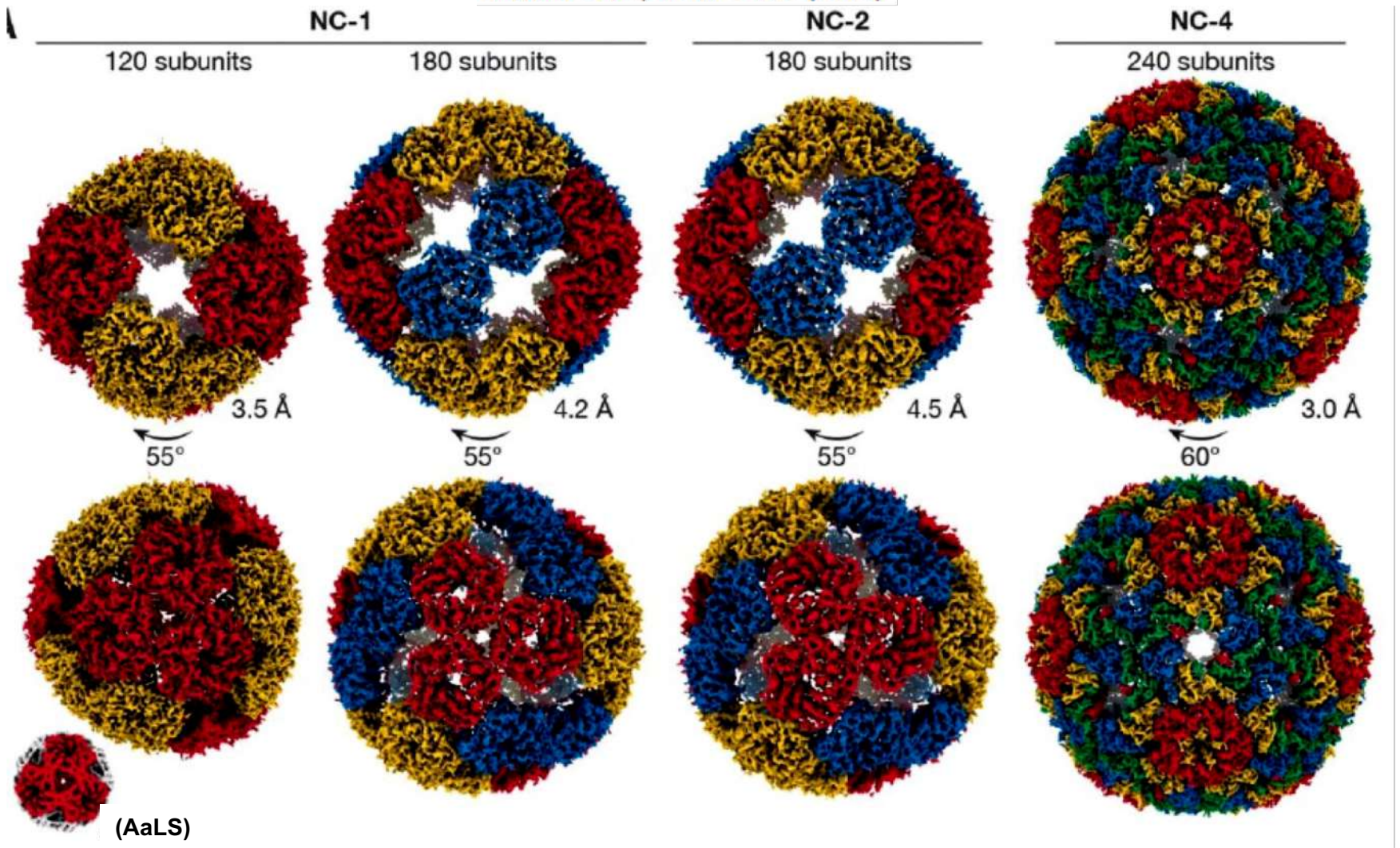
- è in corso uno studio di fase 1b/2 con terapia fagica in pazienti con fibrosi cistica colonizzati con P. aeruginosa.
- è in corso uno studio di fase 2/3 su terapia fagica in infezioni urinarie da E. coli MDR.
- si sta tentando di ottimizzare le terapie fagiche utilizzando la tecnologia CRISPR-Cas3

Il Mondo a RNA



Evolution of a virus-like architecture and packaging mechanism in a repurposed bacterial protein

Science **372**, 1220–1224 (2021)



Aquifex aeolicus lumazine synthase (**AaLS**), a bacterial enzyme, was redesigned appending the arginine-rich peptide lN+, which tightly binds his own RNAm.

An Update on Eukaryotic Viruses Revived from Ancient Permafrost

Viruses **2023**, *15*, 564.

Description	Isolated Virus
Surface soil, Shapina river bank, Kamchatka Modern	*Cedratvirus kamchatka* (strain P5)
Lena river, Yakutsk Modern	*Cedratvirus lena* (strain DY0) *Pandoravirus lena* (strain DY0)
Talik, −6.5 m below a lake, Yukechi Alas [43] Isolation: >53 y BP	*Pandoravirus talik* (strain Y4)
Melting ice wedge Duvanny yar [23,44] Mixed ages	*Cedratvirus duvanny* (strain DY1) *Pandoravirus duvanny* (strain DY1)
−16 m below a lake, Yukechi Alas [43] Isolation: >48,500 y BP	*Pandoravirus yedoma* (strain Y2)
Woolly mammoth stomach content, Maly Lyakhovsky Island [45] Isolation: >28,600 y BP	*Pandoravirus mammoth* (strain Mm38)
Soil with mammoth wool RHS paleolithic site, Yana river left bank [46,47] Isolation: >27,000 BP	*Megavirus mammoth* (strain Yana14) *Pithovirus mammoth* (strain Yana14) *Pandoravirus mammoth* (strain Yana14)
Fossil wolf (Canis lupus) intestinal content, RHS paleolithic site [46,47] Isolation: >27,000 y BP	*Pandoravirus lupus* (strain Tums1)
Fossil wolf (Canis lupus) intestinal content, RHS paleolithic site [46,47] Isolation: >27,000 y BP	*Pacmanvirus lupus* (strain Tums2)

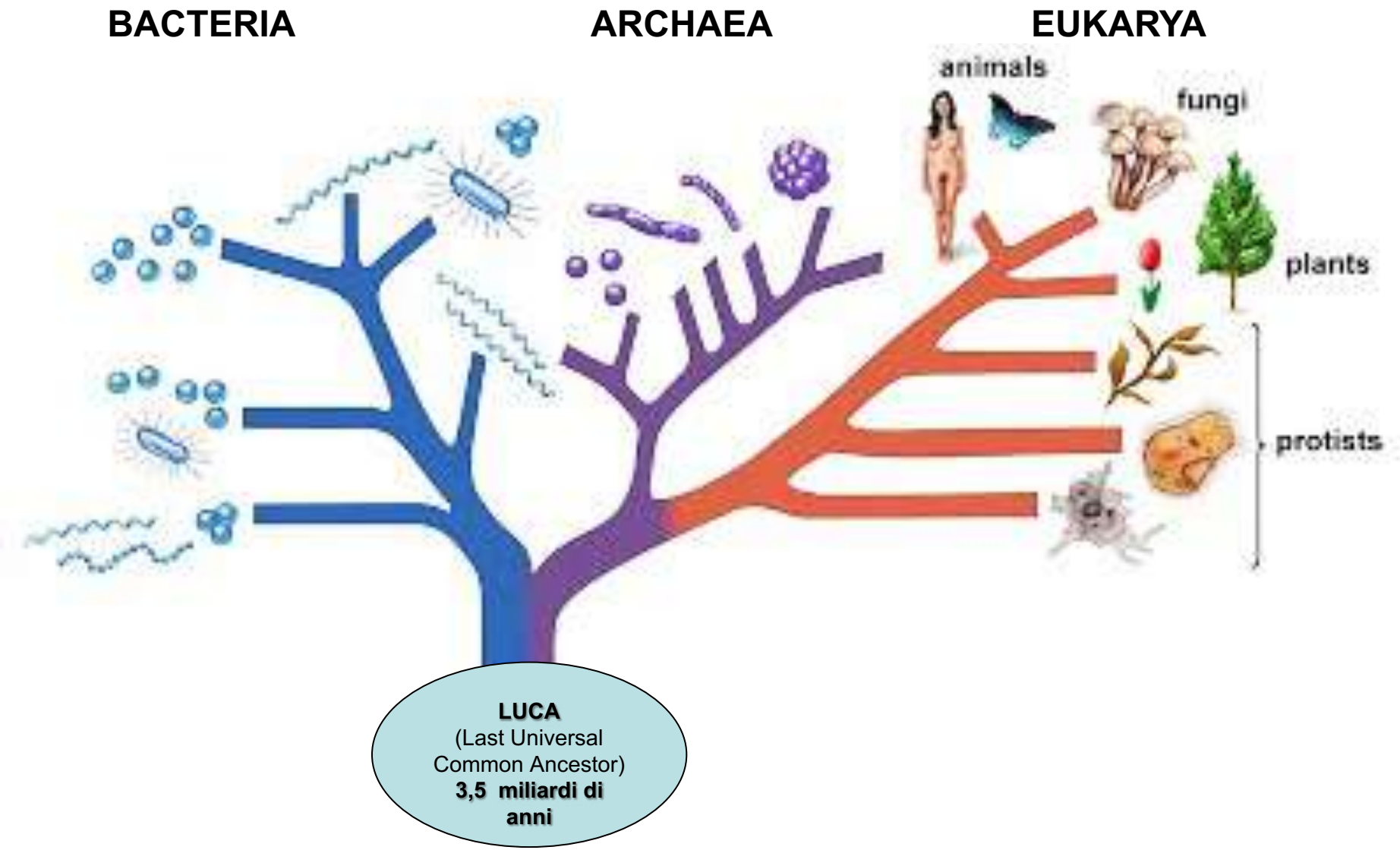
An Update on Eukaryotic Viruses Revived from Ancient Permafrost

Viruses 2023, *15*, 564.

Description	Isolated Virus
Surface soil, Shapina river bank, Kamchatka Modern	*Cedratvirus kamchatka* (strain P5)
Lena river, Yakutsk Modern	*Cedratvirus lena* (strain DY0) *Pandoravirus lena* (strain DY0)
Talik, −6.5 m below a lake, Yukechi Alas [43] Isolation: >53 y BP	*Pandoravirus talik* (strain Y4)
Melting ice wedge Duvanny yar [23,44] Mixed ages	*Cedratvirus duvanny* (strain DY1) *Pandoravirus duvanny* (strain DY1)
−16 m below a lake, Yukechi Alas [43] Isolation: >48,500 y BP	*Pandoravirus yedoma* (strain Y2)
Woolly mammoth stomach content, Maly Lyakhovsky Island [45] Isolation: >28,600 y BP	*Pandoravirus mammoth* (strain Mm38)
Soil with mammoth wool RHS paleolithic site, Yana river left bank [46,47] Isolation: >27,000 BP	*Megavirus mammoth* (strain Yana14) *Pithovirus mammoth* (strain Yana14) *Pandoravirus mammoth* (strain Yana14)
Fossil wolf (Canis lupus) intestinal content, RHS paleolithic site [46,47] Isolation: >27,000 y BP	*Pandoravirus lupus* (strain Tums1)
Fossil wolf (Canis lupus) intestinal content, RHS paleolithic site [46,47] Isolation: >27,000 y BP	*Pacmanvirus lupus* (strain Tums2)

- Many studies have documented the presence of a large diversity of bacteria in ancient permafrost, a significant proportion of which are thought to be alive…
- Fortunately, we can reasonably hope that an epidemic caused by a revived prehistoric pathogenic bacterium could be quickly controlled by the modern antibiotics at our disposal.
- The situation would be much more disastrous in the case of plant, animal, or human diseases caused by the revival of an ancient unknown virus..
- The first one was the isolation of Influenza RNA from one frozen biopsy of the lung of a victim buried in permafrost…
- Another one was the detection of smallpox virus DNA in a 300-year-old Siberian mummy buried in Permafrost. Probably for safety/regulatory reasons, there were not follow-up studies attempting to “revive” these viruses (fortunately)…
- The first isolation of two fully infectious eukaryotic viruses from 30,000 y old permafrost was performed in our laboratory and published in 2015.
- **Tupanvirus possess the most complete gene set related to the protein synthesis, including 20 aminoacyl-tRNA synthetases, tRNAs, and genes involved in maturation and modification of tRNA and mRNA (Archives of Virology 164:325–331; 2019)**

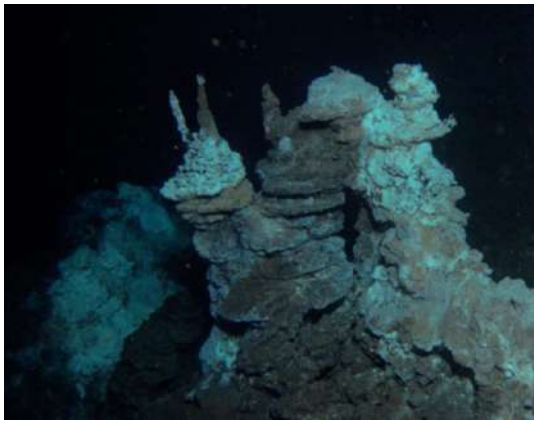
Phylogenetic Tree of Life



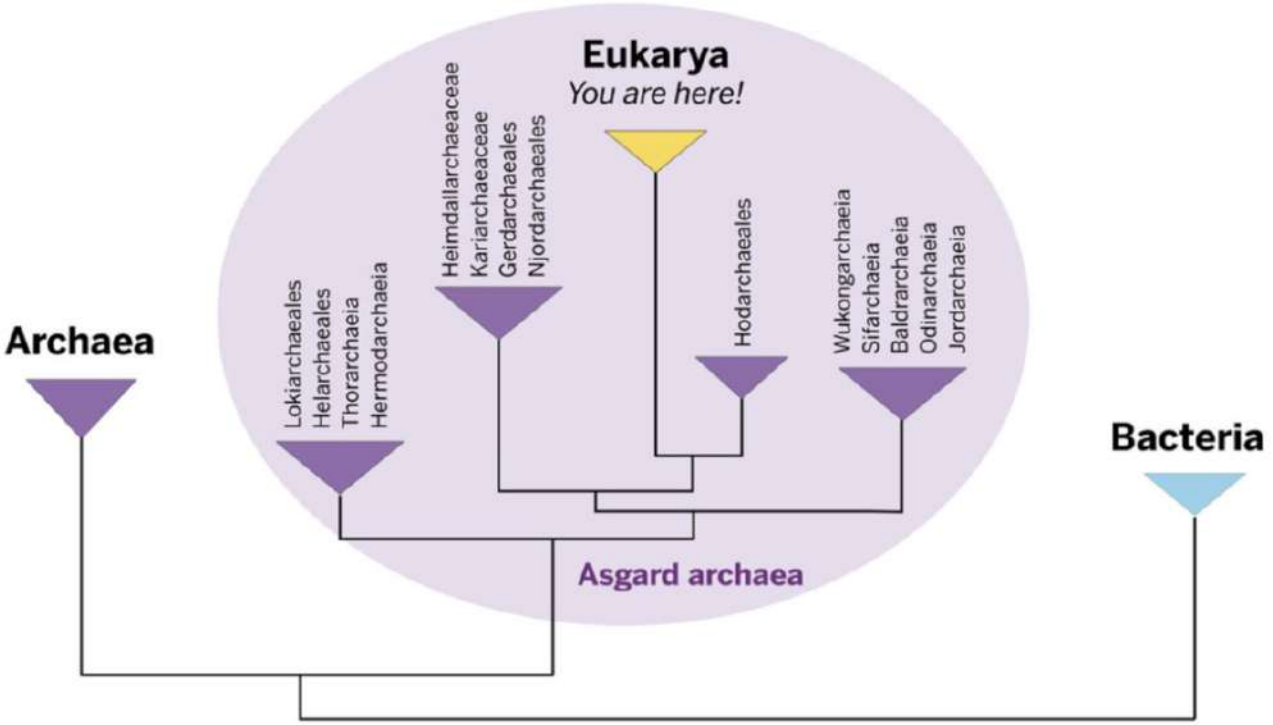
Inference and reconstruction of the heimdallarchaeial ancestry of eukaryotes

Nature | Vol 618 | 29 June 2023

….we infer that the last common ancestor of Asgard archaea was probably a thermophilic chemolithotroph and that the lineage from which eukaryotes evolved adapted to mesophilic conditions and acquired the genetic potential to support a heterotrophic lifestyle.



Il "castello di Loki" è una sorgente idrotermale scoperta nel mar glaciale Artico dai ricercatori del centro di geobiologia di Norvegia

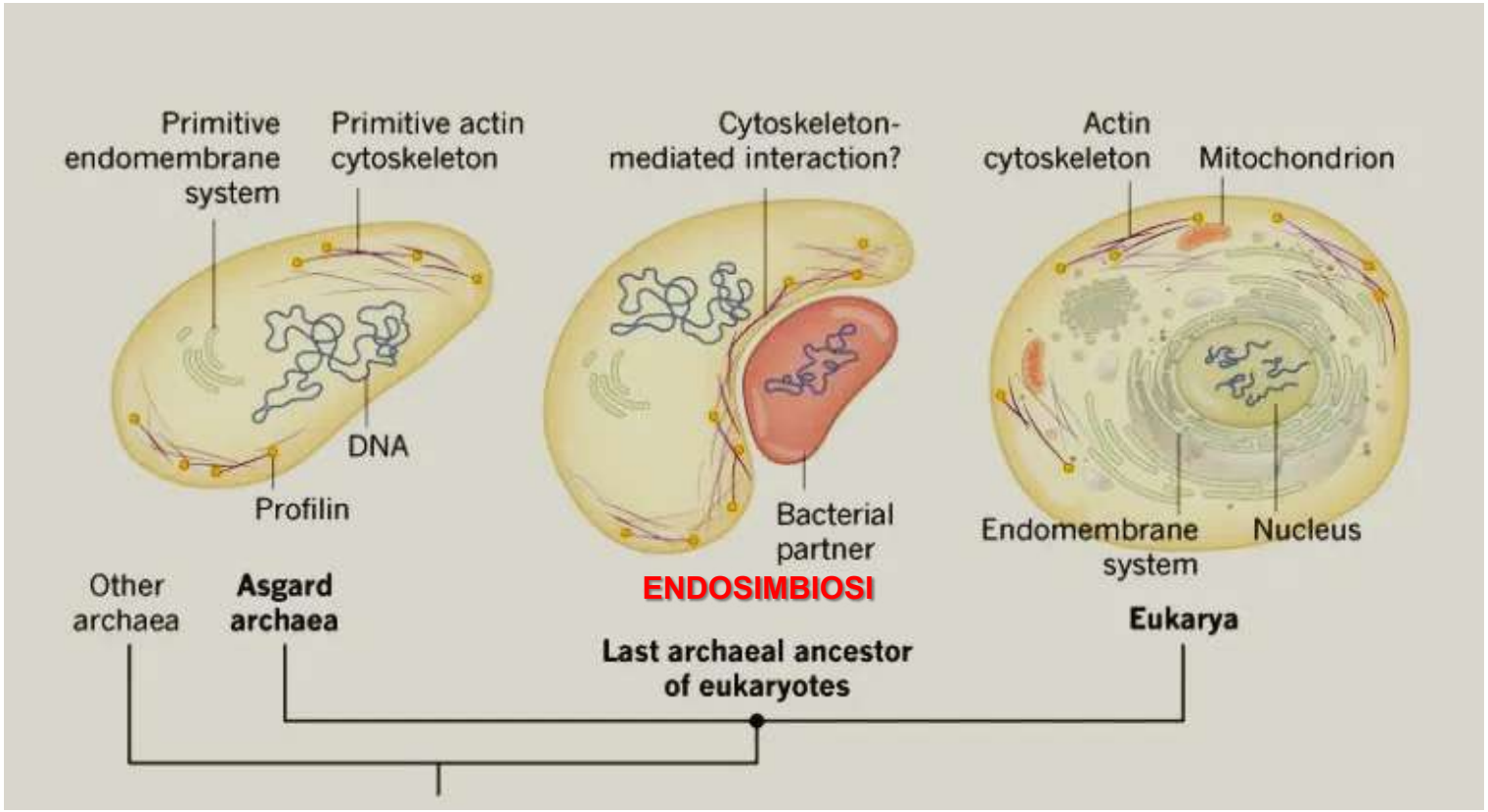


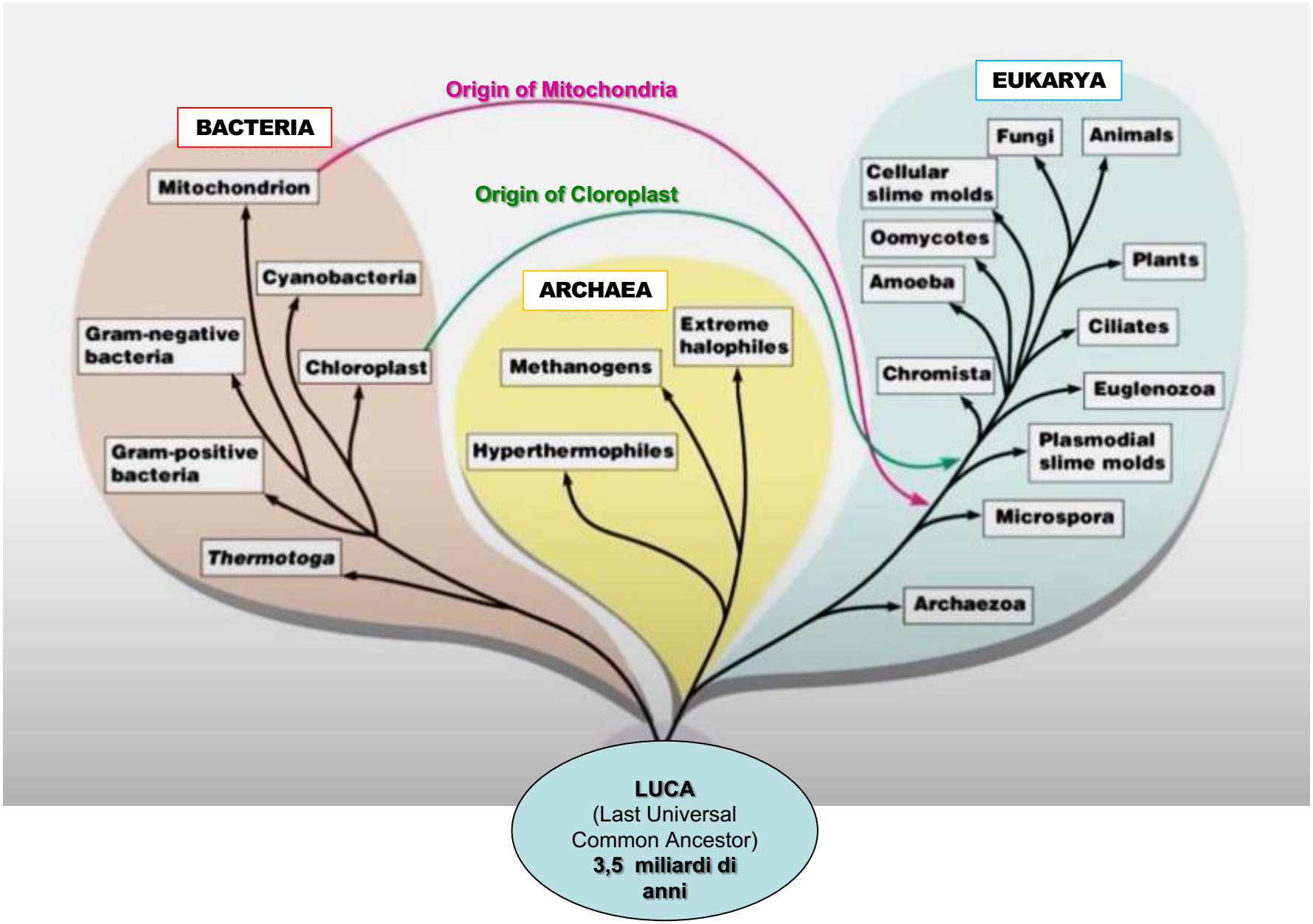
In the ongoing debates about eukaryogenesis members of the Asgard Archaea play a key part as the closest archaeal relatives of eukaryotes.

The eukaryotic ancestor shapes up

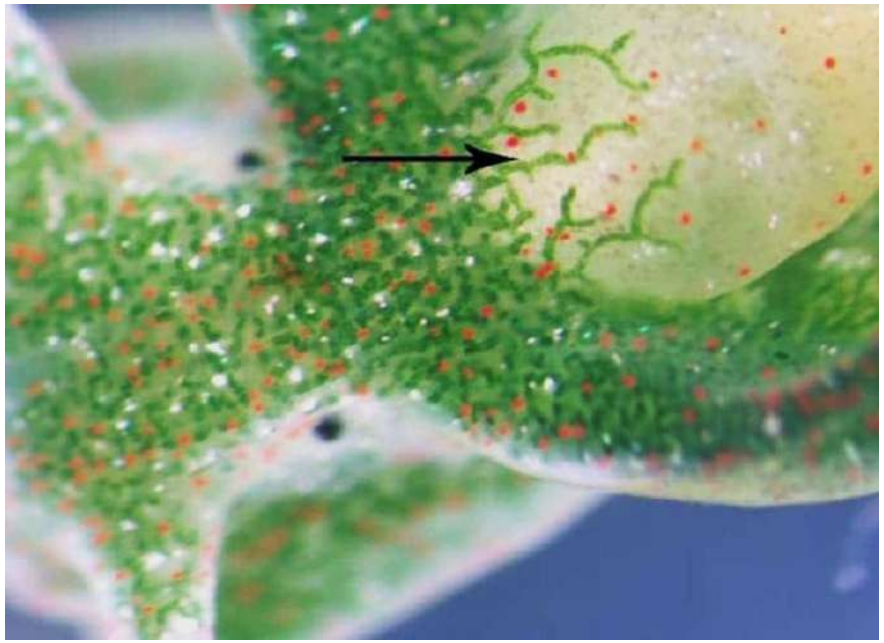
nature | 03 October 2018

Asgard Archaea are the closest known relatives of nucleus-bearing organisms called eukaryotes.
A study indicates that these archaea have a dynamic network of actin protein — a trait thought of as eukaryote-specific.




Origin of Mitochondria
BACTERIA
Mitochondrion
Origin of Cloroplast
Cyanobacteria
ARCHAEA
Gram-negative bacteria
Chloroplast
Extreme halophiles
Methanogens
Gram-positive bacteria
Hyperthermophiles
Thermotoga
EUKARYA
Fungi
Animals
Cellular slime molds
Oomycotes
Plants
Amoeba
Ciliates
Chromista
Euglenozoa
Plasmodial slime molds
Microspora
Archaezoa
LUCA
(Last Universal Common Ancestor)
3,5 miliardi di anni

Elysia chlorotica: la lumaca fotosintetica



Dettaglio: si vedono i diverticoli digestivi con i cloroplasti integrati.

Costasiella Kuroshimae

Toxoneuron nigriceps

insects

MDPI

Article

Role of Ovarian Proteins Secreted by *Toxoneuron nigriceps* (Viereck) (Hymenoptera, Braconidae) in the Early Suppression of Host Immune Response

Rosanna Salvia [1,2], Carmen Scieuzo [1,2], Annalisa Grimaldi [3], Paolo Fanti [1], Antonio Moretta [1], Antonio Franco [1,2], Paola Varricchio [4], S. Bradleigh Vinson [5] and Patrizia Falabella [1,2,*]

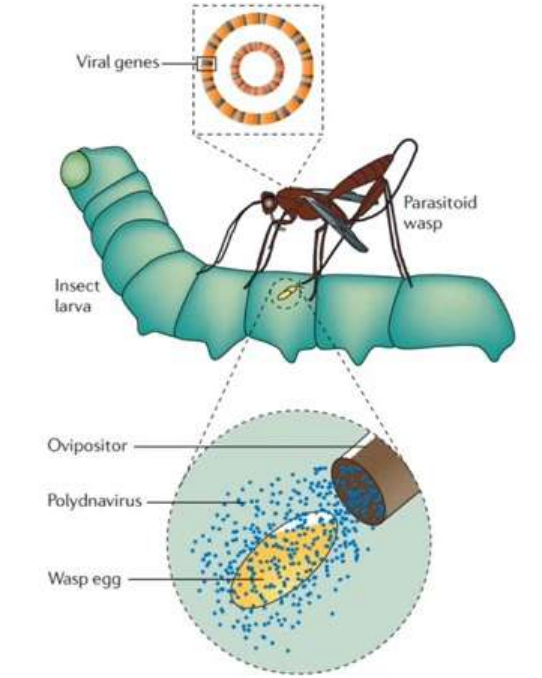
Insects **2021**, *12*, 33. https://doi.org/10.3390/insects12010033

Un Polydnavirus (PDV) il cui genoma è ormai integrato nel DNA della vespa

e si reproduce <u>solo</u> nell'organo ovodepositore

da cui infetta il "verme del tabacco"

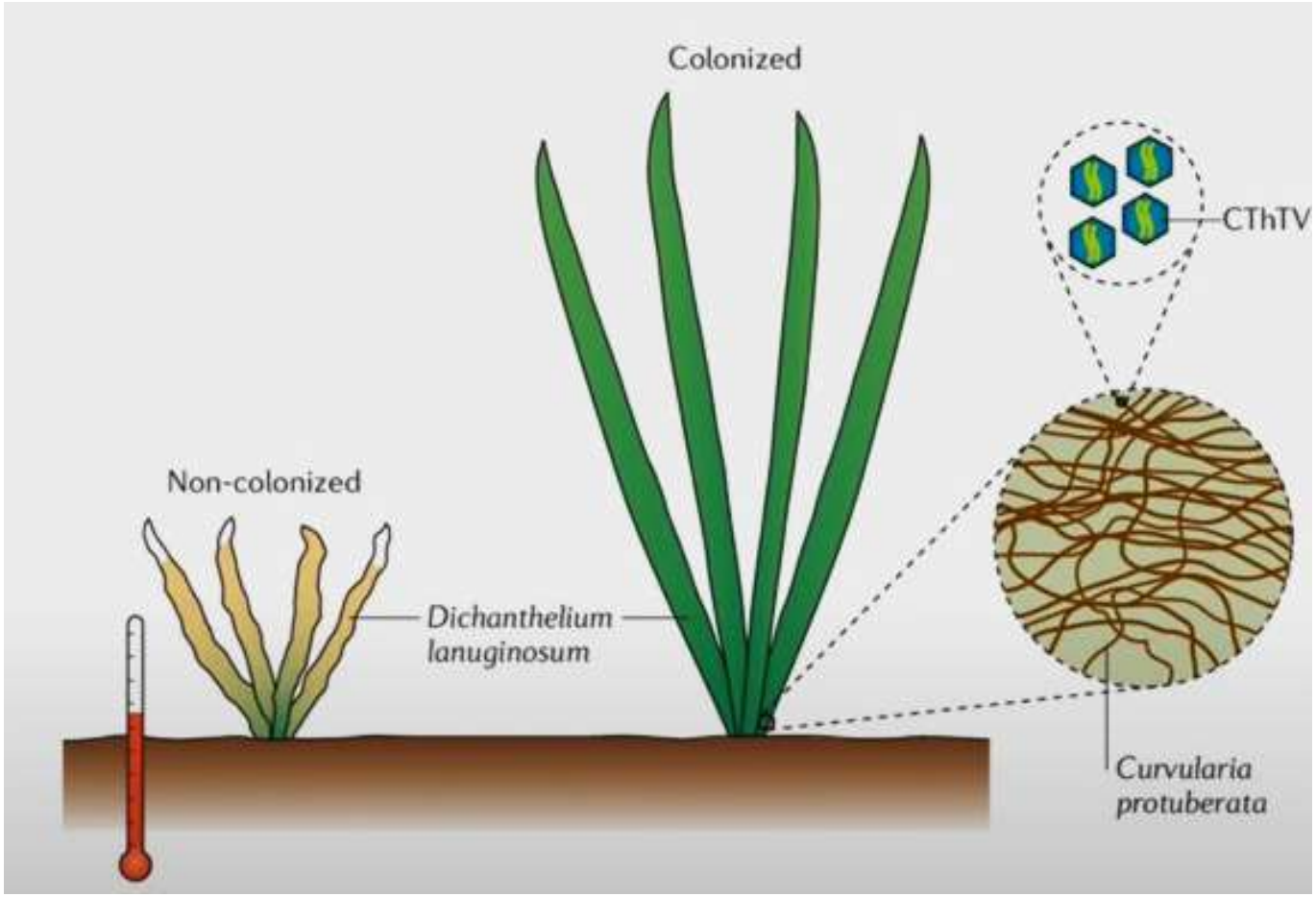
che è la larva di una falena



A Virus in a Fungus in a Plant: Three-Way Symbiosis Required for Thermal Tolerance

LUIS M. MÁRQUEZ, REGINA S. REDMAN, RUSSELL J. RODRIGUEZ, AND MARILYN J. ROOSSINCK Authors Info & Affiliations

SCIENCE • 26 Jan 2007 • Vol 315, Issue 5811 • pp. 513-515 • DOI: 10.1126/science.1136237



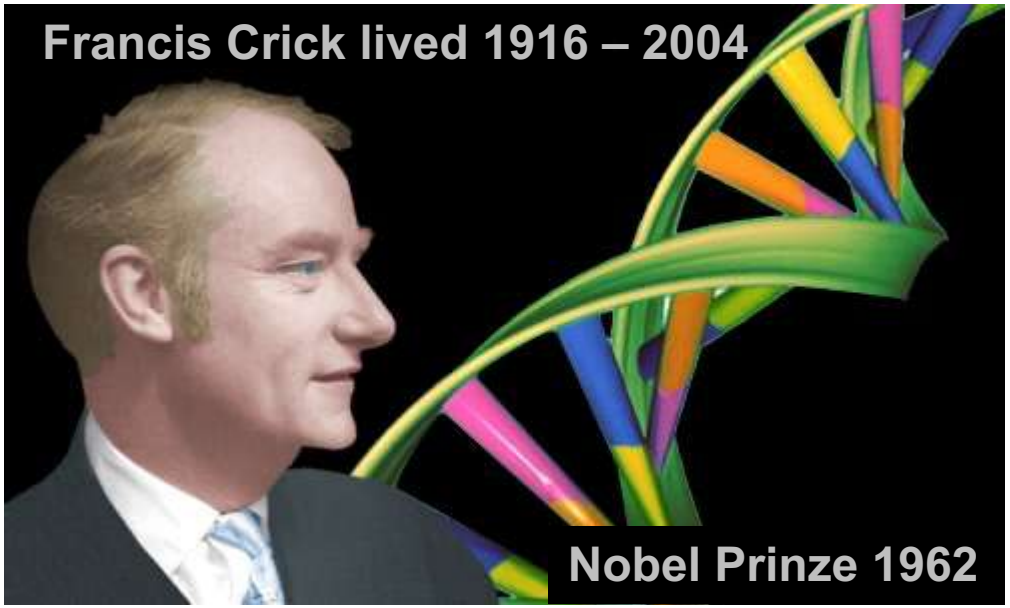
When it is colonised by the fungus Curvularia protuberata

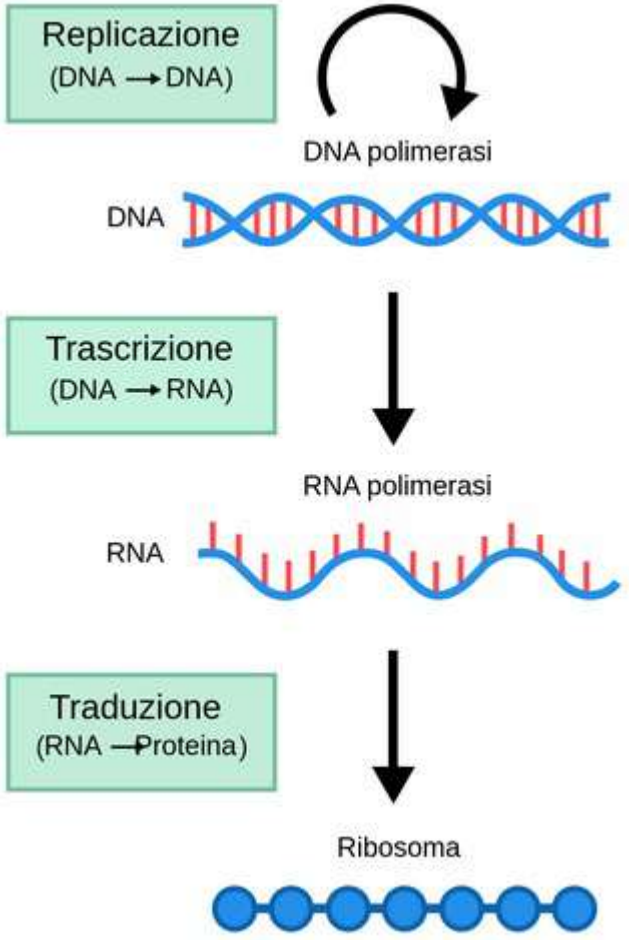
and the fungus is in turn colonised by a particular virus

the grass is able to tolerate soil temperatures of up to 65 °C that would otherwise be lethal.

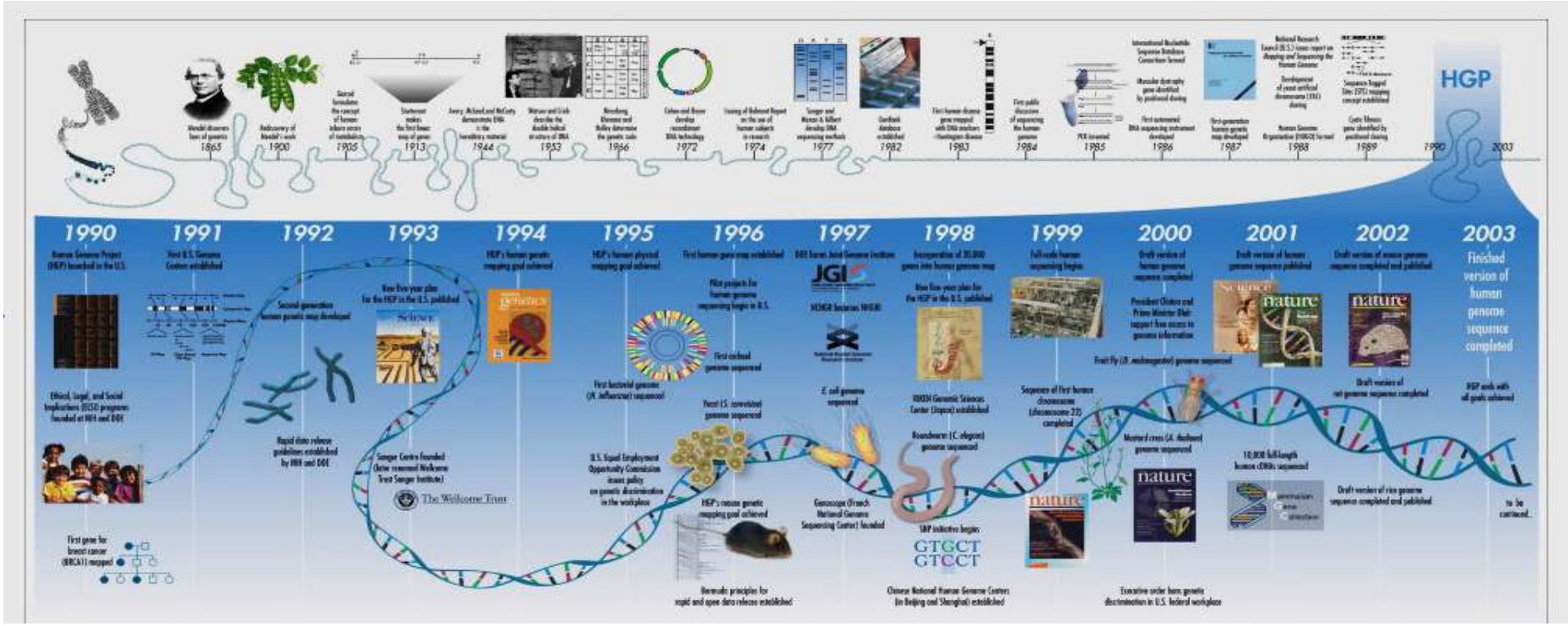
Dogma centrale della genetica

(postulato da Crick nel 1958)





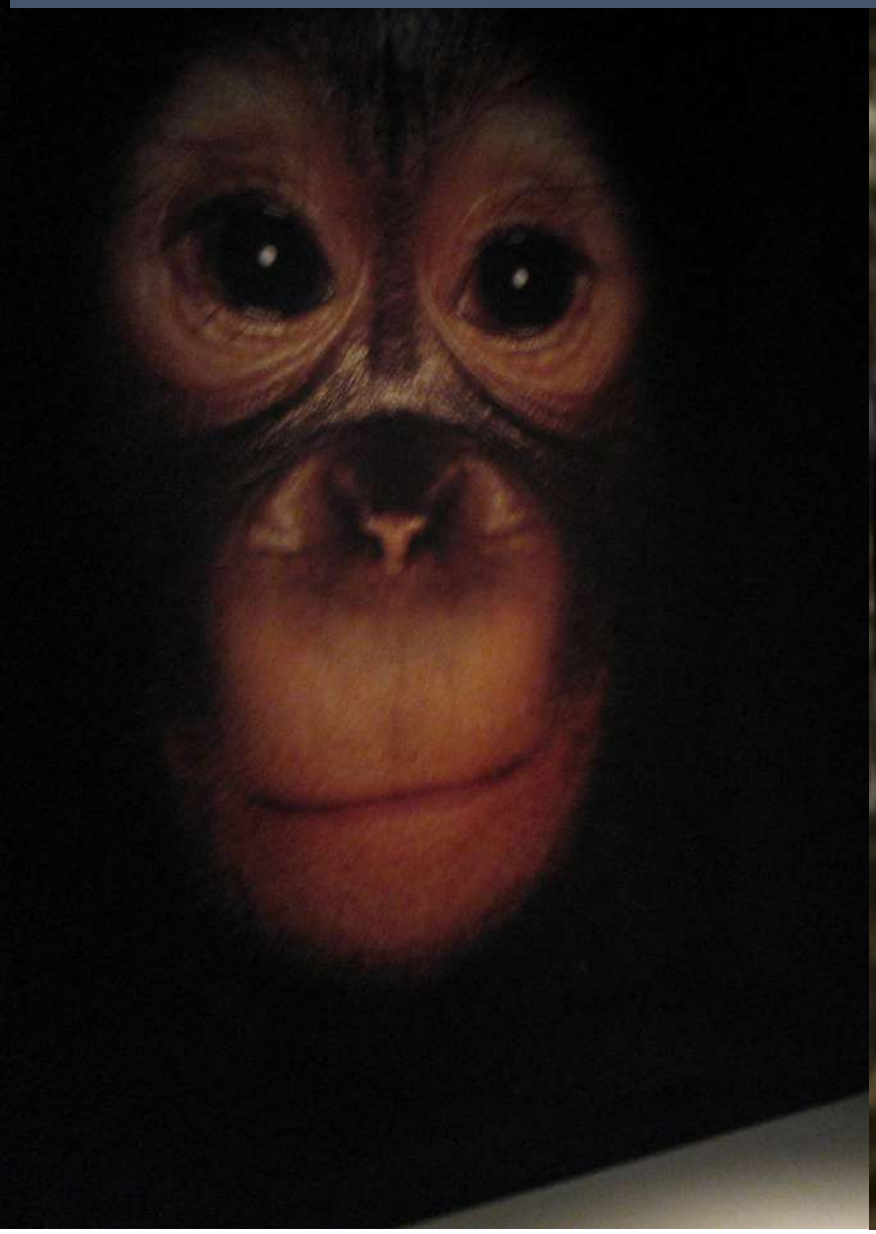
HUGO (HUman Genome Organization) 1990-2003: il colpo di grazia al dogma centrale della genetica



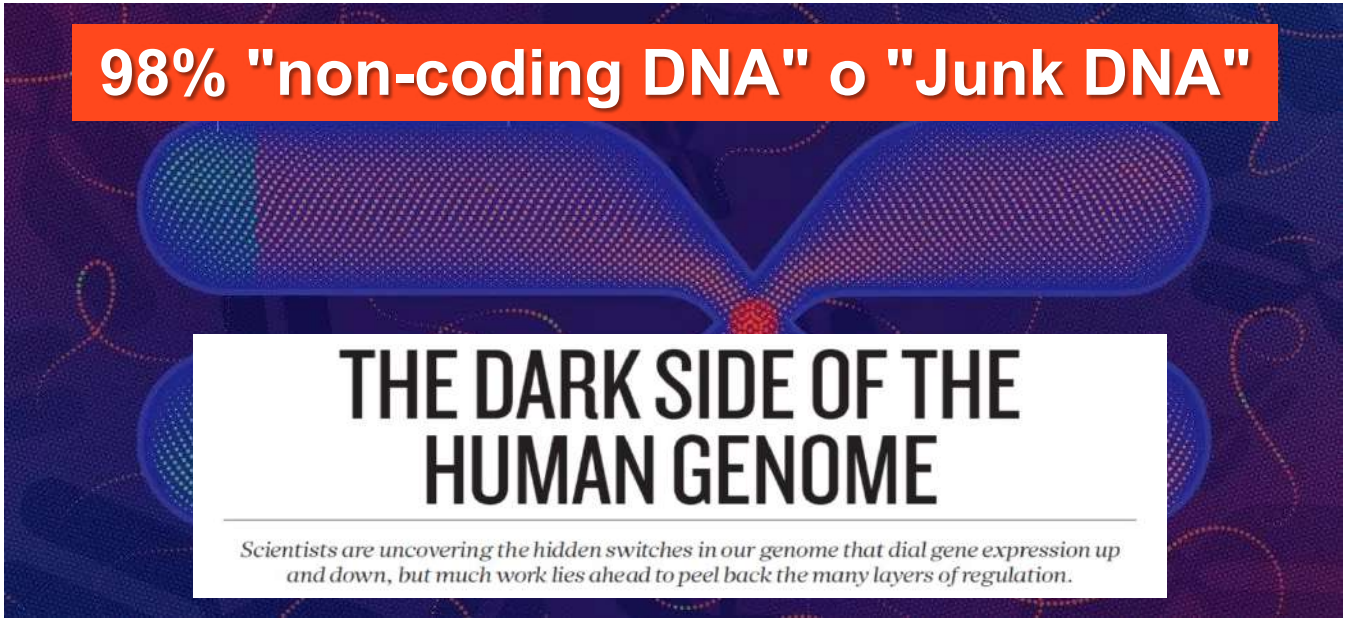
HUGO (HUman Genome Organization):
è durato 13 anni, ha coinvolto migliaia di ricercatori di tutto il mondo ed e costato oltre 3 bilioni di dollari.
Oggi un'analisi di genoma costa meno di mille dollari e la effettua un tecnico.

Il 26 Giugno 2000: Conferenza stampa alla Casa Bianca di Clinton con i rappresentanti della Celera Genomics

Ci rendiamo conto di condividere con lo scimpanzè oltre il 98% del corredo genetico …



Scopriamo che solo il 2% del nostro DNA codifica proteine
e il numero di geni codificanti proteine
è lo stesso di un verme o del pesce palla ….



Prokaryotes (E. Coli)	*circa 7.000 geni codificanti*
Drosophila	*circa 14.000 geni codificanti*
Worm (C. Elegans)	*circa 20.000 geni codificanti*
Humans	*circa 22.000 geni codificanti*
Pufferfish (Fugu rubripes)	*circa 26.000 geni codificanti*

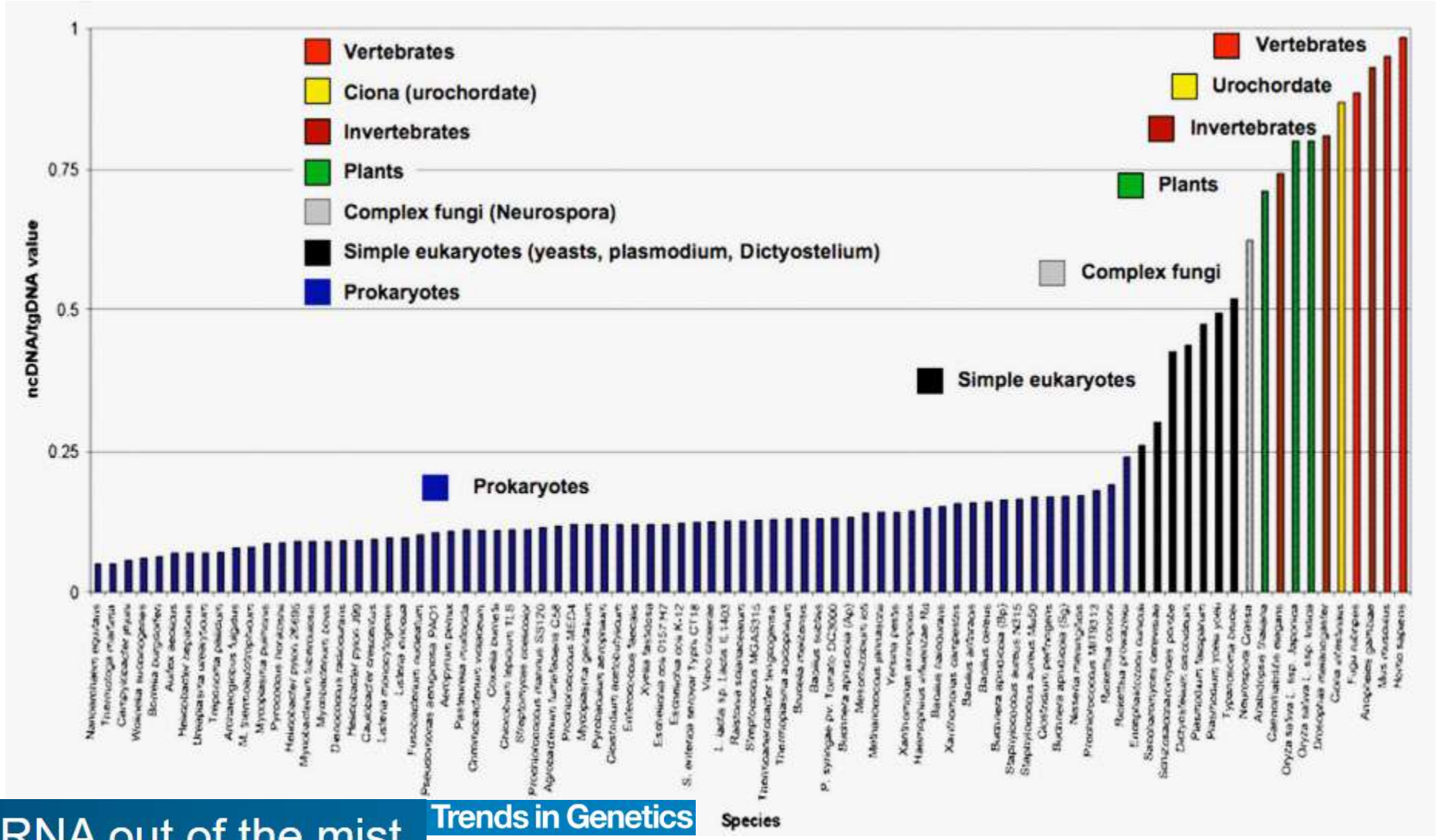
Per di più molte proteine sono omologhe e hanno funzioni simili !!

…. ma al contrario del verme
scopriamo di avere moltissimi geni non codificanti,
geni che si trascrivono in RNA non codificante.

	E. Coli 	*C. Elegans* 	*Humans* 
Cromosomi	**1**	**6**	**23**
Geni codificanti proteine	**6692**	**20541**	**21995**
DNA non codificante proteine	**5%**	**60%**	**98%**
Geni non codificanti proteine	**15**	**224**	**> 40000**
miRNA	**0**	**1522**	**10616**

Il ***non-coding DNA*** contiene sequenze trascritte ad RNA ma non tradotte a proteina:
il numero di questi ***non-coding RNA*** supera di gran lunga il numero di geni codificanti.

Ci accorgiamo così che il ncDNA aumenta con l'aumentare della complessità,
che riveste il ruolo principale nel modulare l'espressione genica,
che contribuisce in modo determinante anche al controllo epigenetico….



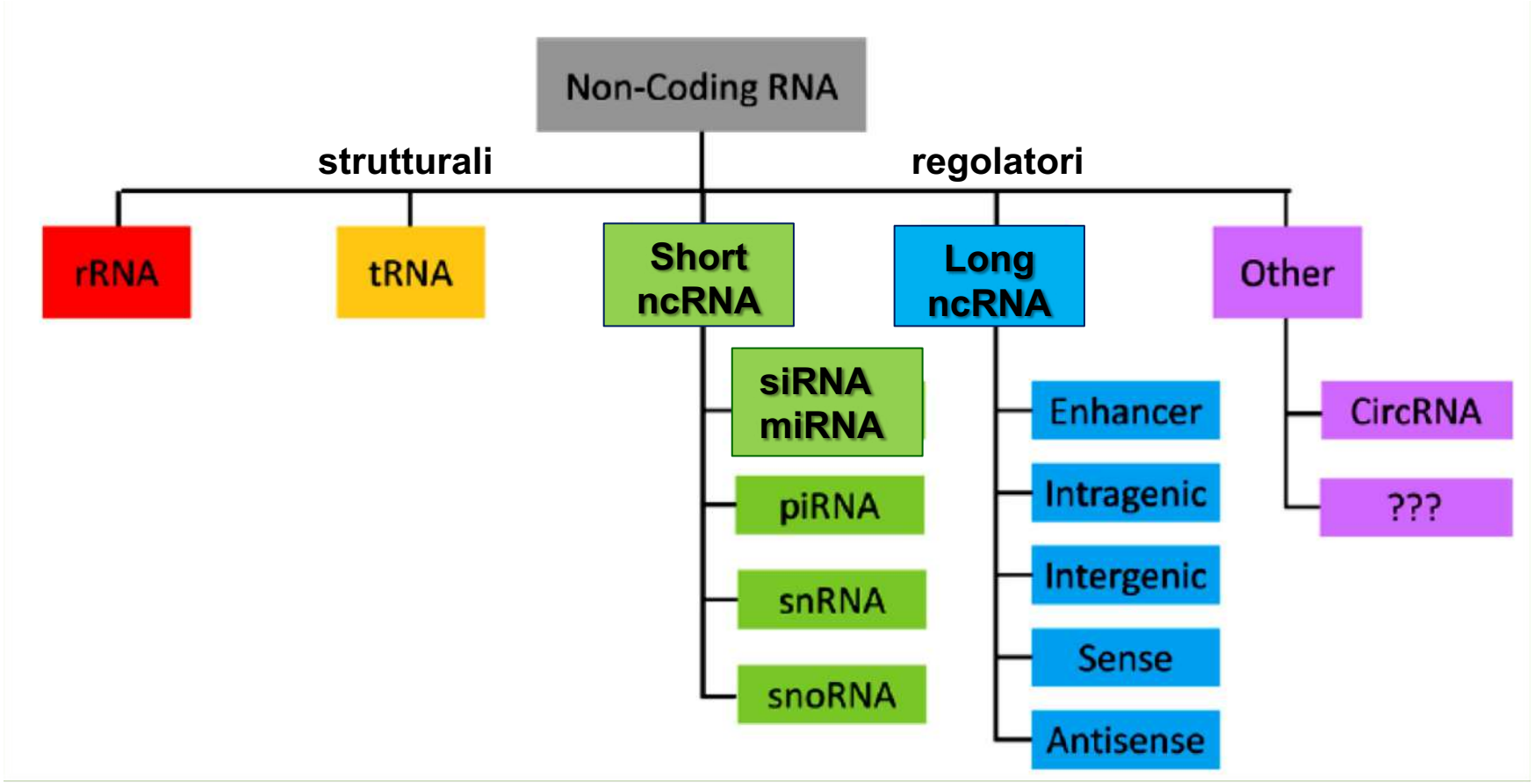
RNA out of the mist

Trends in Genetics

December 16, 2022

To code or not to code?

That is the question for RNA in timekeeping



A number of nuclear **lncRNAs** appear to regulate both their neighboring environment and act in distant genomic loci. In other words, **they may be involved in the specific repression of a promoter or transcription activation**.

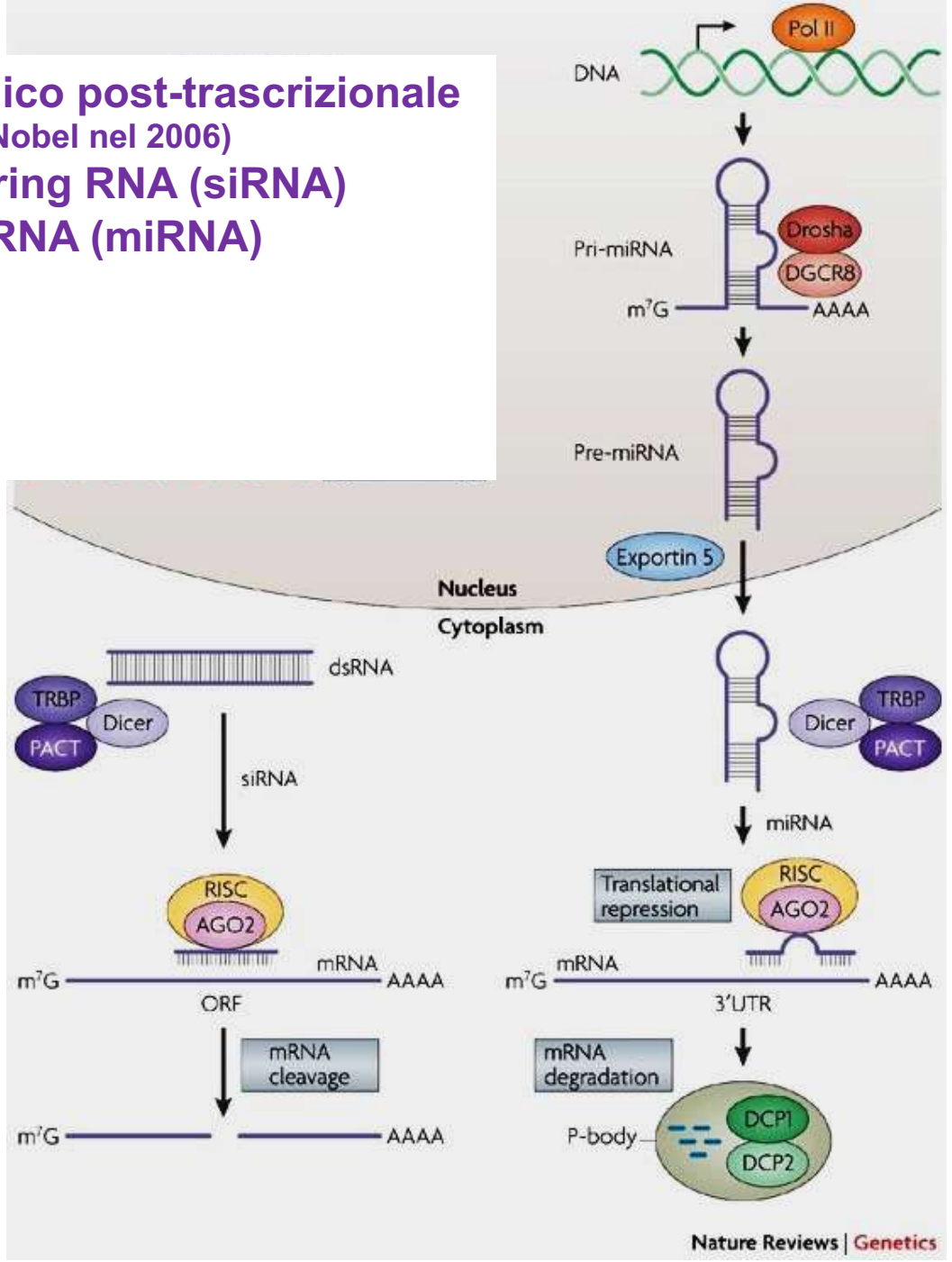
INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 51: 8, 2023

Silenziamento genico post-trascrizionale
(Premio Nobel nel 2006)
small interfering RNA (siRNA) e micro RNA (miRNA)

Attraverso una endonucleasi (*Dicer*) il dsRNA è tagliato in frammenti di 19-21 paia di basi.

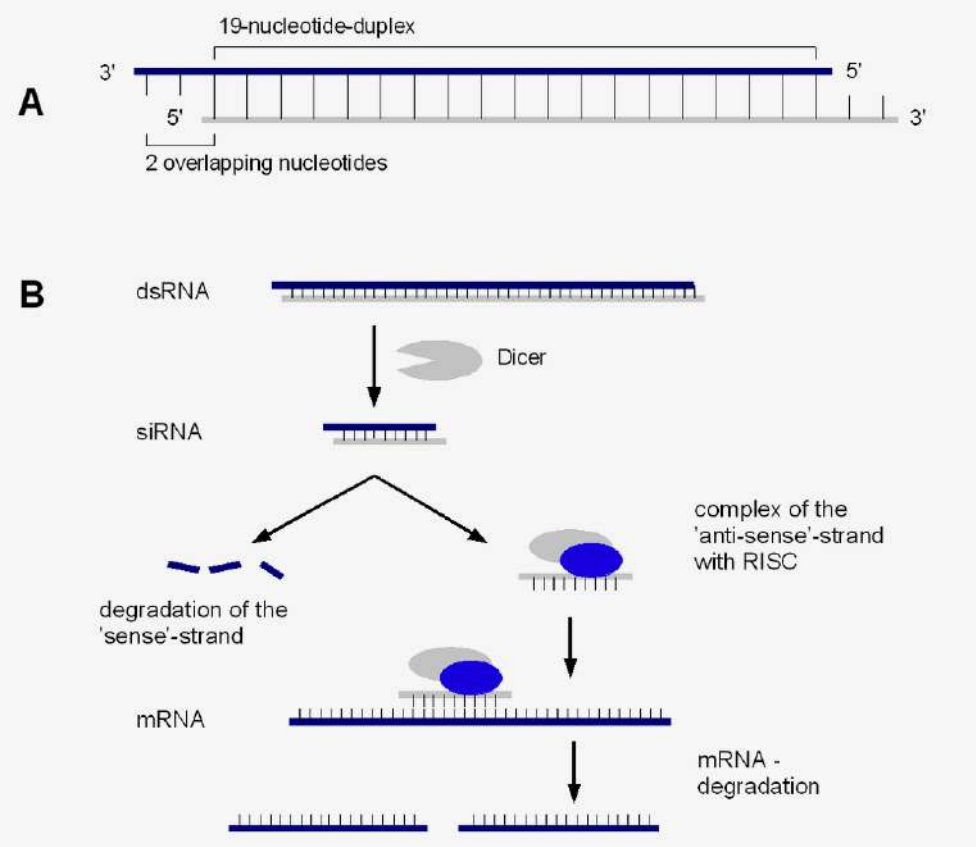
Il filamento *antisenso*, il *siRNA* (filamento guida) rimane legato a RISC, quello *senso* (filamento passeggero) viene degradato.

Un componente del RISC (*argonaute*) taglia mRNA che deve essere inattivato



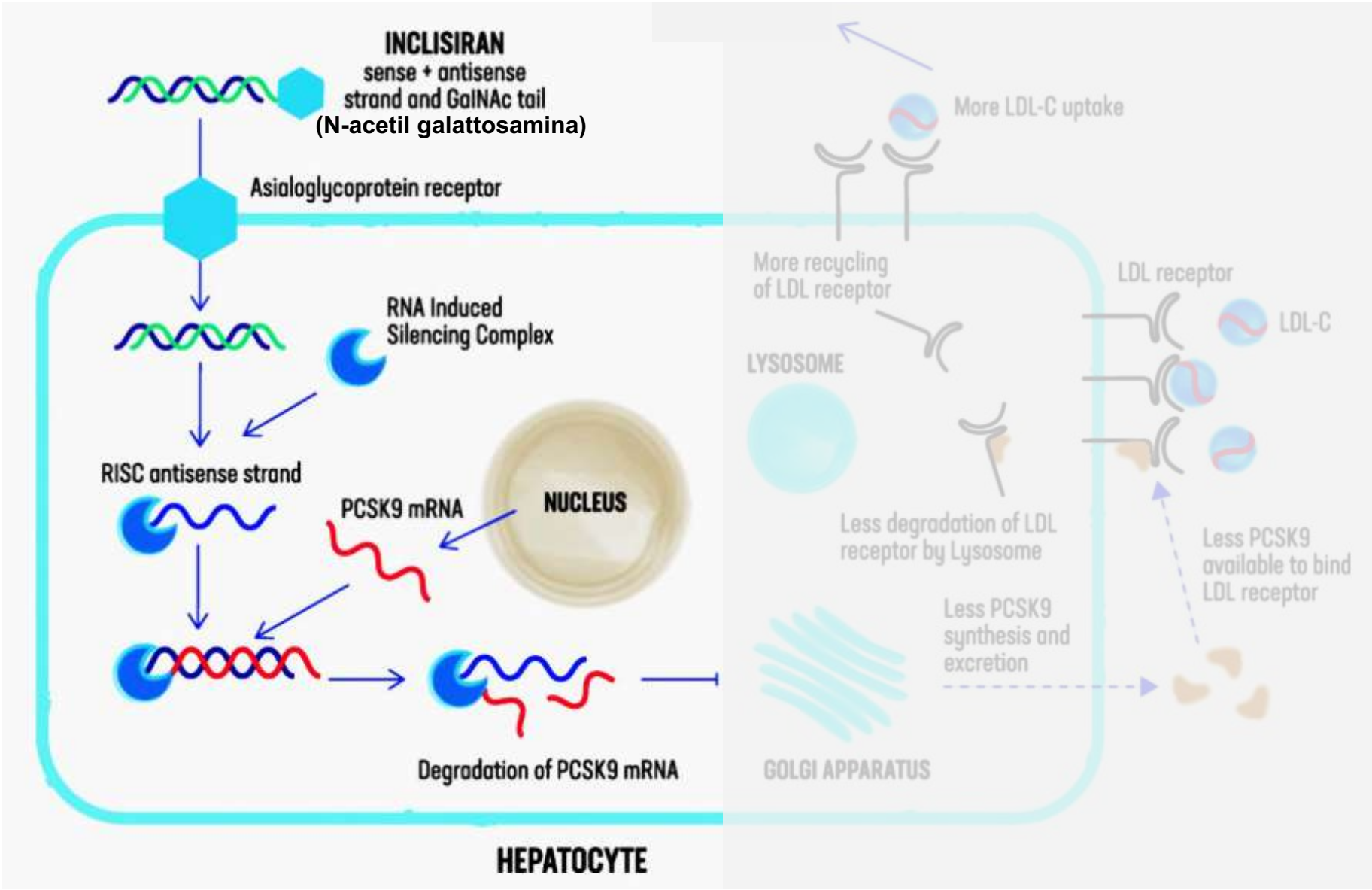
siRNA pathway

miRNA pathway



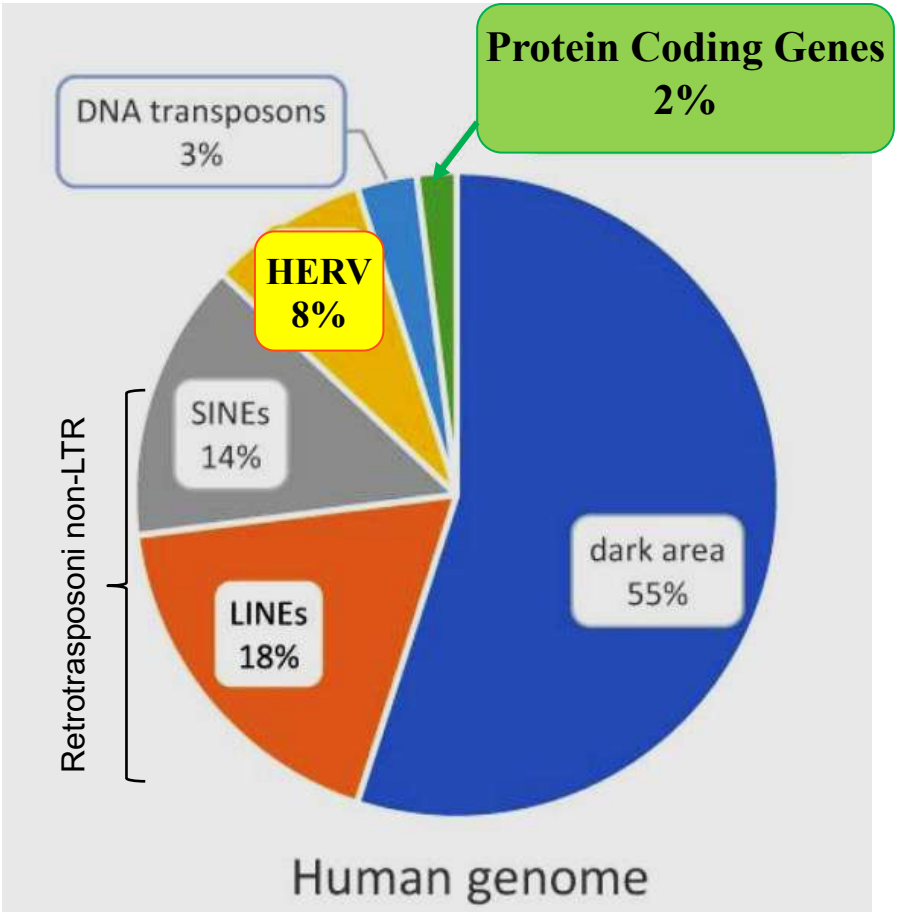
- By identifying and mapping the mRNA of Angiotensinogen, researchers have developed a dsRNA medication called Zilbesiran.
- By identifying and mapping the mRNA of the Apo(a) of Lp(a) researchers have developed a dsRNA medication called Olpasiran.
- Finally by identifying and mapping the mRNA of the PCSK9 researchers have developed a dsRNA medication called **Inclisiran.**

Inclisiran (Leqvio®)



Infine scopriamo con stupore che l'8% del nostro DNA è costituito da HERV (Human Endogenous RetroVirus), in sostanza virus "endogenizzati".

The mystery of the human genome's dark matter

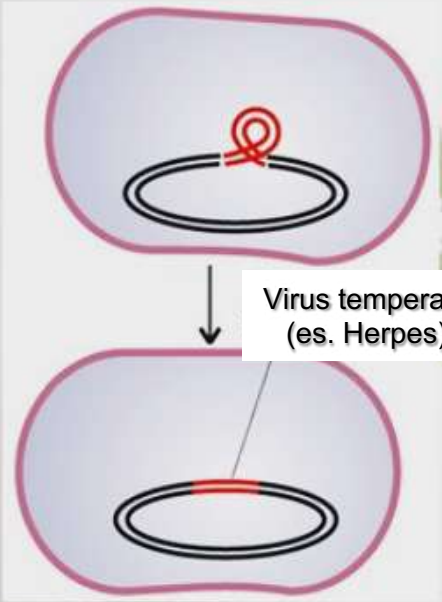


Non-coding DNA:
jumping genes (o trasposoni)
HERV e tutto il resto…

- Il 26 % del DNA non codificante sono introni
- 5-6% sequenze altamente ripetitive, non trascritte in mRNA e prive di funzioni note
- Il 2-3% trasposoni a DNA
- L'**8% retrotrasposoni di HERV** (Human Endogenous RetroVirus) **o trasposoni LTR**
- Oltre il 30% retrotrasposoni non-LTR

elementi trasponibili

CICLO LISOGENO



NEL CICLO LISOGENO IL **GENOMA VIRALE** SI INTEGRA NEL CROMOSOMA OSPITE

- IL GENOMA VIRALE SI DUPLICA DURANTE IL PROCESSO DI DUPLICAZIONE DELLA CELLULA OSPITE. QUANDO QUESTA SI DIVIDE, QUINDI, TRASMETTE ALLE CELLULE FIGLIE ANCHE IL DNA VIRALE (CHE PERÒ NON È ESPRESSO)
- DOPO MIGLIAIA DI DIVISIONI CELLULARI, IL DNA VIRALE PUÒ ATTIVARSI, STACCANDOSI DAL CROMOSOMA E DANDO INIZIO A UN CICLO LITICO

- Nel corso dei tempi alcune particelle virali che si erano integrate nel DNA (ciclo lisogeno) hanno perso la capacità di replicarsi autonomamente, staccarsi e uscire dalla cellula ospite.
- Molte sono sequenze inattive e rappresentano una sorta di «fossili» che ricordano antiche infezioni.
- Molte, ma non tutte…….

La scoperta dei "jumping genes"

For much of the 20th century, genes were considered to be stable entities arranged in an orderly linear pattern on chromosomes, like beads on a string (1). In the late 1940s, Barbara McClintock challenged existing concepts of what genes were capable of when she discovered that some genes could be mobile. Her studies of chromosome breakage in maize led her to discover a chromosome-breaking locus that could change its position within a chromosome. McClintock went on to discover other such mobile elements, now known as transposons. She also found that depending on where they inserted into a chromosome these mobile elements could reversibly alter the expression of other genes. She summarized her data on the first transposable elements she discovered, *Ac* and *Ds*, in a 1950 PNAS Classic Article, "The origin and behavior of mutable loci in maize" (2). Although their existence was accepted relatively soon after by maize geneticists, the widespread nature of mobile genetic elements and the implications of McClintock's discovery took decades to be widely recognized.

By the 1970s the great strides made in molecular biology led to the discovery of transposons in other organisms, starting with viruses and bacteria. We now know that transposons constitute more than 65% of our genomes and approximately

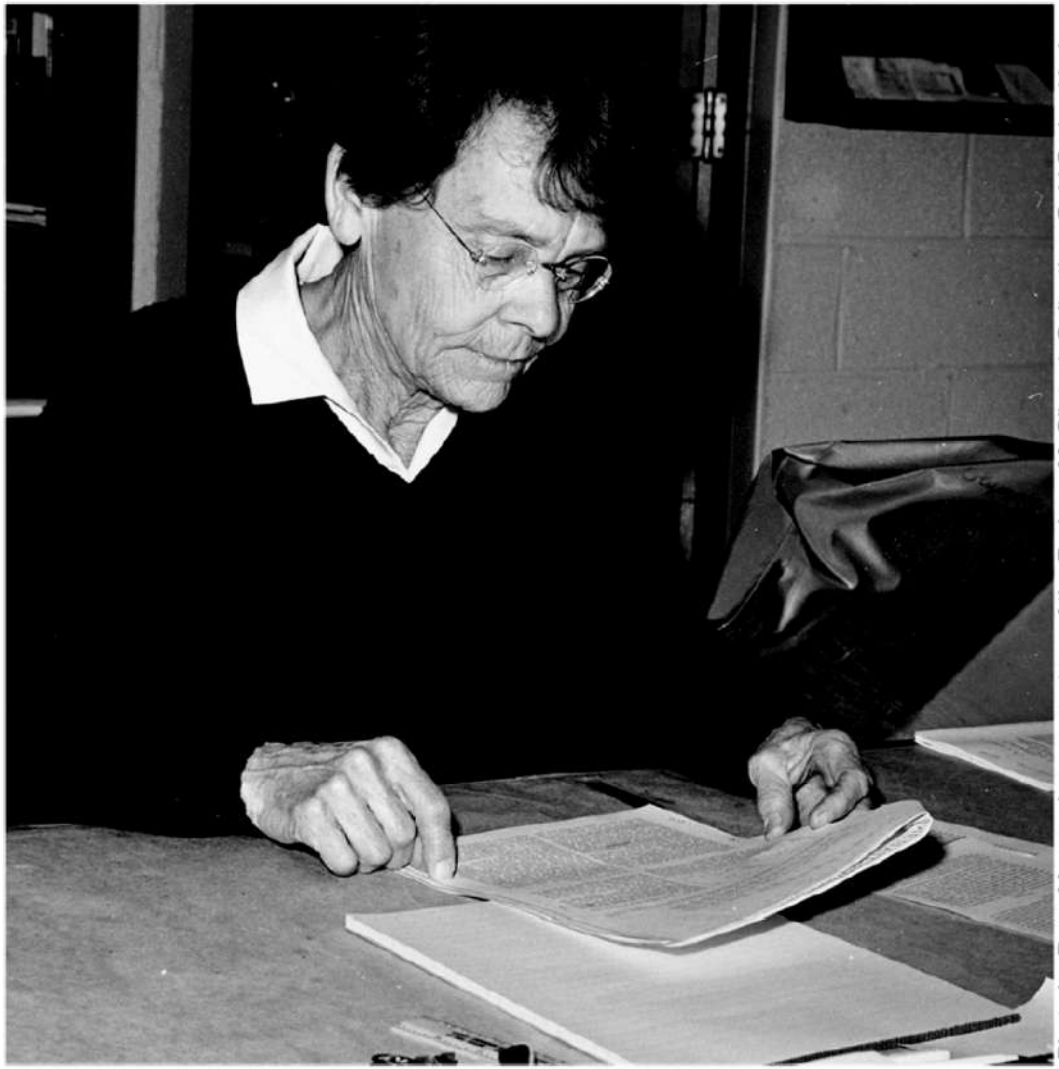


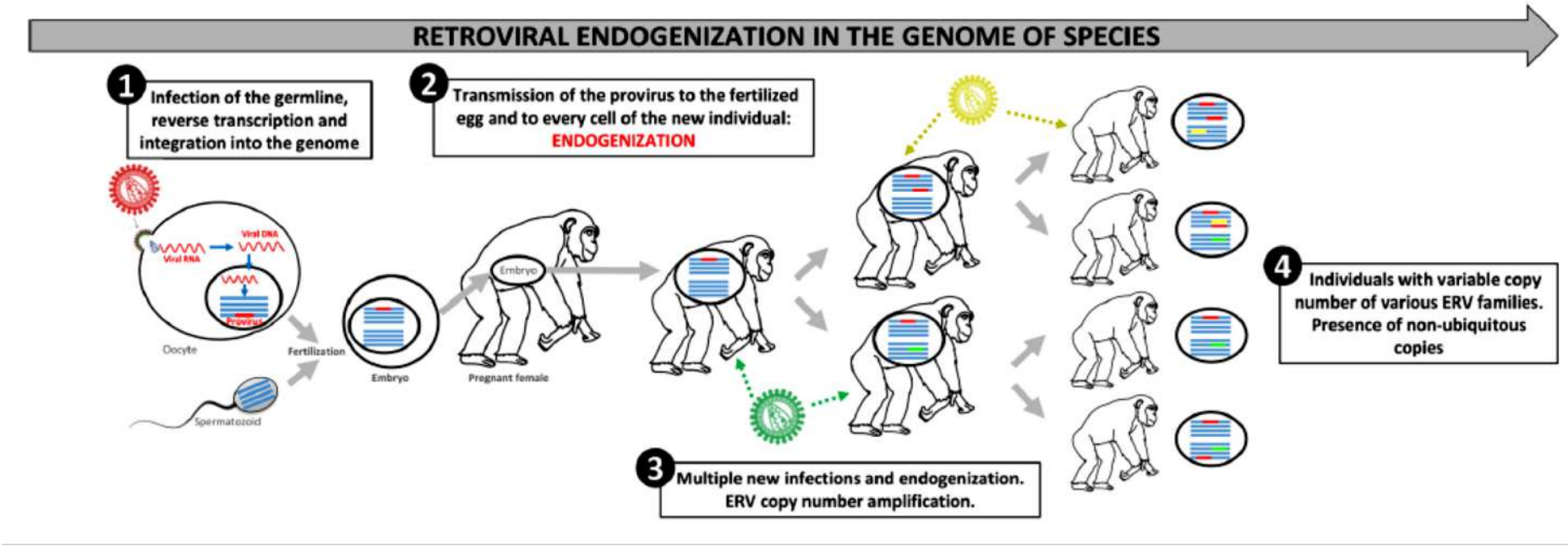
Photo by Ross Meurer. Image courtesy of the Barbara McClintock Collection, Cold Spring Harbor Laboratory Library and Archives.

Premio Nobel per la medicina nel 1983: 35 anni dopo !!

chromosomes—set the stage for her

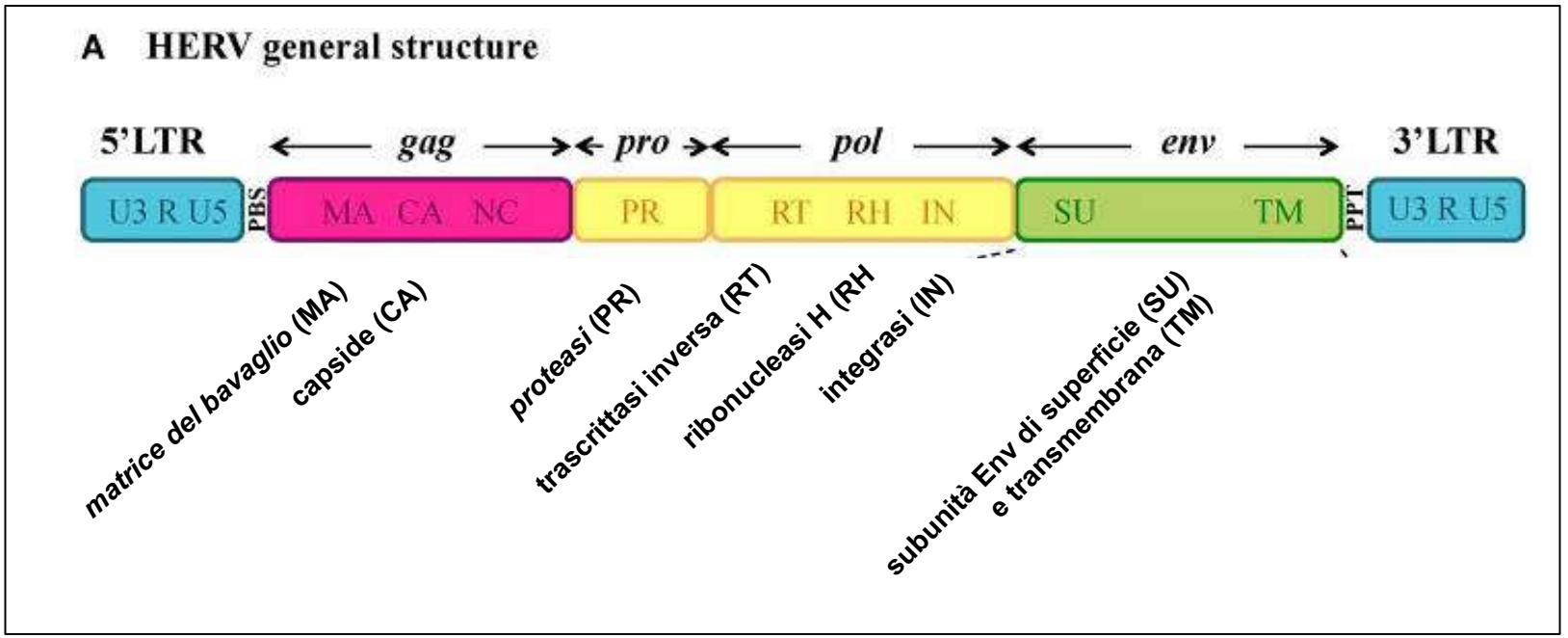
had refined these techniques sufficiently

Retrovirus Umani Endogeni (HERV) o retrotrasposoni LTR



I retrotrasposoni LTR cioè quelli che presentano lunghe ripetizioni terminali (LTRs, Long Terminal Repeats), sono gli **HERV**s (Human Endogenous Retrovirus), elementi di tipo retrovirale che costituiscono l'8% del genoma.

Si sono inseriti nel DNA *della linea germinale* circa 25 milioni di anni fa.



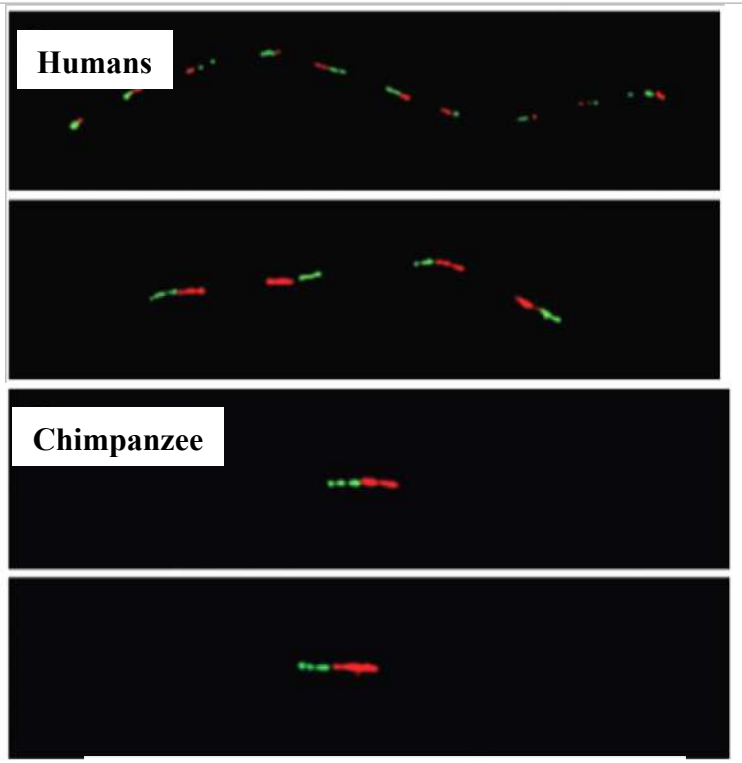
Le loro lunghe ripetizioni terminali (LTR), sequenze regolative importanti per la mobilità,

fiancheggiano la regione centrale codificante tutte le proteine generalmente prodotte dai genomi (retro)virali

fatta eccezione per quelle costituenti il capside virale.

Nat Genet. 2007 October ; 39(10): 1256–1260.

Diet and the evolution of human amylase gene copy number variation



Endogenous retroviral sequences are required for tissue-specific expression of a human salivary amylase gene

GENES & DEVELOPMENT 6:1457–1465 © 1992

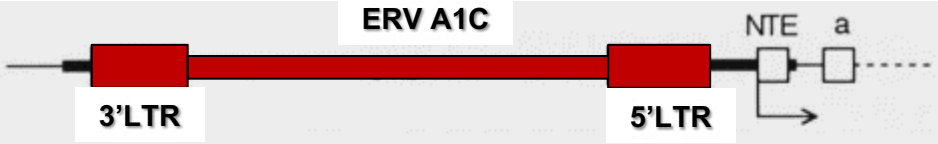
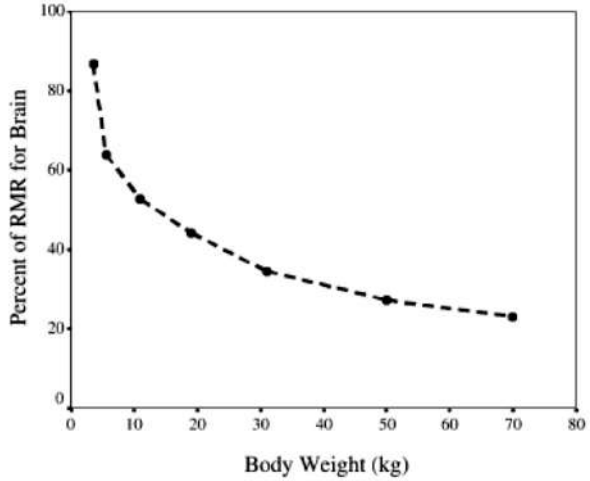
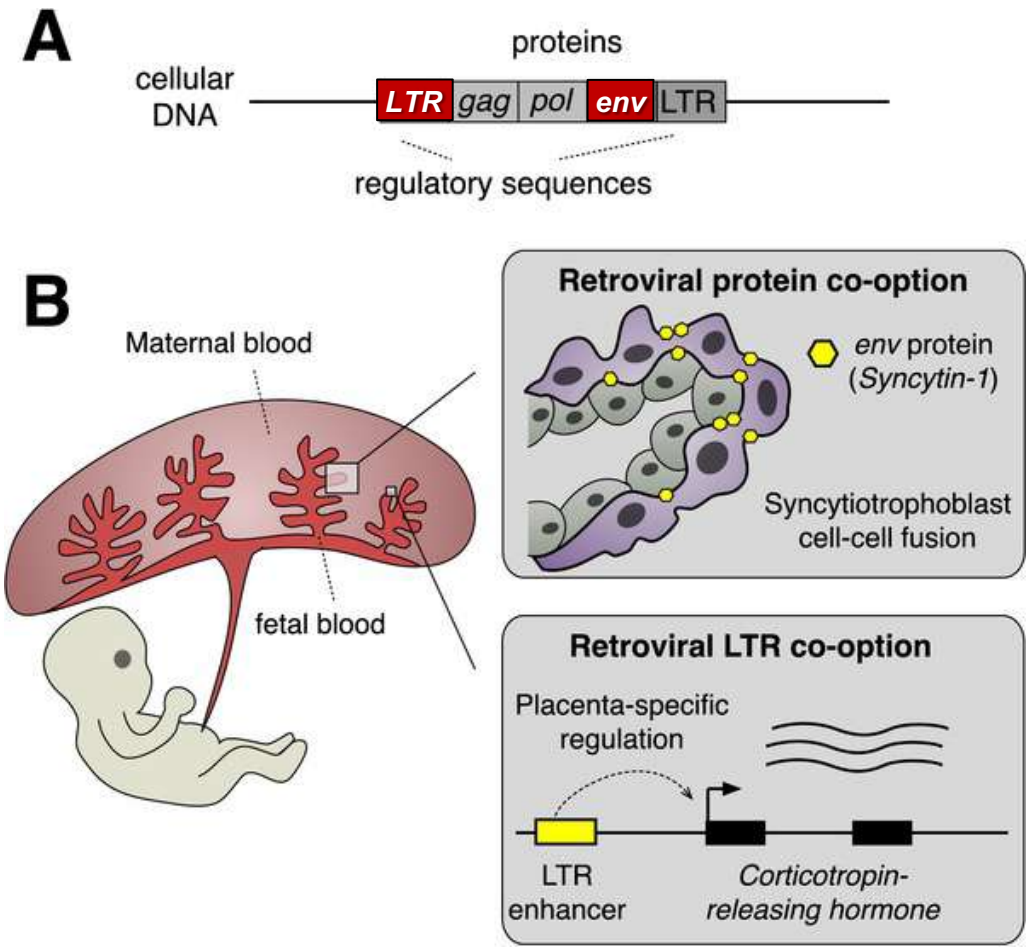


Figure 1. Structure of the human salivary amylase gene AMY1C. Insertions of the γ-actin pseudogene (solid bar) and the retrovirus ERVA1C occurred ~40 million years ago (Samuelson et al. 1990). (□)Exon a and the NTE; the rest of the gene is not shown. The major start site for transcription is indicated by an arrow. Insertion of the retrovirus apparently activated a cryptic promoter within the γ-actin pseudogene.



The placenta goes viral: Retroviruses control gene expression in pregnancy

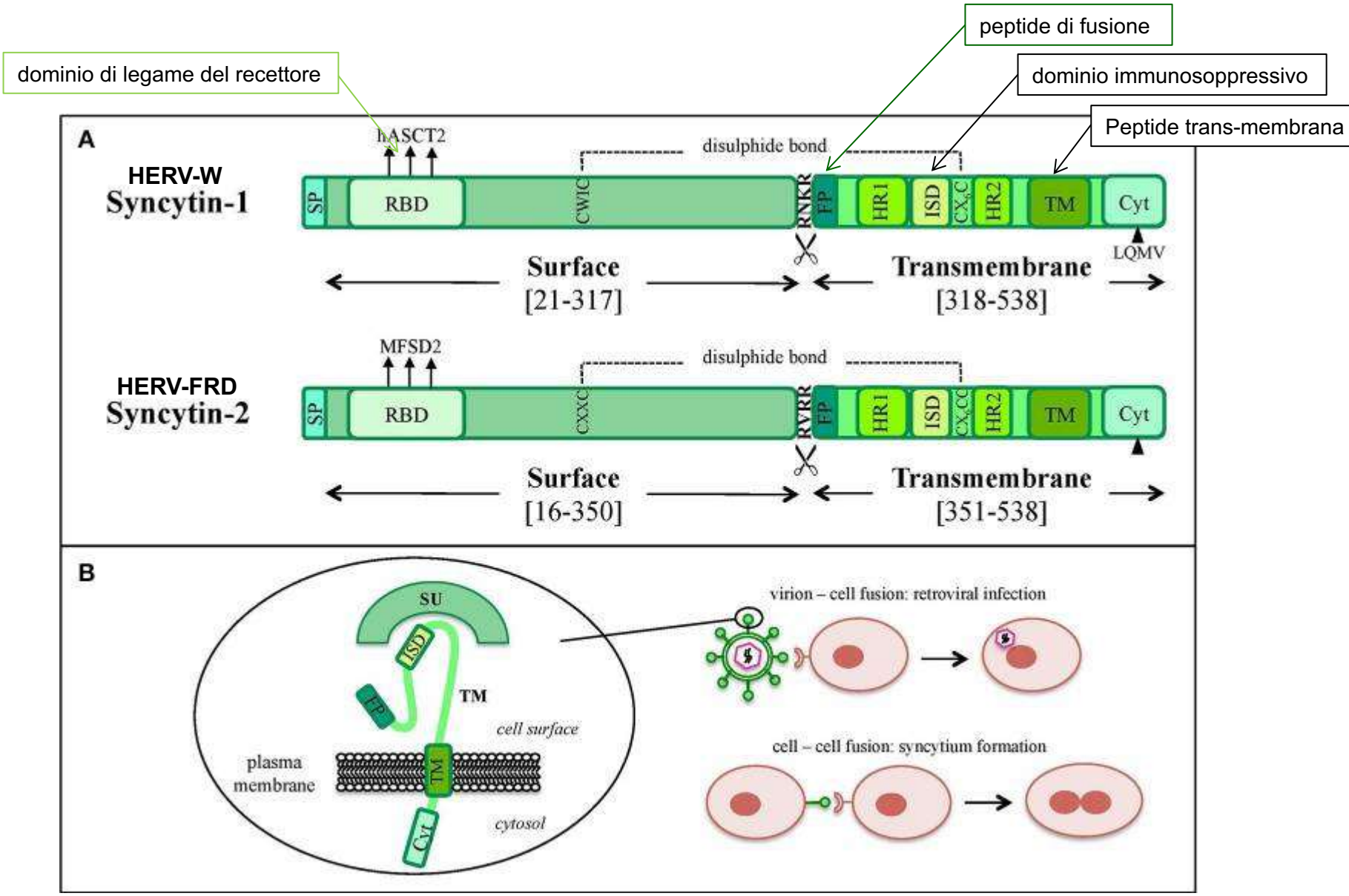
PLOS Biology October 9, 2018



env: attivazione fusione sinciziale

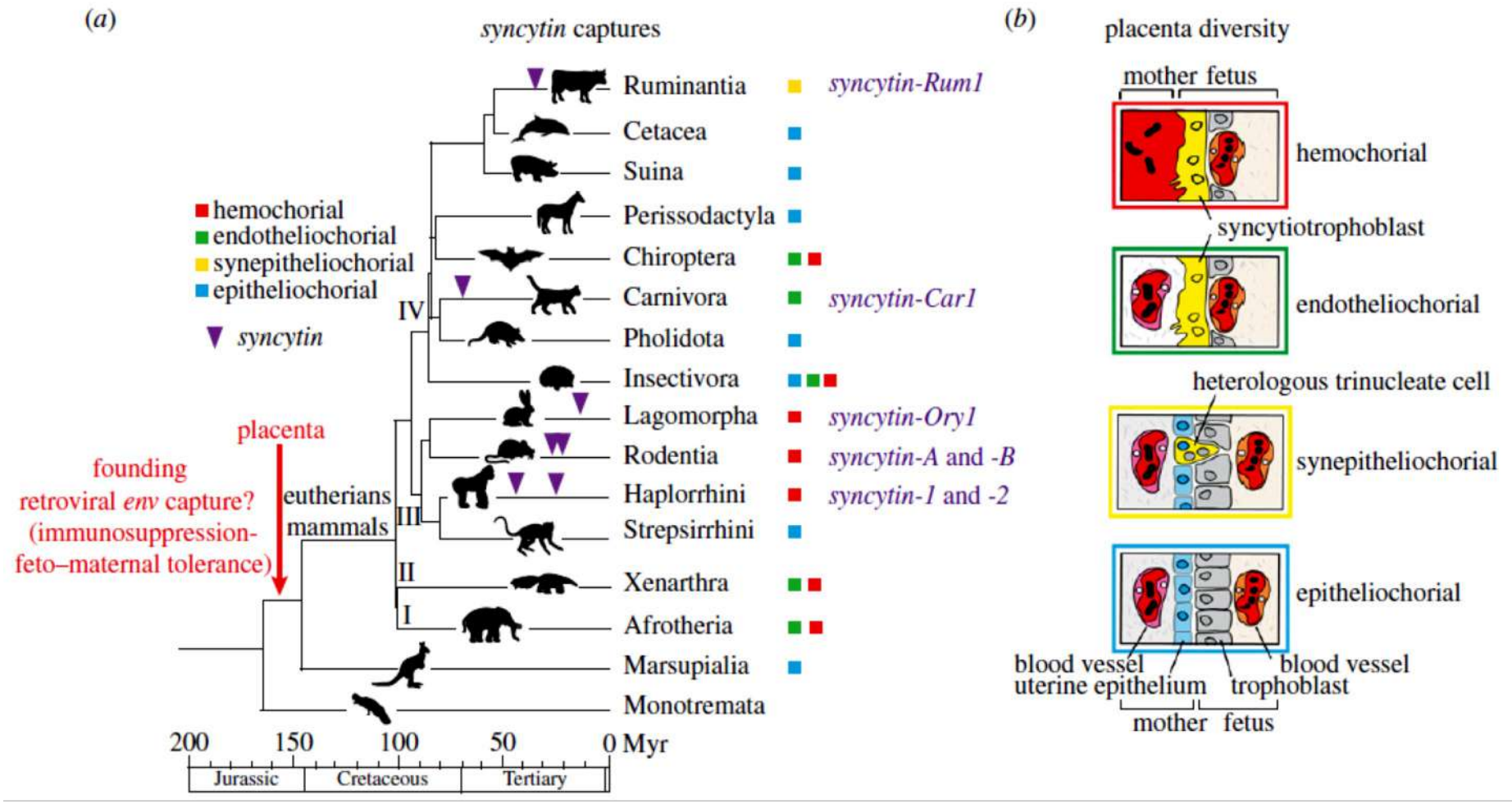
LTR: enhancer espressione placentare del Corticotropin Releasing Hormone

Fig 1. A) Schematic of an endogenous retrovirus upon integration in the host genome. B) Examples of retrovirus protein-coding [13] and regulatory sequence [24] co-option in the placenta. LTR, long terminal repeat.


dominio di legame del recettore
peptide di fusione
dominio immunosoppressivo
Peptide trans-membrana
A
HERV-W
Syncytin-1
hASCT2
disulphide bond
SP
RBD
CWIC
RNKR
FP
HR1
ISD
CX6C
HR2
TM
Cyt
LQMV
Surface
[21-317]
Transmembrane
[318-538]
HERV-FRD
Syncytin-2
MFSD2
disulphide bond
SP
RBD
CXXC
RVRR
FP
HR1
ISD
CX6CC
HR2
TM
Cyt
Surface
[16-350]
Transmembrane
[351-538]
B
SU
ISD
FP
TM
cell surface
plasma
membrane
TM
Cyt
cytosol
virion – cell fusion: retroviral infection
cell – cell fusion: syncytium formation

Baton pass hypothesis: successive incorporation of unconserved endogenous retroviral genes for placentation during mammalian evolution

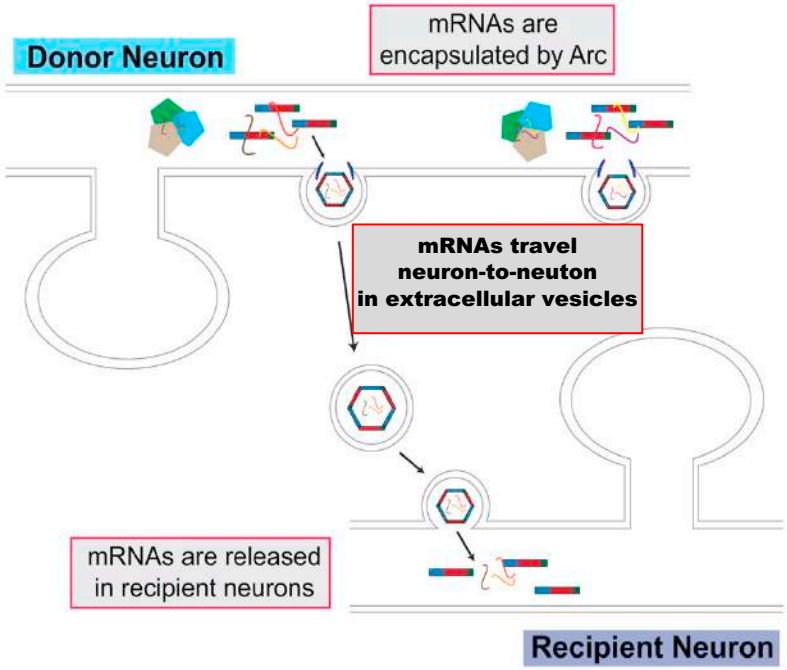
Genes to Cells (2015)

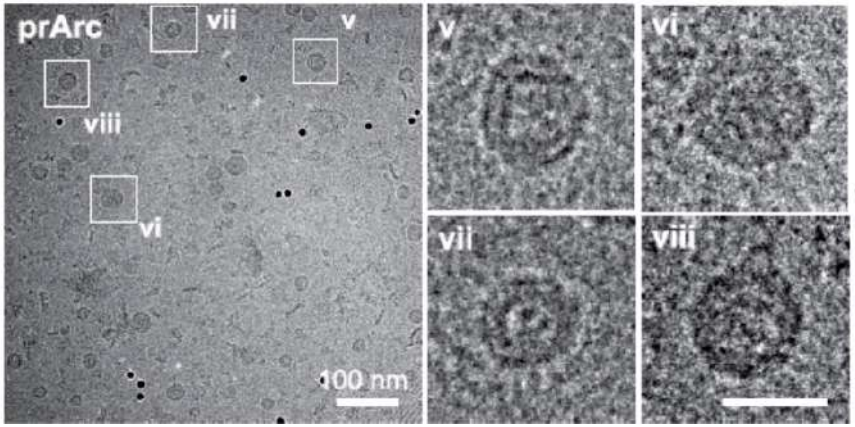


Cell

Pastuzyn et al., 2018,

The Neuronal Gene *Arc* Encodes a Repurposed Retrotransposon Gag Protein that Mediates Intercellular RNA Transfer



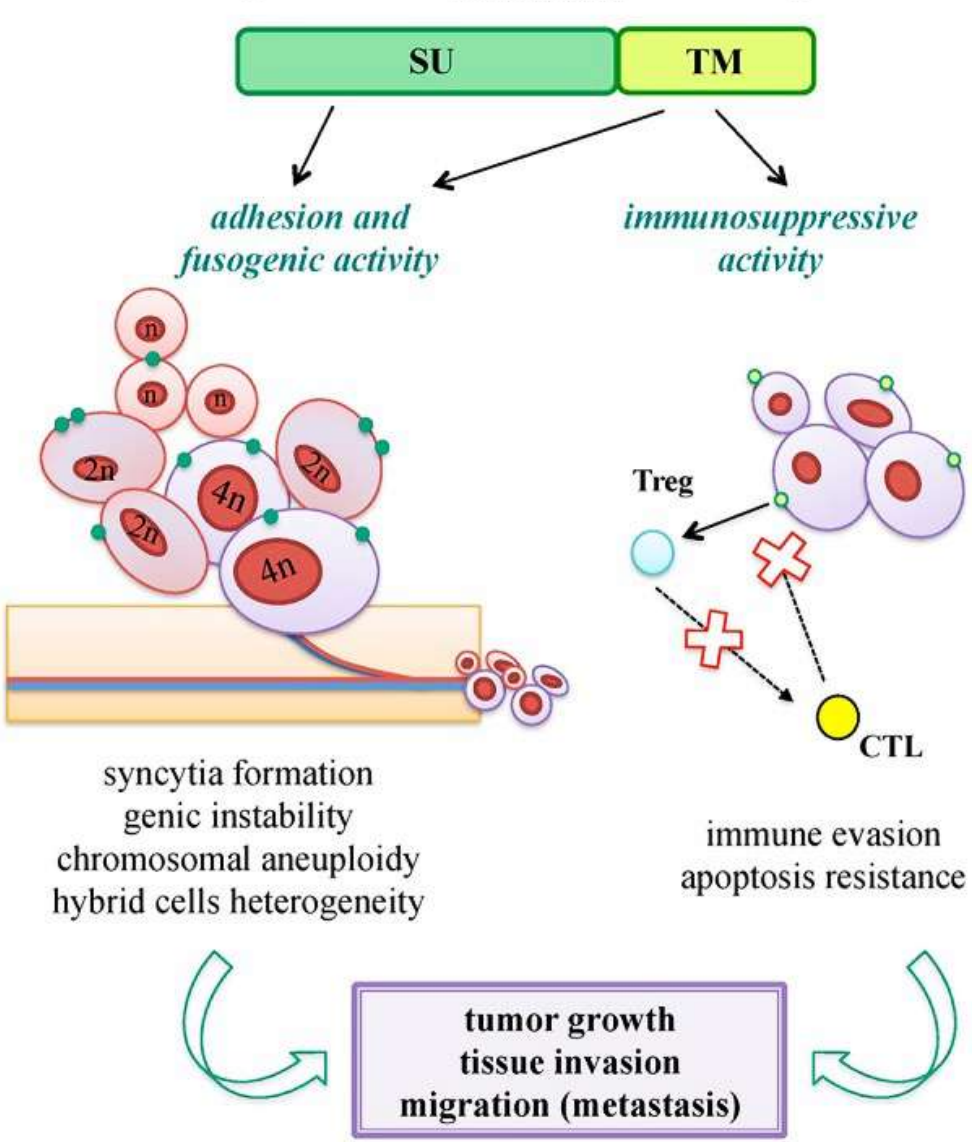


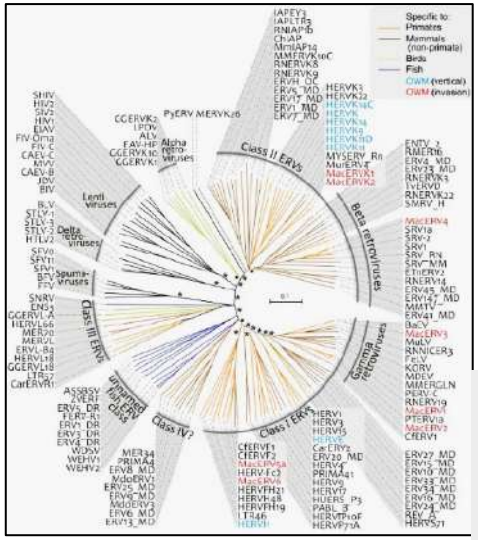
SUMMARY

The neuronal gene *Arc* is essential for long-lasting information storage in the mammalian brain, mediates various forms of synaptic plasticity, and has been implicated in neurodevelopmental disorders. However, little is known about Arc's molecular function and evolutionary origins. Here, we show that Arc self-assembles into virus-like capsids that encapsulate RNA. Endogenous Arc protein is released from neurons in extracellular vesicles that mediate the transfer of *Arc* mRNA into new target cells, where it can undergo activity-dependent translation. Purified Arc capsids are endocytosed and are able to transfer *Arc* mRNA into the cytoplasm of neurons. These results show that Arc exhibits similar molecular properties to retroviral Gag proteins. Evolutionary analysis indicates that Arc is derived from a vertebrate lineage of Ty3/*gypsy* retrotransposons, which are also ancestors to retroviruses. These findings suggest that Gag retroelements have been repurposed during evolution to mediate intercellular communication in the nervous system.

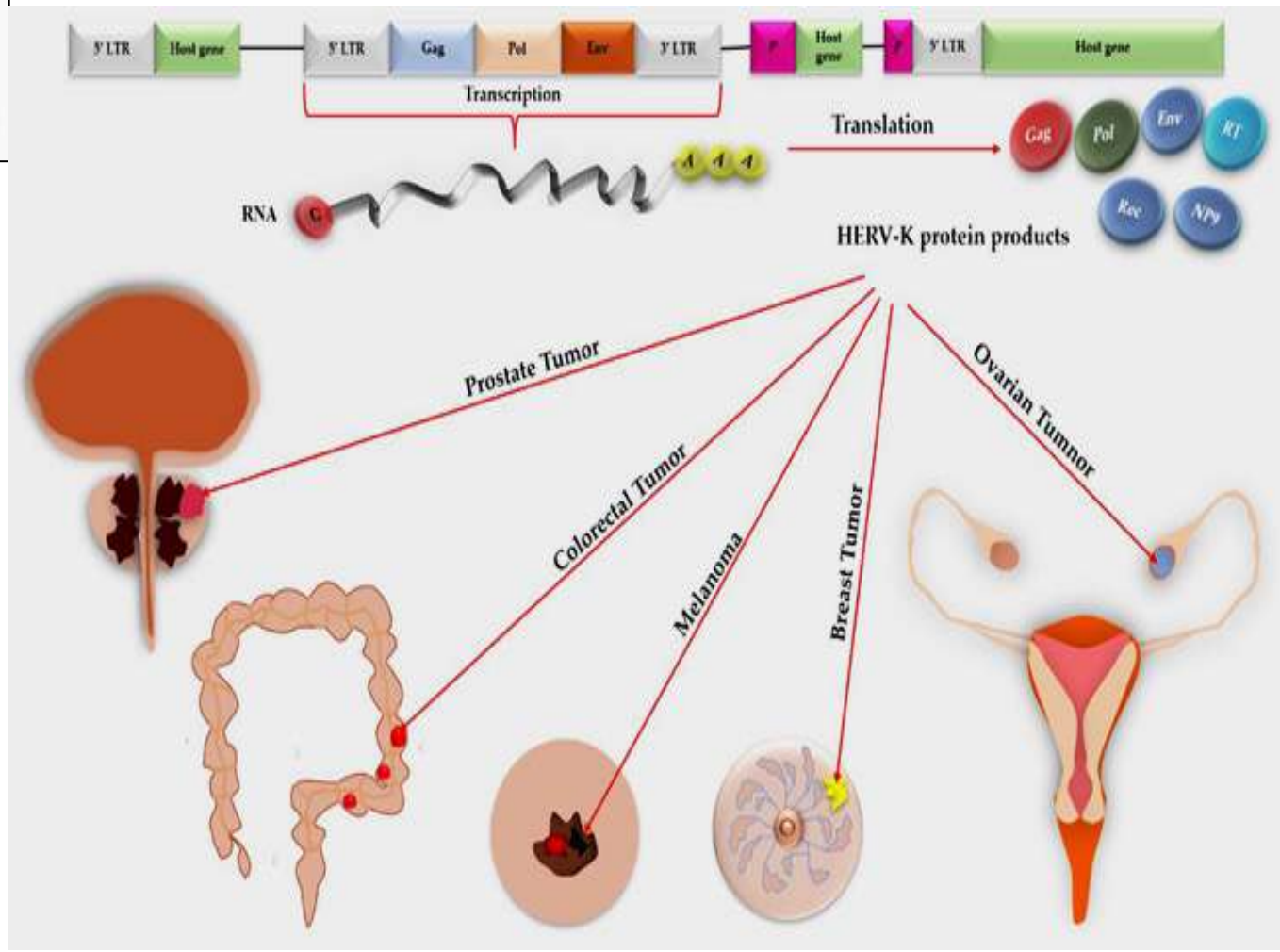
HERV Envelope Proteins: Physiological Role and Pathogenic Potential in Cancer and Autoimmunity

Frontiers in Microbiology
March 2018 | Volume 9 |



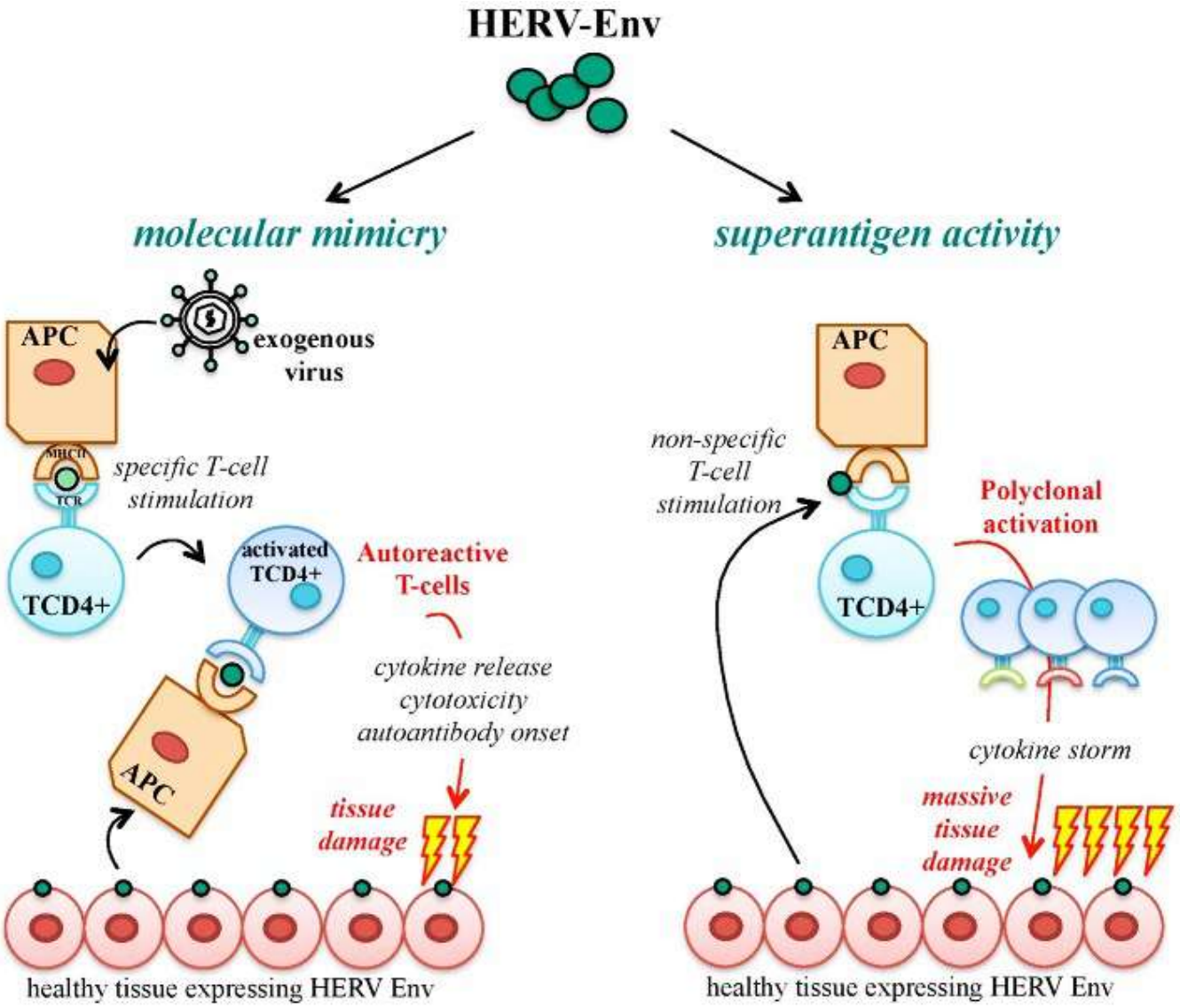

Specific to:
Primates
Mammals (non-primate)
Birds
Fish
OWM (vertical)
OWM (invasion)
Class II ERVs
Class I ERVs
Class III ERVs
Class IV
Lenti viruses
Beta retroviruses
Gamma retroviruses
Delta viruses
Spuma-viruses
Alpha retroviruses
Unassigned

HERV-K


5' LTR
Host gene
5' LTR
Gag
Pol
Env
3' LTR
Host gene
5' LTR
Host gene
Transcription
Translation
RNA
Gag
Pol
Env
RT
Rec
NPro
HERV-K protein products
Prostate Tumor
Colorectal Tumor
Melanoma
Breast Tumor
Ovarian Tumnor

HERV Envelope Proteins: Physiological Role and Pathogenic Potential in Cancer and Autoimmunity

Frontiers in Microbiology
March 2018 | Volume 9 |



Human endogenous retroviruses (HERV) and non-HERV viruses incorporated into the human genome and their role in the development of autoimmune diseases

Journal of Translational Autoimmunity 4 (2021)

Table 1

Most representative human endogenous retroviruses (HERVs) associated with some autoimmune diseases and their most probable pathogenetic role.

Disease	HERVs Involved	Predominant Autoimmunity mechanism	References
Type 1 diabetes mellitus (T1DM)	HERV-H HERV-K HERVW	Molecular mimicry Superantigens	47,48,49
Multiple sclerosis (MS)	MSRV HERV-K HERVW	Superantigen	53, 56,57,58
Systemic Lupus Erythematosus (SLE)	HRES-1 HERV-K HERV-E	DNA hypomethylation Molecular mimicry	37,45,66
Rheumatoid Arthritis	HML-2	Molecular mimicry	68,69,70

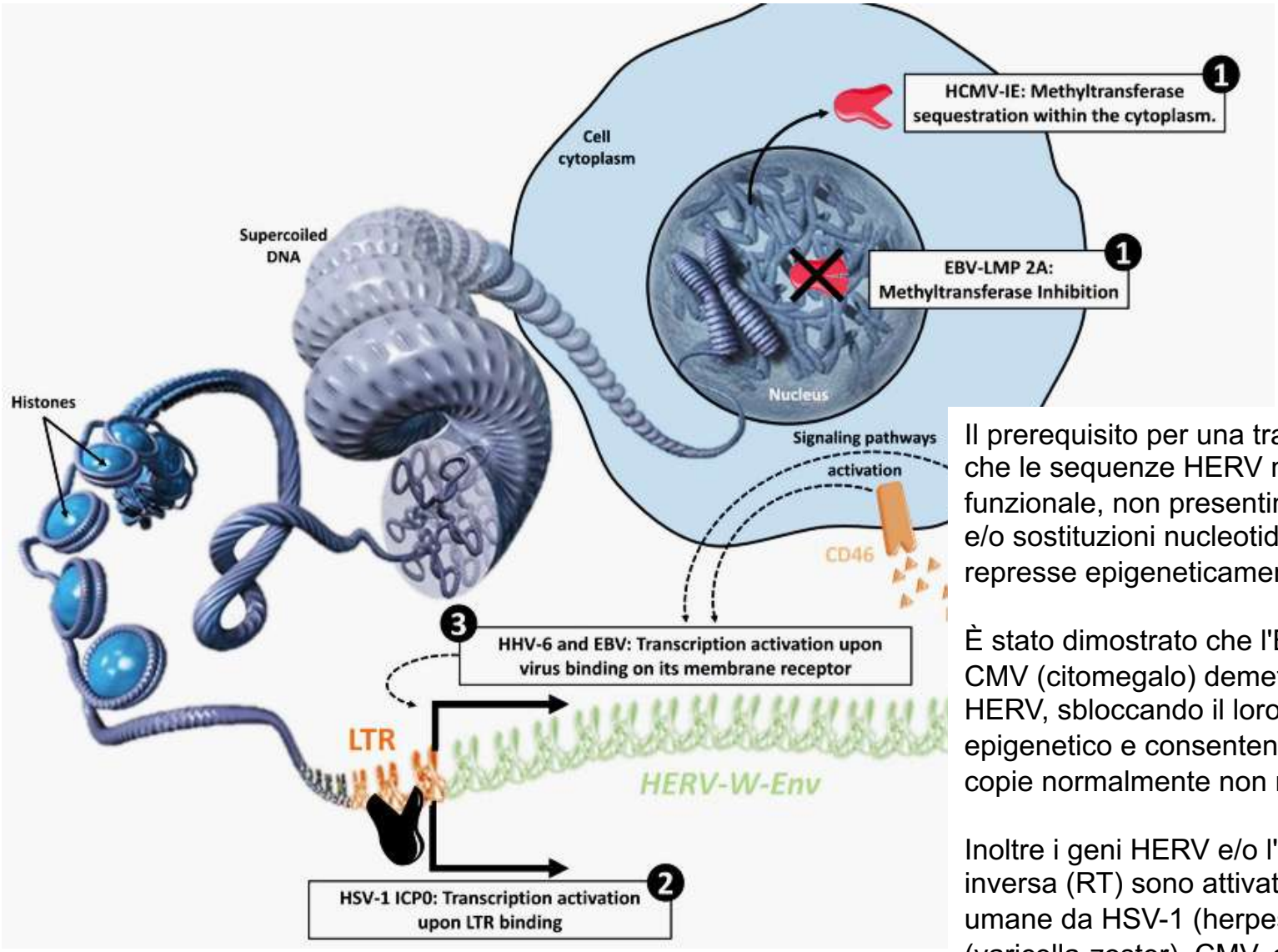
SUPERANTIGENS Some HERVs can encode proteins that can act as superantigens, activating cells of the immune system, especially CD4 T lymphocytes: Epstein-Barr virus (EBV) could transactivate the HERV-K18 that encodes a superantigen. This creation of superantigens induced by EBV could be a mechanism by which this virus generates autoimmunity.

MOLECULAR MIMICRY HERVs sequences can code for proteins that have regions like proteins encoded by the host genome. Antibodies generated against HERVs proteins cross-reactive against a self-antigen is generated: in systemic lupus erythematosus (SLE) antibodies against an endogenous retroviral element-encoded nuclear protein autoantigen, HRES-1, were more present in patients with SLE vs. controls (52% vs. 3%)

DNA HYPOMETHYLATION Methylation is an epigenetic mechanism of DNA regulation. DNA hypomethylation has been described in multiple diseases, especially neoplastic, but it has been shown that can play a role in the pathogenesis of SLE.

Human Endogenous Retroviruses and Type 1 Diabetes

Current Diabetes Reports (2019)



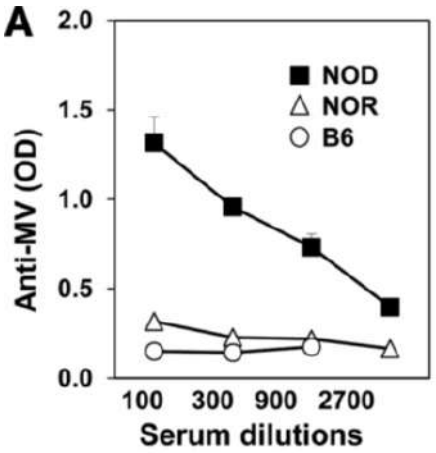
Il prerequisito per una trascrizione efficace è che le sequenze HERV mantengano un LTR funzionale, non presentino delezioni maggiori e/o sostituzioni nucleotidiche e siano de-represse epigeneticamente.

È stato dimostrato che l'EBV (Epstein Barr) e il CMV (citomegalo) demetilano sequenze di HERV, sbloccando il loro silenziamento epigenetico e consentendo l'attivazione ulteriori copie normalmente non risponsive.

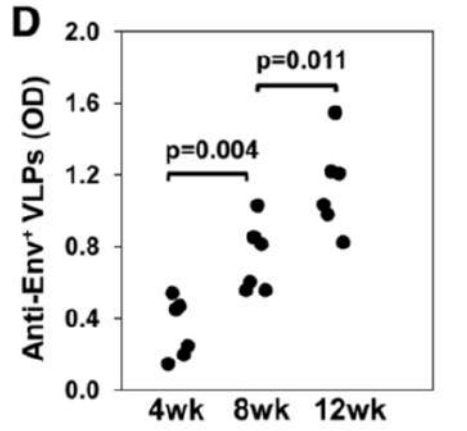
Inoltre i geni HERV e/o l'attività della trascrittasi inversa (RT) sono attivati in vari tipi di cellule umane da HSV-1 (herpes simplex tipo 1),VZV (varicella-zoster), CMV, e EBV.

Type 1 diabetes pathogenesis is modulated by spontaneous autoimmune responses to endogenous retrovirus antigens in NOD mice

Eur. J. Immunol. 2017. 47: 575–584



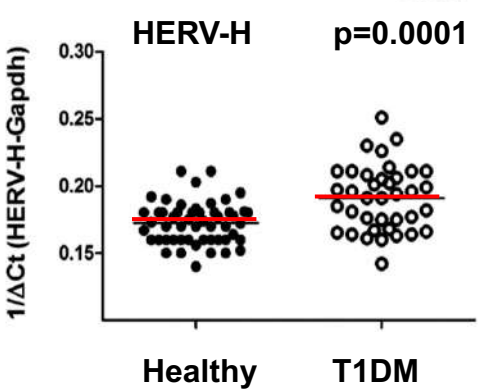
The Authors demonstrated that NOD but not diabetes-resistant mice developed anti-Env autoantibodies

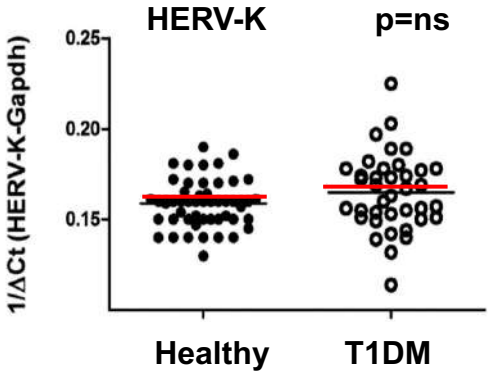


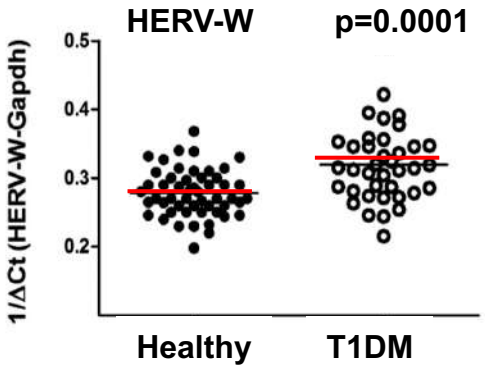
and that increase in titer as disease progresses

1. **Islet cell-released microvesicles (MVs) are potent inflammatory triggers that stimulate autoreactive B and T cells, causing Type 1 Diabetes in NOD mice.**
2. **Proteomic analysis of purified MVs released from islet cells detected the presence of endogenous retrovirus (ERV) antigens, including Env and Gag.**
3. **Gag and Env are expressed in NOD islet-derived primary mesenchymal stem cells (MSCs).**
4. **However, MSCs derived from the islets of diabetes-resistant mice do not express the antigens.**

Taken together, abnormal ERV activation and secretion of MVs may induce anti-retroviral responses to trigger autoimmunity.







Enhanced expression of human endogenous retroviruses in new-onset type 1 diabetes: Potential pathogenetic and therapeutic implications

Autoimmunity Volume 53, 2020 -

HERV-W-Env

Study design

The researchers assessed the transcription levels of pol genes of HERV-H, HERV-K, and HERV-W in peripheral leucocytes from 37 children and adolescents with new-onset T1DM and 50 age-matched control subjects.

Results

The expression levels of the HERV-H and HERV-W pol gene were significantly higher in diabetic patients than in control subjects.

Discussion

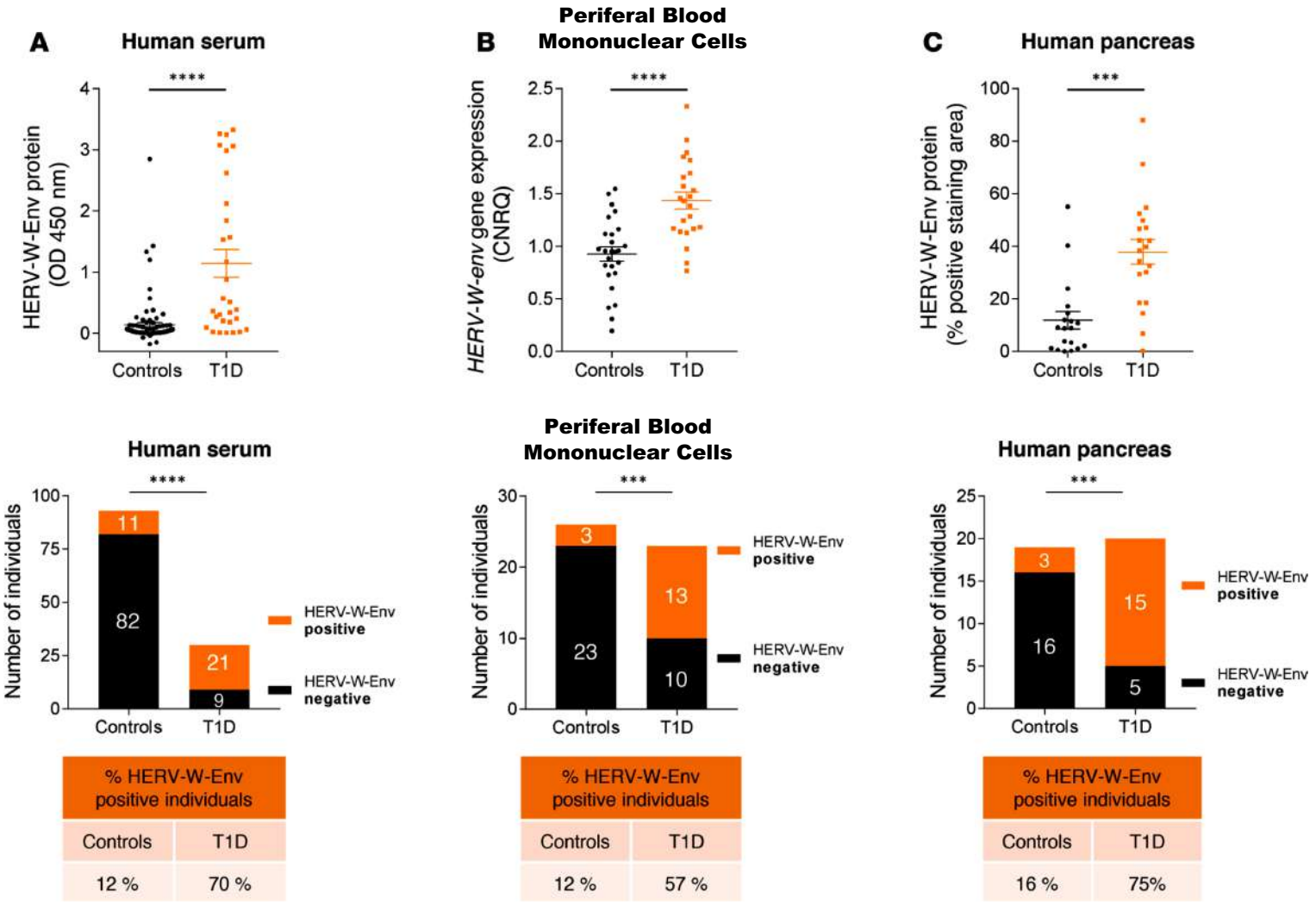
….. HERVs are particularly transactivated in HIV positive individuals and antiretroviral therapy reduces the viral load not only of HIV but also of HERVs….

Therefore, the administration of antiretroviral drugs in new-onset T1D appears an exciting speculation potentially heralding a new therapeutic strategy.

An ancestral retroviral protein identified as a therapeutic target in type-1 diabetes

JCI insight 2017;2(17)

HERV-W-Env



% HERV-W-Env positive individuals	
Controls	T1D
12 %	70 %

% HERV-W-Env positive individuals	
Controls	T1D
12 %	57 %

% HERV-W-Env positive individuals	
Controls	T1D
16 %	75%

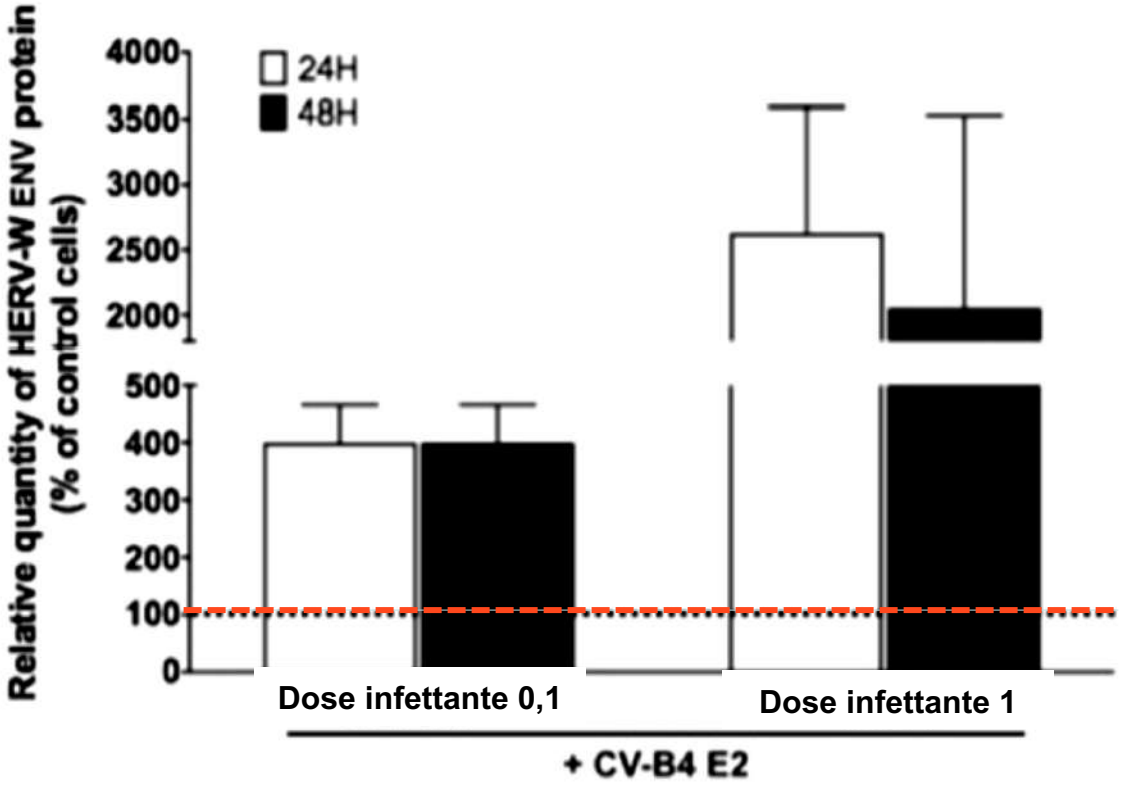
Coxsackievirus-B4 Infection Can Induce the Expression of Human Endogenous Retrovirus W in Primary Cells

Microorganisms 2020,

HERV-W-Env

Levels of Human Endogenous Retrovirus W Envelope (HERV-W ENV) protein in primary pancreatic ductal cells infected by Coxsackievirus B4 (CV-B4).

Dotted line indicates the non-specific background signal in mock-infected cultures (controls).



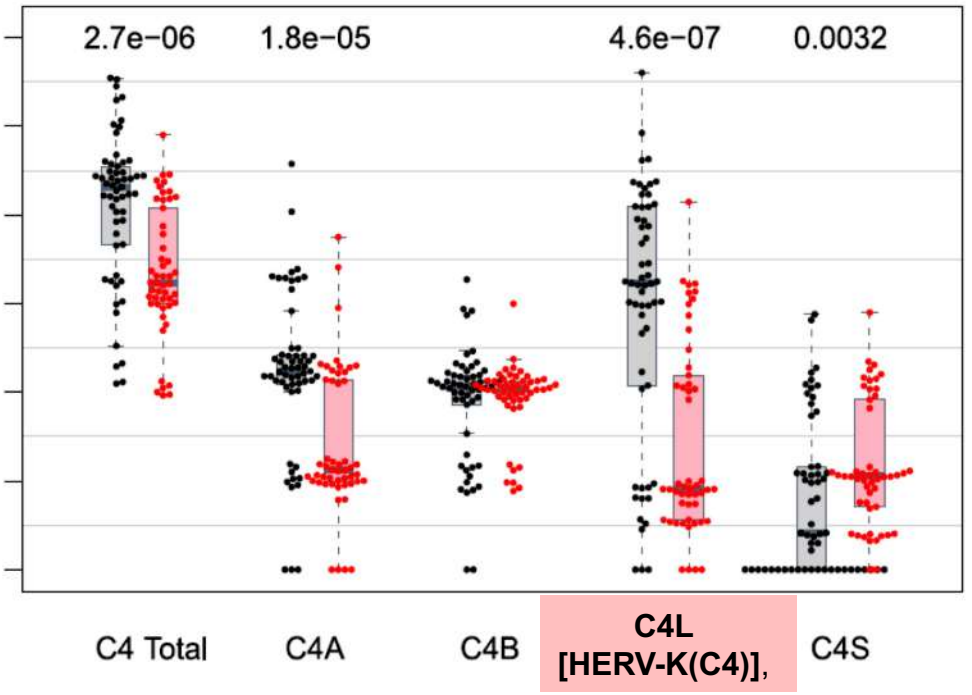
These observations open up new avenues of reflection to investigate the viral pathogenesis of T1D. Infection with a virus, such as CV-B4, can result in the activation of an endogenous factor, in this case HERV-W ENV, whose pathogenic effects have been reported: inhibition of insulin secretion by cells, induction of autoimmunity by molecular mimicry, and superantigen-like activity exacerbating the immune response against pancreatic cells.

Low HERV-K(C4) Copy Number Is Associated With Type 1 Diabetes

Diabetes Volume 63, May 2014

HERV-K

Complement component C4 (C4) is a highly variable complement pathway gene situated ~500 kb from DRB1 and DQB1, the genes most strongly associated with many autoimmune diseases. Variations in C4 copy number (CN), length, and isotype create a highly diverse gene cluster in which insertion of an endogenous retrovirus in the ninth intron of C4, termed HERV-K(C4), is a notable component. We investigated the relationship between C4 variation/CN and type 1 diabetes. We found that individuals with type 1 diabetes have significantly fewer copies of HERV-K(C4)



As an intronic insertion, HERV-K(C4) may influence C4 RNA processing, ultimately affecting the complement pathway.

We show that HERV-K(C4) is a novel marker of type 1 diabetes that accounts for the disease association previously attributed to some key HLA-DQB1 alleles.

Positional Cloning of Zinc Finger Domain Transcription Factor *Zfp69*, a Candidate Gene for Obesity-Associated Diabetes Contributed by Mouse Locus *Nidd/SJL*

PLoS Genetics July 2009

Author Summary

Type 2 diabetes in humans as well as in obese mice is caused by a combination of adipogenic and diabetogenic gene variants. We have identified a gene that appears to be involved in the pathogenesis of hyperglycaemia in obese mice: in some mouse strains, the gene *Zfp69* is disrupted by a retroviral transposon (IAPLTR1a), which generates a truncated mRNA. Disruption of the gene was associated with a reduced susceptibility for diabetes, whereas the normal allele enhanced hyperglycaemia in obese mice. *Zfp69* encodes a transcription factor which appears to interfere with lipid storage in adipose tissue, and thereby enhances lipid deposition in liver. In humans with type 2 diabetes, mRNA levels of the human orthologue of *Zfp69* (*ZNF642*) were increased in adipose tissue. Thus, the transcription factor ZFP69/ZNF642 may be involved in the pathogenesis of obesity-associated diabetes.

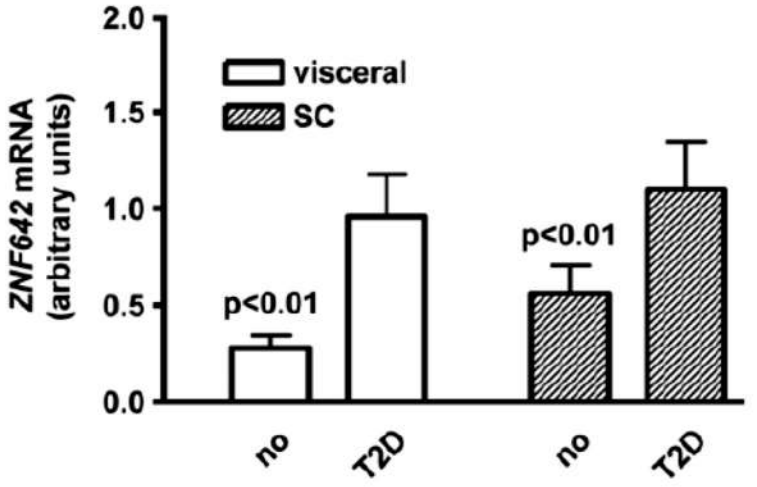
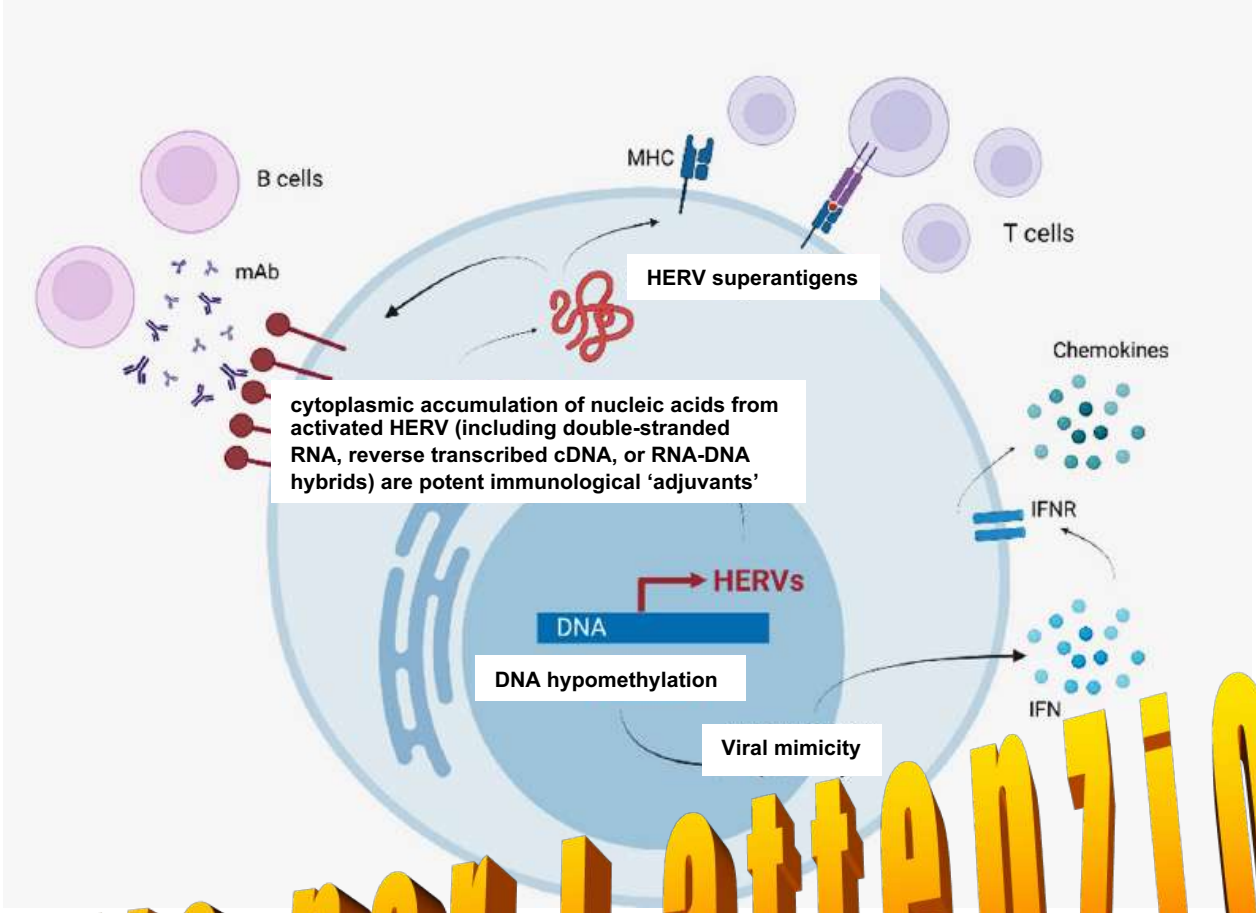


Figure 7. Expression of *ZNF642* in adipose tissue of human individuals with (T2D) and without (no) type 2 diabetes. mRNA

Review

Contribution of Retrotransposons to the Pathogenesis of Type 1 Diabetes and Challenges in Analysis Methods

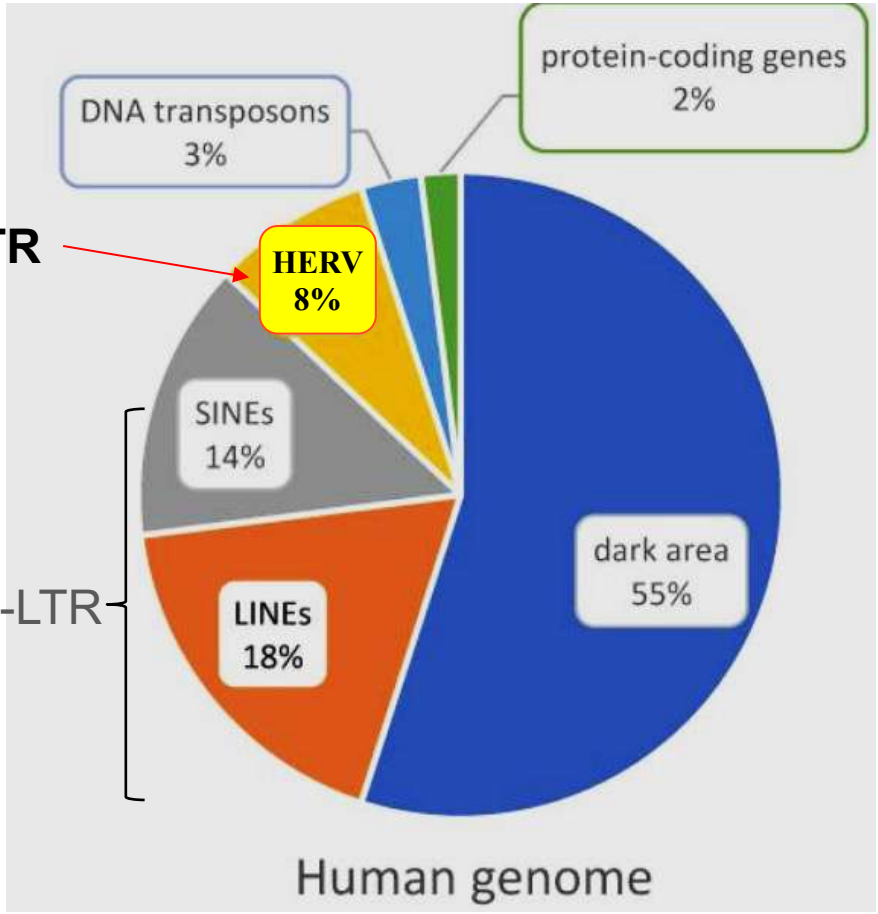
Int. J. Mol. Sci. 2023,



grazie per l attenzione

Retrotrasposoni LTR

Retrotrasposoni non-LTR



Quindi il genoma umano, costituito per circa il 45% da elementi trasponibili.

I Trasposoni a DNA sono stati attivi agli inizi dell'evoluzione dei Primati, fino a 37,000 YBP. Molti non sono più in grado di trasporsi e/o di replicarsi durante la trasposizione.

I **Retrotrasposoni LTR o HERVs** (Human Endogenous Retrovirus), si traspongono in un nuovo locus genomico duplicandosi attraverso la formazione di un intermedio a RNA. Possono così aumentare di numero e questo ha consentito di espandersi nel genoma.