# Genomic Medicine: Basic Molecular Biology

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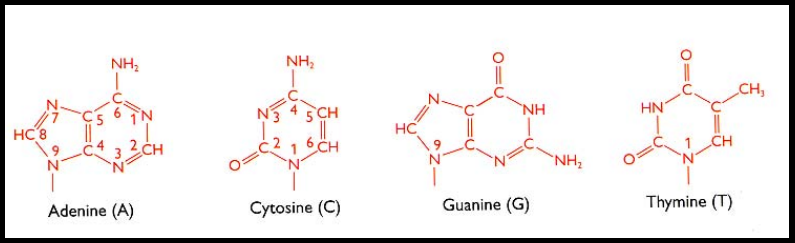
# Basic Biology

- Organisms need to produce proteins for a variety of functions over a lifetime
  - Enzymes to catalyze reactions
  - Structural support
  - Hormone to signal other parts of the organism
- Problem one: how to encode the instructions for making a specific protein
- Step one: nucleotides



## Basic Biology

- Complementary nucleotides form base pairs
- Base pairs are put together in chains (strands)
- Naturally form double helixes
- Redundant information in each strand

## Chromosomes

- We do not know exactly how strands of DNA wind up to make a chromosome
- Each chromosome has a single double-strand of DNA
- 22 human chromosomes are paired
- In human females, there are two X chromosomes
- In males, one X and one Y

# What does a gene look like?

- Each gene encodes instructions to make a single protein
- DNA before a gene is called upstream, and can contain regulatory elements
- Introns may be within the code for the protein
- There is a code for the start and end of the protein coding portion
- Theoretically, the biological system can determine promoter regions and intron-exon boundaries using the sequence syntax alone

# Area between genes

- The human genome contains 3 billion base pairs (3000 Mb) but only 35 thousand genes
- The coding region is 90 Mb (only 3% of the genome)
- Over 50% of the genome is repeated sequences
  - Long interspersed nuclear elements
  - Short interspersed nuclear elements
  - Long terminal repeats
  - Microsatellites
- Many repeated sequences are different between individuals

# Genome size

- We're the smartest, so we must have the largest genome, right?
- Not quite
- Our genome contains 3000 Mb (~750 megabytes)

- E. coli has 4 Mb
- Yeast has 12 Mb
- Pea has 4800 Mb
- Maize has 5000 Mb
- Wheat has 17000 Mb

# Genomes of other organisms

- *Plasmodium falciparum* chromosome 2

Please see Figure 1 of Science. 1998 Nov 6; 282(5391): 1126-32. Chromosome 2 sequence of the human malaria parasite Plasmodium falciparum. Gardner MJ, et al.

# mRNA is made from DNA

- Genes encode instructions to make proteins
- The design of a protein needs to be duplicable
- mRNA is transcribed from DNA within the nucleus
- mRNA moves to the cytoplasm, where the protein is formed

# Digitizing amino acid codes

- Proteins are made of 20 (21) amino acids
- Yet each position can only be one of 4 nucleotides
- Nature evolved into using 3 nucleotides to encode a single amino acid
- A chain of amino acids is made from mRNA

# Genetic Code

Please see figure 34 of Nature. 2001 Feb 15; 409(6822): 860-921.
Initial sequencing and analysis of the human genome.
Lander ES, et al.

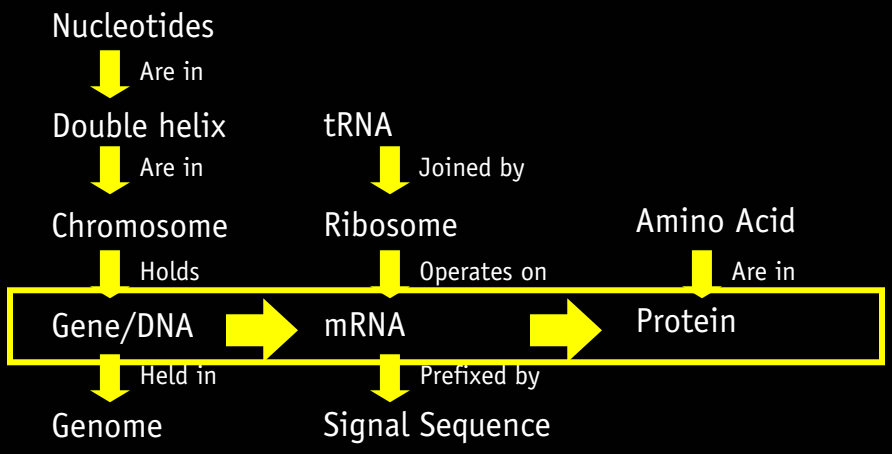
# Molecular Biology

Nucleotides
↓ Are in
Double helix
↓ Are in
Chromosome
↓ Holds
Gene/DNA
↓ Held in
Genome

tRNA
↓ Joined by
Ribosome
↓ Operates on
mRNA
↓ Prefixed by
Signal Sequence

Amino Acid
↓ Are in
Protein

# Central Dogma




# Protein targeting

- The first few amino acids may serve as a signal peptide
- Works in conjunction with other cellular machinery to direct protein to the right place

# Transcriptional Regulation

- Amount of protein is roughly governed by RNA level
- Transcription into RNA can be activated or repressed by transcription factors

# What starts the process?

- Transcriptional programs can start from
  - Hormone action on receptors
  - Shock or stress to the cell
  - New source of, or lack of nutrients
  - Internal derangement of cell or genome
  - Many, many other internal and external stimuli

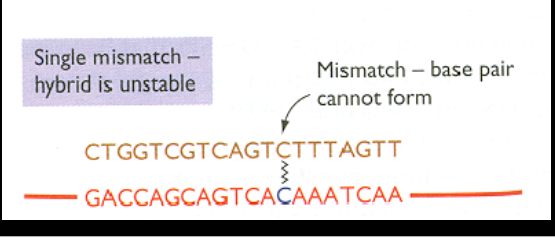
# Temporal Programs

- Segmentation versus Homeosis: same two houses at different times

Please see Figure 1 of Cell. 2000 Jan 7; 100(1): 27-40. Development: the natural history of genes. Scott MP.

# mRNA

- mRNA can be transcribed at up to several hundred nucleotides per minute
- Some eukaryotic genes can take many hours to transcribe
  - Dystrophin takes 20 hours to transcribe
- Most mRNA ends with poly-A, so it is easy to pick out
- Can look for the presence of specific mRNA using the complementary sequence



## Periodic Table for Biology

- Knowing all the genes is the equivalent of knowing the periodic table of the elements
- Instead of a table, our periodic table may read like a tree

## More Information

- Department of Energy Primer on Molecular Genetics http://www.ornl.gov/hgmis/publicat/primer/primer.pdf
- T. A. Brown, Genomes, John Wiley and Sons, 1999.

## Gene Measurement Techniques

**DNA**

- Sequencing
- Polymorphisms

**RNA**

- Serial analysis of gene expression
- DNA Microarrays
- Wafers

**Protein**

- 2D-PAGE
- Mass spectrometry
- Protein arrays

## Sequencing Reactions

Please refer to Annu Rev Biomed Eng. 1999; 1: 649-78.

Instrumentation for the genome project.

Jaklevic JM, Garner HR, Miller GA.

## Sanger Chain Termination

Please see Figure 1 of J Biotechnol. 2000 Jan 7; 76(1): 1-31.

Sequence analysis of genes and genomes.

Sterky F, Lundeberg J.

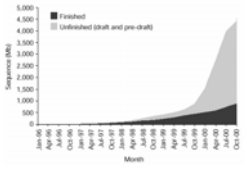
## Sequencing Reactions

Please see Buetow KH, et al. Nature Genetics 21: 323 (1999).

## Sequencing Reactions

- PHRAP: assembles sequence data using base-quality scores into sequence contigs
- Assembly-quality scores

- Most of the genome was sequenced over 12 months
- Highest throughput center at Whitehead: 100,000 sequencing reactions per 12 hours
- Robots pick 100,000 colonies, sequence 60 million nucleotides per day



## Assembly

- Contamination from non-human sequences removed
- Clones overlaid on physical map
- High-quality semiautomatic sequencing from both ends of very large numbers of numbers of human genome fragments
- Overlaps take memory: Drosophila 600 GB RAM
- Human 10 4-processor 4 GB and 16-processor 64 GB, 10K CPU hrs

## Genome Browsers

- Genome browsers: University of California at Santa Cruz and EnsEMBL
- Overlap sequence, cytogenetic, SNP, genetic maps
- Overlap annotations, disease genes

## Single Nucleotide Polymorphisms

- Three step approach
- First, find the genes you are interested in
- Second, catalog all the polymorphisms in a gene (by sequencing)
- Third, measure those polymorphisms in a larger population

## Clinical use of SNPs

- New publication with association of SNP with disease is almost a daily occurrence

Please see Gao, X. et al.
Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. *N Engl J Med* 344,
1668-75 (2001).

## SNPs and pharmacogenomics

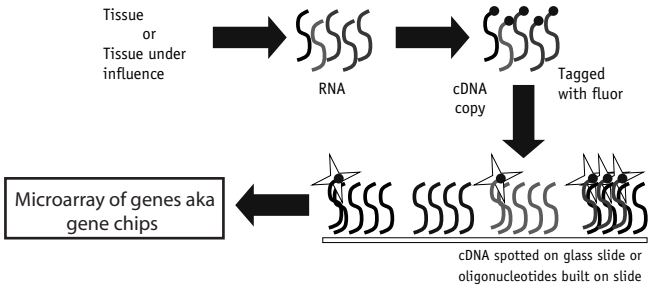
- Genes will help us determine which drugs to use in particular disease subtypes
- Genes will help us predict those who get side-effects

Please see Sesti F. PNAS 97: 10613, 2000.

## Serial Analysis of Gene Expression

Please refer to Madden, S. L., Wang, C.J. & Landes, G. Serial analysis of gene expression: from gene discovery to target identification. Drug Discov Today 5, 415-425 (2000).

## RNA expression detection chips




- Quantitative, absolute or relative
- Genes chosen arbitrarily
- Needs functional tissue

Schena M, et al. PNAS 93:10614 (1996).
Nature Genetics, 21: supplement (Jan 1999).

Please see Lockhart, DJ. Winzeler, EA. Nature 405, 827-36 (2000).

## Experiment Design

- Quantitate specific RNA expression before and after an intervention
- Compare expression between two tissue types
- Compare expression between different strains or constructed organisms
- Compare expression between neighboring cells

Please see Luo L, et al. Nature Medicine; 5: 117(1999).

## Validation

- In situ hybridization
- Real-time Polymerase Chain Reaction

## Microarrays in Diagnosis

Please see Figure 3b of Science. 1999 Oct 15; 286(5439): 531-7. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, Bloomfield CD, Lander ES

- Difficulty distinguishing between leukemias
- Microarrays can find genes that help make the diagnosis easier

## Microarrays in Prognosis

Please see Nature. 2000 Feb 3; 403 (6769): 503-11. Distinct types of diffuse large B-cell lymphoma identified by gene expression profilling. Alizadeh AA, et al.

- Patients with seemingly the same B-cell lymphoma
- Looking at pattern of activated genes helped discover two subsets of lymphoma
- Big differences in survival

## After microarrays comes wafers...

- Chromosome 21 has 21 million base-pairs
- Each 5 inch square wafers (Perlegen) hold 60 million probes
- Can sequence an entire chromosome in one experiment
- Each scan takes up around 10 terabytes
- Can sequence all SNPs within a human in 10 days

Please see Patil N. Science 2001, 294: 1719.

## 2D-PAGE

- Two axis = two properties of proteins: pH versus mass
- Global view of proteins
- Patterns can be scanned, saved and searched
- Spots need to be picked for identification
- Unfortunately, not very quantitative

Please see Gygi, S. P., Rochon, Y., Franza, B. R. & Aebersold, R. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19, 1720-30 (1999).

Please see Gygi, S. P. & Aebersold, R. Proteomics: A Trends Guide. (2000)

Please see Gygi, S. P. & Aebersold, R. Mass spectrometry and proteomics.
Curr Opin Chem Biol 4, 489-94 (2000).

## Clinical uses for proteomics

- Petricoin, et al., used this technique on serum
- Finding markers distinguishing ovarian cancer versus non-neoplasia
- Quest for biomarkers

Please see Petricoin, E. F. et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet 359, 572-7. (2002).

## Quantitative proteomics

- The examples so far demonstrate identification, not quantification
- One can take advantage of the extreme sensitivity of detection of mass spectrometry
- Add to the proteins a known amount of label

## Protein chips

- Detection vs. Function
- Kinase chips

Please see Williams, D. M. & Cole, P. A. Kinase chips hit the proteomics era. Trends Biochem Sci 26, 271-3 (2001).

## Protein Detection

- Specific antibodies
- Antibodies need to be available

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