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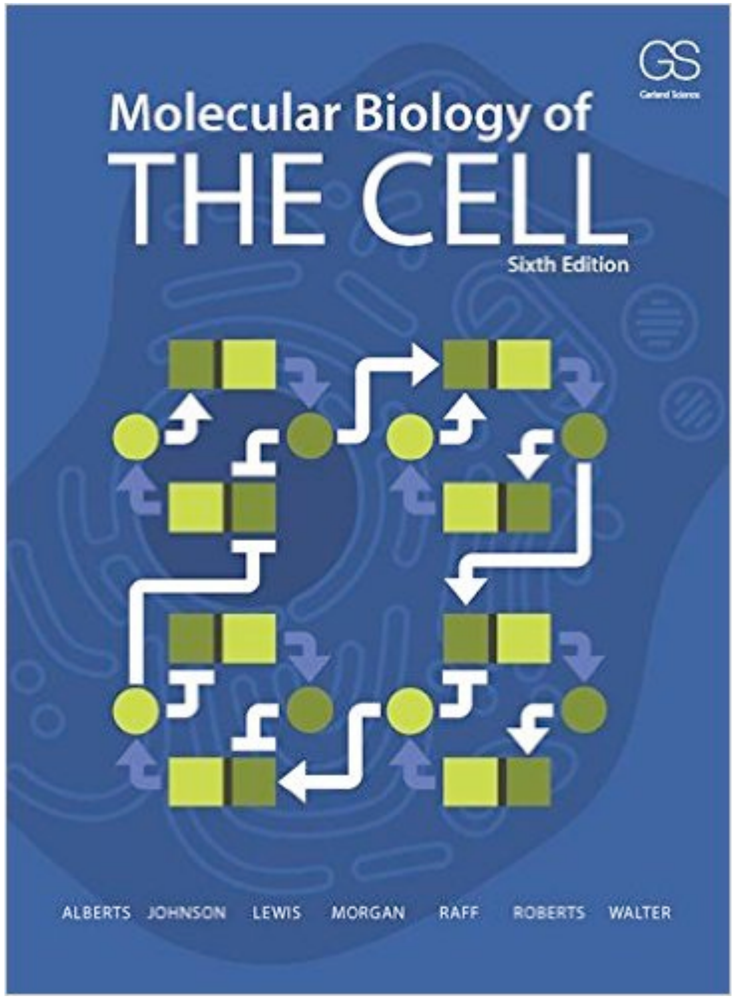
Biologia Molecolare
AA 2020-2021



B. Alberts et al.
Molecular Biology of the Cell
Garland Publishing

F. Amaldi
BIOLOGIA MOLECOLARE
Casa Editrice Ambrosiana

B. Alberts et al.
Biologia Molecolare della cellula
Zanichelli

Per alcune lezioni che tratteranno argomenti particolarmente innovativi e non sufficientemente descritti nei libri di testo, sara' fornito agli studenti opportuno materiale didattico.

ESAME

45’ CON 18 DOMANDE A RISPOSTA MULTIPLA E 4 DOMANDE APERTE

ESAME

Che cosa studieremo?

Dove e' scritta l'informazione genica:
Replicazione

Come si esprime l'informazione genica:
Trascrizione e Traduzione

In
PROCARIOTI
ed
EUCARIOTI

Meccanismi di CONTROLLO dell'espressione genica:
piccoli RNA regolatori

Come l'ambiente influenza l'espressione genica:
Epigenetica

La Biologia Molecolare nel 2020

Tecniche di Biologia Molecolare

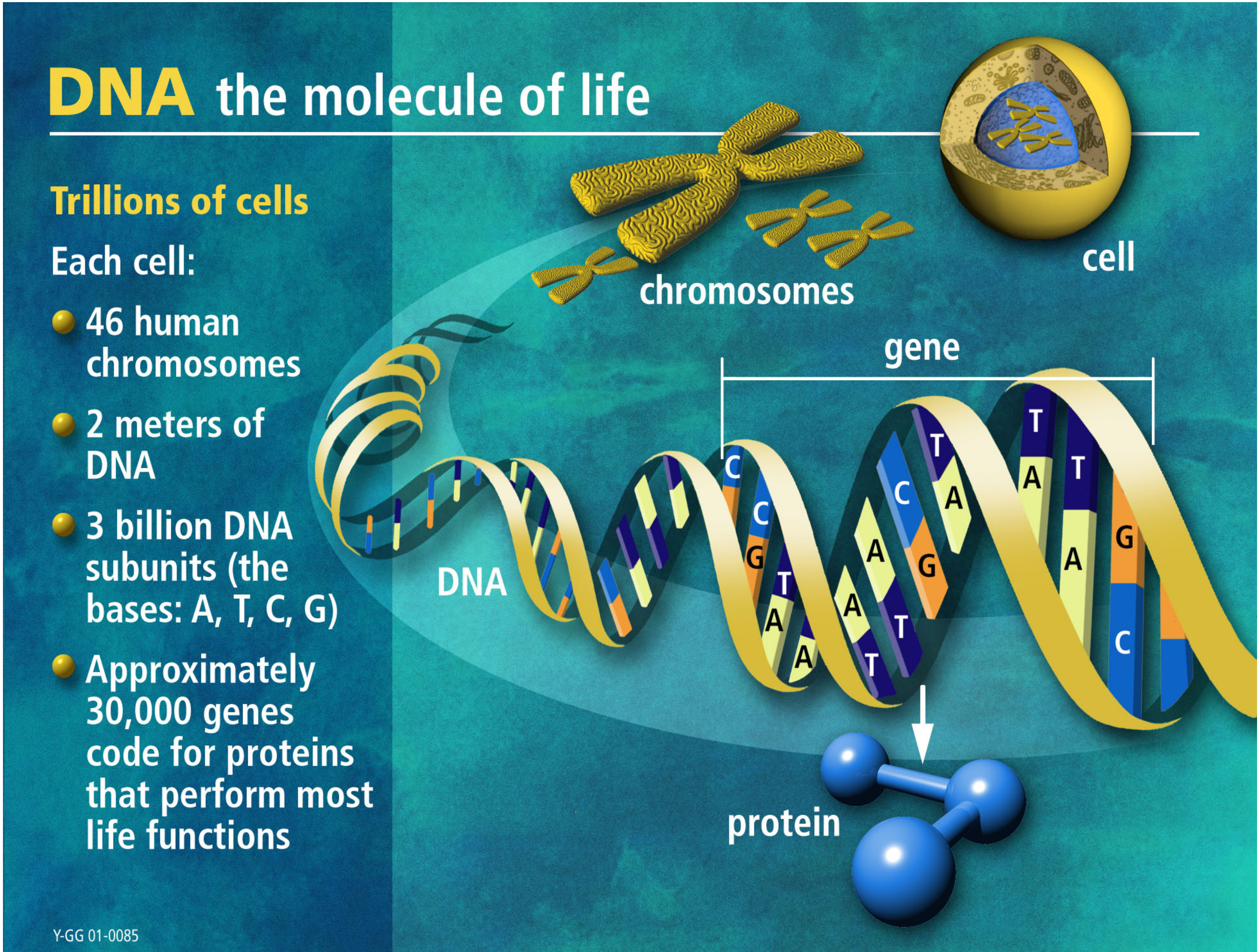
Ingegneria Genetica

Terapia Genica

Gene e Genome Editing

Molecular Medicine

Dove e' scritta l'informazione genica?



Cosa e’ l’informazione genica?

L’informazione genica consiste in una sequenza lineare di nucleotidi (=DNA) che viene decodificata allo stesso modo in tutte le cellule di tutti gli organismi.

Il genoma:

- il complesso dell'informazione genica di una cellula
- la massa totale del DNA cellulare
- il patrimonio ereditario dell'organismo a cui appartiene

COMPLESSITA' DEL GENOMA

Servono informazioni proporzionali alla complessità dell'organismo ?

Paradosso della ***non proporzionalita'*** tra complessita' genoma e complessita' dell'organismo

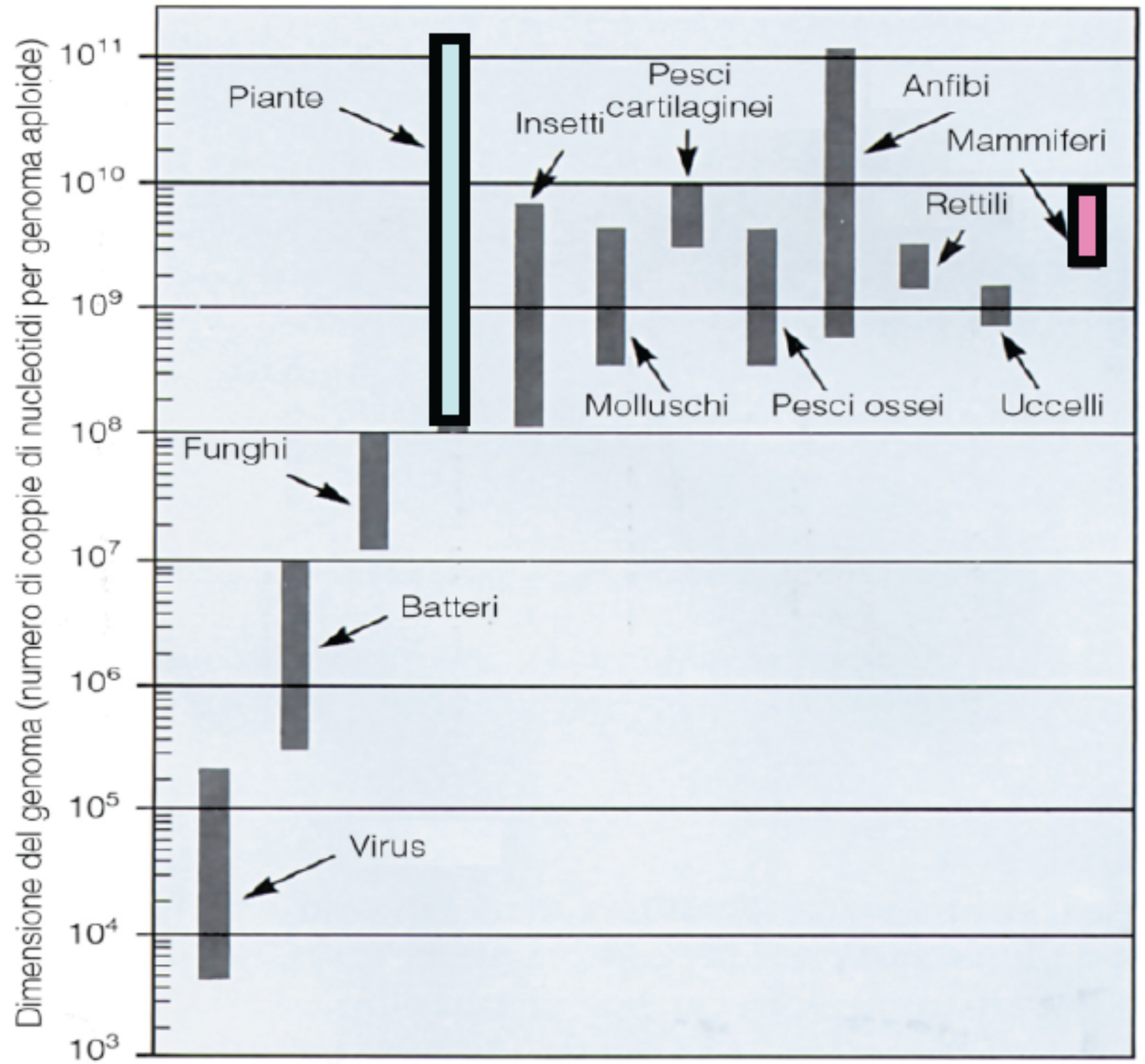


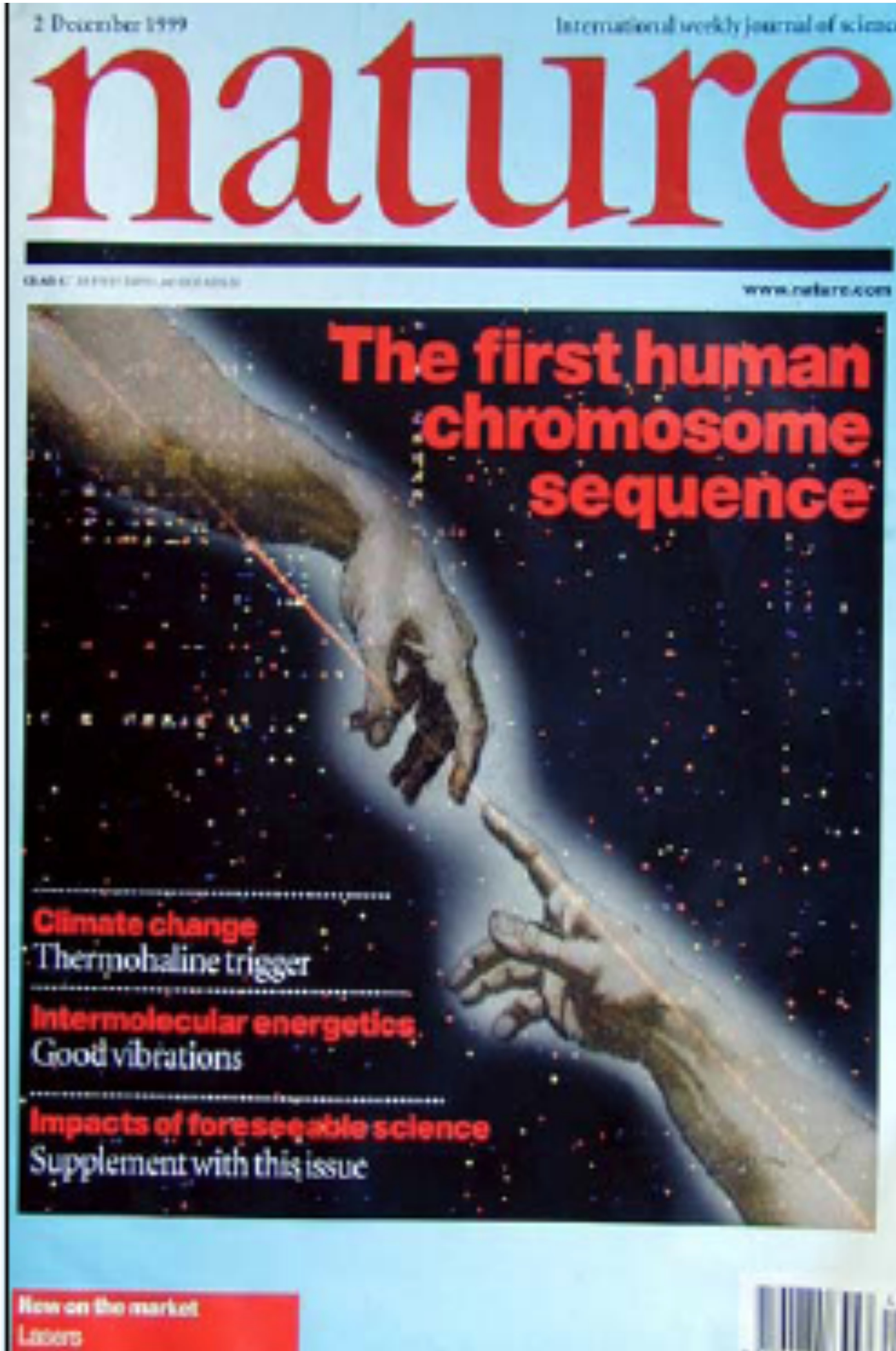
Figura 16-11 Correlazione fra dimensione del genoma e tipo di organismo. Per ogni gruppo di organismi la barra rappresenta la variabilità approssimativa della dimensione del genoma, misurata come quantità di coppie di nucleotidi per genoma aploide.

Technical advancement that made human genome sequencing feasible

1977	DNA sequencing by chain-termination nucleotide analogs (di-deoxy sequencing method) - Sanger
1986	DNA sequencing using the Sanger method with fluorescent dyes attached to nucleotides - Hood
1987	First automated DNA sequencer (Applied Biosystems, California)
1998	PE Biosystems (now Applied Biosystems) develops ABI PRIMS 3700 DNA analyzer, an automated high throughput capillary DNA sequencer

Human Genome Sequencing Projects

- Human genome sequencing project first proposed in 1985
- HGP started in 1990 (NIH & DOE) plus International sequencing labs
- By early 1998, only less than 5% of the genome had been sequenced; uncertainty to finish before 2005
- Celera shot gun sequencing intitated Sept 1999 and completed June 2000 (!)



The Sequence of the Human Genome

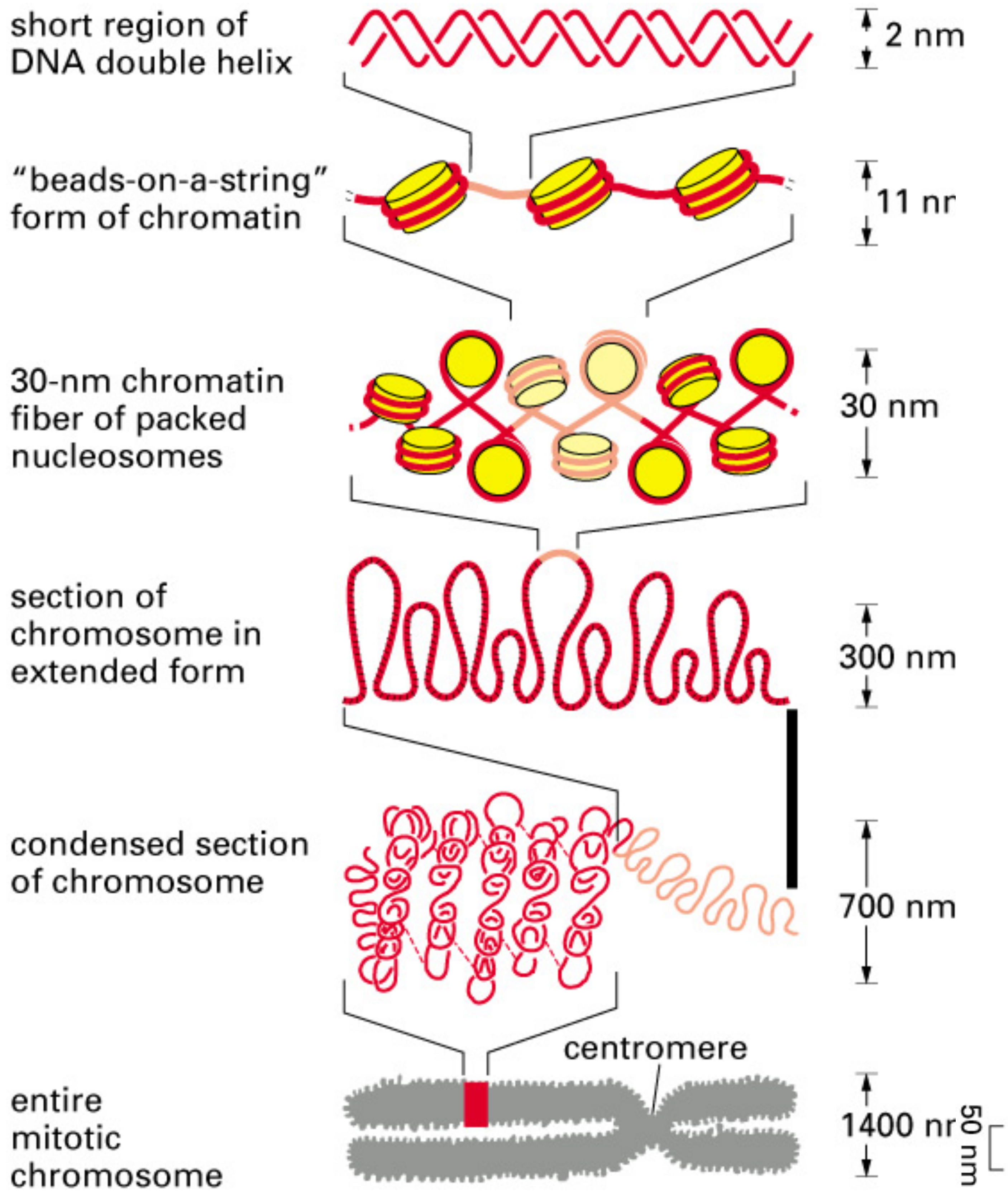
J. Craig Venter,[1,*] Mark D. Adams,[1] Eugene W. Myers,[1] Peter W. Li,[1] Richard J. Mural,[1] Granger G. Sutton,[1] Hamilton O. Smith,[1] Mark Yandell,[1] Cheryl A. Evans,[1] Robert A. Holt,[1] Jeannine D. Gocayne,[1] Peter Amanatides,[1] Richard M. Ballew,[1] Daniel H. Huson,[1] Jennifer Russo Wortman,[1] Qing Zhang,[1] Chinnappa D. Kodira,[1] Xiangqun H. Zheng,[1] Lin Chen,[1] Marian Skupski,[1] Gangadharan Subramanian,[1] Paul D. Thomas,[1] Jinghui Zhang,[1] George L. Gabor Miklos,[2] Catherine Nelson,[3] Samuel Broder,[1] Andrew G. Clark,[4] Joe Nadeau,[5] Victor A. McKusick,[6] Norton Zinder,[7] Arnold J. Levine,[7] Richard J. Roberts,[8] Mel Simon,[9] Carolyn Slayman,[10] Michael Hunkapiller,[11] Randall Bolanos,[1] Arthur Delcher,[1] Ian Dew,[1] Daniel Fasulo,[1] Michael Flanigan,[1] Liliana Florea,[1] Aaron Halpern,[1] Sridhar Hannenhalli,[1] Saul Kravitz,[1] Samuel Levy,[1] Clark Mobarry,[1] Knut Reinert,[1] Karin Remington,[1] Jane Abu-Threideh,[1] Ellen Beasley,[1] Kendra Biddick,[1] Vivien Bonazzi,[1] Rhonda Brandon,[1] Michele Cargill,[1] Ishwar Chandramouliswaran,[1] Rosane Charlab,[1] Kabir Chaturvedi,[1] Zuoming Deng,[1] Valentina Di Francesco,[1] Patrick Dunn,[1] Karen Eilbeck,[1] Carlos Evangelista,[1] Andrei E. Gabrielian,[1] Weiniu Gan,[1] Wangmao Ge,[1] Fangcheng Gong,[1] Zhiping Gu,[1] Ping Guan,[1] Thomas J. Heiman,[1] Maureen E. Higgins,[1] Rui-Ru Ji,[1] Zhaoxi Ke,[1] Karen A. Ketchum,[1] Zhongwu Lai,[1] Yiding Lei,[1] Zhenya Li,[1] Jiayin Li,[1] Yong Liang,[1] Xiaoying Lin,[1] Fu Lu,[1] Gennady V. Merkulov,[1] Natalia Milshina,[1] Helen M. Moore,[1] Ashwinikumar K Naik,[1] Vaibhav A. Narayan,[1] Beena Neelam,[1] Deborah Nusskern,[1] Douglas B. Rusch,[1] Steven Salzberg,[12] Wei Shao,[1] Bixiong Shue,[1] Jingtao Sun,[1] Zhen Yuan Wang,[1] Aihui Wang,[1] Xin Wang,[1] Jian Wang,[1] Ming-Hui Wei,[1] Ron Wides,[13] Chunlin Xiao,[1] Chunhua Yan,[1] Alison Yao,[1] Jane Ye,[1] Ming Zhan,[1] Weiqing Zhang,[1] Hongyu Zhang,[1] Qi Zhao,[1] Liansheng Zheng,[1] Fei Zhong,[1] Wenyan Zhong,[1] Shiaoping C. Zhu,[1] Shaying Zhao,[12] Dennis Gilbert,[1] Suzanna Baumhueter,[1] Gene Spier,[1] Christine Carter,[1] Anibal Cravchik,[1] Trevor Woodage,[1] Feroze Ali,[1] Huijin An,[1] Aderonke Awe,[1] Danita Baldwin,[1] Holly Baden,[1] Mary Barnstead,[1] Ian Barrow,[1] Karen Beeson,[1] Dana Busam,[1] Amy Carver,[1] Angela Center,[1] Ming Lai Cheng,[1] Liz Curry,[1] Steve Danaher,[1] Lionel Davenport,[1] Raymond Desilets,[1] Susanne Dietz,[1] Kristina Dodson,[1] Lisa Doup,[1] Steven Ferriera,[1] Neha Garg,[1] Andres Gluecksmann,[1] Brit Hart,[1] Jason Haynes,[1] Charles Haynes,[1] Cheryl Heiner,[1] Suzanne Hladun,[1] Damon Hostin,[1] Jarrett Houck,[1] Timothy Howland,[1] Chinyere Ibegwam,[1] Jeffery Johnson,[1] Francis Kalush,[1] Lesley Kline,[1] Shashi Koduru,[1] Amy Love,[1] Felecia Mann,[1] David May,[1] Steven McCawley,[1] Tina McIntosh,[1] Ivy McMullen,[1] Mee Moy,[1] Linda Moy,[1] Brian Murphy,[1] Keith Nelson,[1] Cynthia Pfannkoch,[1] Eric Pratts,[1] Vinita Puri,[1] Hina Qureshi,[1] Matthew Reardon,[1] Robert Rodriguez,[1] Yu-Hui Rogers,[1] Deanna Romblad,[1] Bob Ruhfel,[1] Richard Scott,[1] Cynthia Sitter,[1] Michelle Smallwood,[1] Erin Stewart,[1] Renee Strong,[1] Ellen Suh,[1] Reginald Thomas,[1] Ni Ni Tint,[1] Sukyee Tse,[1] Claire Vech,[1] Gary Wang,[1] Jeremy Wetter,[1] Sherita Williams,[1] Monica Williams,[1] Sandra Windsor,[1] Emily Winn-Deen,[1] Keriellen Wolfe,[1] Jayshree Zaveri,[1] Karena Zaveri,[1] Josep F. Abril,[14] Roderic Guigó,[14] Michael J. Campbell,[1] Kimmen V. Sjolander,[1] Brian Karlak,[1] Anish Kejariwal,[1] Huaiyu Mi,[1] Betty Lazareva,[1] Thomas Hatton,[1] Apurva Narechania,[1] Karen Diemer,[1] Anushya Muruganujan,[1] Nan Guo,[1] Shinji Sato,[1] Vineet Bafna,[1] Sorin Istrail,[1] Ross Lippert,[1] Russell Schwartz,[1] Brian Walenz,[1] Shibu Yooseph,[1] David Allen,[1] Anand Basu,[1] James Baxendale,[1] Louis Blick,[1] Marcelo Caminha,[1] John Carnes-Stine,[1] Parris Caulk,[1] Yen-Hui Chiang,[1] My Coyne,[1] Carl Dahlke,[1] Anne Deslattes Mays,[1] Maria Dombroski,[1] Michael Donnelly,[1] Dale Ely,[1] Shiva Esparham,[1] Carl Fosler,[1] Harold Gire,[1] Stephen Glanowski,[1] Kenneth Glasser,[1] Anna Glodek,[1] Mark Gorokhov,[1] Ken Graham,[1] Barry Gropman,[1] Michael Harris,[1] Jeremy Heil,[1] Scott Henderson,[1] Jeffrey Hoover,[1] Donald Jennings,[1] Catherine Jordan,[1] James Jordan,[1] John Kasha,[1] Leonid Kagan,[1] [illegible] Kraft,[1] Alexander Levitsky,[1] Mark Lewis,[1] Xiangjun Liu,[1] John Lopez,[1] Daniel Ma,[1] [illegible] Joe McDaniel,[1] Sean Murphy,[1] Matthew Newman,[1] Trung Nguyen,[1] Ngoc Nguyen,[1] [illegible] Sue Pan,[1] Jim Peck,[1] Marshall Peterson,[1] William Rowe,[1] Robert Sanders,[1] [illegible] Michael Simpson,[1] Thomas Smith,[1] Arlan Sprague,[1] Timothy Stockwell,[1] Russell Turner,[1] Eli Venter,[1] Mei Wang,[1] Meiyuan Wen,[1] David Wu,[1] Mitchell Wu,[1] Ashley Xia,[1] Ali Zandieh,[1] Xiaohong Zhu[1]

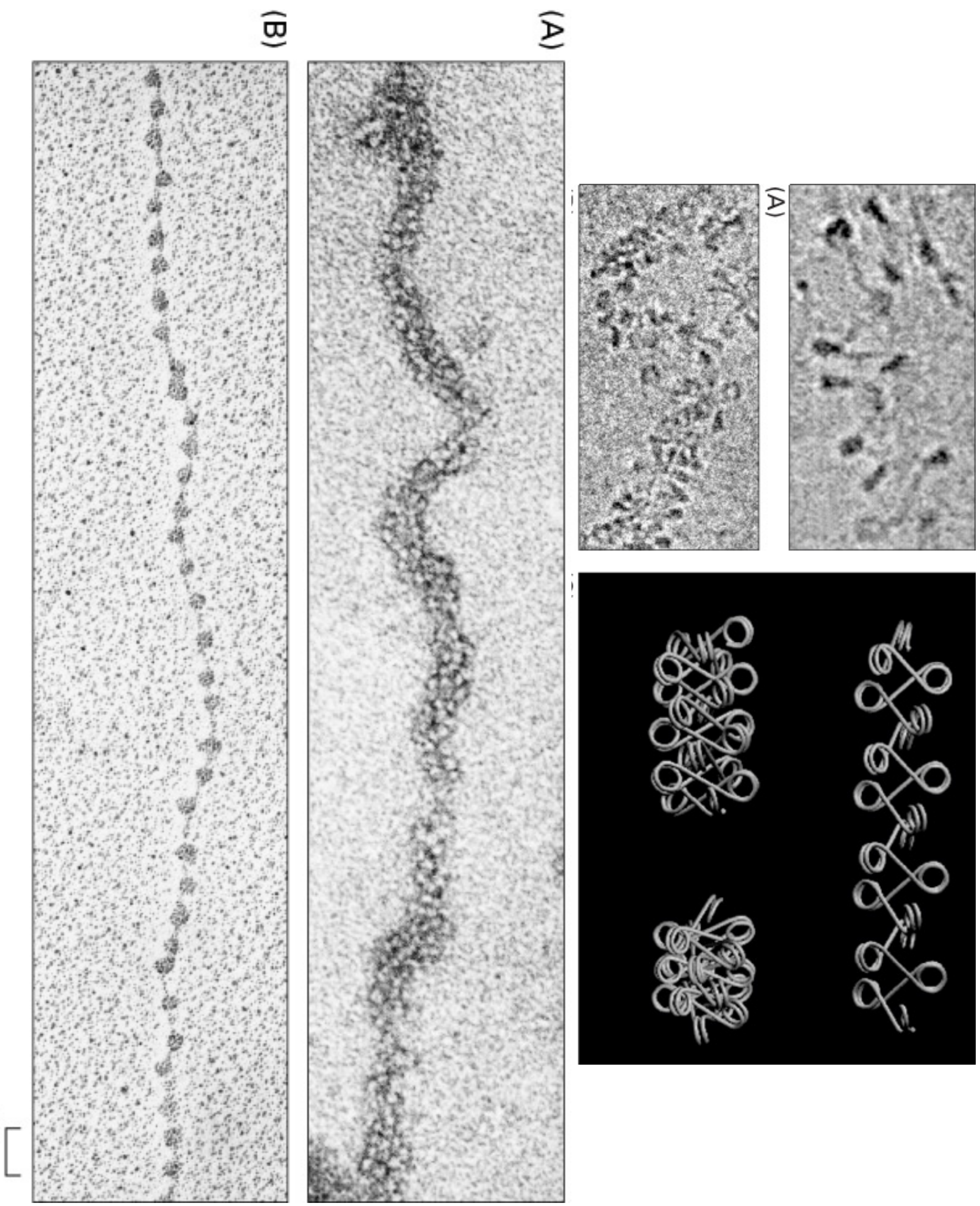
The human genome

Number of chromosomes:	46
Size of haploid genome:	~3x10^9 bp
Estimated number of genes:	~20.000

Genes:	30%
Extragenic DNA	70%
Gene coding regions	<2%
Non coding regions	>98% (!)

Nucleotide polymorphisms:	~3x10^6 (!)



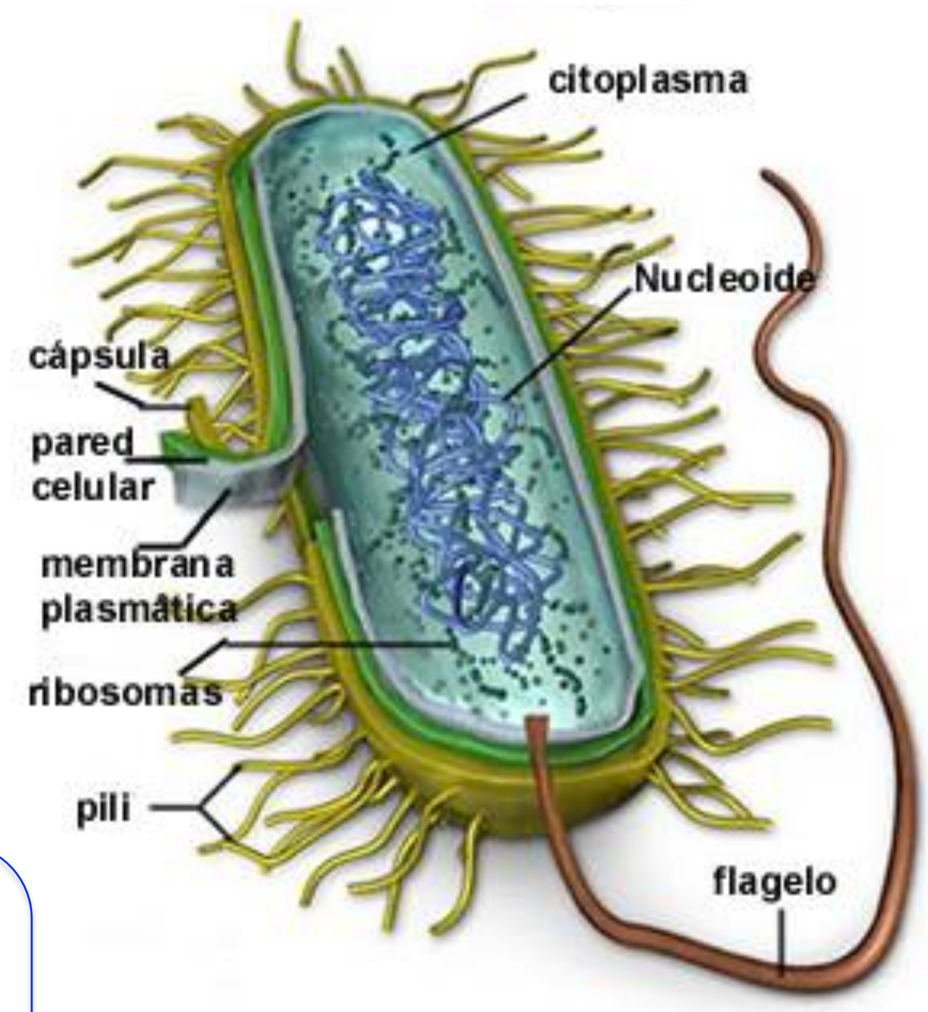


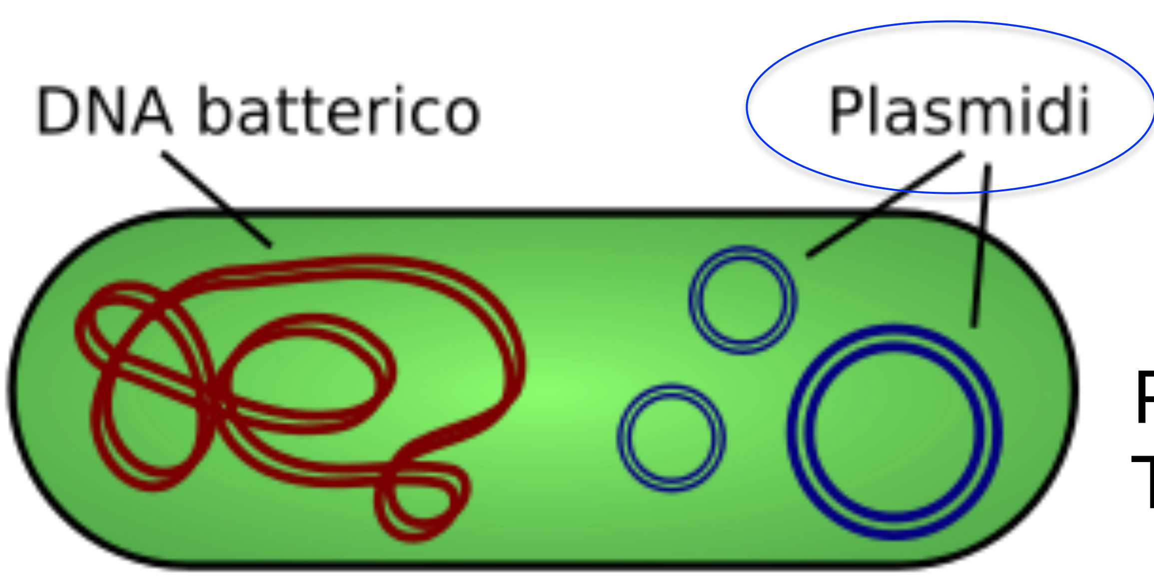
NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

LIVELLI DI COMPATTAZIONE DEL DNA

NEI PROCARIOTI

1 singolo cromosoma circolare organizzato in una struttura ovoidale condensata chiamata NUCLEOIDE





Resistenza agli antibiotici
Tecnologia del DNA ricombinante

ESPRESSIONE/"DECODIFICA" DELL'INFORMAZIONE

Il DNA contiene i "GENI", cioè tratti specifici di nucleotidi responsabili della produzione di un prodotto funzionale

DOGMA CENTRALE DELLA BIOLOGIA MOLECOLARE (1957)

REPLICAZIONE

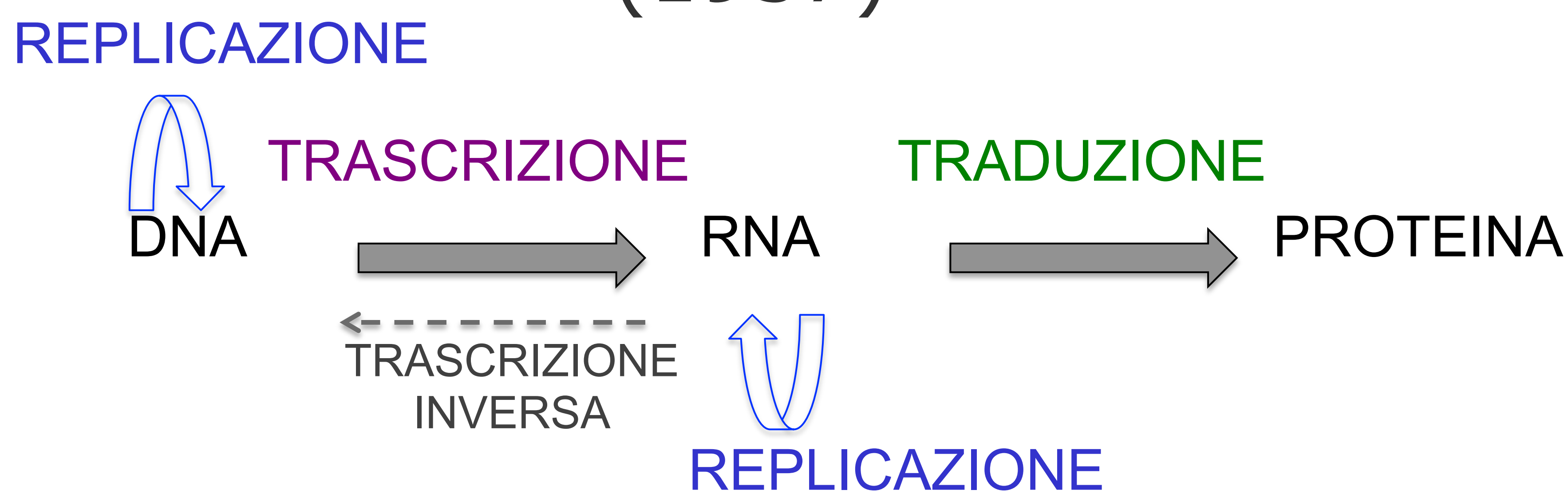
TRASCRIZIONE TRADUZIONE

DNA → RNA → PROTEINA

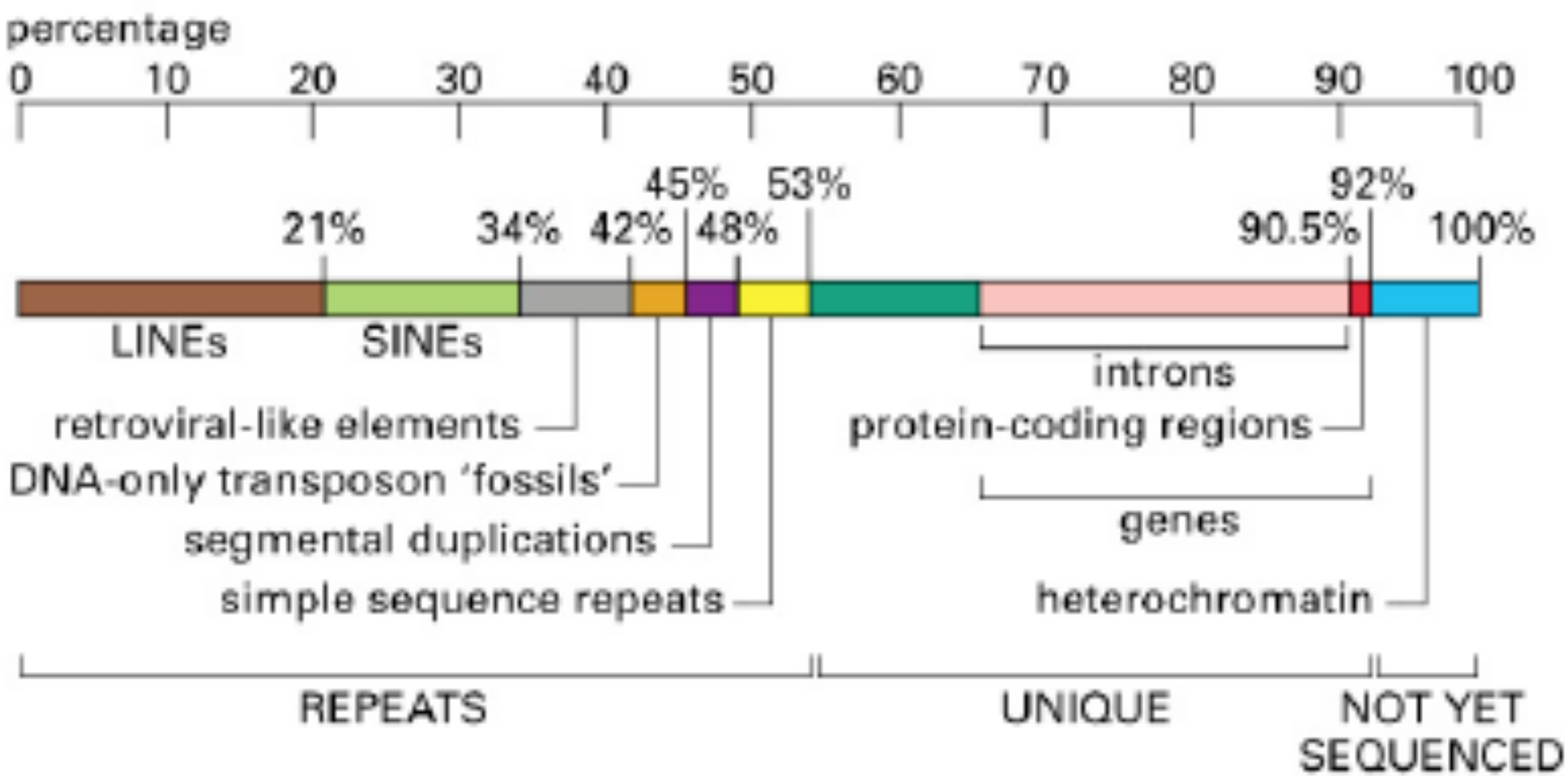
ESPRESSIONE/"DECODIFICA" DELL'INFORMAZIONE

Il DNA contiene i "GENI", cioè tratti specifici di nucleotidi responsabili della produzione di un prodotto funzionale

DOGMA CENTRALE DELLA BIOLOGIA MOLECOLARE (1957)



Anatomy of the human genome



junk DNA or useful RNA?

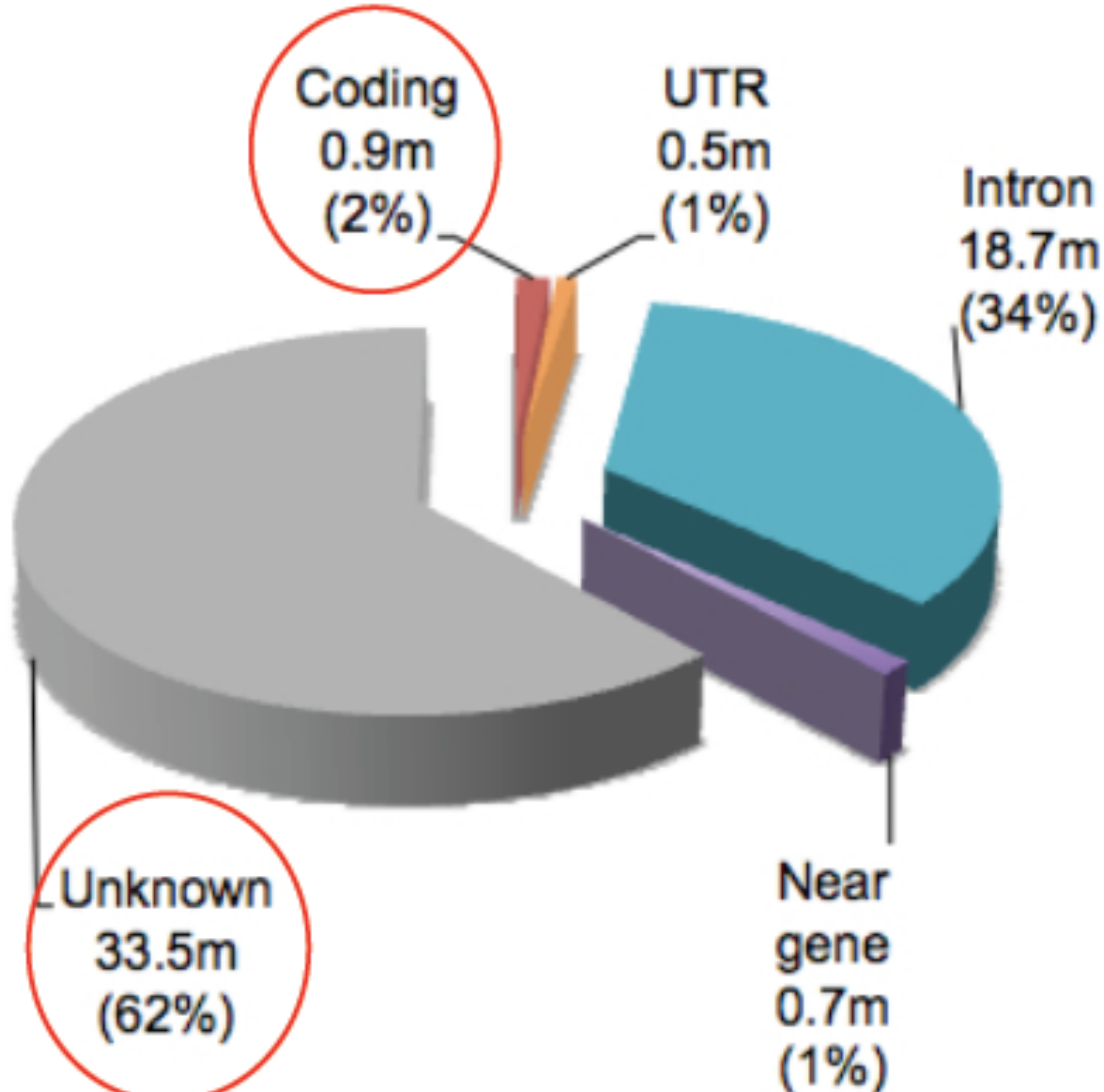
1972

Journal Title: Evolution of genetic systems.
Brookhaven sympoisa in biology, no. 23

SO MUCH "JUNK" DNA IN OUR GENOME

Susumu Ohno
City of Hope National Medical Center

The mammalian genome (haploid chromosome complement) contains roughly 3.0 X 10^{-9} mg of DNA which represents a 3.0 X 10^{9} base pairs. This is at least 750 times the ge size of *E. coli*. If we take the simplistic assumption t the number of genes contained is proportional to the ger



from massgenomics.org

Small RNAs and Gene Silencing

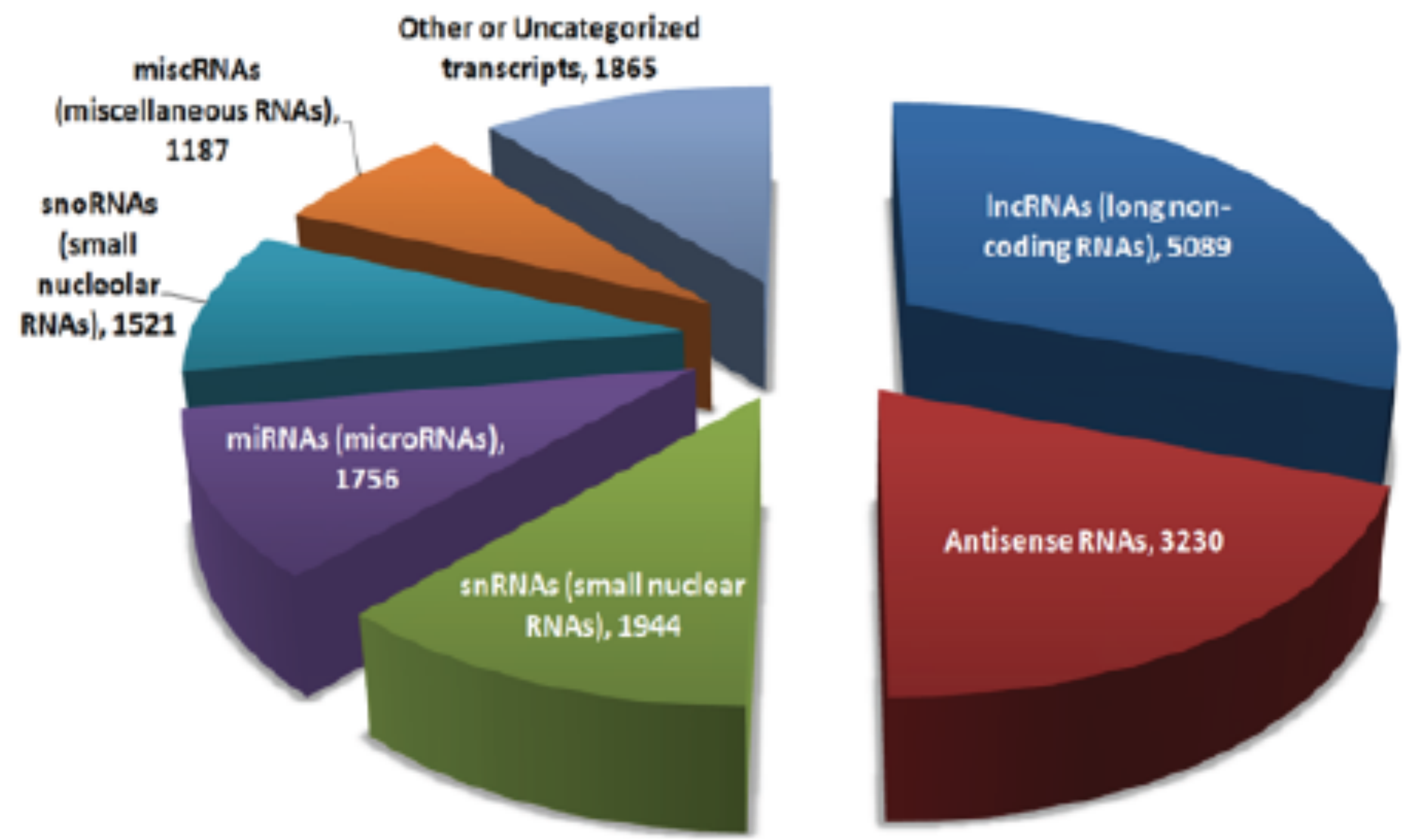
Name	Organism	Length (nt)	Proteins	Source of trigger	Function
miRNA	Plants, algae, animals, viruses, protists	20–25	Drosha (animals only) and Dicer	Pol II transcription (pri-miRNAs)	Regulation of mRNA stability, translation
casiRNA	Plants	24	DCL3	Transposons, repeats	Chromatin modification
tasiRNA	Plants	21	DCL4	miRNA-cleaved RNAs from the *TAS* loci	Post-transcriptional regulation
natsiRNA	Plants	22	DCL1	Bidirectional transcripts induced by stress	Regulation of stress-response genes
		24	DCL2		
		21	DCL1 and DCL2		
Exo-siRNA	Animals, fungi, protists	~21	Dicer	Transgenic, viral or other exogenous dsRNA	Post-transcriptional regulation, antiviral defense
	Plants	21 and 24			
Endo-siRNA	Plants, algae, animals, fungi, protists	~21	Dicer (except secondary siRNAs in *C. elegans*, which are products of RdRP transcription, and are therefore not technically siRNAs)	Structured loci, convergent and bidirectional transcription, mRNAs paired to antisense pseudogene transcripts	Post-transcriptional regulation of transcripts and transposons; transcriptional gene silencing
piRNA	Metazoans excluding *Trichoplax adhaerens*	24–30	Dicer-independent	Long, primary transcripts?	Transposon regulation, unknown functions
piRNA-like (soma)	*Drosophila melanogaster*	24–30	Dicer-independent	In *ago2* mutants in *Drosophila*	Unknown
21U-RNA piRNAs	*Caenorhabditis elegans*	21	Dicer-independent	Individual transcription of each piRNA?	Transposon regulation, unknown functions
26G RNA	*Caenorhabditis elegans*	26	RdRP?	Enriched in sperm	Unknown

adapted from Ghildiyal & Zamore. 2009. Nat Rev Genet. 10:94

miRNA: microRNAs; casiRNA: siRNA: short interfering RNA; cis-acting siRNA: short interfering RNAs; tasiRNA: trans-acting siRNA; natsiRNAs: natural antisense transcript-derived siRNA; exo-siRNA: endogenous siRNA; endo-siRNA: endogenous siRNA ; piRNA: PIWI-interacting RNA;

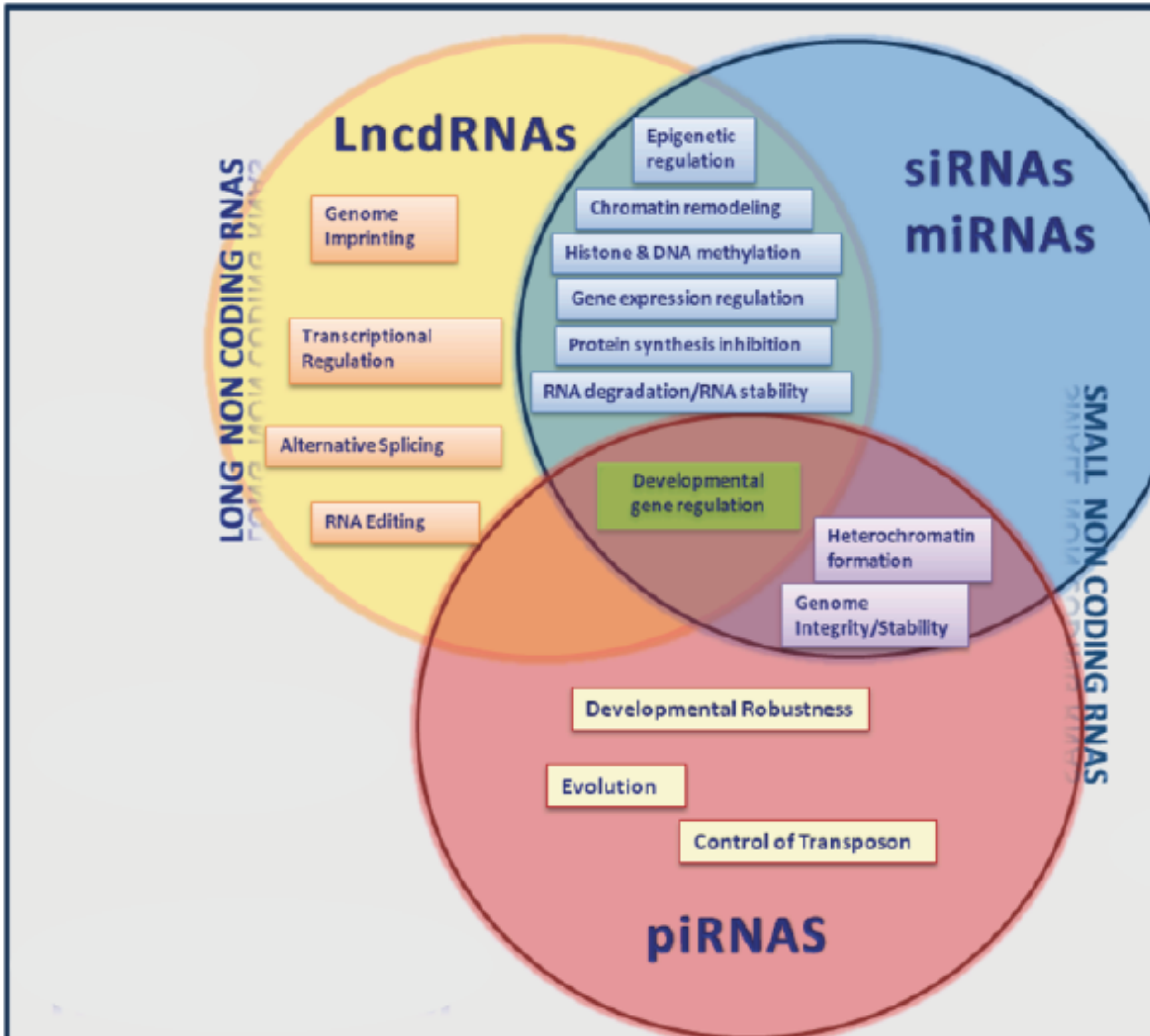
What are lnc RNAs?

- **Non coding** RNAs
- >200 nt in length
- cell-specific function
- poorly conserved among species
- the amount of lnc RNAs in DNA correlates with organism complexity



from Baker M., Long noncoding RNAs: the search for function, *Nature Methods* **8**, 379–383 (2011)

Many different classifications - function

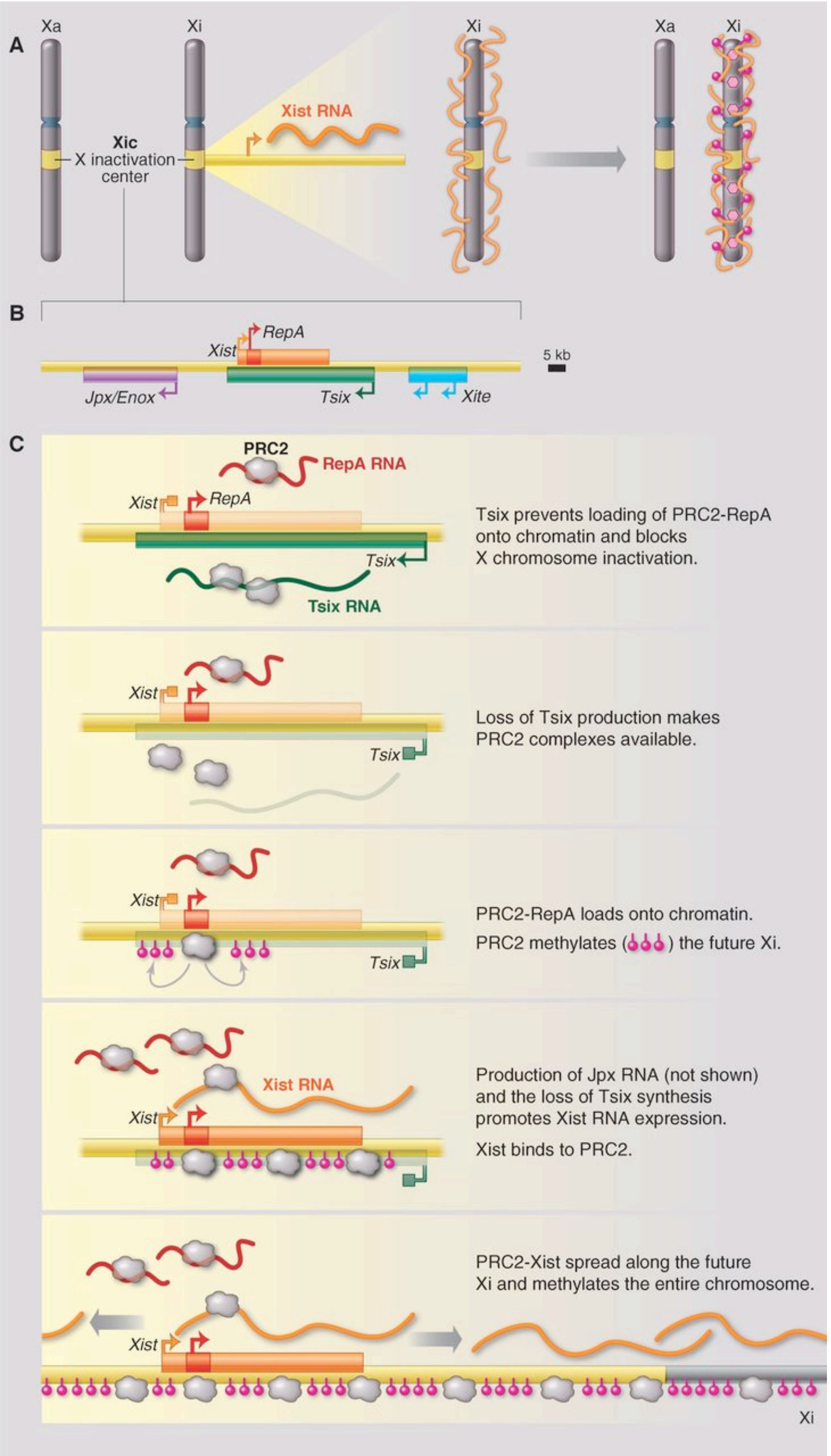


adapted from Gomes Q.A. et al., Non-Coding RNAs: Multi-Tasking Molecules in the Cell, Int. J. Mol. Sci. **2013**, 14(8)

Epigenetic Regulation by Long Noncoding RNAs

Jeannie T. Lee

www.sciencemag.org SCIENCE VOL 338 14 DECEMBER 2012

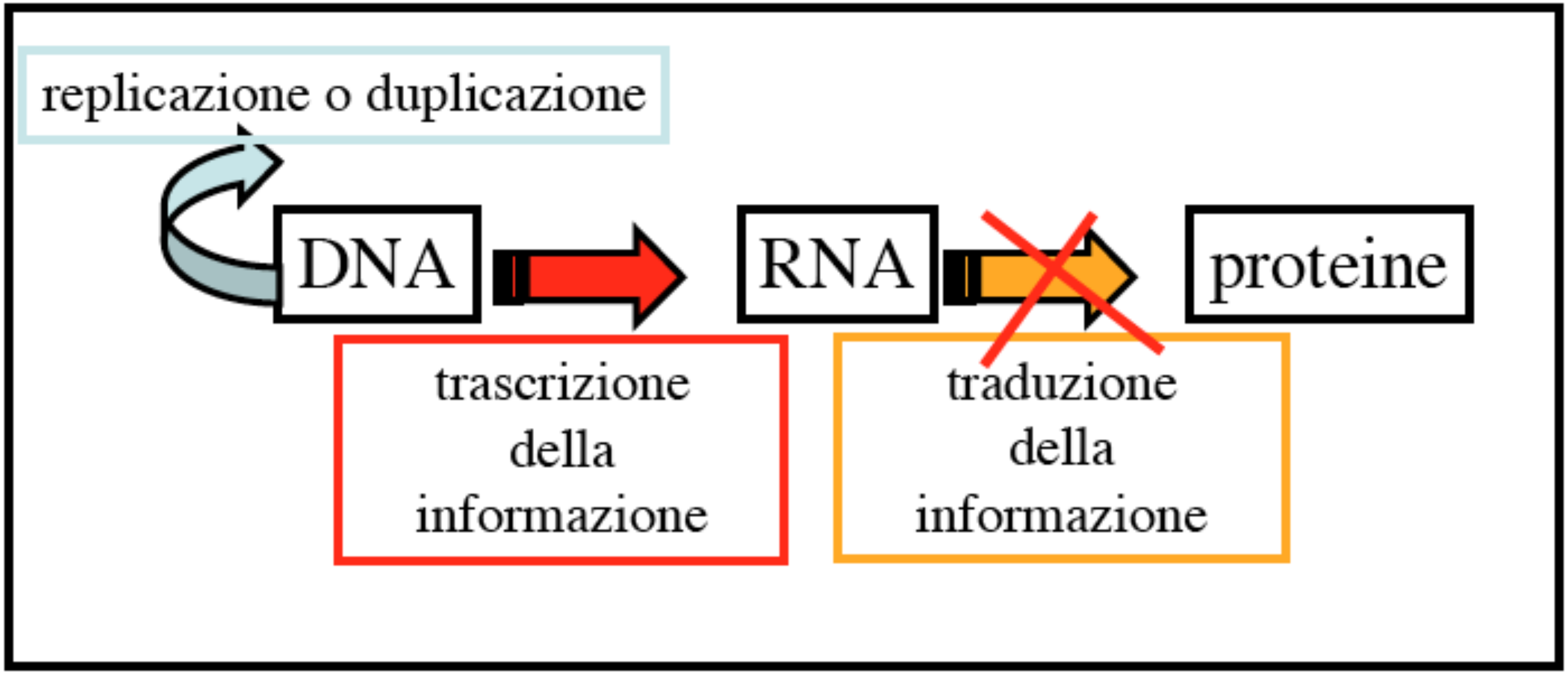


LncRNAs in X-chromosome inactivation.

(A) The lncRNA Xist is transcribed from the Xic of the inactive X chromosome (Xi). Xist RNA covers the entire chromosome and silences gene expression through epigenetic modification of histones and DNA.

(B) The core region of the Xic and its lncRNAs.

(C) LncRNA-protein interactions at the initiation of XCI.



Reintrepetazione
del dogma della biologia molecolare alla luce delle nuove conoscenze sul genoma