Corso di Biofisica (2013-2014)
Struttura e funzione delle proteine

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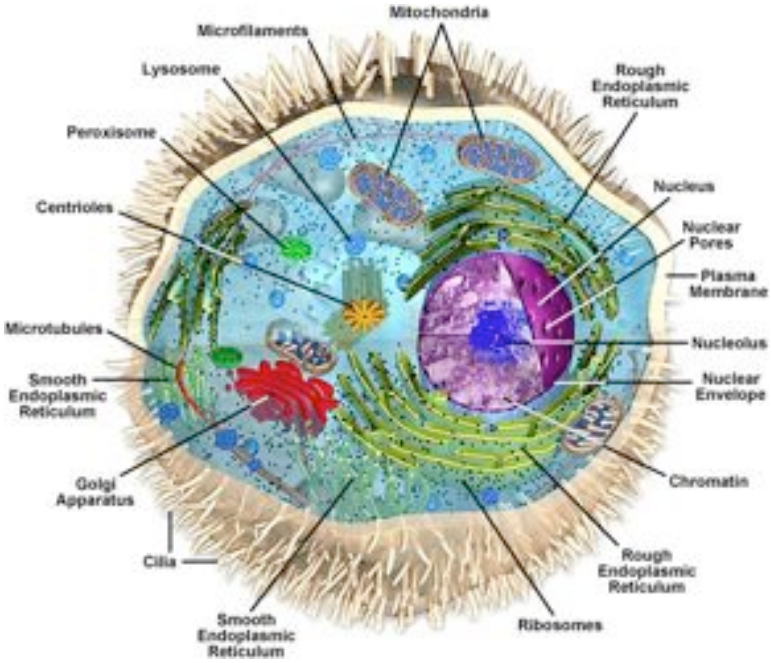
http://users.unimi.it/biolstru/Home.html

Slides dal corso dello scorso anno:
http://digilander.libero.it/mario.milani/teaching.html

1. Amminoacidi: caratteristiche chimiche. Proteine, classificazione strutturale
2. Interazioni non covalenti
3. Rapporto struttura - funzione di proteine: l'esempio dei Virus
4. Espressione e purificazione di proteine ricombinanti
5. Cristallografia di proteine: cristallogenesi. Simmetrie nei cristalli, gruppi puntuali e gruppi spaziali
6. Esperimento di diffrazione e raccolta dati
7. Problema della fase e tecniche di fasatura: molecular replacement, multiple isomorfous replacement, multiple anomalous diffraction
8. Raffinamento e validazione delle strutture
9. Interazione onda elettromagnetica - materia
10. Transizioni elettroniche e assorbimento
11. Strutture quaternarie e caratteristiche strutturali a bassa risoluzione: dynamic light scattering (DLS) e small angle X-ray diffraction scattering (SAXS)

La cellula

eukaryotic cell



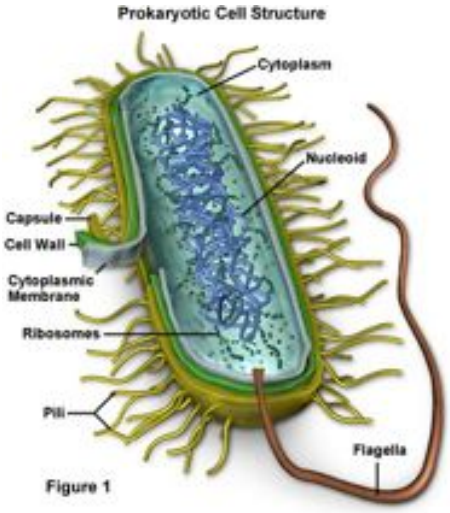
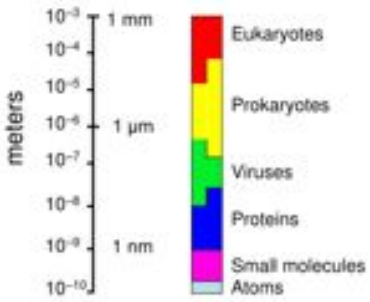


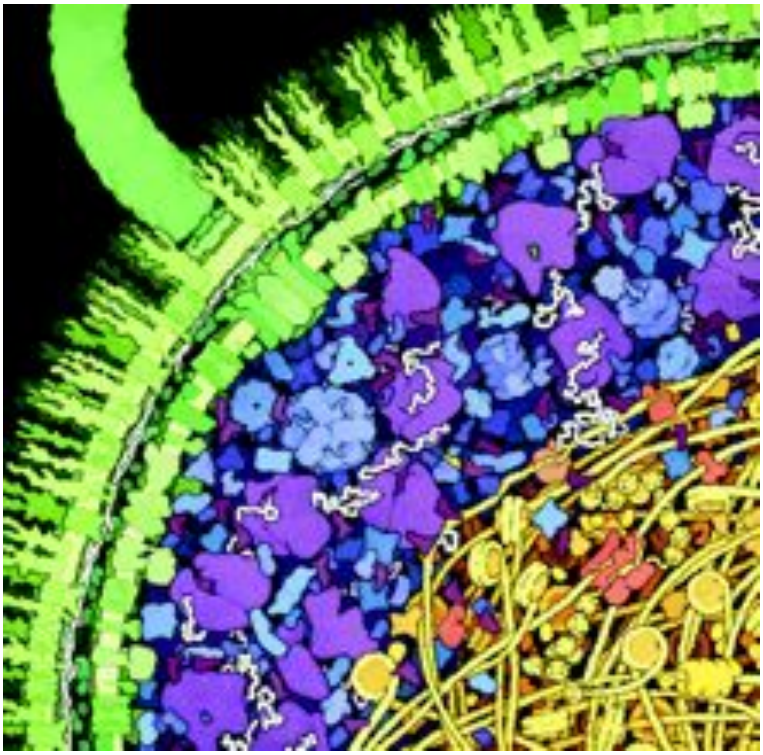
Figure 1

macromolecular crowding

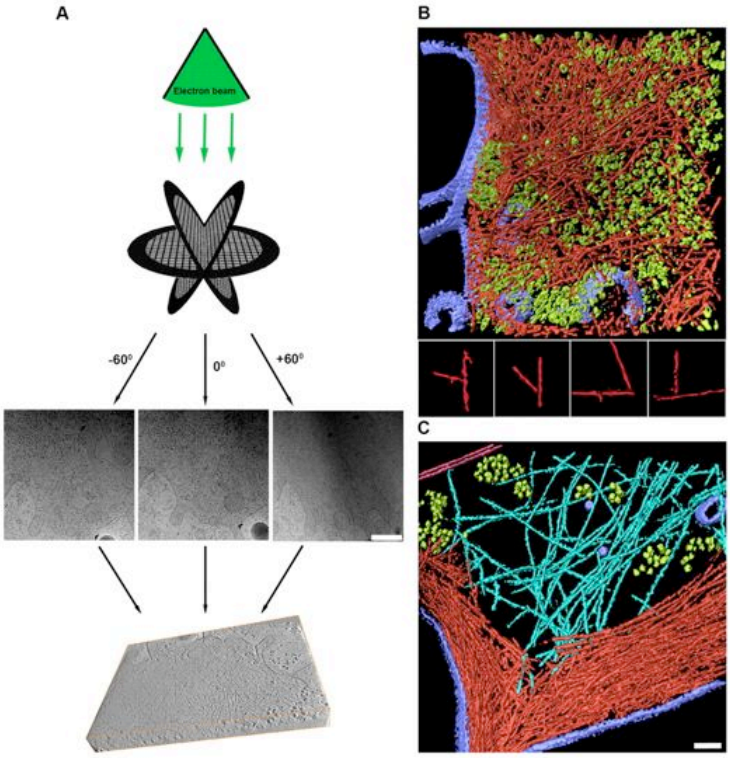


Escherichia coli cell. Cell wall, membranes, transmembrane proteins, and a flagellum with its motor
Cytoplasm is shown in blue and purple. Nucleic acids are shown in yellow.

The Role of Biomacromolecular Crowding, Ionic Strength, and Physicochemical Gradients in the Complexities of Life's Emergence Microbiol. Mol. Biol. Rev. 2009 73: 371-388.



Electron cryotomography



(A) 2D projections at different tilt angles for individual 3D objects are recorded by tilting the specimen holder

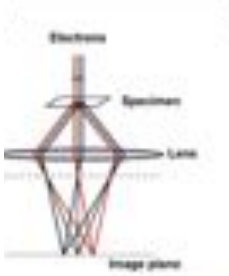
(B) Surface-rendering view of the reconstructed volume shown in A shows the **actin filaments**, **cell membrane** **and** **large macromolecular complexes, mostly ribosomes**.

(C) Surface-rendering view of the reconstructed volume of a human fibroblast cell shows all cytoskeletal elements. Cortical actin is located along the cell membrane, whereas intermediate filaments are localized further into the cell interior; these present a wider diameter (~10 nm), a different texture and a lower persistence length than actin. In addition, one microtubule (Medalia et al., 2002).

Scale bar: 100 nm

Ben-Harush et al., Journal of Cell Science 123, 7-12 (2010)

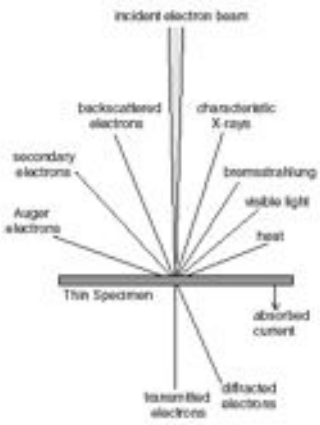
Cryo-electron microscopy



Imaging biological objects in an electron microscope is, in principle, analogous to light microscopic imaging.

After passing through the specimen, scattered electrons are focused by the electromagnetic lenses of the microscope.

The resolution possible with visible light (wavelengths of approximately 400–700 nm) is significantly less than that achieved with electron sources in a typical transmission electron microscope (wavelength of ≈ 0.02 Å for operation at 300 kV).



Transmission Electron Microscopy, TEM

TEM exploits three different interactions of electron beam-specimen; Unscattered electrons (transmitted beam), elastically scattered electrons (diffracted beam) and inelastically scattered electrons.

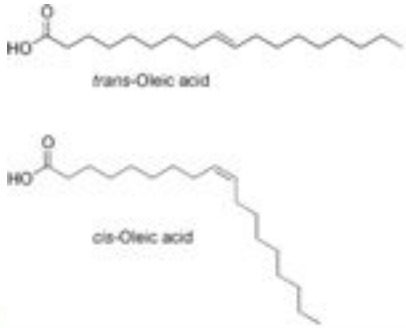
All electrons follow Bragg's Law $n\lambda=2d\sin(\theta)$

The scattered electrons can be collated using magnetic lenses to form a pattern of spots; each spot corresponding to a specific atomic spacing. This pattern can then yield information about the orientation, atomic arrangements and phases present in the area being examined.

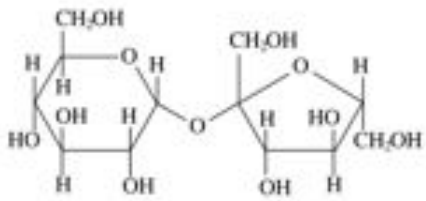
the objective and selected area aperture are used to choose of the elastically scattered electrons that will form the image of the microscope.

Molecole

Acidi grassi: membrane

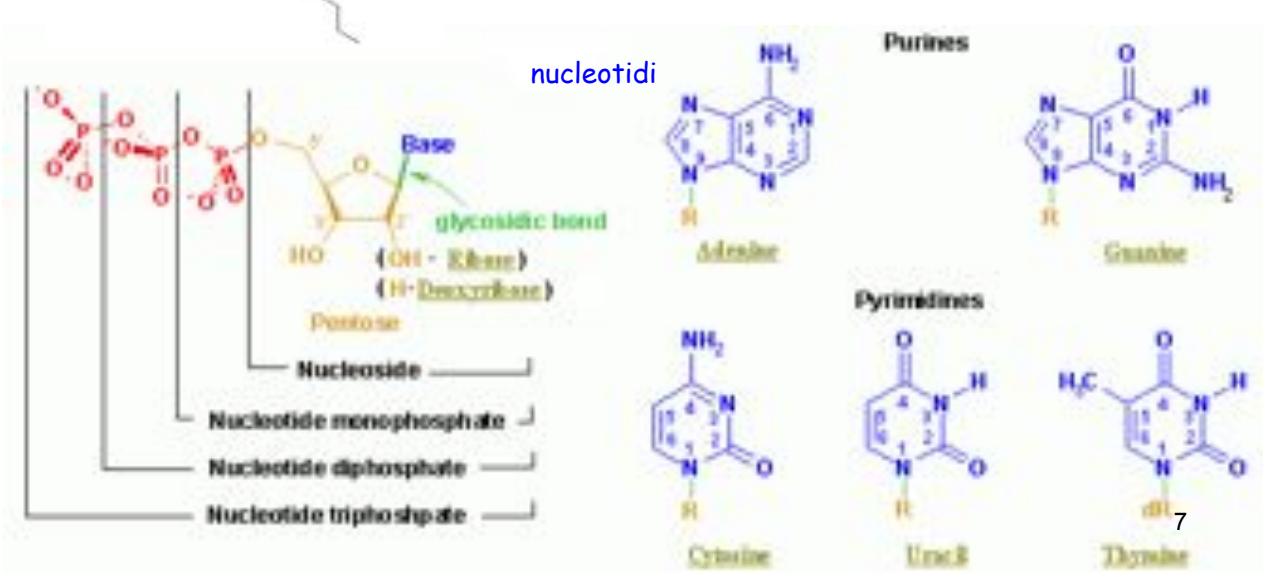


zuccheri



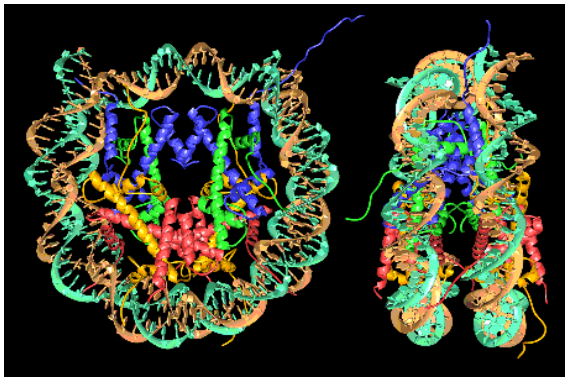
Sucrose: a disaccharide of glucose (left) and fructose (right)

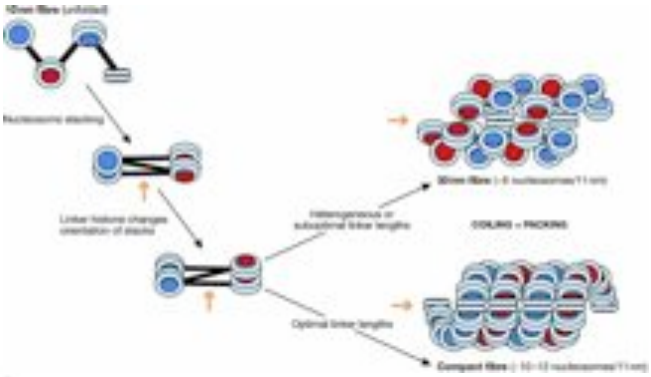
nucleotidi

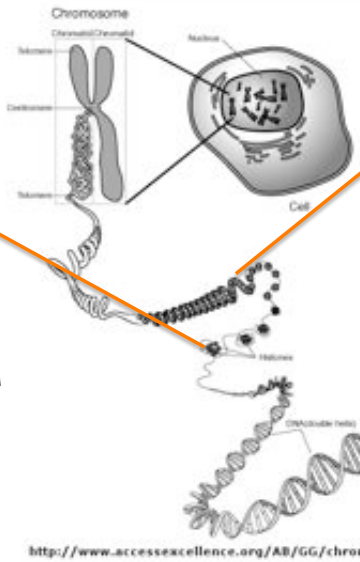


DNA

DNA (nucleosoma)



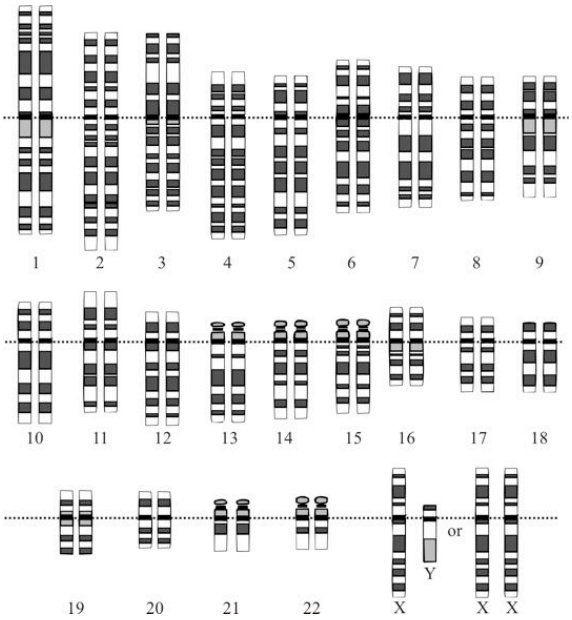




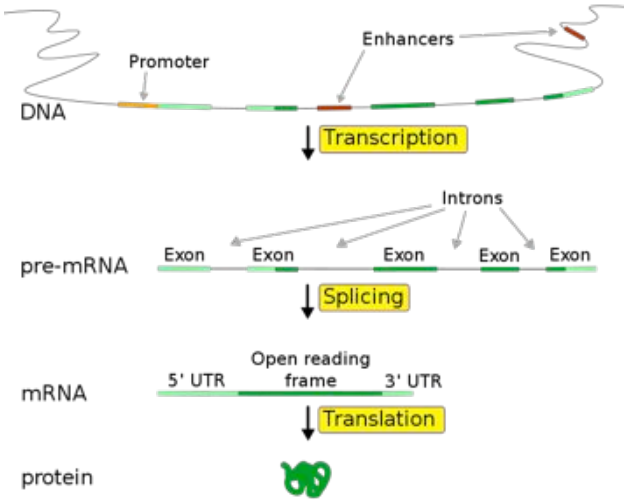
Proposal for a folding pathway for chromatin fibres.

Lunghezza del DNA umano ~ 2-3 m

genoma umano: 23 coppie di cromosomi



There are estimated to be between 20,000 and 25,000 human protein-coding genes.



million base pairs (Mbp): 3100

localizzazione dei geni nel genoma

http://www.ncbi.nlm.nih.gov/gene/55328

http://genome.ucsc.edu/

Chromosome 10

Region Displayed: 89,80 - 90,58 Mbp

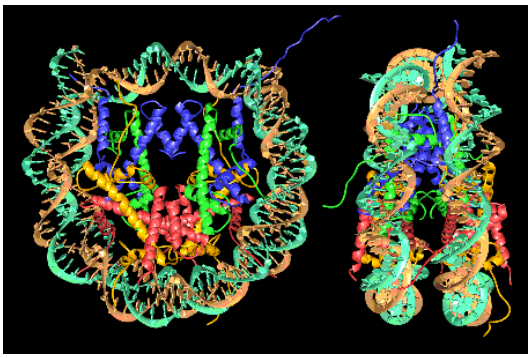
136 Mbp

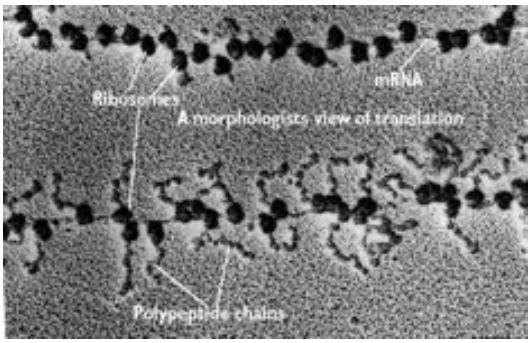
E	Cyto	Description
best RefSeq	10q23-q24	3'-phosphoadenosine 5'-phosphosulfate synthase 2
best RefSeq	10	ATPase family, AAA domain containing 1
best RefSeq	10	cofilin pseudogene 1
best RefSeq	10q23	killin protein
best RefSeq	10q23.3	phosphatase and tensin homolog
best RefSeq	10q23.3	ribosomal protein L11 pseudogene 3
protein	10	similar to mediator of RNA polymerase II transcription, subunit 6 homolog
best RefSeq	10	renalase, FAD-dependent amine oxidase
best RefSeq	10	lipase, family member J
best RefSeq	10	ribosomal protein L7 pseudogene 34
best RefSeq	10	lipase, gastric
protein	10	hypothetical LOC100288843
best RefSeq	10	lipase, family member K
best RefSeq	10	lipase, family member N
protein	10	similar to regulator of chromosome condensation and BTB domain containing protein
best RefSeq	10	lipase, family member M
best RefSeq	10	ankyrin repeat domain 22
protein	10	hypothetical LOC100132487
best RefSeq	10	STAM binding protein-like 1
protein	10	hypothetical LOC100132116
best RefSeq	10q23.3	actin, alpha 2, smooth muscle, aorta
best RefSeq	10q24.1	Fas (TNF receptor superfamily, member 6)
protein	10	hypothetical LOC100289238

http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9606

geni=proteine

DNA

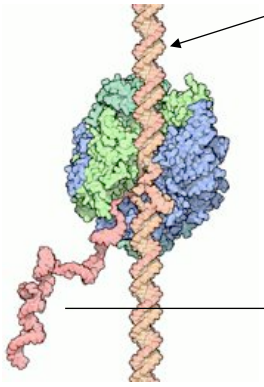




proteine

(traduzione)

DNA: AGTA…

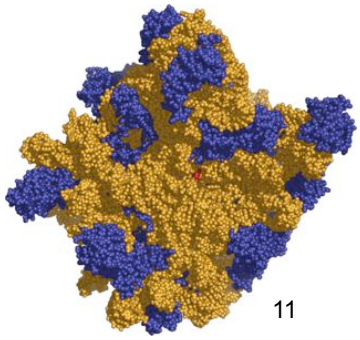


RNA polimerasi
(trascrizione)

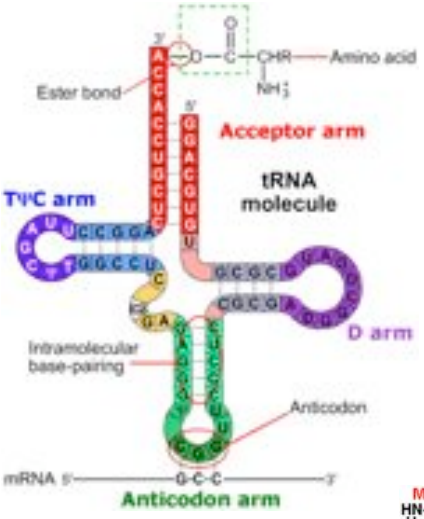
mRNA

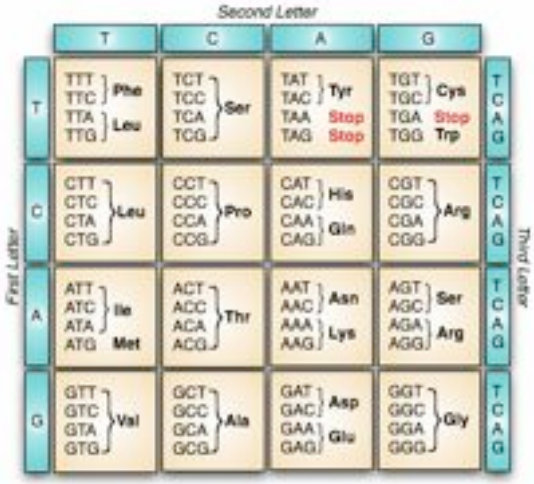
AGUA…

Ribosoma

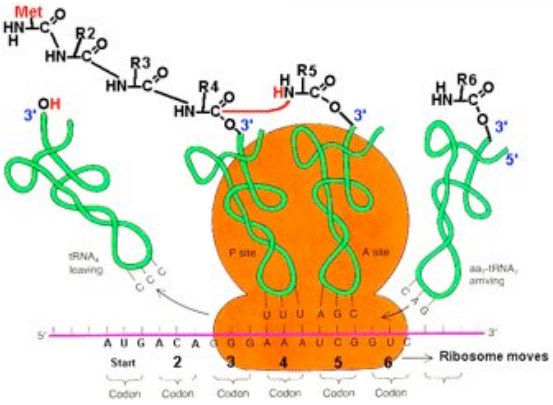


Codice genetico

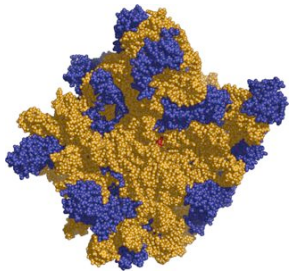




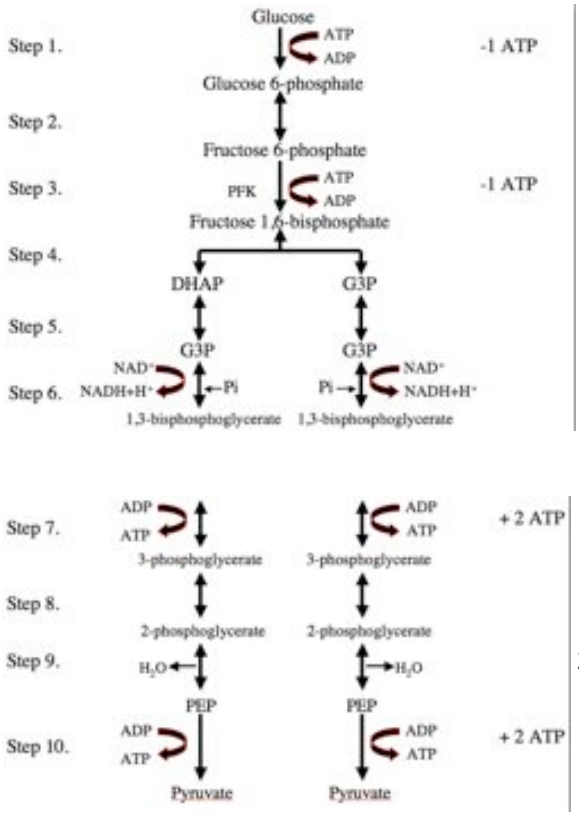
First Letter	Second Letter: T	C	A	G	Third Letter
T	TTT, TTC Phe; TTA, TTG Leu	TCT, TCC, TCA, TCG Ser	TAT, TAC Tyr; TAA Stop; TAG Stop	TGT, TGC Cys; TGA Stop; TGG Trp	T C A G
C	CTT, CTC, CTA, CTG Leu	CCT, CCC, CCA, CCG Pro	CAT, CAC His; CAA, CAG Gln	CGT, CGC, CGA, CGG Arg	T C A G
A	ATT, ATC, ATA Ile; ATG Met	ACT, ACC, ACA, ACG Thr	AAT, AAC Asn; AAA, AAG Lys	AGT, AGC Ser; AGA, AGG Arg	T C A G
G	GTT, GTC, GTA, GTG Val	GCT, GCC, GCA, GCG Ala	GAT, GAC Asp; GAA, GAG Glu	GGT, GGC, GGA, GGG Gly	T C A G



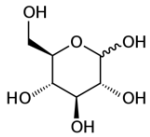
Modified from Griffiths et al., AN INTRODUCTION TO GENETIC ANALYSIS, 6th Ed., W.H. Freeman & Co., 1996.

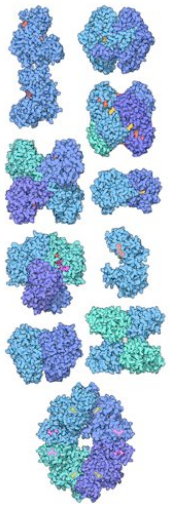


Glicolisi: produzione di energia



Alcune proteine sono enzimi



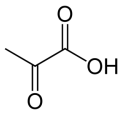


$C_6H_{12}O_6$

[Glucose] + 2 [NAD]$^+$ + 2 [ADP] + 2 [P]

$2C_3H_4O_3$

2 [Pyruvate] + 2 [NADH] + 2 H^+ + 2 [ATP] + 2 H_2O



BIOCHIMICA

Geni e DNA "spazzatura"

Circa il 98.5% del genoma umano è composto di sequenze non codificanti.

Types of noncoding DNA sequences

Noncoding functional RNA	MicroRNAs are predicted to control the translational activity
Cis-regulatory elements	are sequences that control the transcription of a gene
Introns	Some introns do appear to have significant biological function
Pseudogenes	"genomic fossils" that are nonfuctional

Repeat sequences, transposons and viral elements

Telomeres	Telomeres are regions of repetitive DNA at the end of a chromosome, which provide protection from chromosomal deterioration during DNA replication.

Origine della vita e evoluzione

Ipotesi di un mondo primordiale ad RNA

→ Conservare e trasmettere l'informazione, come il DNA

→ Operare come un enzima, come le proteine

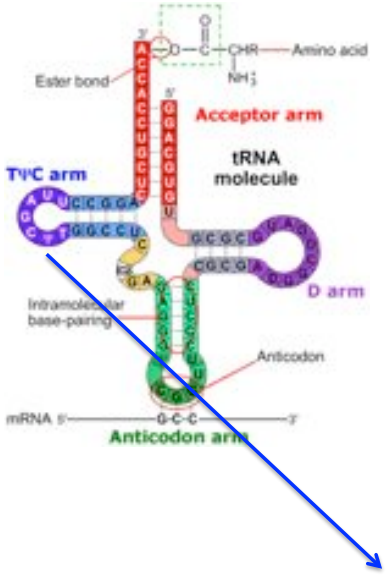
Approfondimenti

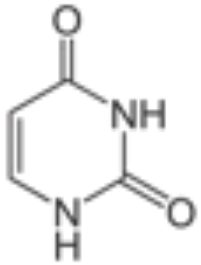
Ritter, Fondamenti di biochimica
Descrizione *Fondamenti di biochimica / Peck Ritter. - Bologna : Zanichelli, 1998.
ISBN 8808172562
Anno di pubblicazione 1998
Lo trovi Agraria ,Biologica , Tutte

Fondamenti di biochimica / Donald Voet, Judit J. Voet, Charlotte W. Pratt. - 2. ed. - Bologna : Zanichelli, 2007
ISBN 9788808068798
Anno di pubblicazione 2007
Lo trovi a Agraria , Medicina veterinaria , Biologica , Tutte

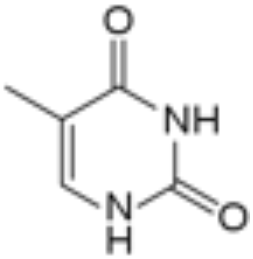
L'essenziale di biologia molecolare della cellula / Bruce Alberts... [et al.]. - Bologna : Zanichelli, 1999. - Anno di pubblicazione 1999
ISBN 8808176304
ISBN 8808230503
Lo trovi a Agraria , Farmacia , Biologica , Tutte

http://opac.unimi.it/SebinaOpac/Opac?sysb=

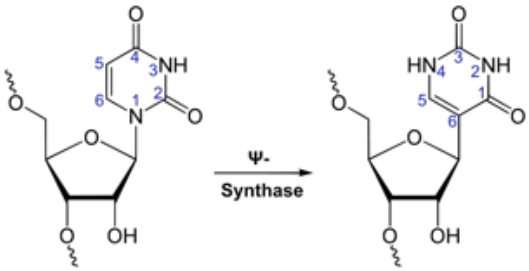




uracil



thymine



Ψ=Pseudouridine