



International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy



Recapito docente

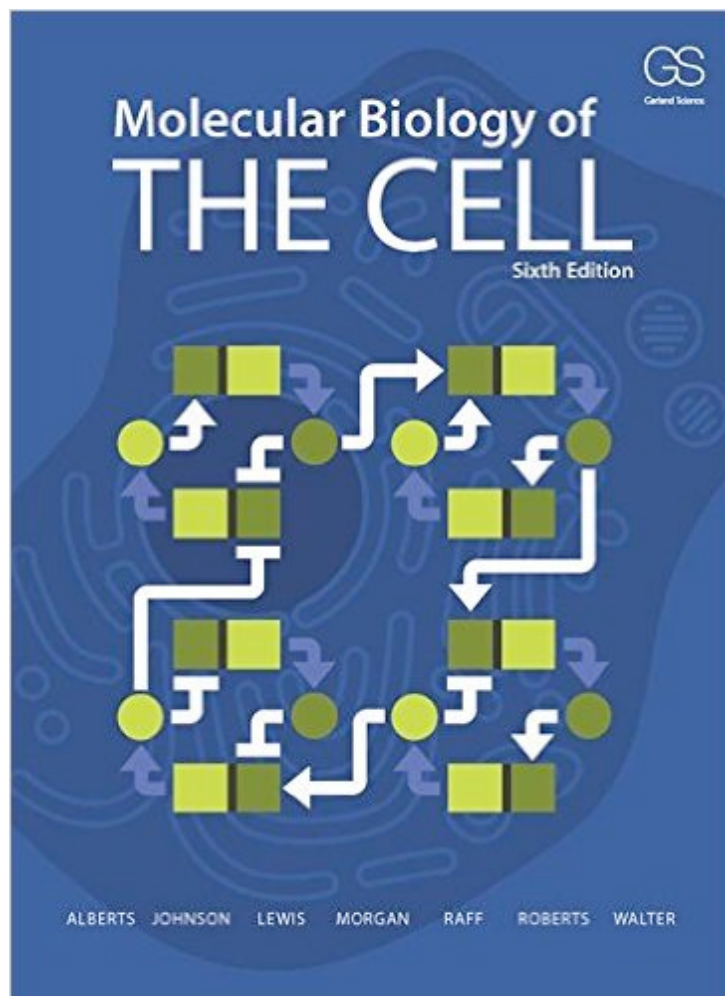
Chiara Collesi

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Molecular Medicine Unit

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

Biologia Molecolare ***AA 2021-2022***



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, sarà fornito agli studenti opportuno materiale didattico.



ESAME

18 DOMANDE A RISPOSTA MULTIPLA E 4 DOMANDE APERTE



ESAME

**DON'T
PANIC**

ESEMPI DOMANDE CHIUSE

Quali delle seguenti proprietà caratterizzano gli mRNA degli istoni:

1. sono trascritti durante la fase S del ciclo cellulare
2. sono trascritti dalla RNA polimerasi II
3. non sono poliadenilati
4. sono presenti solo nelle cellule eucariotiche

La proteina Dicer:

1. sta nel nucleo
2. è una ribonucleasi
3. è una endonucleasi che taglia RNA a doppio filamento
4. riconosce preferenzialmente gli mRNA dei geni housekeeping

ESEMPI DOMANDE APERTE

- ✓ Cosa sono i frammenti di Okazaki?
- ✓ Illustrare schematicamente I meccanismi di controllo della fedeltà di duplicazione del DNA
- ✓ Illustrare brevemente come viene stabilito il corretto inizio della traduzione

Che cosa studieremo?

Dove e' scritta l'informazione genica:

Replicazione

Come si esprime l'informazione genica:

Trascrizione e Traduzione



In
PROCARIOTI
ed
EUCARIOTI

Che cosa studieremo?

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Meccanismi di CONTROLLO dell'espressione genica:

piccoli RNA regolatori

Come l'ambiente influenza l'espressione genica:

Epigenetica

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La Biologia Molecolare nel 2022

Tecniche di Biologia Molecolare

Ingegneria Genetica

Terapia Genica

Gene e Genome Editing

Molecular Medicine

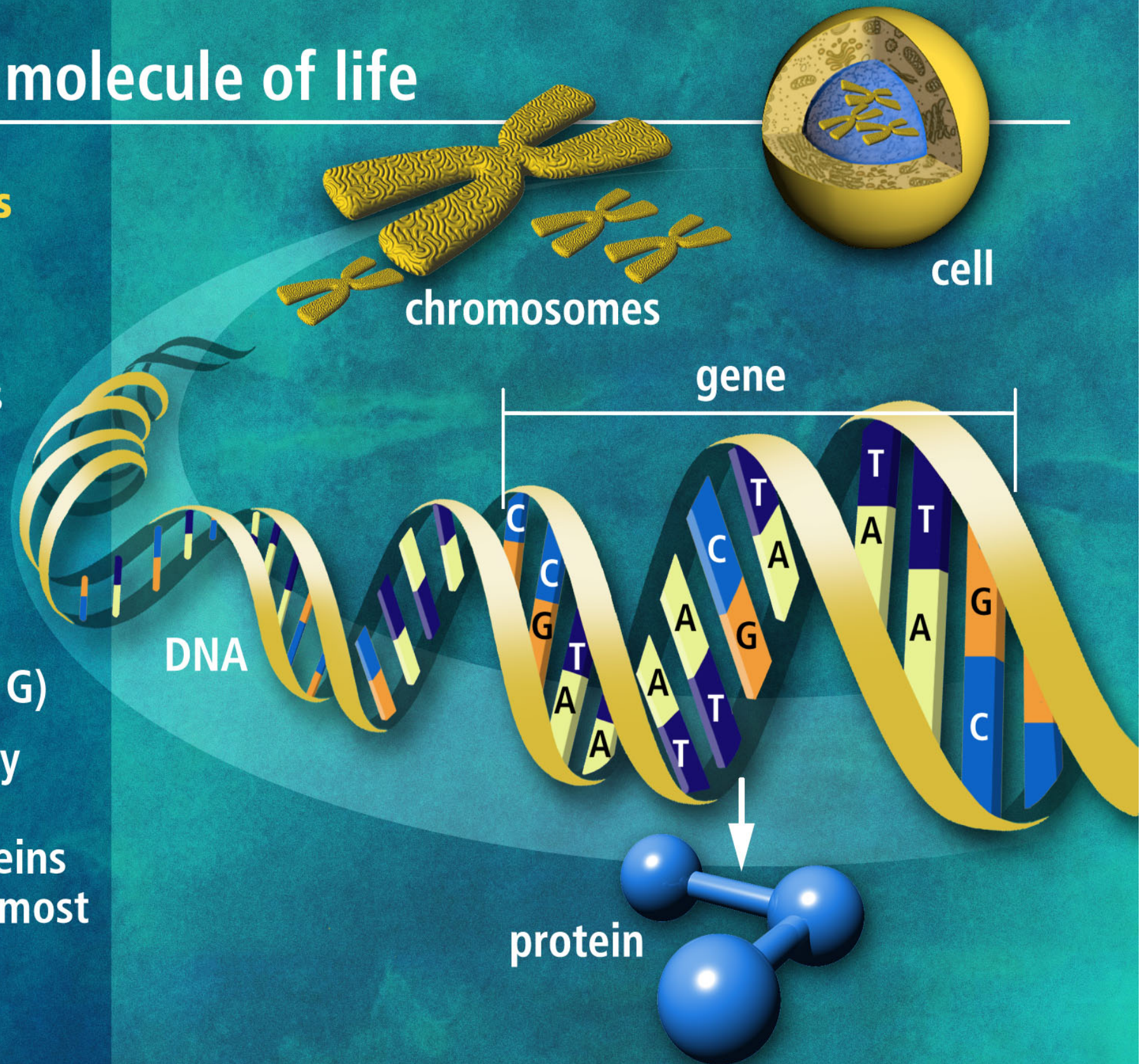
Dove e' scritta l'informazione genica?

DNA the molecule of life

Trillions of cells

Each cell:

- 46 human chromosomes
- 2 meters of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- Approximately 30,000 genes code for proteins that perform most life functions



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 proporzionalità*** tra complessità' genoma e complessità' dell'organismo

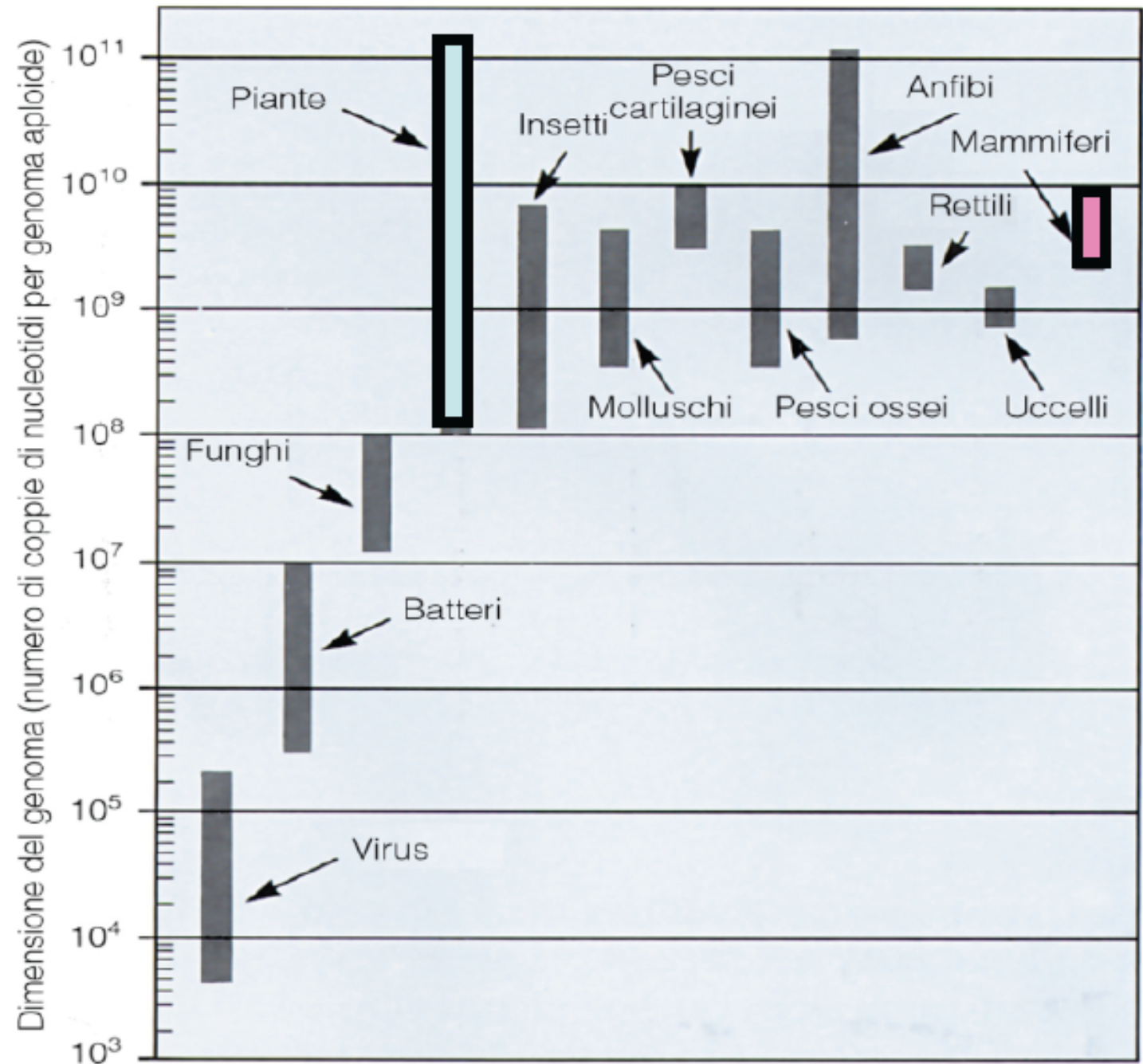


Figura 16-11 Correlazione fra dimensione del genoma e tipo di organismo. Per ogni gruppo di organismi la barra rappresenta la variabilità approssimativa della dimensione del genoma, misurata come quantità di coppie di nucleotidi per genoma aploide.

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 shotgun sequencing initiated Sept 1999 and completed June 2000 (!)

nature

CLASSIC ART BY MICHELANGELO

www.nature.com



Now on the market
Lasers

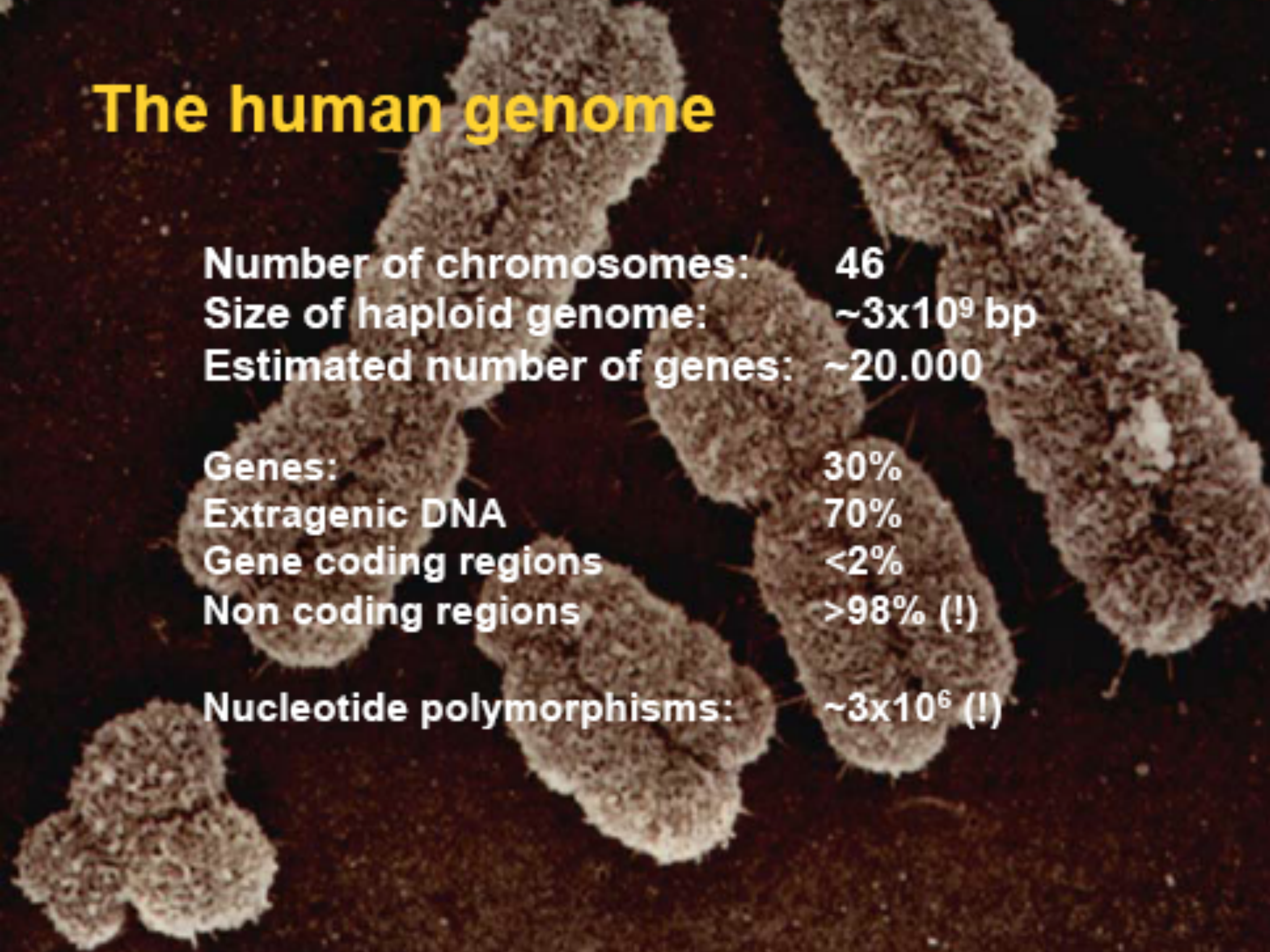


The Sequence of the Human Genome

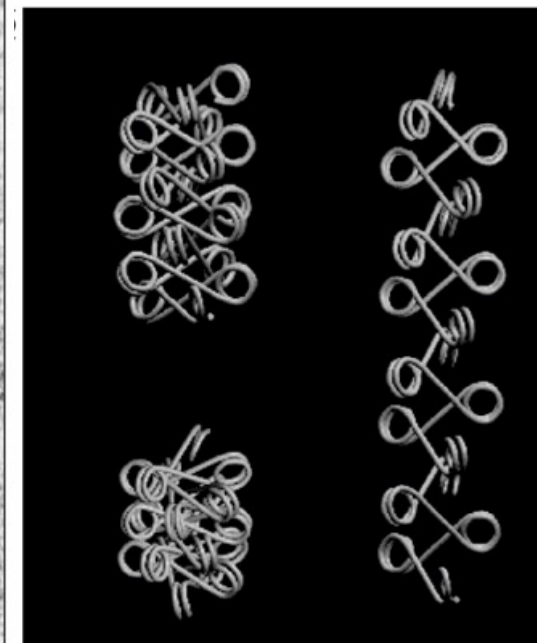
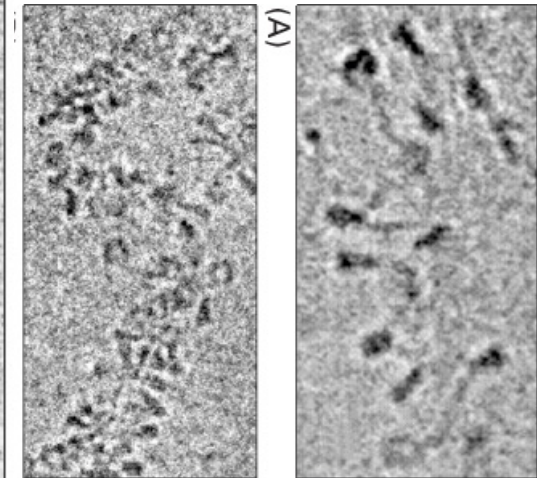
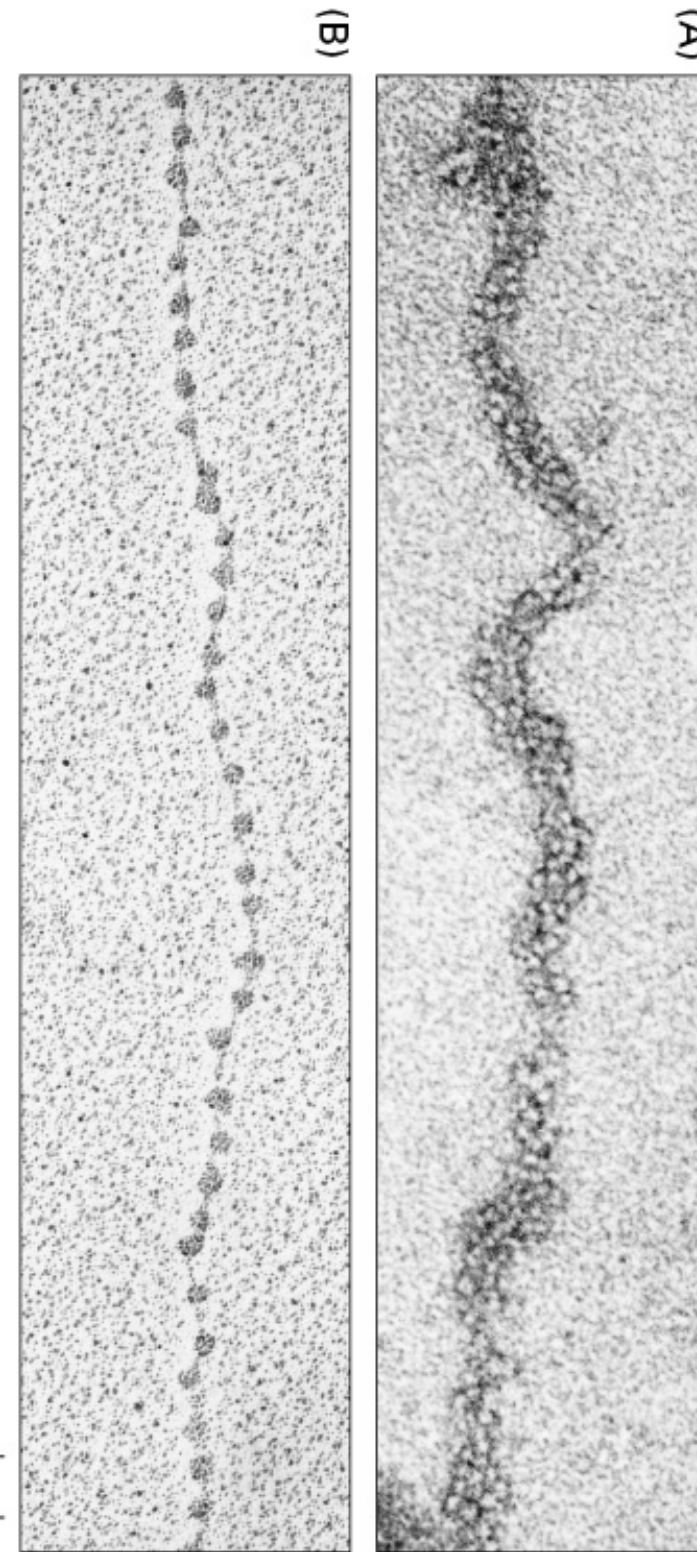
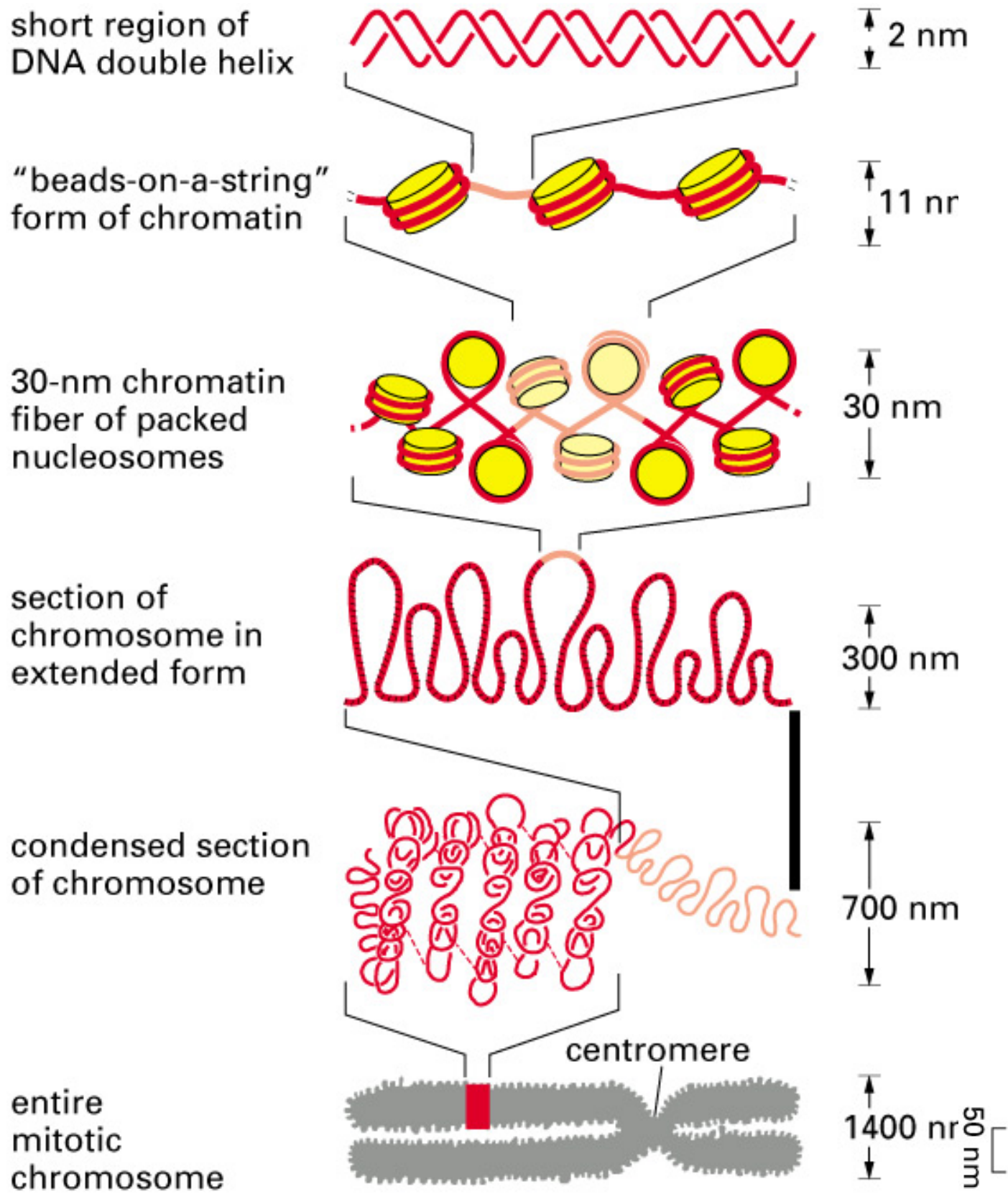
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 Sue Pan,¹ Jim Peck,¹ Marshall Peterson,¹ William Rowe,¹ Robert Sanders,¹ John Searles,¹
 Michael Simpson,¹ Thomas Smith,¹ Arlan Sprague,¹ Timothy Stockwell,¹ Russell Turner,¹ Ali Venter,¹
 Mei Wang,¹ Malyuan Wen,¹ David Wu,¹ Mitchell Wu,¹ Ashley Xia,¹ Ali Zandieh,¹ Xiaohong Zhu¹



The human genome

A scanning electron micrograph showing several human chromosomes. They appear as thick, textured, brownish structures against a dark background. The chromosomes are arranged in a somewhat diagonal line from the top left towards the bottom right.

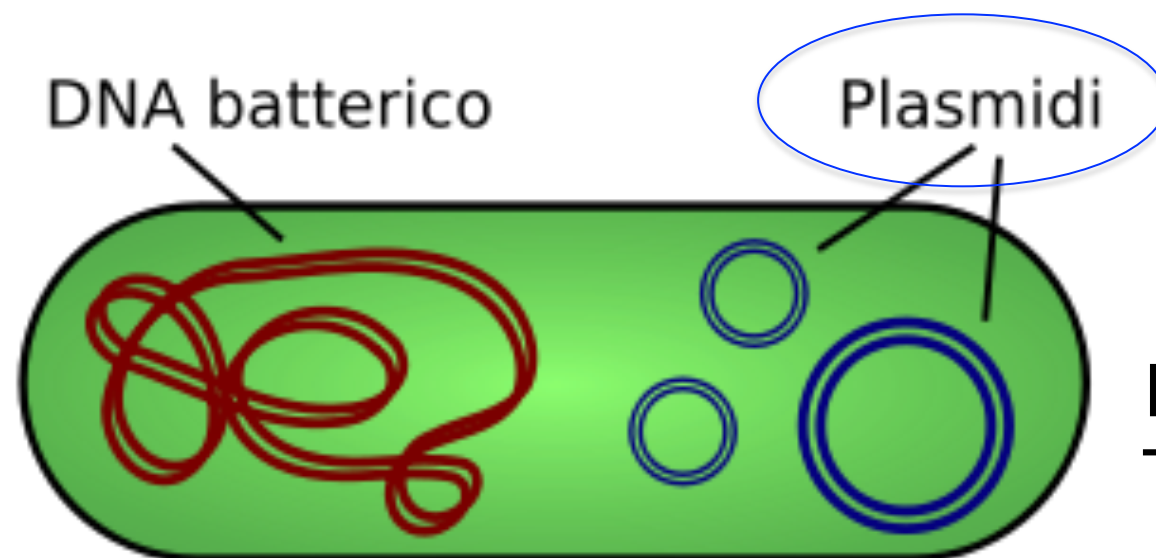
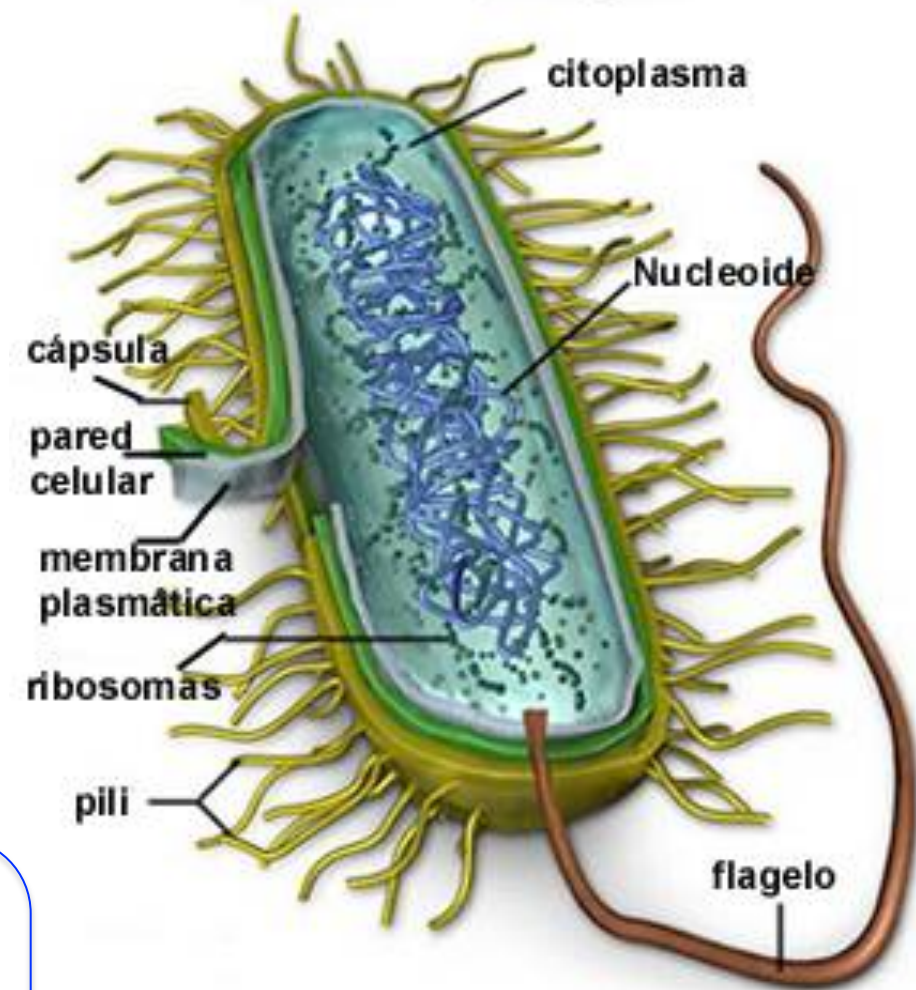
Number of chromosomes:	46
Size of haploid genome:	$\sim 3 \times 10^9$ bp
Estimated number of genes:	$\sim 20,000$
Genes:	30%
Extragenic DNA	70%
Gene coding regions	<2%
Non coding regions	>98% (!)
Nucleotide polymorphisms:	$\sim 3 \times 10^6$ (!)



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

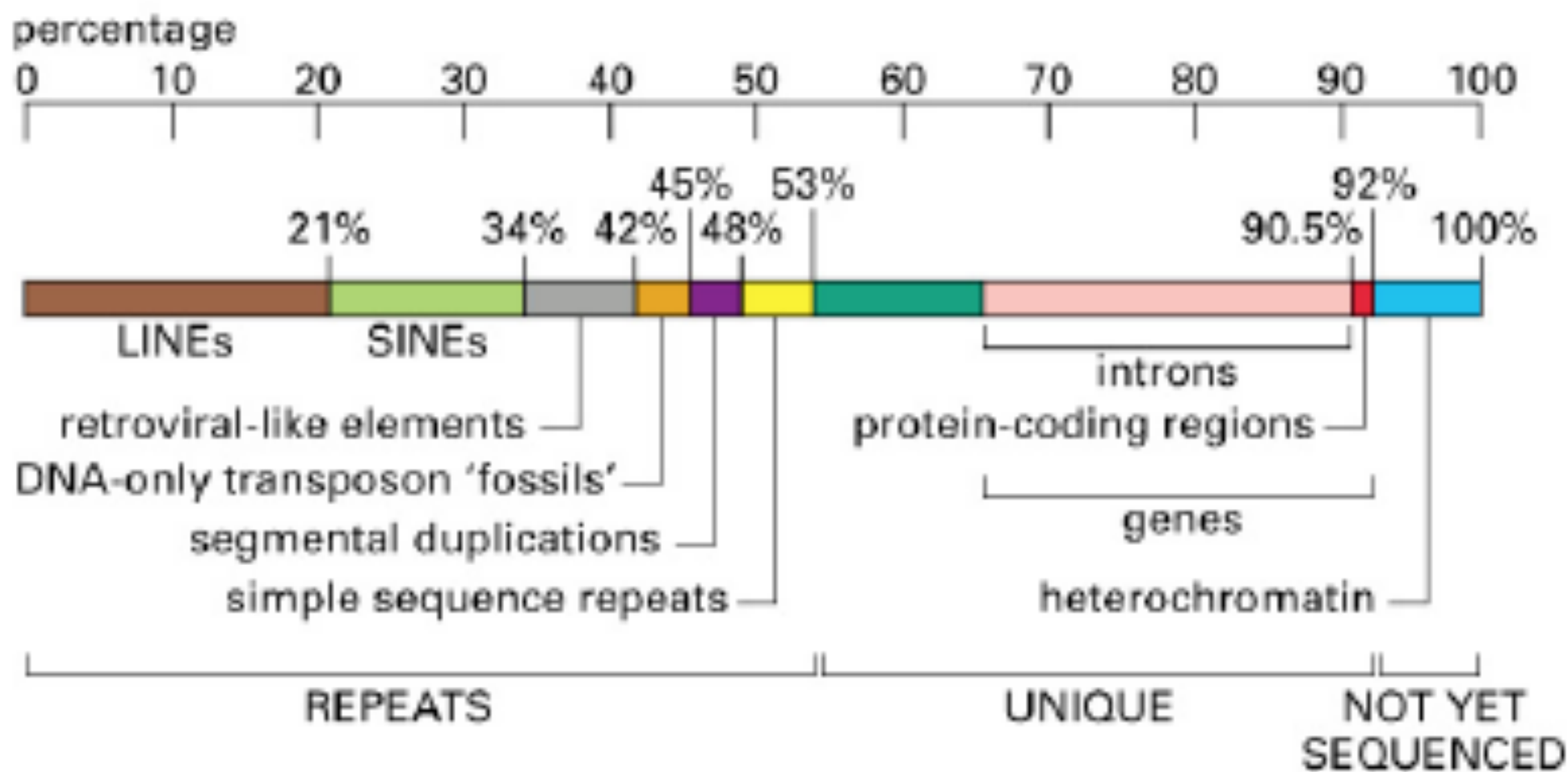
LIVELLI DI COMPATTAZIONE DEL DNA NEI PROCARIOTI

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



Resistenza agli antibiotici
Tecnologia del DNA ricombinante

Anatomy of the human genome



junk DNA or useful RNA?

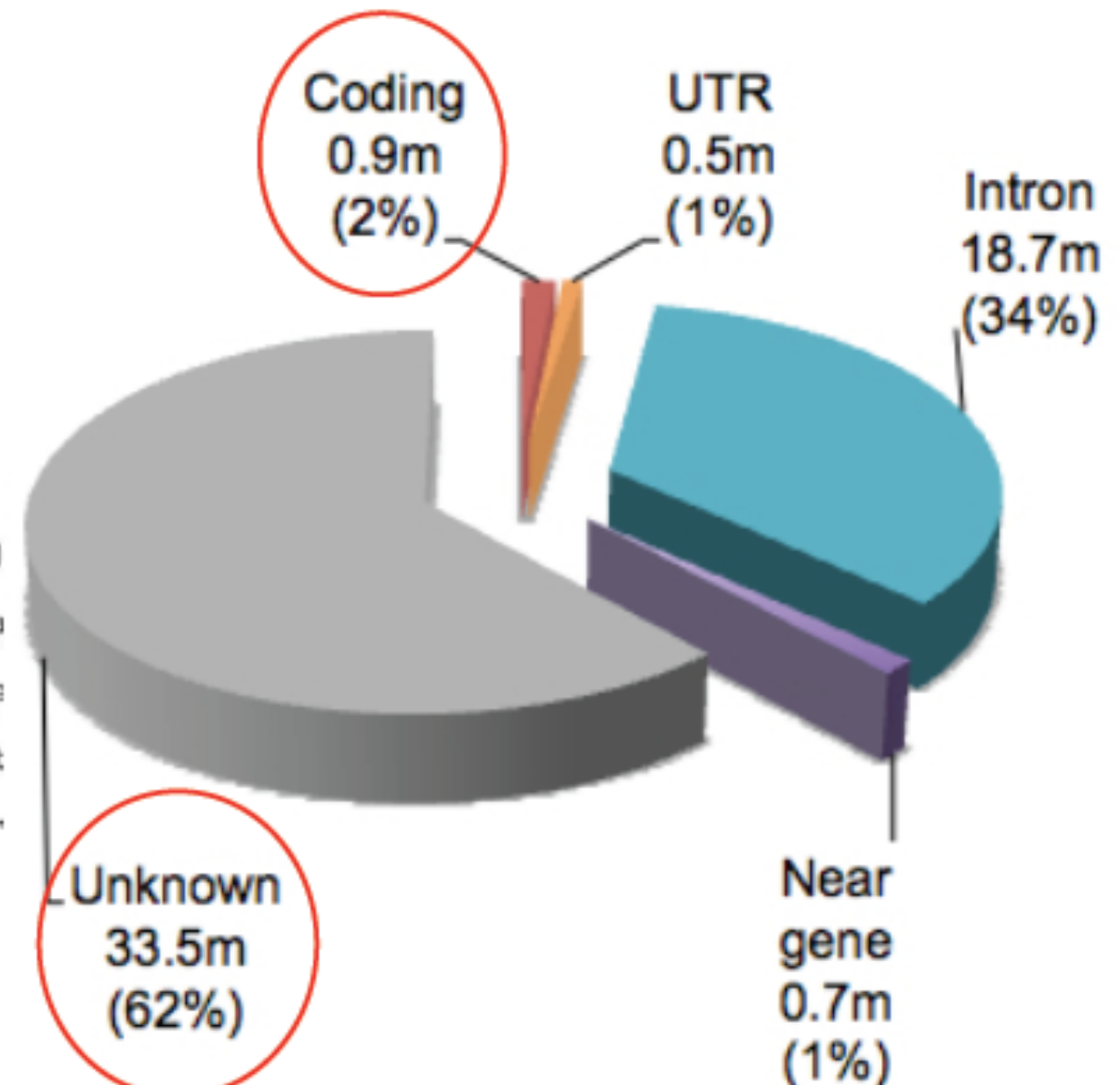
1972

Journal Title: Evolution of genetic systems.
Brookhaven symposia 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 3.0×10^{-9} mg of DNA which represents a 3.0×10^9 base pairs. This is at least 750 times the genome size of *E. coli*. If we take the simplistic assumption that the number of genes contained is proportional to the genome size, the mammalian genome contains at least 750 times as many genes as *E. coli*.



from massgenomics.org

Small RNAs and Gene Silencing

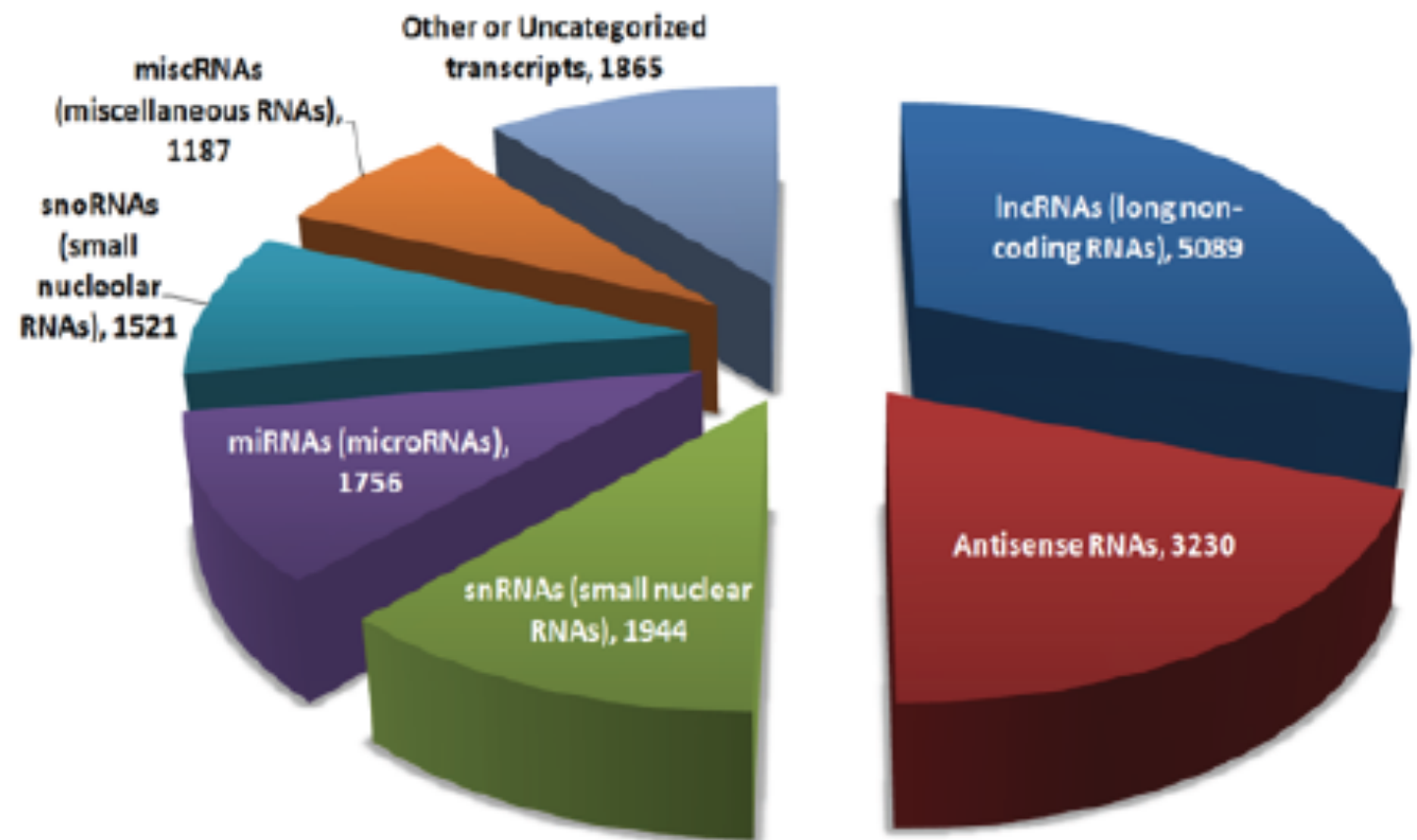
Name	Organism	Length (nt)	Proteins	Source of trigger	Function
miRNA	Plants, algae, animals, viruses, protists	20–25	Drosha (animals only) and Dicer	Pol II transcription (pri-miRNAs)	Regulation of mRNA stability, translation
casiRNA	Plants	24	DCL3	Transposons, repeats	Chromatin modification
tasiRNA	Plants	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 <i>C. elegans</i> , 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 <i>Trichoplax adhaerens</i>	24–30	Dicer-independent	Long, primary transcripts?	Transposon regulation, unknown functions
piRNA-like (soma)	<i>Drosophila melanogaster</i>	24–30	Dicer-independent	In <i>ago2</i> mutants in <i>Drosophila</i>	Unknown
21U-RNA piRNAs	<i>Caenorhabditis elegans</i>	21	Dicer-independent	Individual transcription of each piRNA?	Transposon regulation, unknown functions
26G RNA	<i>Caenorhabditis elegans</i>	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;

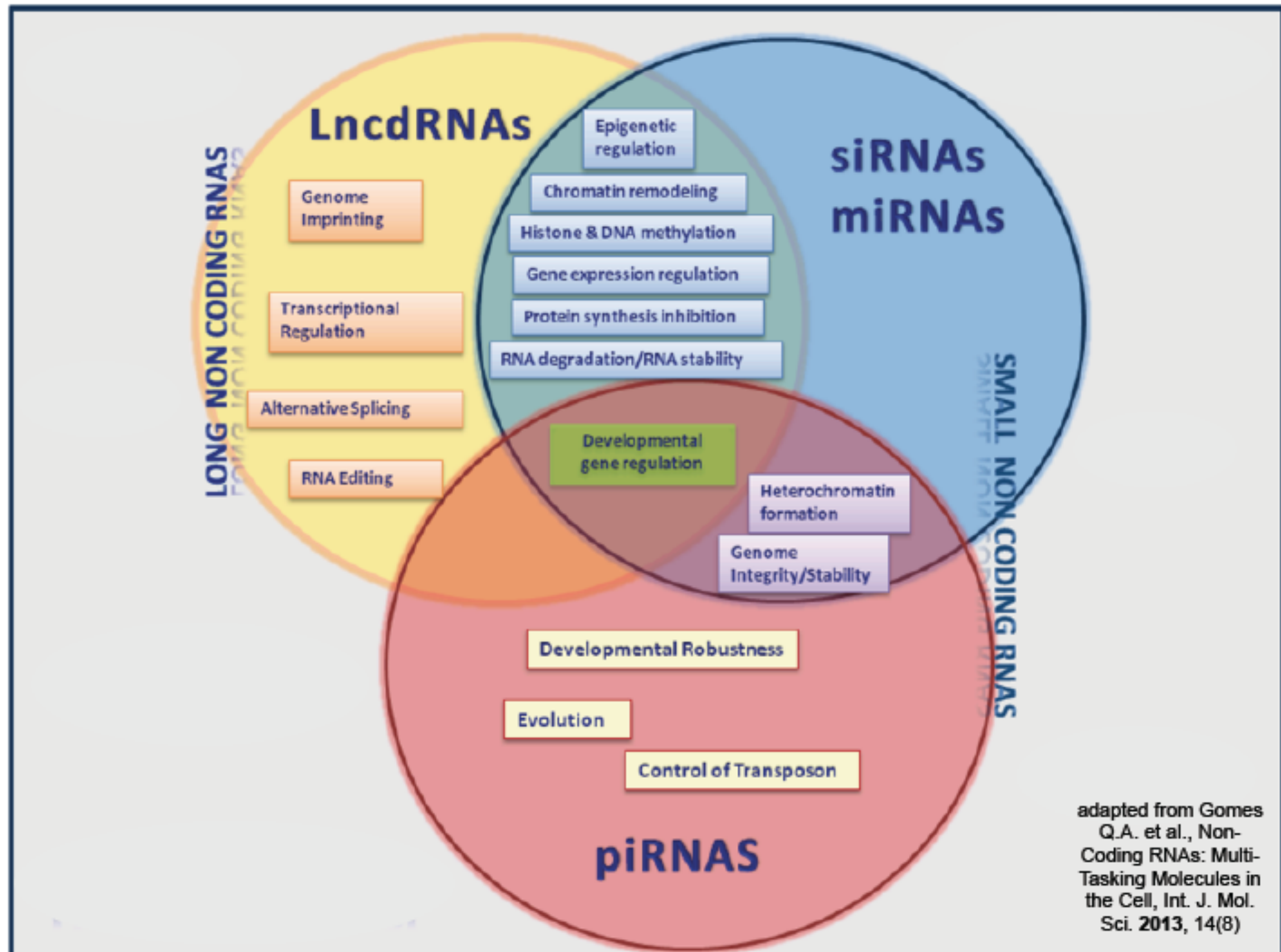
What are Inc RNAs?

- **Non coding** RNAs
- >200 nt in length
- cell-specific function
- poorly conserved among species
- the amount of Inc 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



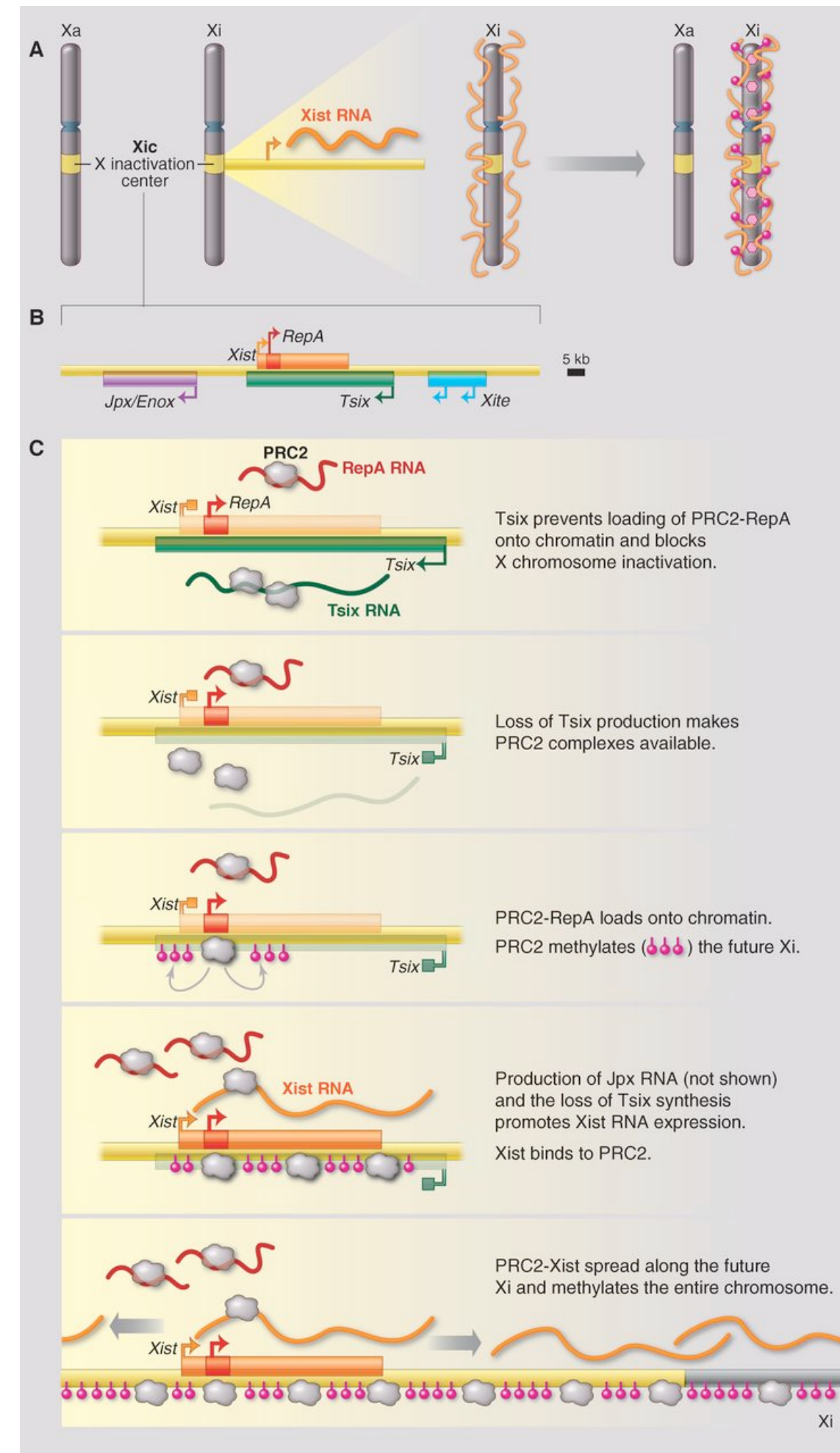
Epigenetic Regulation by Long Noncoding RNAs

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.

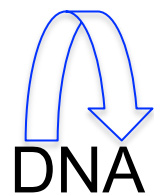


ESPRESSIONE/"DECODIFICA" DELL'INFORMAZIONE

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

DOGMA CENTRALE DELLA BIOLOGIA MOLECOLARE (1957)

REPLICAZIONE



DNA

TRASCRIZIONE



RNA

TRADUZIONE



PROTEINA

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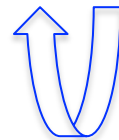
RNA

TRADUZIONE

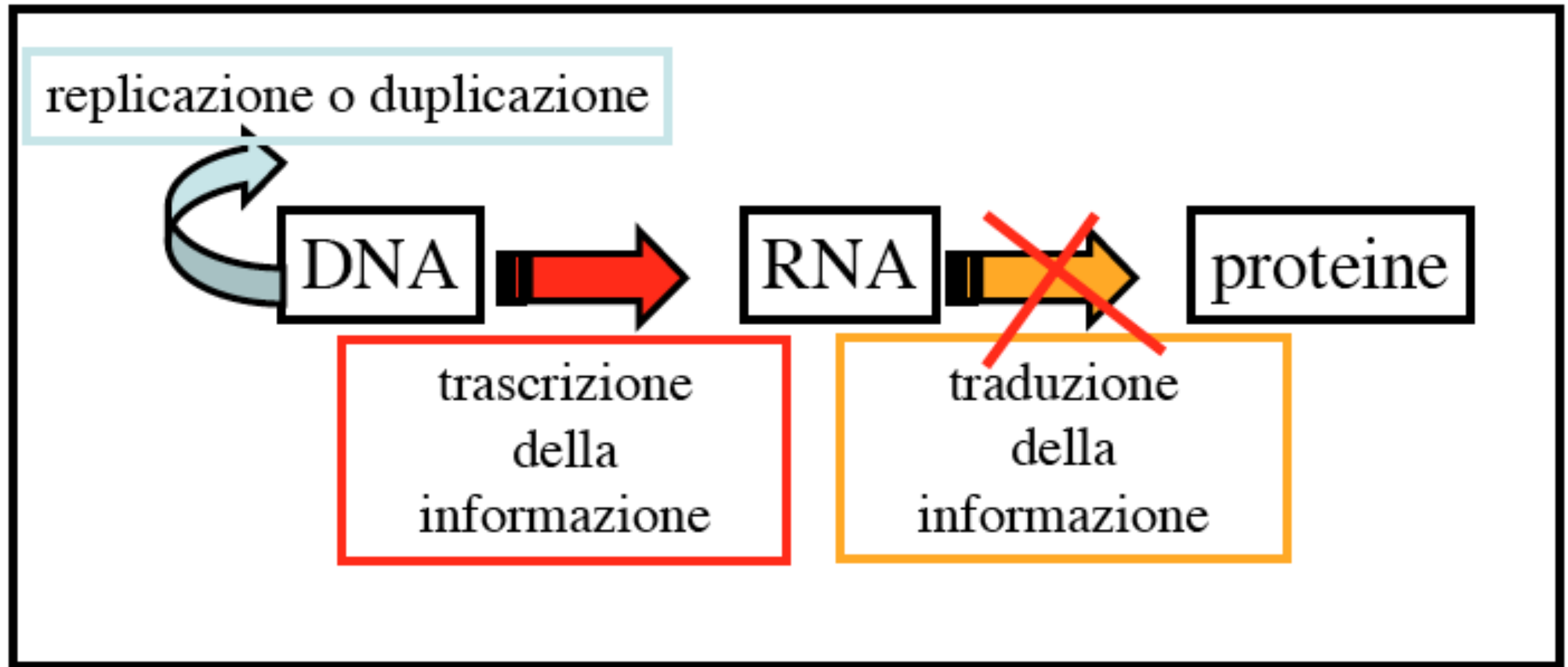


PROTEINA

←
TRASCRIZIONE
INVERSA



REPLICAZIONE



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