CSE427
Computational Biology

http://www.cs.washington.edu/427

Larry Ruzzo
Winter 2008

UW CSE Computational Biology Group

He who asks is a fool for five minutes, but he who does not ask remains a fool forever.

-- Chinese Proverb

This week

Admin

Why Comp Bio?

The world’s shortest Intro. to Mol. Bio.

Admin Stuff

Course Mechanics & Grading

Reading

In class discussion

Homeworks

- reading
- paper exercises
- programming

Small Project?

No exams

Digression: Evolution & scientific literacy

“human beings, as we know them, developed from earlier species of animals”
(avoiding the now politically charged word “evolution”)

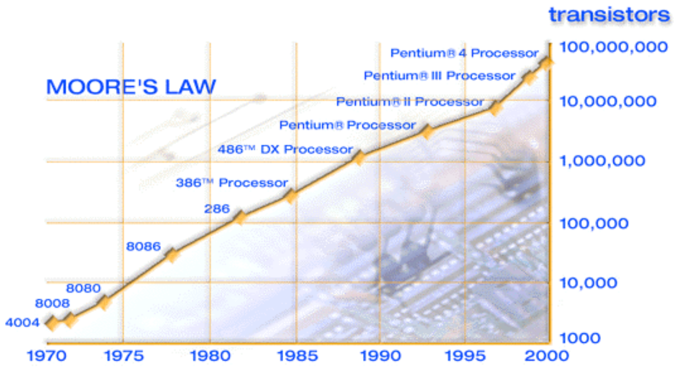
from 1985 to 2005, the % of Americans

- rejecting: declined from 48% to 39%
- accepting: also declined 45% to 40
- uncertain: increased 7% to 21%

In a 2005 survey,the proportion of adults who accept evolution in 34 European countries and Japan, the United States ranked 33rd, just above Turkey.

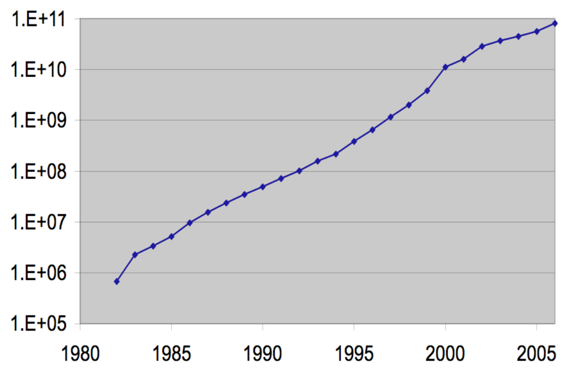
http://biology.plosjournals.org/perlserv/?request=get-document&doi=10.1371/journal.pbio.0040167

Background & Motivation



Source: http://www.intel.com/research/silicon/mooreslaw.htm

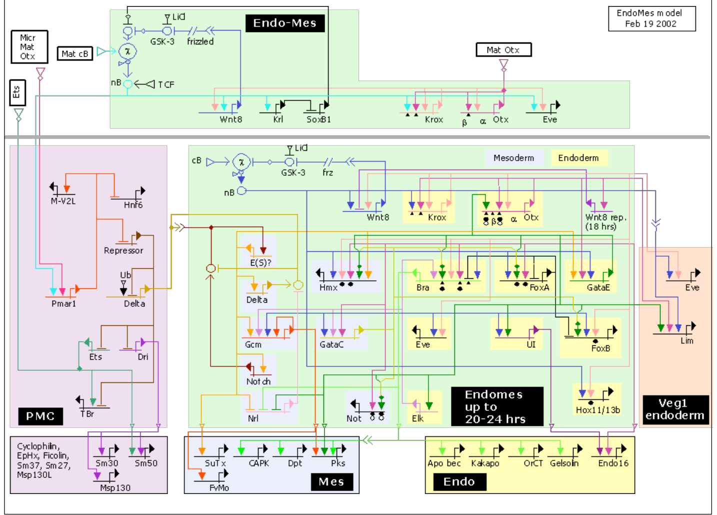
Growth of GenBank (Base Pairs)



Source: http://www.ncbi.nlm.nih.gov/Genbank/genbankstats.html

The Human Genome Project

```
   1 gagcccggcc cgggggacgg gcggcgggat agcgggaccc cggcgcggcg gtgcgcttca
  61 gggcgcagcg gcggccgcag accgagcccc gggcgcggca agaggcggcg ggagccggtg
 121 gcggctcggc atcatgcgtc gagggcgtct gctggagatc gccctgggat ttaccgtgct
 181 tttagcgtcc tacacgagcc atggggcgga cgccaatttg gaggctggga acgtgaagga
 241 aaccagagcc agtcgggcca agagaagagg cggtggagga cacgacgcgc ttaaaggacc
 301 caatgtctgt ggatcacgtt ataatgctta ctgttgccct ggatggaaaa ccttacctgg
 361 cggaaatcag tgtattgtcc ccatttgccg gcattcctgt ggggatggat tttgttcgag
 421 gccaaatatg tgcacttgcc catctggtca gatagctcct tcctgtggct ccagatccat
 481 acaacactgc aatattcgct gtatgaatgg aggtagctgc agtgacgatc actgtctatg
 541 ccagaaagga tacataggga ctcactgtgg acaacctgtt tgtgaaagtg gctgtctcaa
 601 tggaggaagg tgtgtggccc caaatcgatg tgcatgcact tacggattta ctggacccca
 661 gtgtgaaaga gattacagga caggcccatg ttttactgtg atcagcaacc agatgtgcca
 721 gggacaactc agcgggattg tctgcacaaa acagctctgc tgtgccacag tcggccgagc
 781 ctggggccac ccctgtgaga tgtgtcctgc ccagcctcac ccctgccgcc gtggcttcat
 841 tccaaatatc cgcacgggag cttgtcaaga tgtggatgaa tgccaggcca tccccgggct
 901 ctgtcaggga ggaaattgca ttaatactgt tgggtctttt gagtgcaaat gccctgctgg
 961 acacaaactt aatgaagtgt cacaaaaatg tgaagatatt gatgaatgca gcaccattcc
1021 ...
```



The sea urchin *Strongylocentrotus purpuratus*

Goals

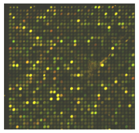
Basic biology

Disease diagnosis/prognosis/treatment

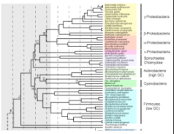
Drug discovery, validation & development

Individualized medicine

…

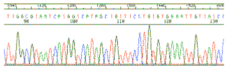


“High-Throughput BioTech”



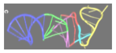
Sensors

- DNA sequencing
- Microarrays/Gene expression
- Mass Spectrometry/Proteomics
- Protein/protein & DNA/protein interaction



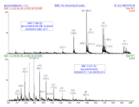
Controls

- Cloning
- Gene knock out/knock in
- RNAi



***Floods* of data**

“Grand Challenge” problems



What’s all the fuss?

The human genome is “finished”…

Even if it were, that’s only the beginning

Explosive growth in biological data is revolutionizing biology & medicine

“All pre-genomic lab techniques are obsolete”

(and computation and mathematics are crucial to post-genomic analysis)

CS Points of Contact & Opportunities

Scientific visualization

- Gene expression patterns

Databases

- Integration of disparate, overlapping data sources
- Distributed genome annotation in face of shifting underlying genomic coordinates

AI/NLP/Text Mining

- Information extraction from journal texts with inconsistent nomenclature, indirect interactions, incomplete/inaccurate models,…

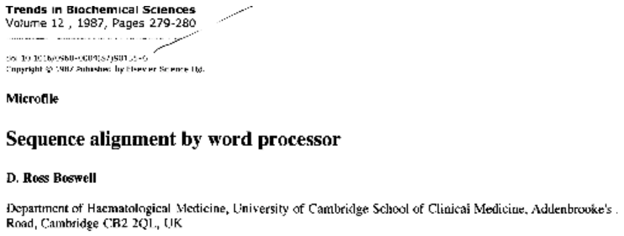
Machine learning

- System level synthesis of cell behavior from low-level heterogeneous data (DNA sequence, gene expression, protein interaction, mass spec,…)

...

Algorithms

Computers in biology: Then & now



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Microfile

Sequence alignment by word processor

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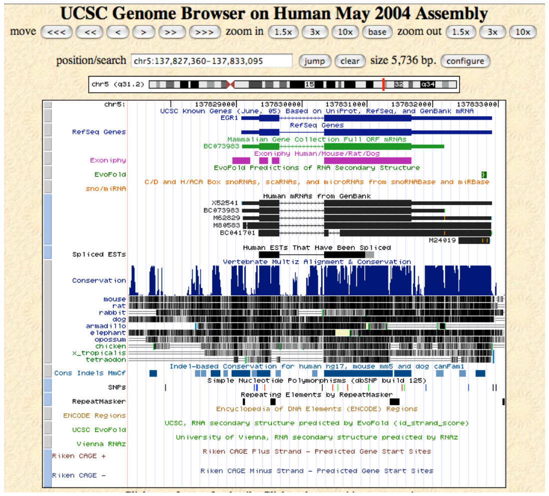
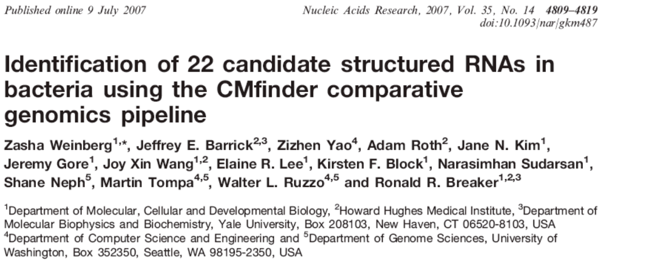


UCSC Genome Browser on Human May 2004 Assembly

position/search chr5:137,827,360-137,833,095 size 5,736 bp.

	UCSC Known Genes (June, 05) Based on UniProt, RefSeq, and GenBank mRNA
RefSeq Genes	RefSeq Genes
	Mammalian Gene Collection Full ORF mRNAs
Exoniphy	Exoniphy Human/Mouse/Rat/Dog
EvoFold	EvoFold Predictions of RNA Secondary Structure
sno/miRNA	C/D and H/ACA Box snoRNAs, scaRNAs, and microRNAs from snoRNABase and miRBase
	Human mRNAs from GenBank
Spliced ESTs	Human ESTs That Have Been Spliced
Conservation	Vertebrate Multiz Alignment & Conservation
mouse rat rabbit dog armadillo elephant opossum chicken x_tropicalis tetraodon	
Cons Indels MmCf	Indel-based Conservation for human hg17, mouse mm5 and dog canFam1
SNPs	Simple Nucleotide Polymorphisms (dbSNP build 125)
RepeatMasker	Repeating Elements by RepeatMasker
ENCODE Regions	Encyclopedia of DNA Elements (ENCODE) Regions
UCSC EvoFold	UCSC, RNA secondary structure predicted by EvoFold (id_strand_score)
Vienna RNAz	University of Vienna, RNA secondary structure predicted by RNAz
Riken CAGE +	Riken CAGE Plus Strand - Predicted Gene Start Sites
Riken CAGE -	Riken CAGE Minus Strand - Predicted Gene Start Sites



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doi:10.1093/nar/gkm487

Identification of 22 candidate structured RNAs in bacteria using the CMfinder comparative genomics pipeline

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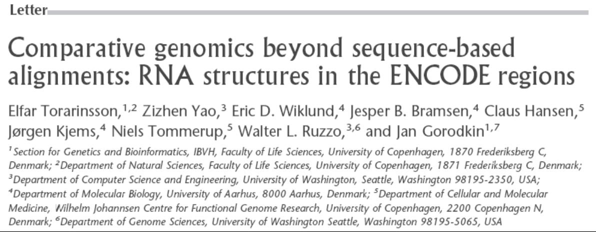

Letter

Comparative genomics beyond sequence-based alignments: RNA structures in the ENCODE regions

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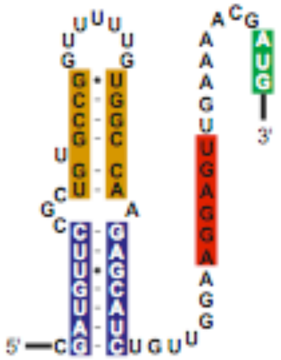
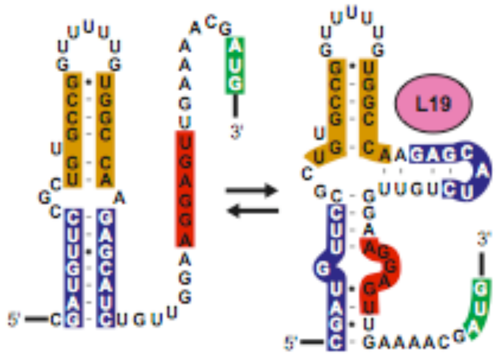
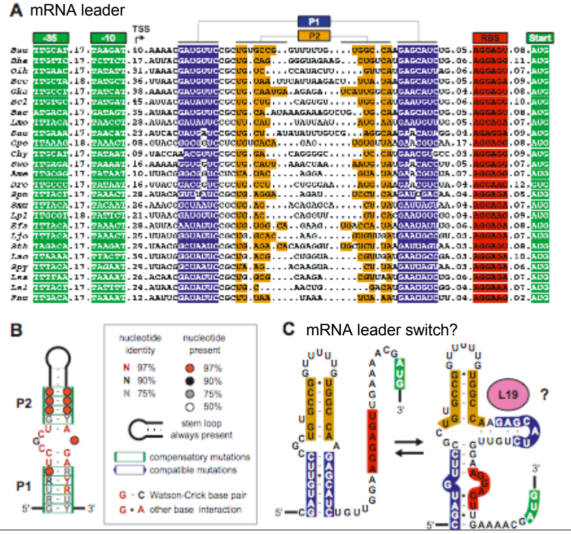
An RNA Structure



An RNA Sensor & On/Off Switch



L19 absent: Gene On L19 present: Gene Off



An RNA Grammar

$S \to LS \quad | \; L$

$L \to s \qquad | \; \text{“}dFd\text{”}$

$F \to LS \quad | \; \text{“}dFd\text{”}$

“dFd” means Watson-Crick base pair:

aFu | uFa | gFc | cFg

paren-like nesting

a) $S \to LS \to LLLLLLLS \to LLLLLLLL$

$\to ssLsssss \to ssdFdsssss$

$\to ssdddFdddsssss$

$\to ssdddLSdddsssss$

$\to ssdddLLLLdddsssss$

$\to ssdddssssdddsssss$

b)

$$\begin{array}{c} s^{ss}s \\ d\text{-}d \\ d\text{-}d \\ ss^{d\text{-}d}sssss \end{array}$$

c) $F \to dFd \to ddFdd \to ddLSdd$

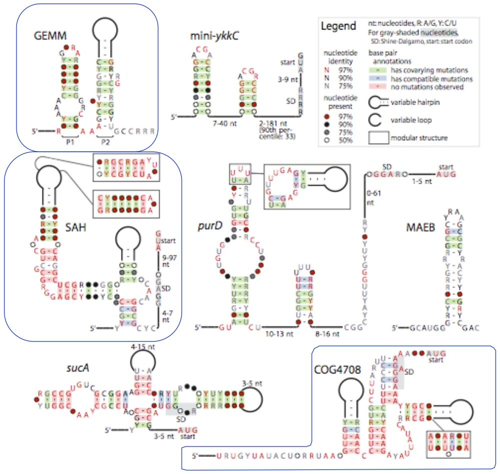
$\to ddLLdd \to ddLsdd \to dddFdsdd$

Actually, a *Stochastic* CFG

Associate probabilities with rules:

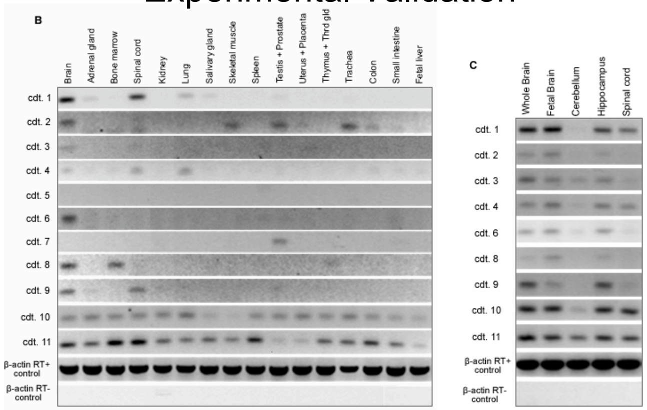
$S \to LS$	(0.87)	$\| L$	(0.13)
$L \to S$	(0.89*p(s))	$\| dFd$	(0.11*p(dd))
$F \to LS$	(0.21)	$\| dFd$	(0.79*p(dd))

Where p(s) & p(dd) are the probabilities of the specific single/paired nucleotides, perhaps from empirical data or a model of sequence evolution



boxed = confirmed riboswitch (+2 more)

Experimental Validation



Bottom Line

- CFG technology is a key tool for RNA description, discovery and search
- A very active research area. (Some call RNA the "dark matter" of the genome.)
- Huge compute hog: results above represent hundreds of CPU-years, and smart algorithms can have a big impact

An Algorithm Example: ncRNAs

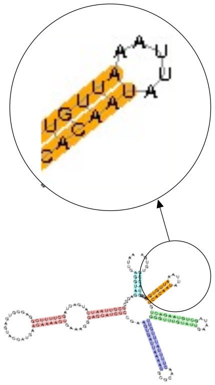
The "Central Dogma":
DNA -> messenger RNA -> Protein

Last ~5 years: 100s – 1000s of examples of functionally important ncRNAs

Much harder to find than protein-coding genes

Main method - Covariance Models (based on stochastic context free grammars)

Main problem - Sloooow … $O(nm^4)$



“Rigorous Filtering” - Z. Weinberg

- Convert CM to HMM
  (AKA: stochastic CFG to stochastic regular grammar)
- Do it so HMM score *always* ≥ CM score
- Optimize for most aggressive filtering subject to constraint that score bound maintained
  - A large convex optimization problem
- Filter genome sequence with (fast) HMM, run (slow) CM only on sequences above desired CM threshold; guaranteed not to miss anything
- Newer, more elaborate techniques pulling in key secondary structure features for better searching
  (uses automata theory, dynamic programming, Dijkstra, more optimization stuff,…)

Details
CENSORED
(but stay tuned…)
Plenty of CS here

Results

- Typically 200-fold speedup or more
- Finding dozens to hundreds of new ncRNA genes in many families
- Has enabled discovery of many new families

- Newer, more elaborate techniques pulling in key secondary structure features for better searching
  (uses automata theory, dynamic programming, Dijkstra, more optimization stuff,…)

More Admin

Course Focus & Goals

- Mainly sequence analysis
- Algorithms for alignment, search, & discovery
- Specific sequences, general types (“genes”, etc.)
- Single sequence and comparative analysis
- Techniques: HMMs, EM, MLE, Gibbs, Viterbi…
- Enough bio to motivate these problems, including very light intro to modern biotech supporting them
- Math/stats/cs underpinnings thereof
- Applied to real data

A *VERY* Quick Intro To Molecular Biology

The Genome

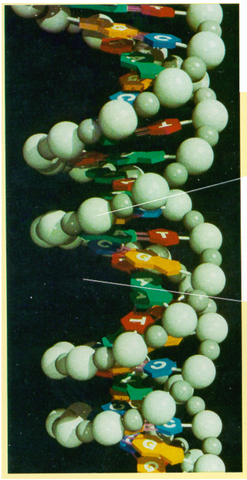
The hereditary info present in every cell

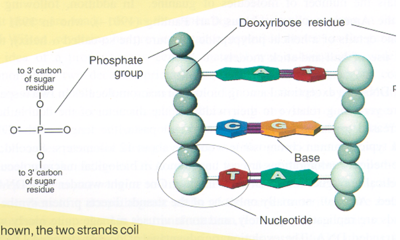
DNA molecule -- a long sequence of *nucleotides* (A, C, T, G)

Human genome -- about 3 x 10^9 nucleotides

The genome project -- extract & interpret genomic information, apply to genetics of disease, better understand evolution, …

The Double Helix





As shown, the two strands coil about each other in a fashion such that all the bases project inward toward the helix axis. The two strands are held together by hydrogen bonds (pink rods) linking each base projecting from one backbone to its so-called complementary base projecting from the other backbone. The base A always bonds to T (A and T are comple-

Shown in (b) is an uncoiled fragment of (a three complementary base pai chemist's viewpoint, each strai a polymer made up of four re called deoxyribonucleotides

Los Alamos Science

DNA

Discovered 1869

Role as carrier of genetic information - much later

The Double Helix - Watson & Crick 1953

Complementarity

A ←→ T C ←→ G

Visualizations:

http://www.rcsb.org/pdb/explore.do?structureId=123D

Genetics - the study of heredity

A *gene* -- classically, an abstract heritable attribute existing in variant forms (*alleles*)

Genotype vs *phenotype*

Mendel

- Each individual two copies of each gene
- Each parent contributes one (randomly)
- Independent assortment

Cells

Chemicals inside a sac - a fatty layer called the *plasma membrane*

Prokaryotes (bacteria, archaea) - little recognizable substructure

Eukaryotes (all multicellular organisms, and many single celled ones, like yeast) - genetic material in nucleus, other organelles for other specialized functions

Chromosomes

1 pair of (complementary) DNA molecules (+ protein wrapper)

Most prokaryotes have just 1 chromosome

Eukaryotes - ~~all~~ most cells have same number of chromosomes, e.g. fruit flies 8, humans & bats 46, rhinoceros 84, …

Mitosis/Meiosis

Most “higher” eukaryotes are *diploid* - have homologous pairs of chromosomes, one maternal, other paternal (exception: sex chromosomes)

Mitosis - cell division, duplicate each chromosome, 1 copy to each daughter cell

Meiosis - 2 divisions form 4 *haploid* gametes (egg/sperm)

- *Recombination/crossover* -- exchange maternal/paternal segments

Proteins

Chain of amino acids, of 20 kinds

Proteins:the major functional elements in cells

- Structural/mechanical
- Enzymes (catalyze chemical reactions)
- Receptors (for hormones, other signaling molecules, odorants,…)
- Transcription factors
- …

3-D Structure is crucial: the protein folding problem

The “Central Dogma”

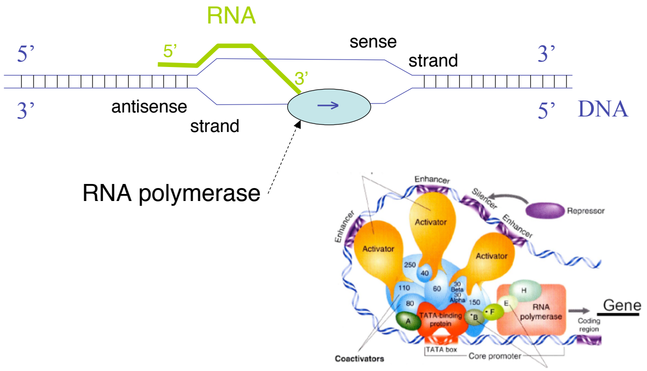
Genes encode proteins

DNA transcribed into messenger RNA

mRNA translated into proteins

Triplet code (codons)

Transcription: DNA → RNA

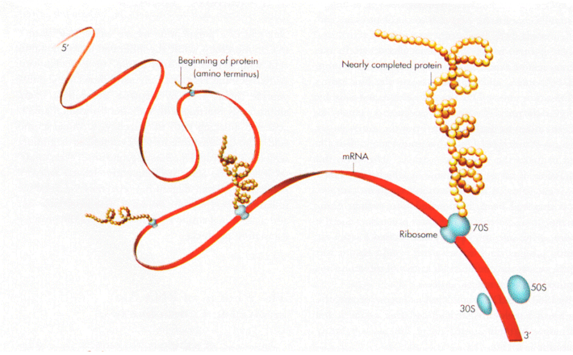


Codons & The Genetic Code

		Second Base				
First Base		U	C	A	G	Third Base
	U	Phe	Ser	Tyr	Cys	U
		Phe	Ser	Tyr	Cys	C
		Leu	Ser	Stop	Stop	A
		Leu	Ser	Stop	Trp	G
	C	Leu	Pro	His	Arg	U
		Leu	Pro	His	Arg	C
		Leu	Pro	Gln	Arg	A
		Leu	Pro	Gln	Arg	G
	A	Ile	Thr	Asn	Ser	U
		Ile	Thr	Asn	Ser	C
		Ile	Thr	Lys	Arg	A
		Met/Start	Thr	Lys	Arg	G
	G	Val	Ala	Asp	Gly	U
		Val	Ala	Asp	Gly	C
		Val	Ala	Glu	Gly	A
		Val	Ala	Glu	Gly	G

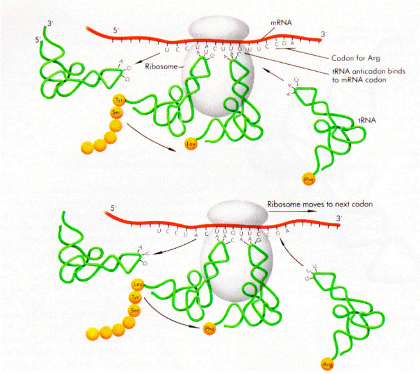
Ala	: Alanine
Arg	: Arginine
Asn	: Asparagine
Asp	: Aspartic acid
Cys	: Cysteine
Gln	: Glutamine
Glu	: Glutamic acid
Gly	: Glycine
His	: Histidine
Ile	: Isoleucine
Leu	: Leucine
Lys	: Lysine
Met	: Methionine
Phe	: Phenylalanine
Pro	: Proline
Ser	: Serine
Thr	: Threonine
Trp	: Tryptophane
Tyr	: Tyrosine
Val	: Valine

Translation: mRNA → Protein



Watson, Gilman, Witkowski, & Zoller, 1992

Ribosomes



Watson, Gilman, Witkowski, & Zoller, 1992

Gene Structure

Transcribed 5’ to 3’

Promoter region and transcription factor binding sites (usually) precede 5’ end

Transcribed region includes 5’ and 3’ untranslated regions

In eukaryotes, most genes also include *introns*, spliced out before export from nucleus, hence before translation

Genome Sizes

	Base Pairs	Genes
Mycoplasma genitalium	580,073	483
MimiVirus	1,200,000	1,260
E. coli	4,639,221	4,290
Saccharomyces cerevisiae	12,495,682	5,726
Caenorhabditis elegans	95,500,000	19,820
Arabidopsis thaliana	115,409,949	25,498
Drosophila melanogaster	122,653,977	13,472
Humans	3.3×10^9	~25,000

Genome Surprises

Humans have < 1/3 as many genes as expected

But perhaps more proteins than expected, due to *alternative splicing, alt start, alt end*

Protein-wise, all mammals are just about the same

But more individual variation than expected

And many more *non-coding RNAs* -- more than protein-coding genes, by some estimates

Many other non-coding regions are highly conserved, e.g., across all vertebrates

90% of DNA is transcribed (< 2% coding)

Complex, subtle "epigenetic" information

… and much more …

Read one of the many intro surveys or books for much more info.