BSC 4934: Q'BIC Capstone Workshop

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http://www.cis.fiu.edu/~giri/teach/BSC4934_Su09.html

24 June through 7 July, 2009

Short Homework

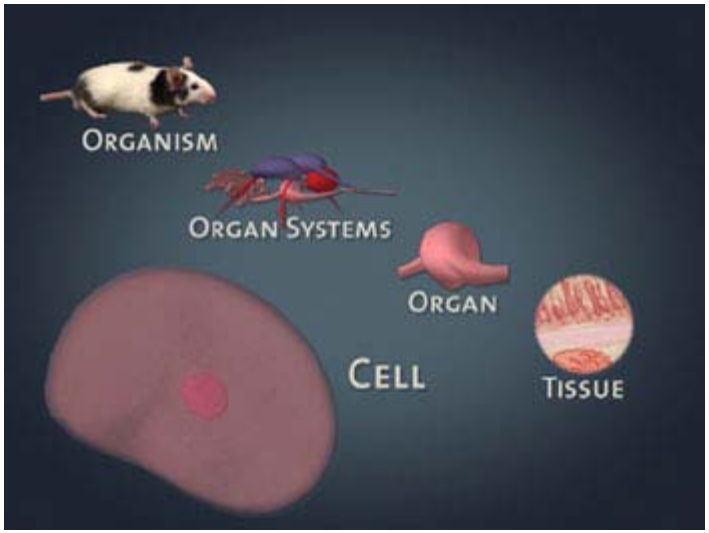
- Change all the numbers on the previous slide with up-to-date information.
- What was the latest large genome to be sequenced?

Short Homework

- Find the organism with the largest genome known! How many chromosomes does it have?
- Do you think a larger genome implies a "more evolved" organism or a "less evolved" organism?

Molecular Biology Background

Cell



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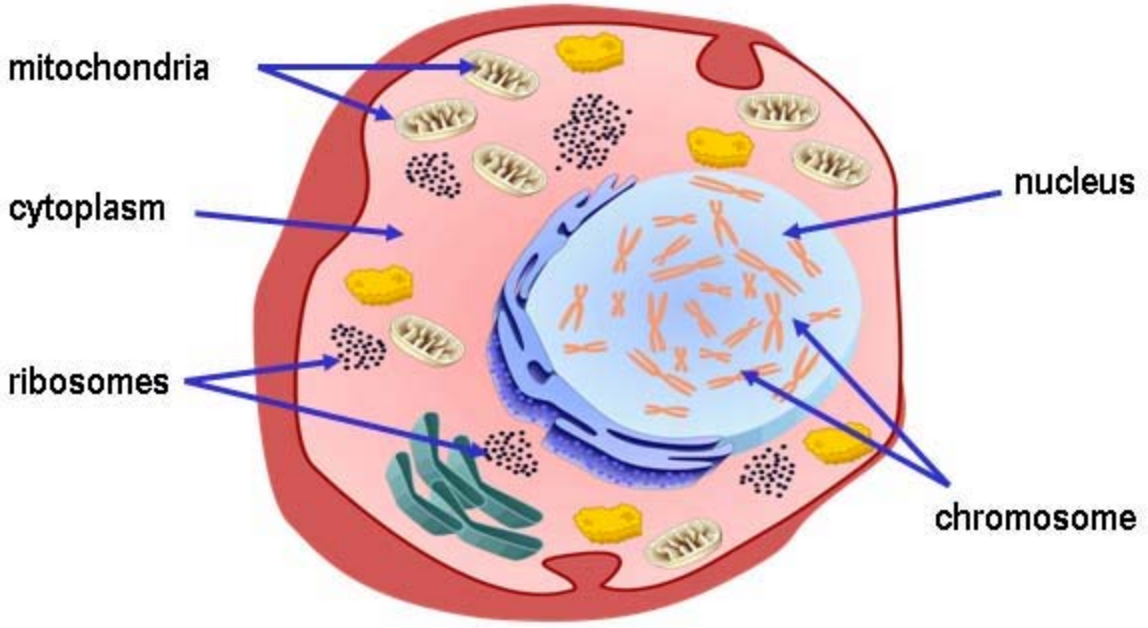
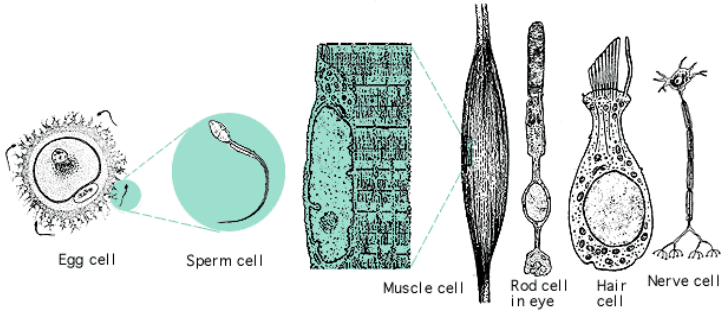
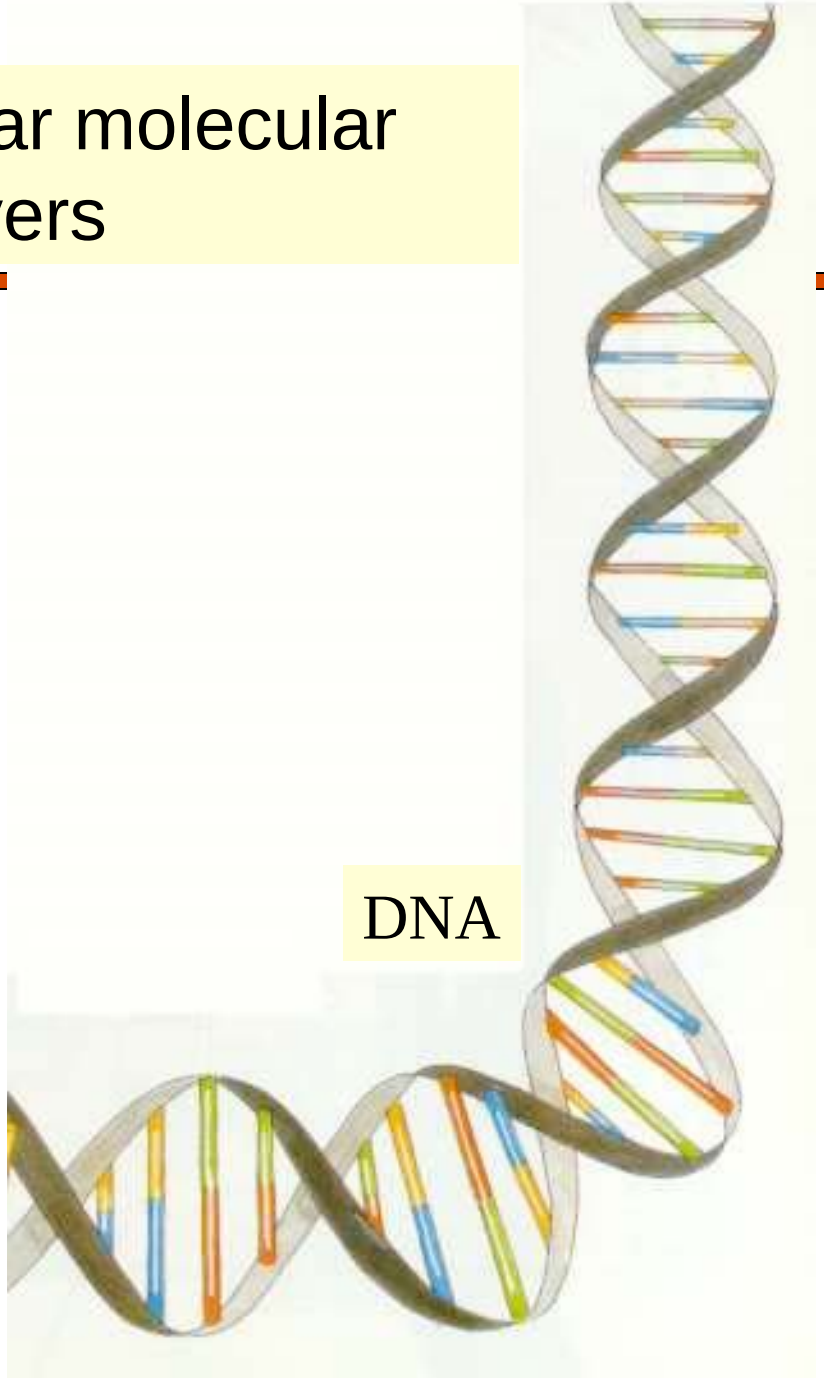


http://www.biotechnologyonline.gov.au/popups/img_cellwithlabels.cfm

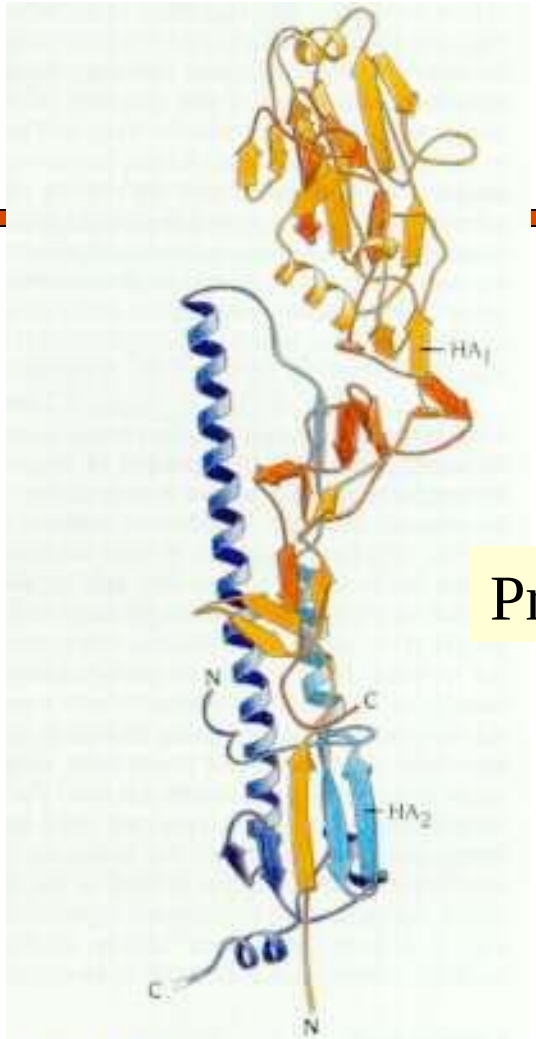


http://www.biology.eku.edu/RITCHISO/301notes1.htm

2 star molecular players



DNA



Protein

Figure 5.21 Schematic diagram of the subunit structure of hemagglutinin from influenza virus. The structure comprises about 550 amino acids arranged in two chains HA_1 (red) and HA_2 (blue). The first half of each chain has a lighter color in the diagram. The subunit is very elongated with a long stemlike region built up by residues from both chains and includes one of the longest α helices known in a globular structure, about 75 Å long. The globular head is formed by residues only from HA_1. (Courtesy of Don Wiley, Harvard University.)

The Polymeric Players

DNA

String with alphabet {A, C, G, T} Nucleotides/ Bases

RNA

String with alphabet {A, C, G, U} Bases

Protein

String with 20-letter alphabet Amino acids/ Residues

Typical DNA Sequence

```
  1 gggagaacac ccggagaagg aggaggaggc gaagaaaagc aacagaagcc cagttgctgc
 61 tccaggtccc tcggacagag ctttttccat gtggagactc tctcaatgga cgtgccccct
121 agtgcttctt agacggactg cggtctccta aaggtcgacc atggtggccg ggacccgctg
181 tcttctagtg ttgctgcttc cccaggtcct cctgggcggc gcggccggcc tcattccaga
241 gctgggccgc aagaagttcg ccgcggcatc cagccgaccc ttgtcccggc cttcggaaga
301 cgtcctcagc gaatttgagt tgaggctgct cagcatgttt ggcctgaagc agagacccac
361 ccccagcaag gacgtcgtgg tgccccccta tatgctagat ctgtaccgca ggcactcagg
421 ccagccagga gcgcccgccc cagaccaccg gctggagagg gcagccagcc gcgccaacac
481 cgtgcgcagc ttccatcacg aagaagccgt ggaggaactt ccagagatga gtgggaaaac
541 ggcccggcgc ttcttcttca atttaagttc tgtccccagt gacgagtttc tcacatctgc
601 agaactccag atcttccggg aacagataca ggaagctttg ggaaacagta gtttccagca
661 ccgaattaat atttatgaaa ttataaagcc tgcagcagcc aacttgaaat ttcctgtgac
721 cagactattg gacaccaggt tagtgaatca gaacacaagt cagtgggaga gcttcgacgt
781 caccccagct gtgatgcggt ggaccacaca gggacacacc aaccatgggt ttgtggtgga
841 agtggcccat ttagaggaga acccaggtgt ctccaagaga catgtgagga ttagcaggtc
901 tttgcaccaa gatgaacaca gctggtcaca gataaggcca ttgctagtga cttttggaca
961 tgatggaaaa ggacatccgc tccacaaacg agaaaagcgt caagccaaac acaaacagcg
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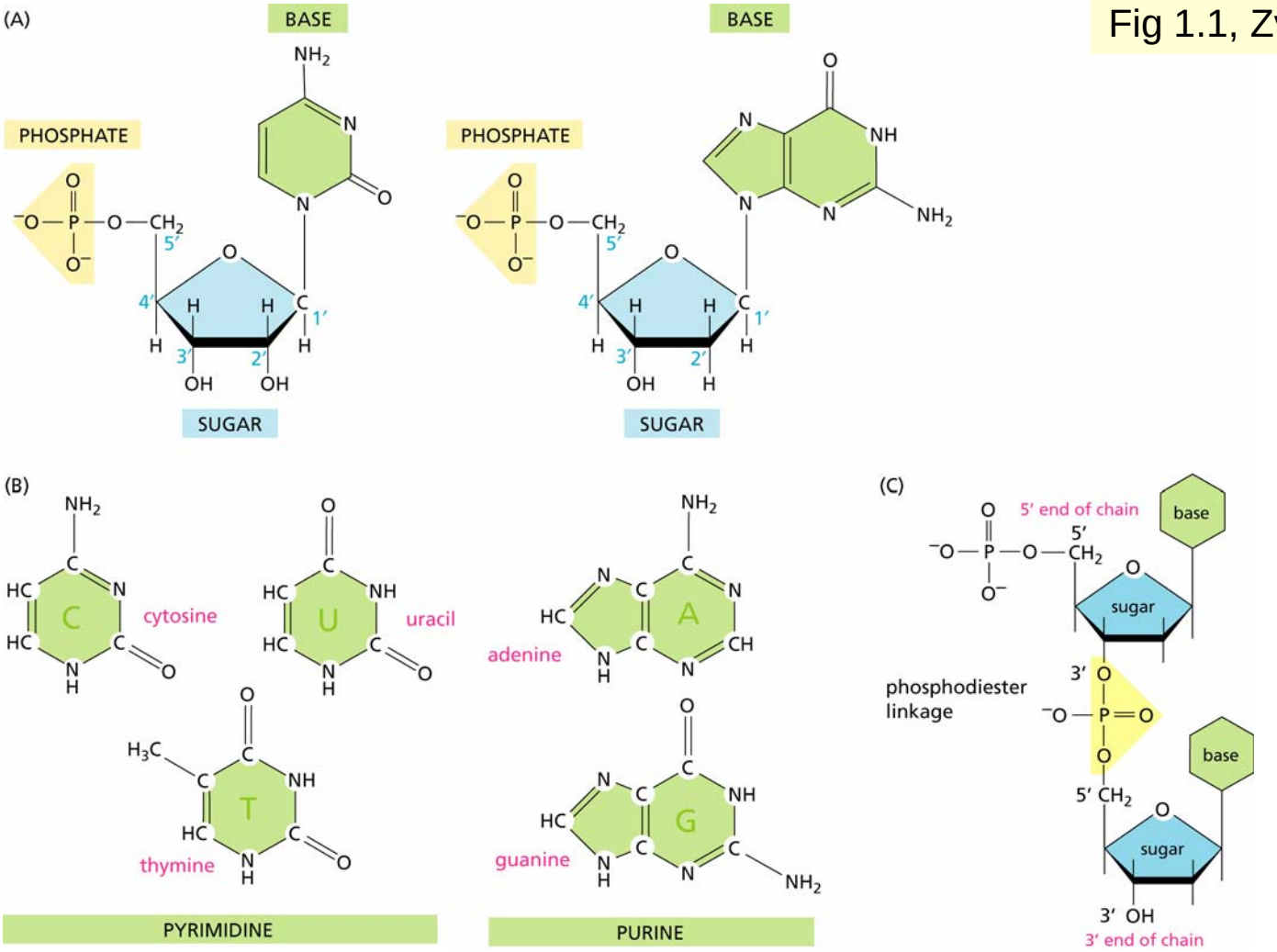
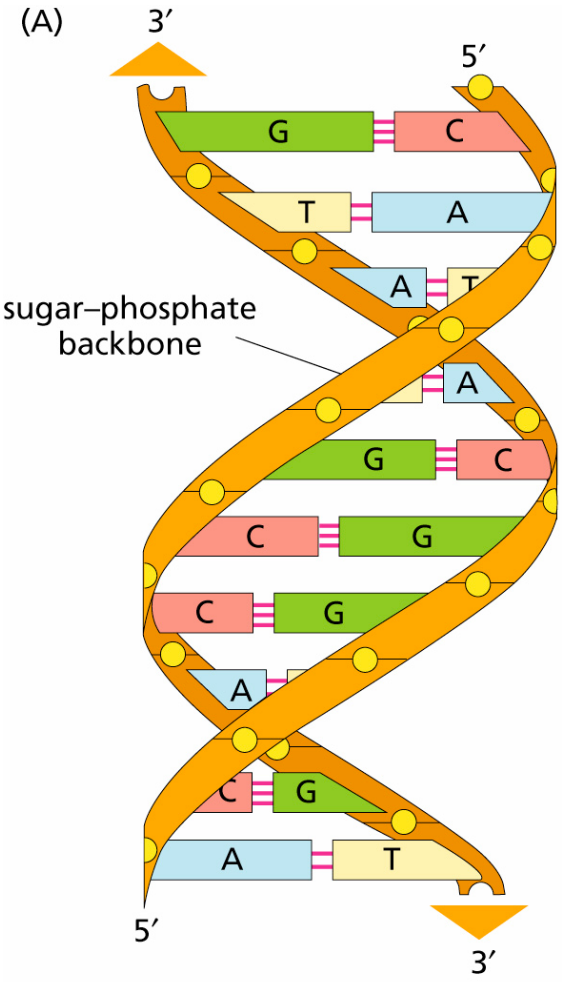
The building blocks of DNA & RNA

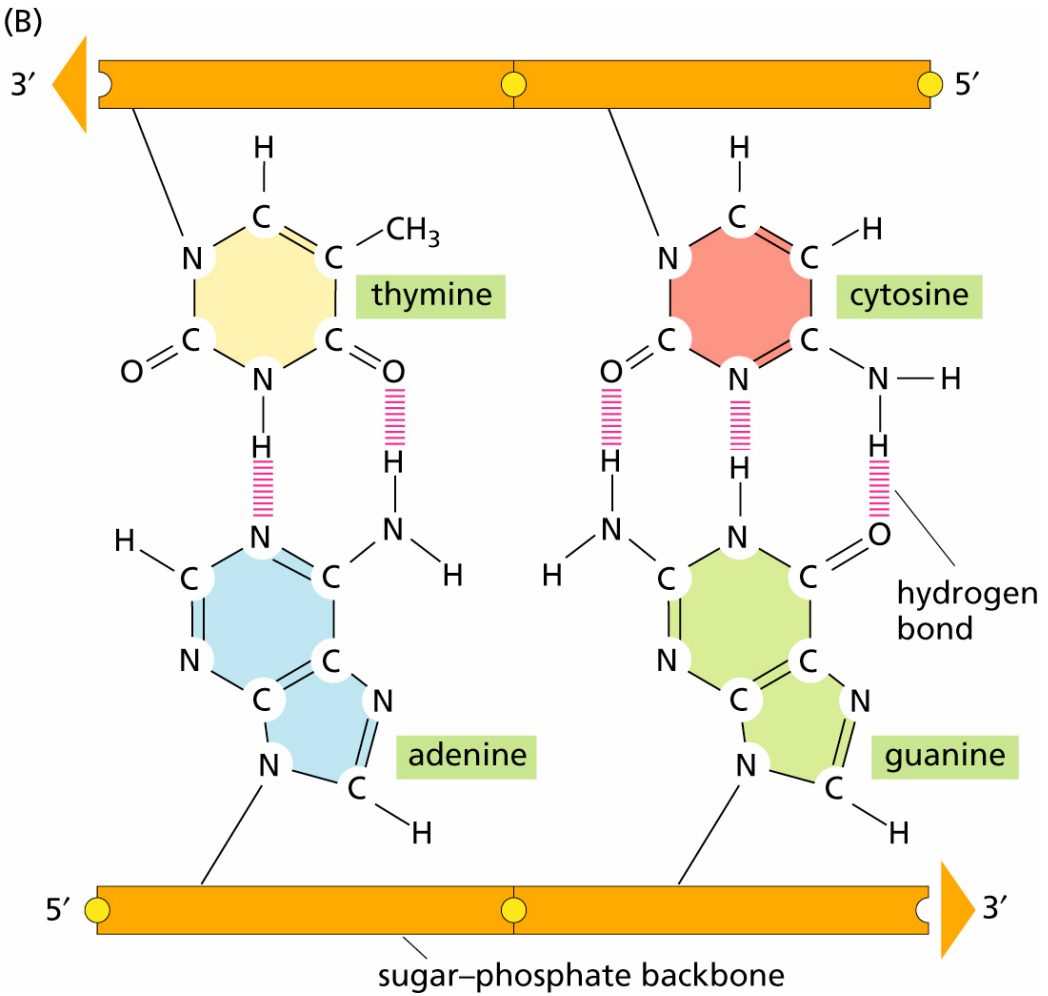
Fig 1.1, Zvelebil/Baum



DNA double helix structure

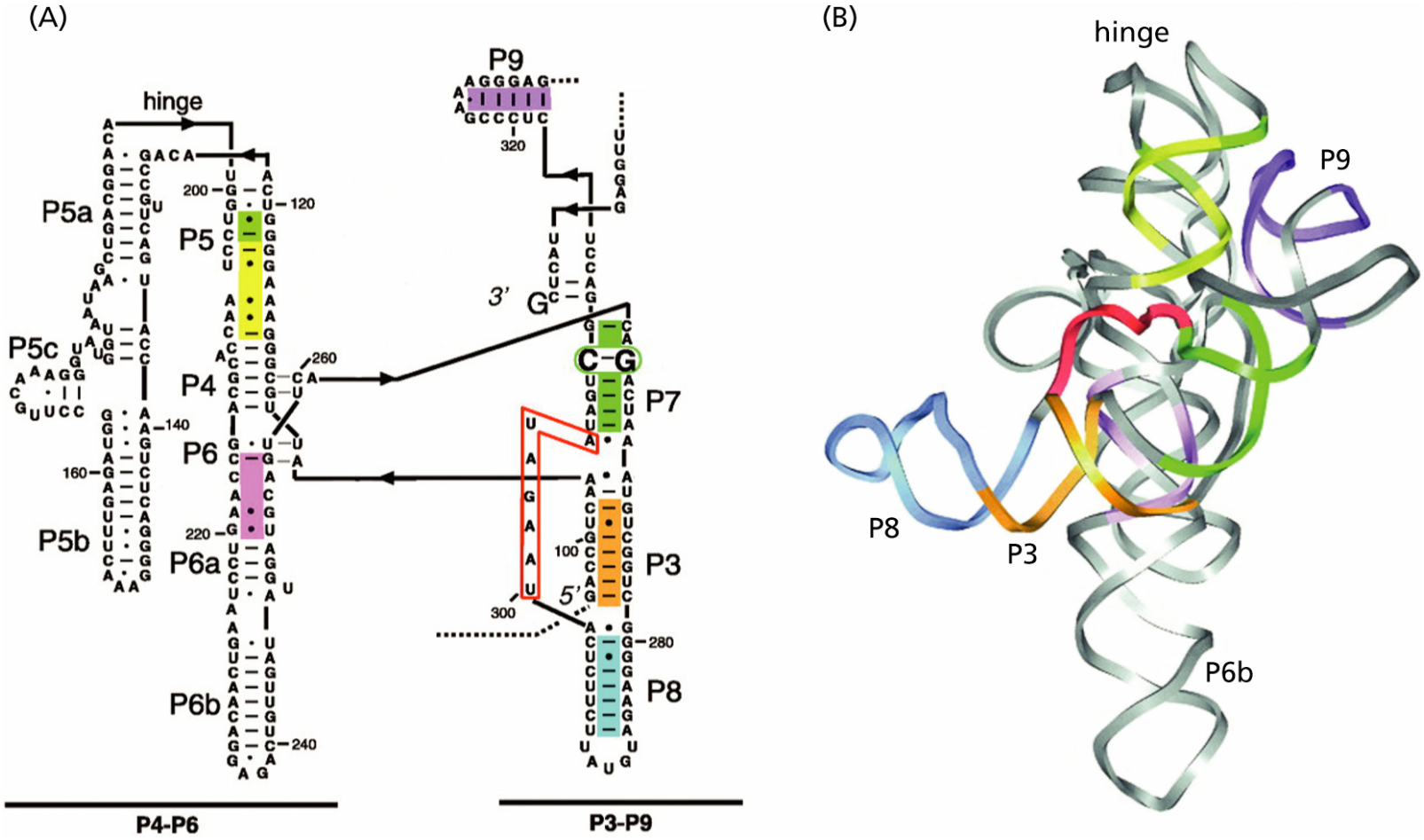
Fig 1.3, Zvelebil/Baum





RNA molecule

Fig 1.5, Zvelebil/Baum



Typical protein sequence

/translation="MVAGTRCLLVLLLPQVLLGGAAGLIPELGRKKFAAASSRPLSRP
SEDVLSEFELRLLSMFGLKQRPTPSKDVVVPPYMLDLYRRHSGQPGAPAPDHRLERAA
SRANTVRSFHHEEAVEELPEMSGKTARRFFFNLSSVPSDEFLTSAELQIFREQIQEAL
GNSSFQHRINIYEIIKPAAANLKFPVTRLLDTRLVNQNTSQWESFDVTPAVMRWTTQG
HTNHGFVVEVAHLEENPGVSKRHVRISRSLHQDEHSWSQIRPLLVTFGHDGKGHPLHK
REKRQAKHKQRKRLKSSCKRHPLYVDFSDVGWNDWIVAPPGYHAFYCHGECPFPLADH
LNSTNHAIVQTLVNSVNSKIPKACCVPTELSAISMLYLDENEKVVLKNYQDMVVEGCG
CR"

Protein 3D Structure

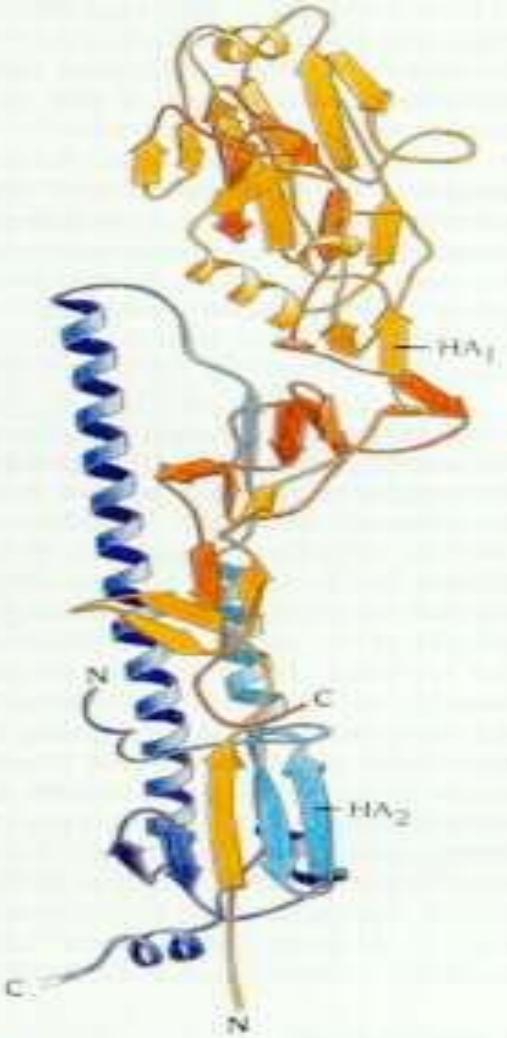
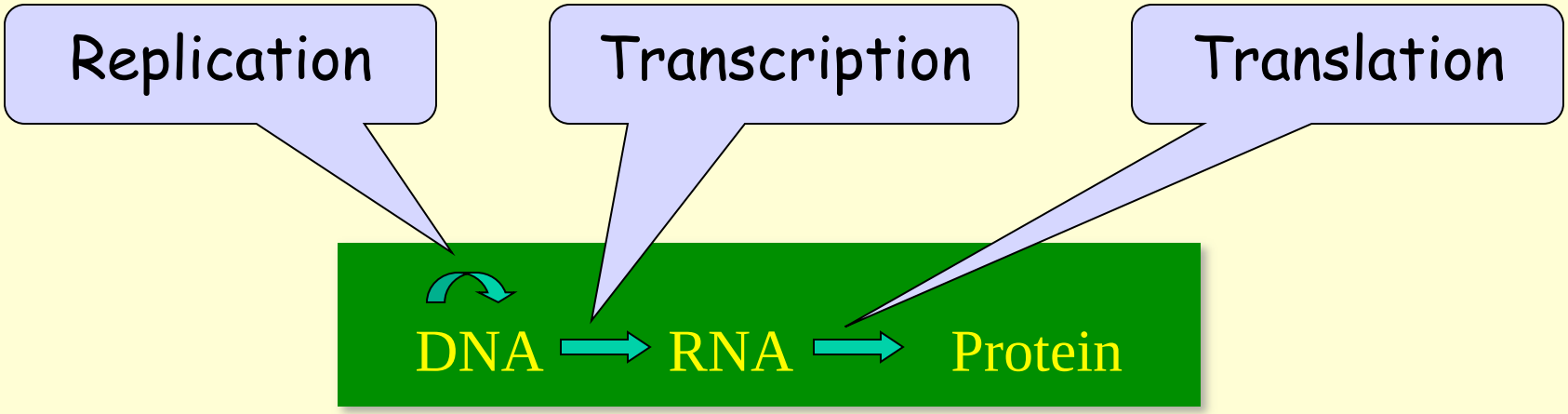


Figure 5.21 Schematic diagram of the subunit structure of hemagglutinin from influenza virus. The structure comprises about 550 amino acids arranged in two chains HA_1 (red) and HA_2 (blue). The first half of each chain has a lighter color in the diagram. The subunit is very elongated with a long stemlike region built up by residues from both chains and includes one of the longest α helices known in a globular structure, about 75Å long. The globular head is formed by residues only from HA_1. (Courtesy of Don Wiley, Harvard University.)

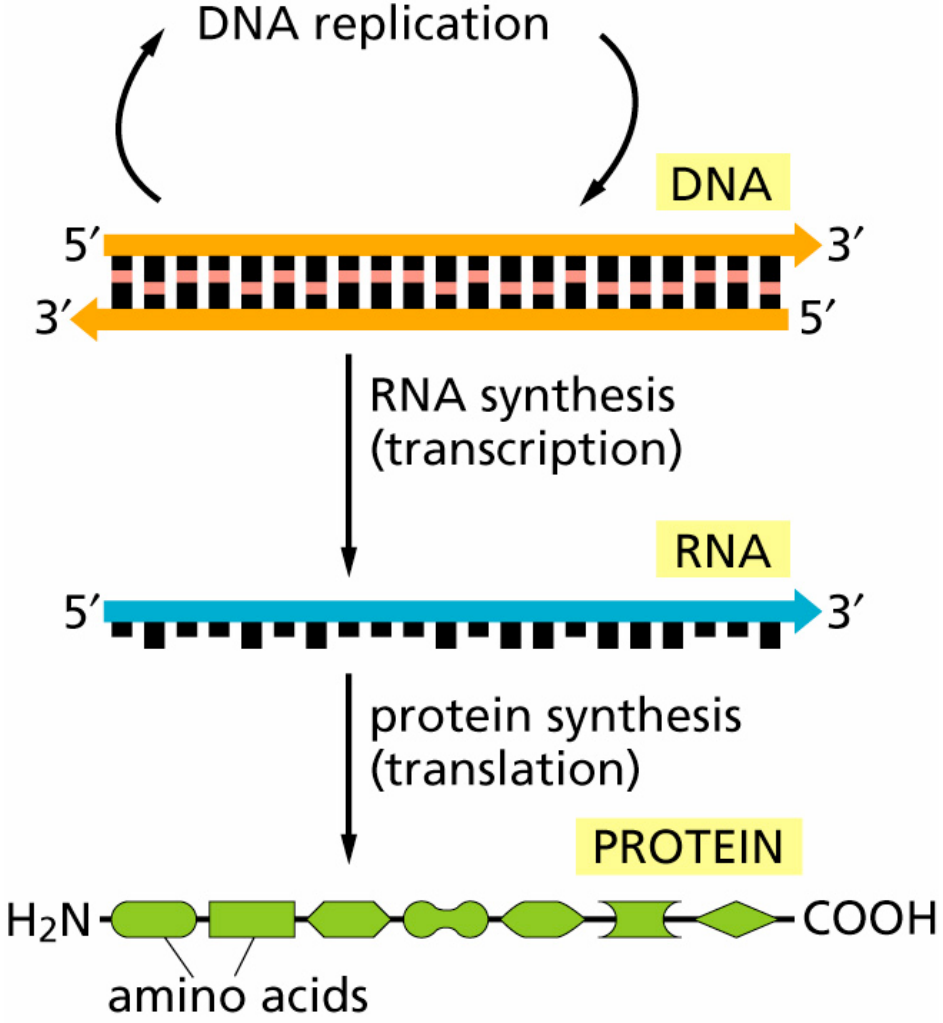
Central Dogma

- DNA acts as a template to replicate itself.
- DNA is transcribed into RNA.
- RNA is translated into **Protein**.



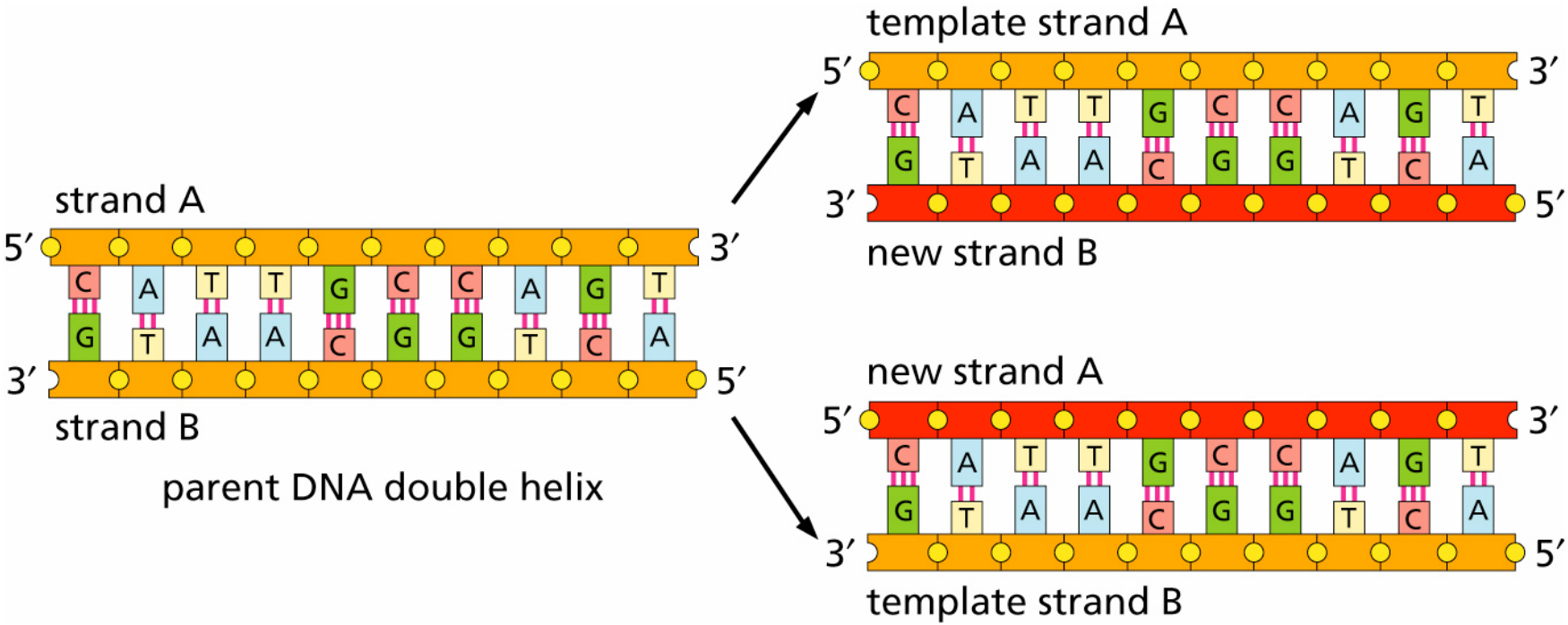
Central Dogma

Fig 1.6, Zvelebil/Baum

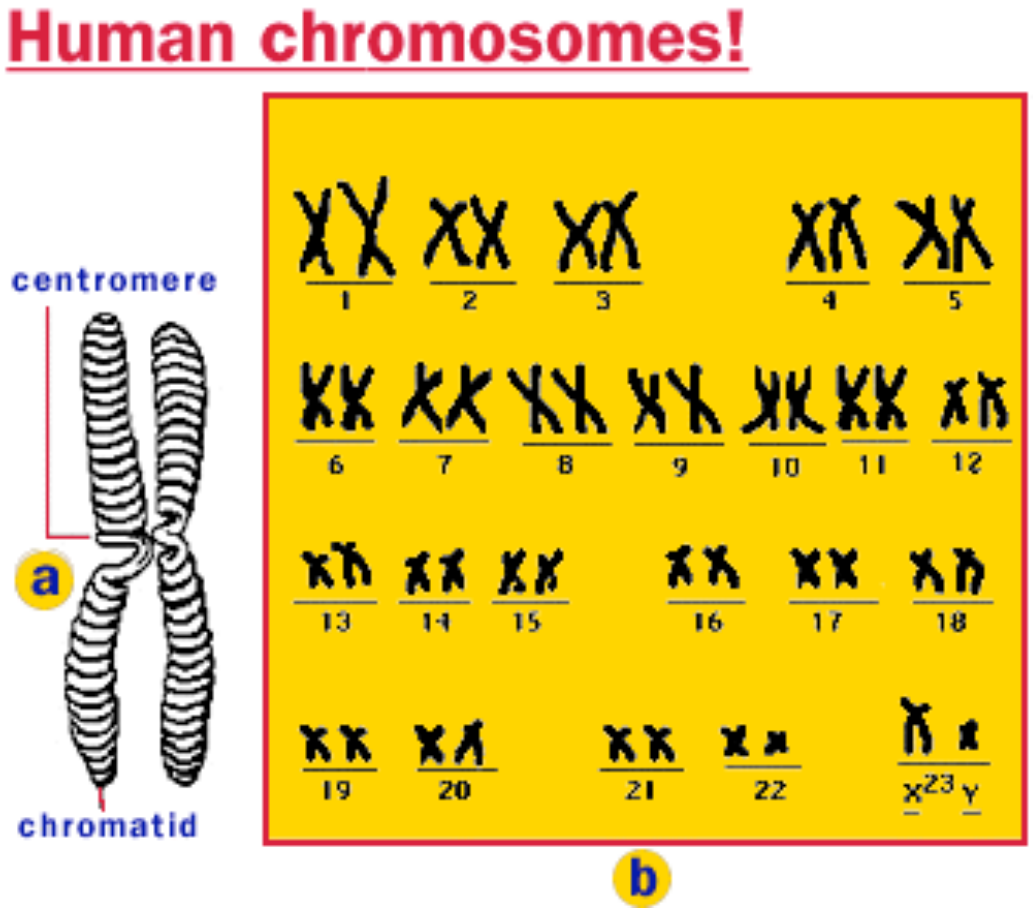


DNA Replication

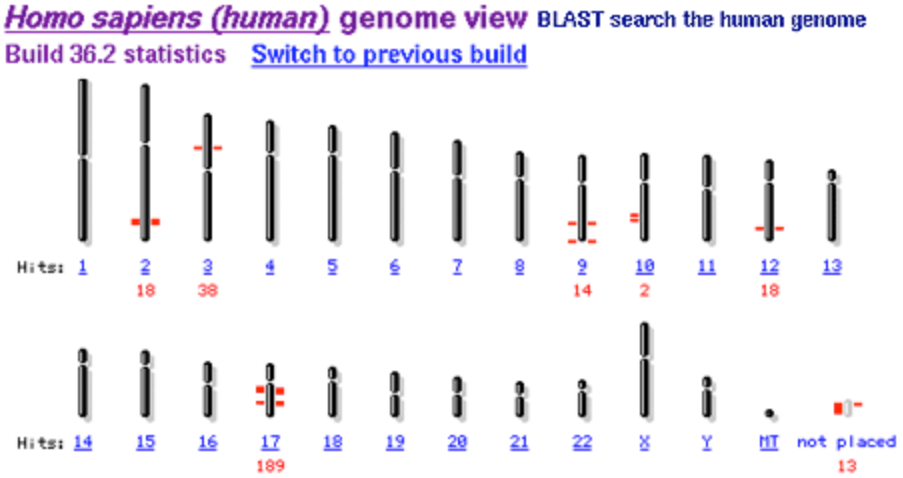
Fig 1.4, Zvelebil/Baum

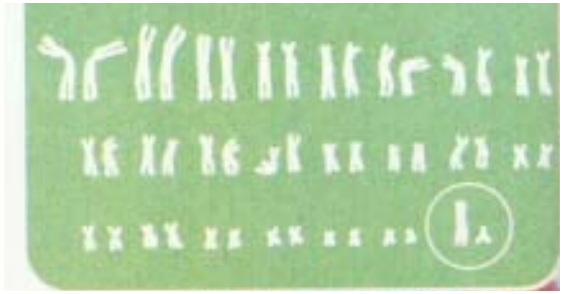


Chromosomes



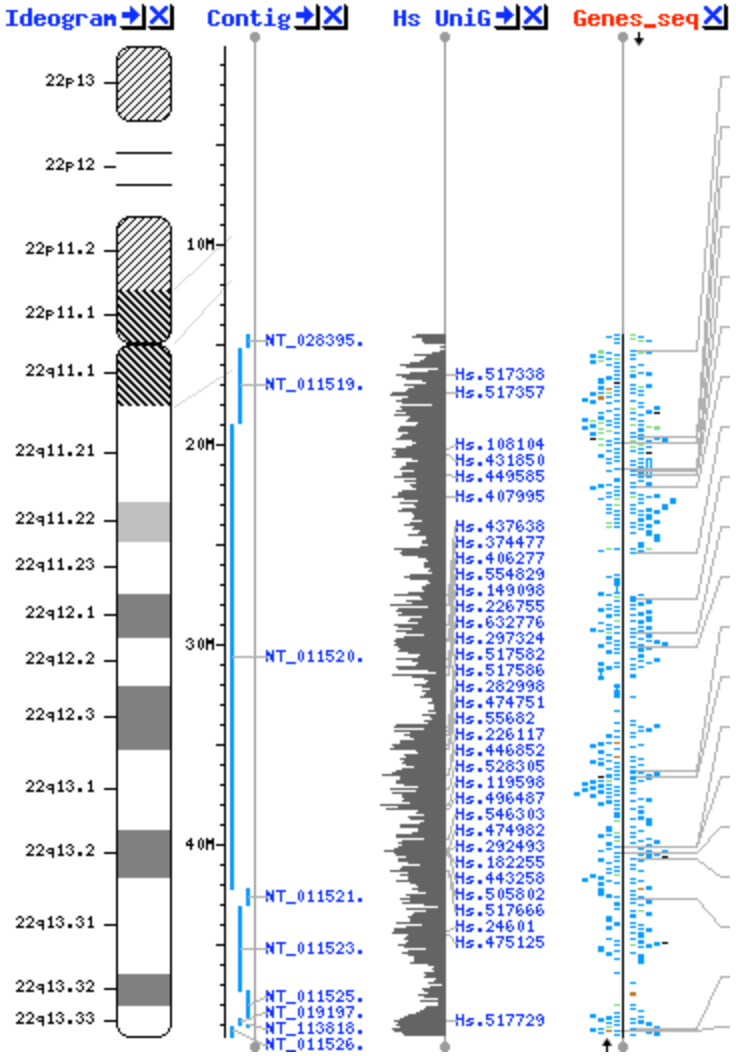
Chromosomes





The chromosomal locations of several genes believed to be associated with the human BRCA1 gene implicated in breast cancer are highlighted.

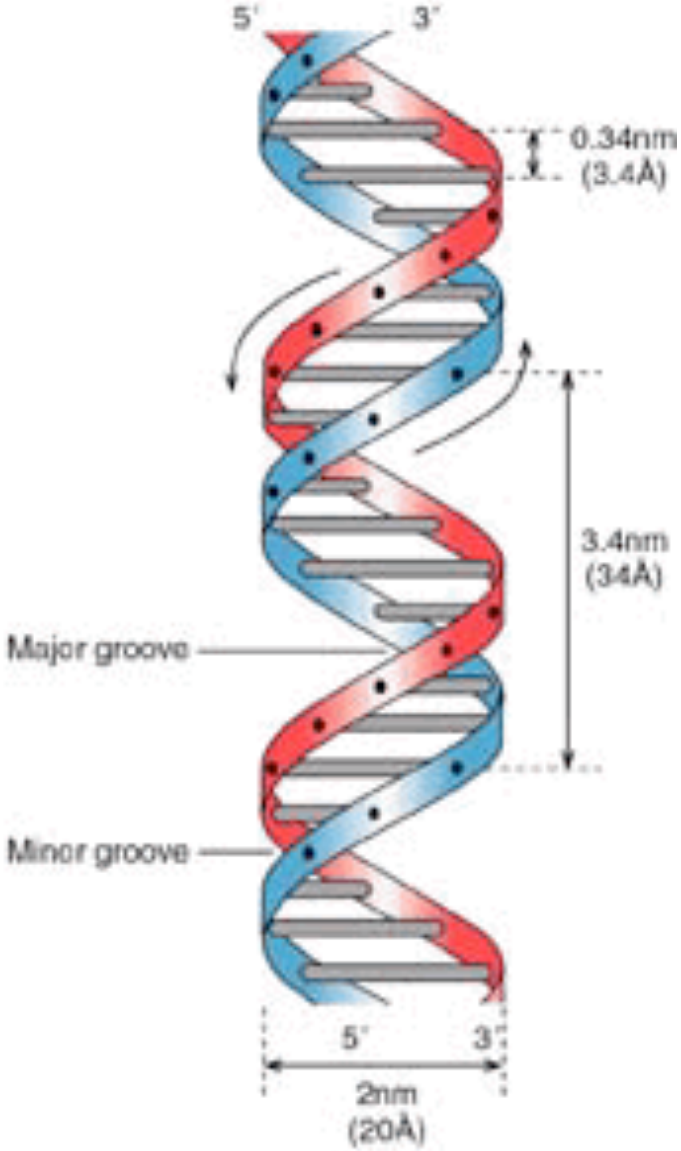
Human Chr 22



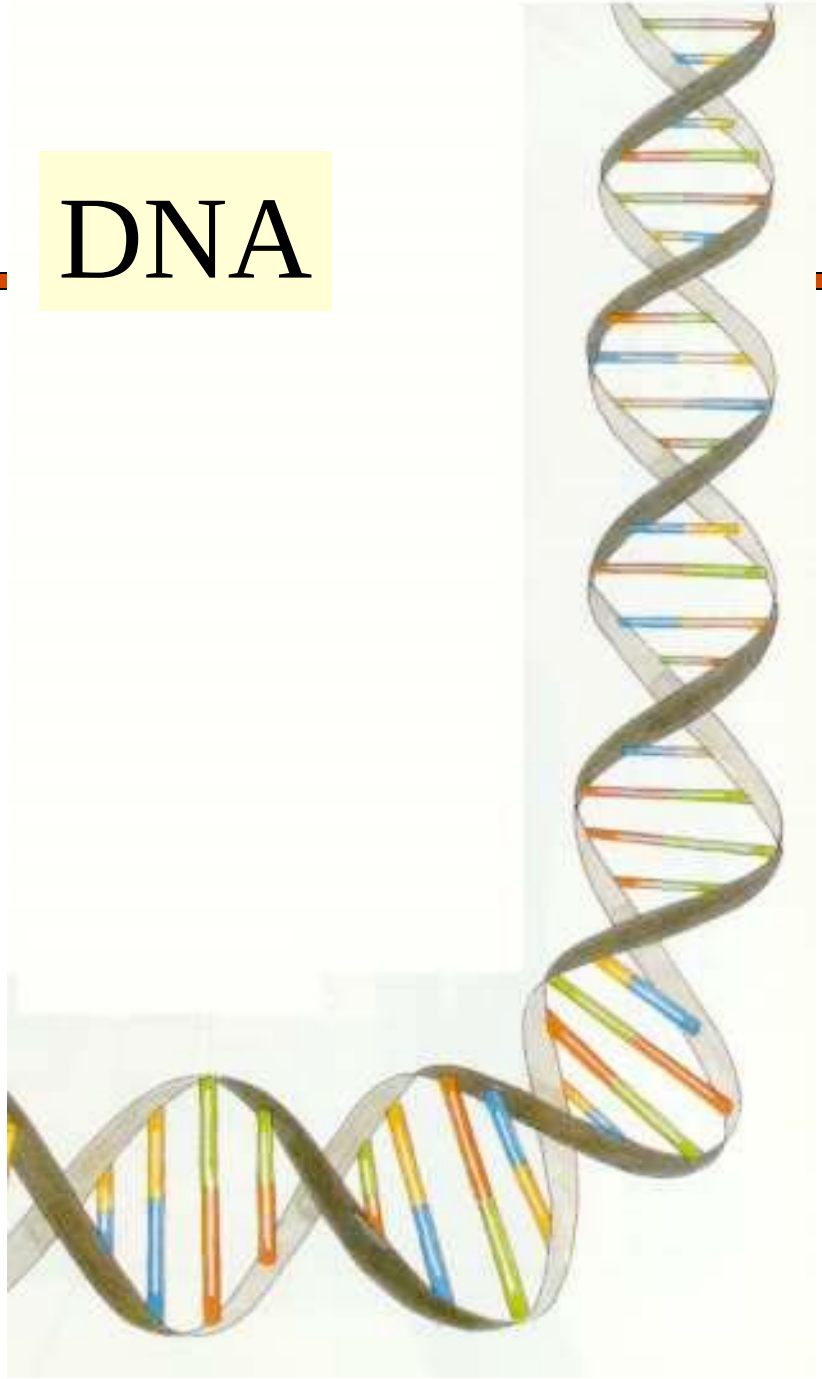
Symbol	Position	Description
ABCD1P4	22q11	ATP-binding cassette, sub-family D (ALD)
SNAP29	22q11.21	synaptosomal-associated protein

-
-
-

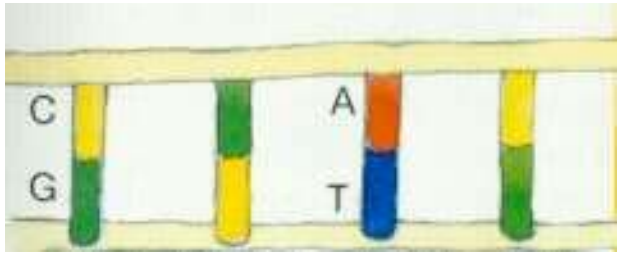
DNA Molecule



DNA



Complementary Bases



Proteins – Amino acids

amino acid	3 letter code	1 letter code
alanine	Ala	A
arginine	Arg	R
aspartic acid	Asp	D
asparginine	Asn	N
cysteine	Cys	C
glutamic acid	Glu	E
glutamine	Gln	Q
glycine	Gly	G
histine	His	H
isoleucine	Ile	I
leucine	Leu	L
lysine	Lys	K
methionine	Met	M
phenylalanine	Phe	F
proline	Pro	P
serine	Ser	S
threonine	Thr	T
tryptophan	Trp	W
tyrosine	Tyr	Y
valine	Val	V

Table 1.1: *Amino acid abbreviations*

RNA

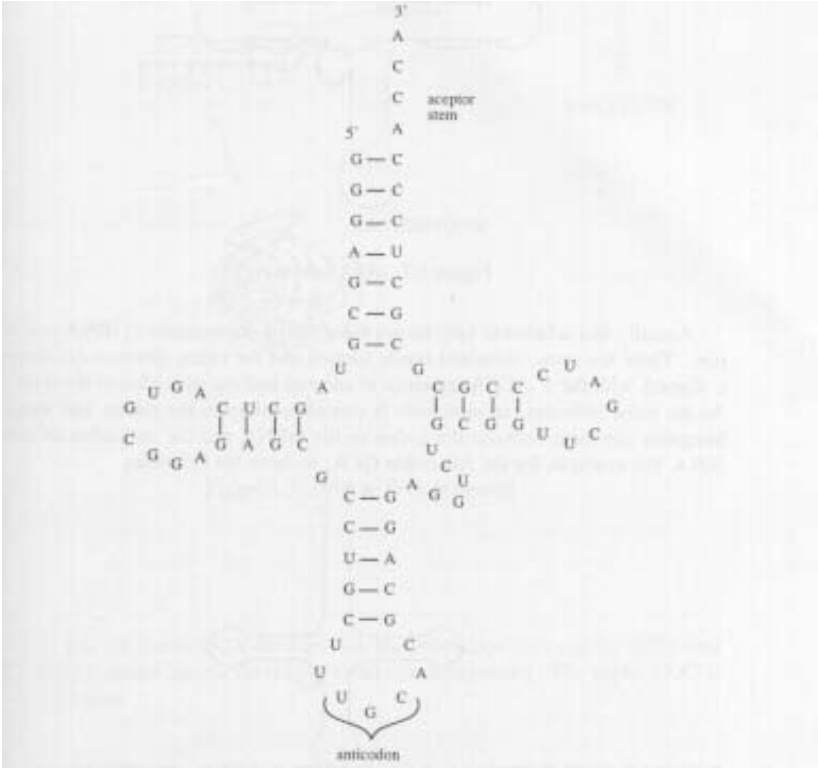


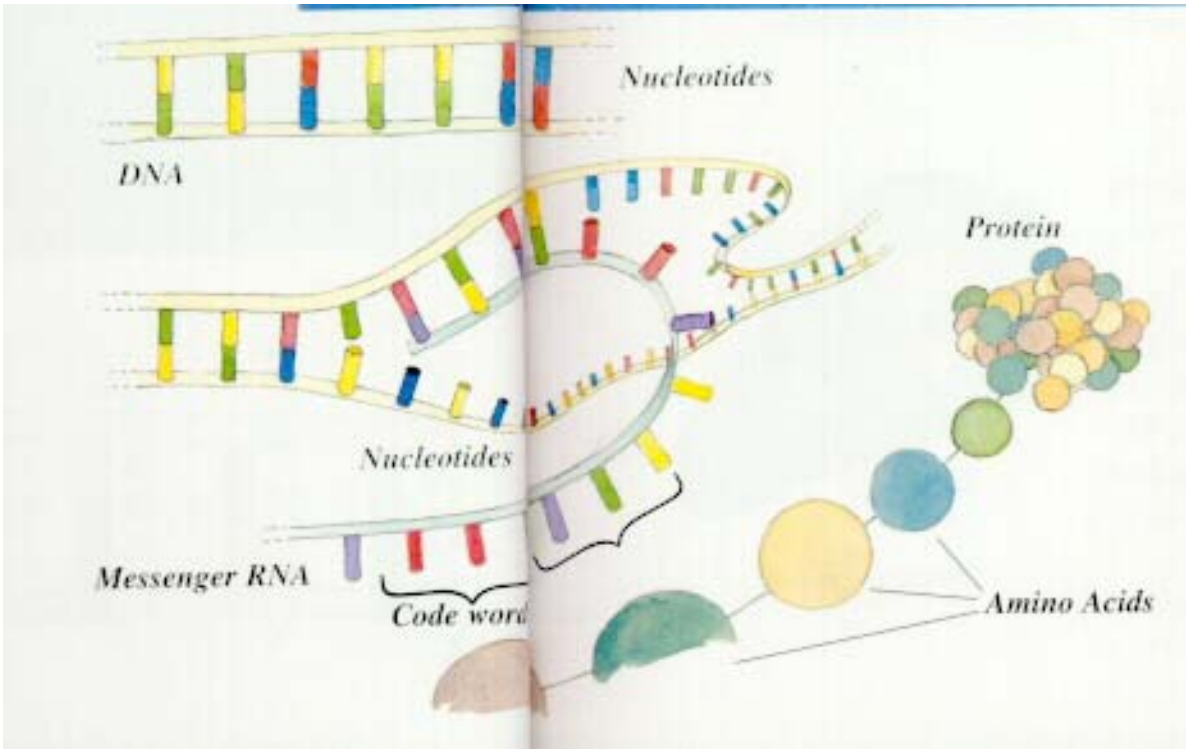
Figure 1.1: E. coli *Ala tRNA*

Genes

DNA

Gene A Gene B Gene C Gene D Gene E

DNA → RNA → Protein



Basic Genetic Processes

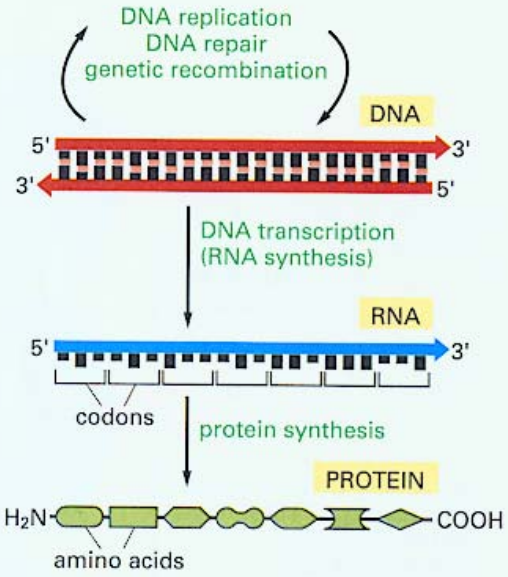
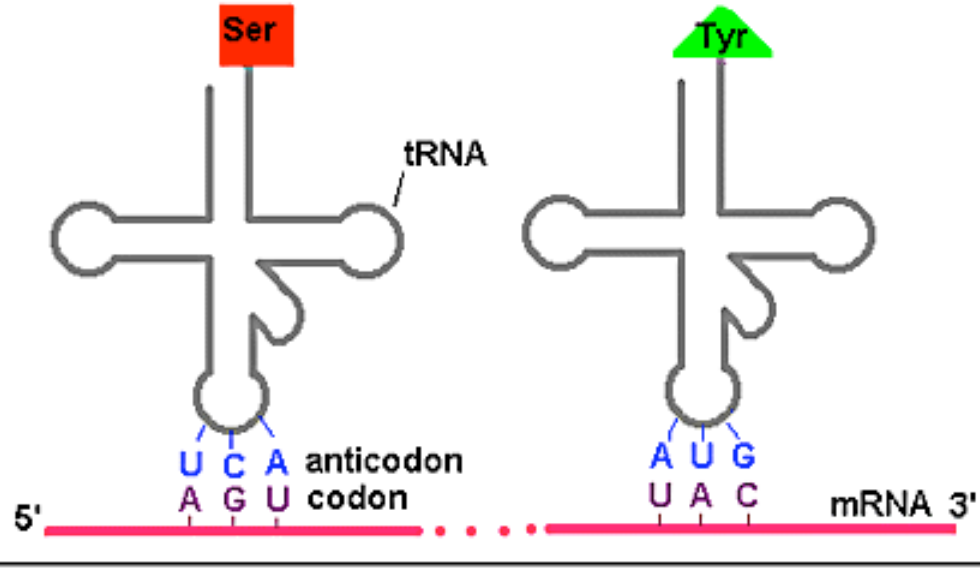


Figure 6–1 The basic genetic processes. The processes shown here are thought to occur in all present-day cells. Very early in the evolution of life, however, much simpler cells probably existed that lacked both DNA and proteins (see Figure 1–11). Note that a sequence of three nucleotides (a codon) in an RNA molecule codes for a specific amino acid in a protein.

The Genetic Code



2nd base in codon

1st base in codon	U	C	A	G	3rd base in codon
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	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

The Genetic Code

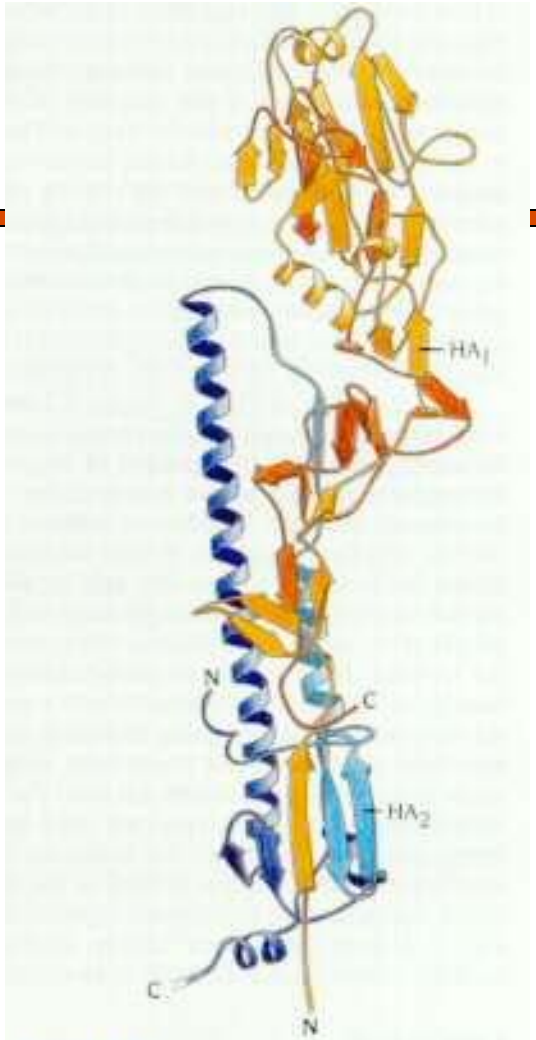
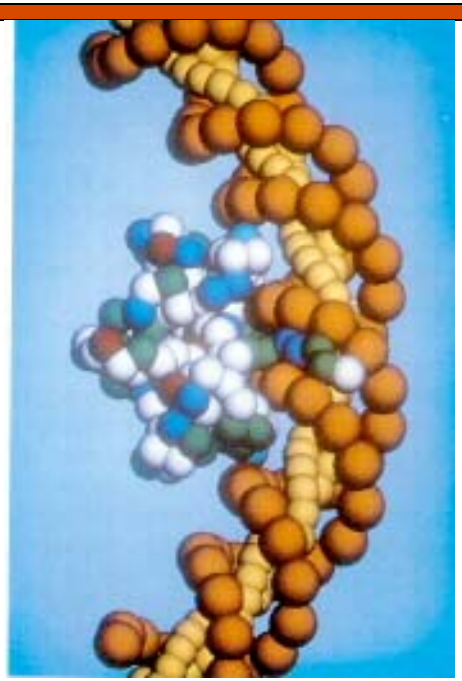
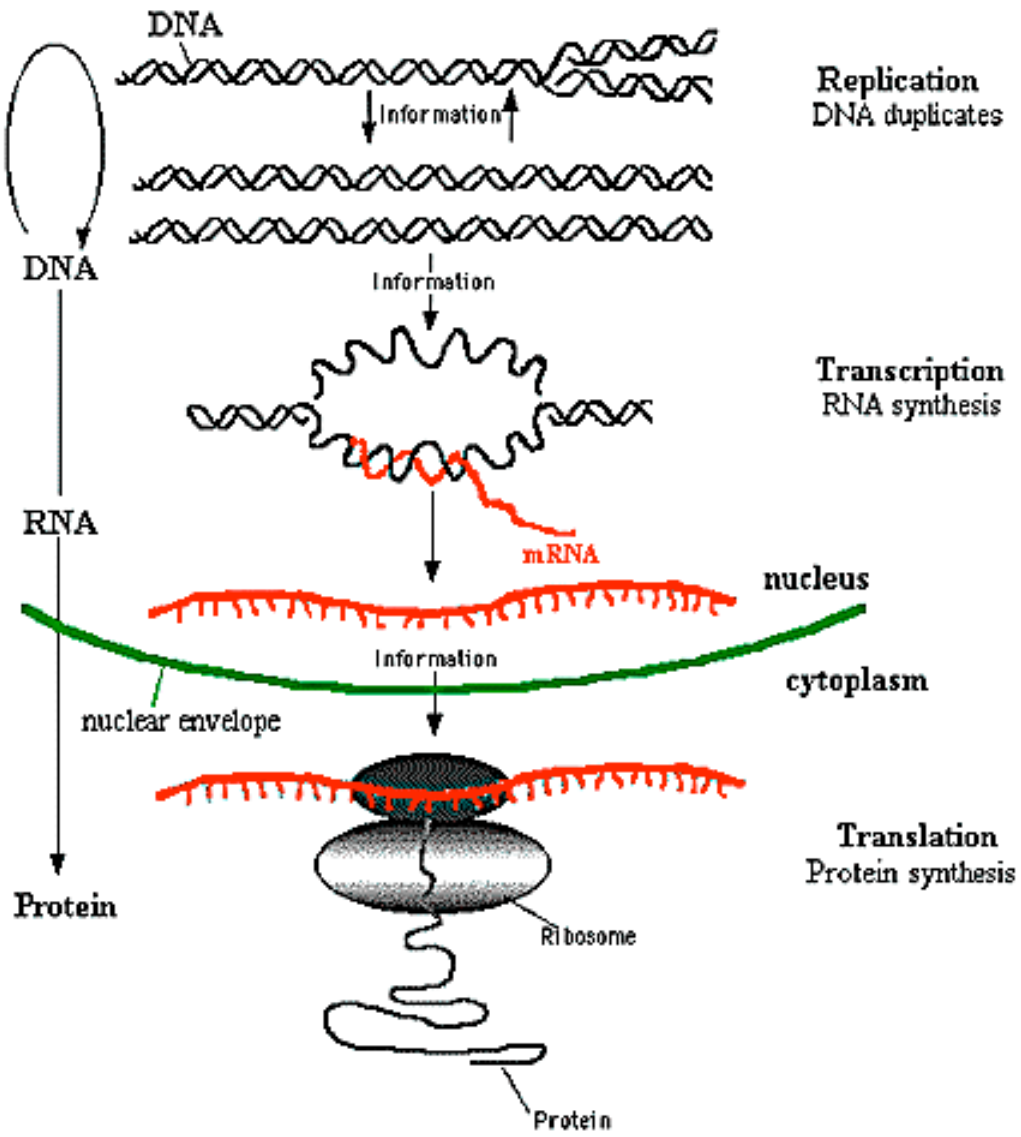


Figure 5.21 Schematic diagram of the subunit structure of hemagglutinin from influenza virus. The structure comprises about 550 amino acids arranged in two chains HA_1 (red) and HA_2 (blue). The first half of each chain has a lighter color in the diagram. The subunit is very elongated with a long stemlike region built up by residues from both chains and includes one of the longest α helices known in a globular structure, about 75 Å long. The globular head is formed by residues only from HA_1. (Courtesy of Don Wiley, Harvard University.)

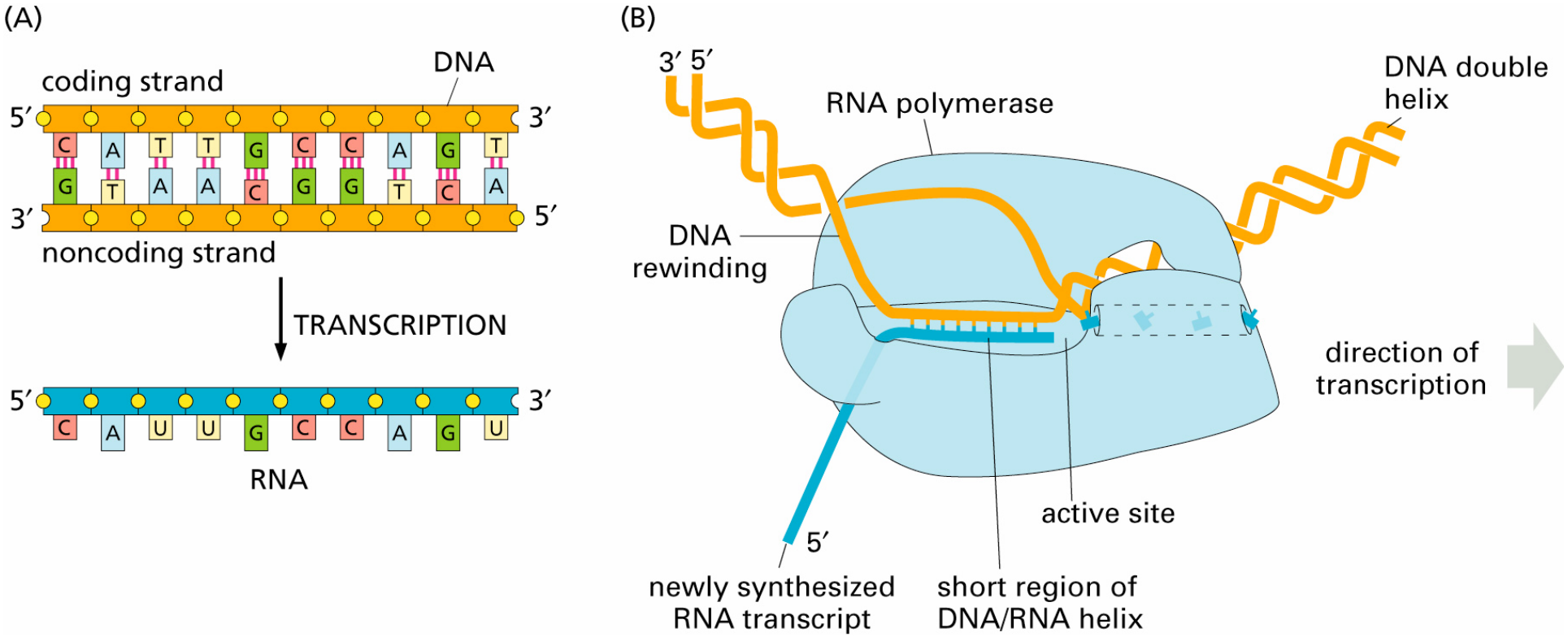


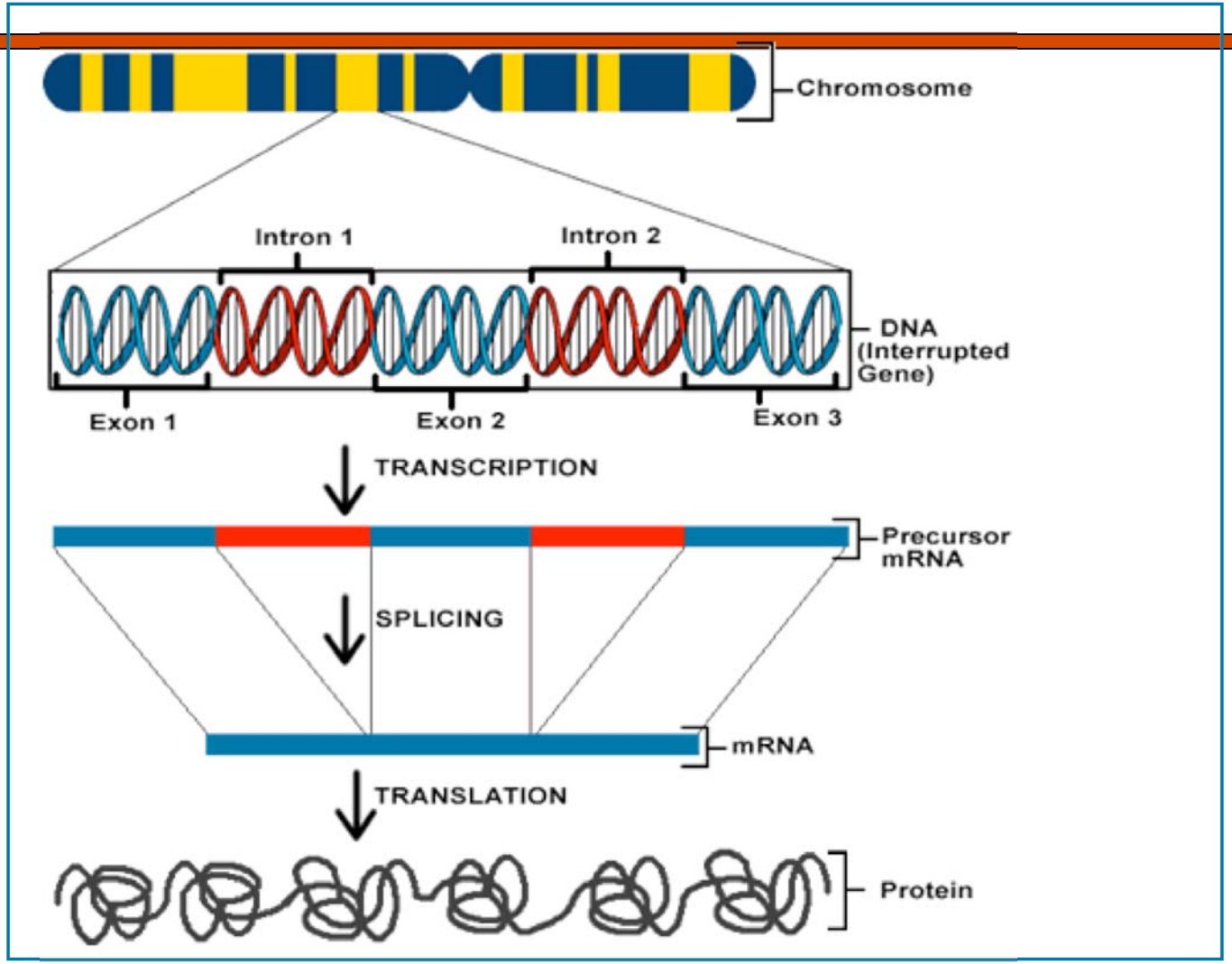


The Central Dogma of Molecular Biology

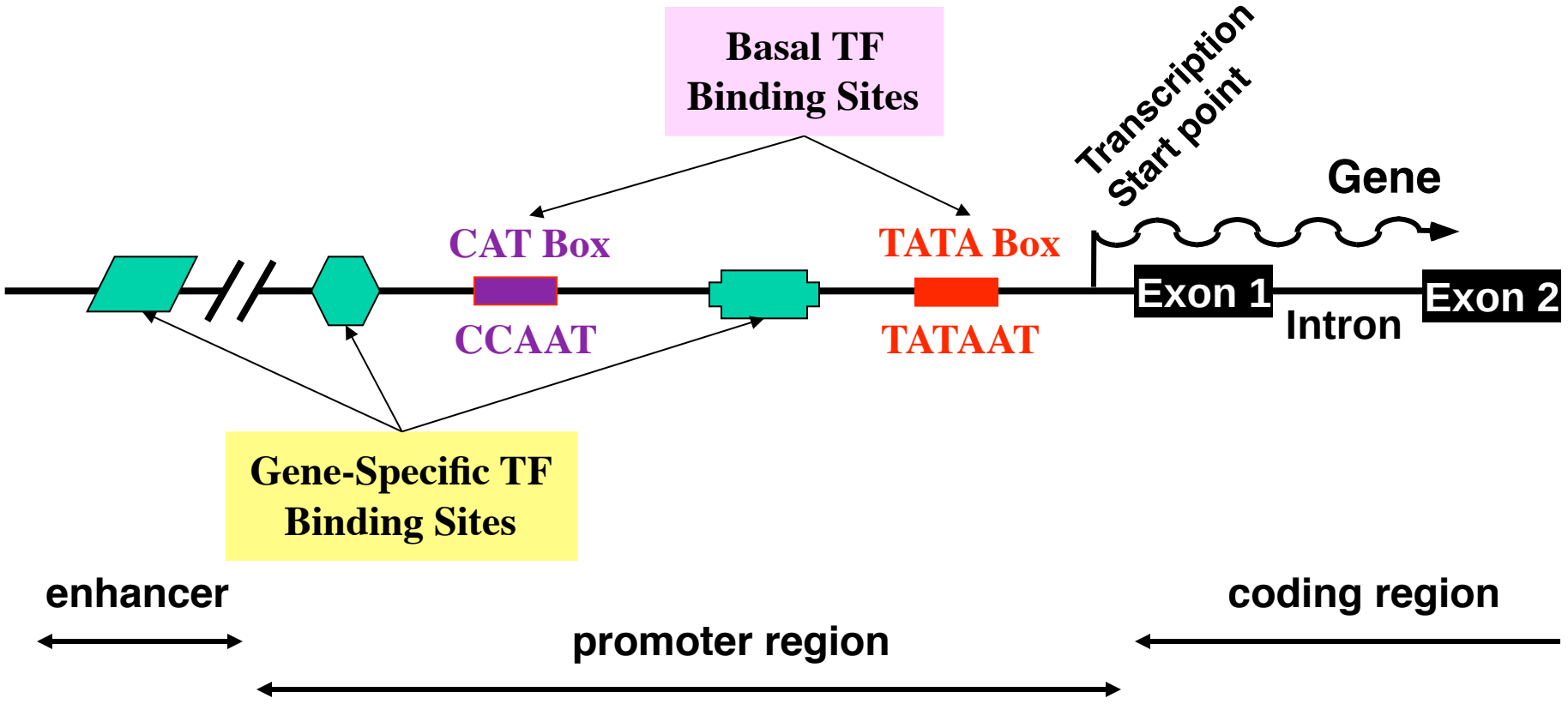
Transcription

Fig 1.7, Zvelebil/Baum



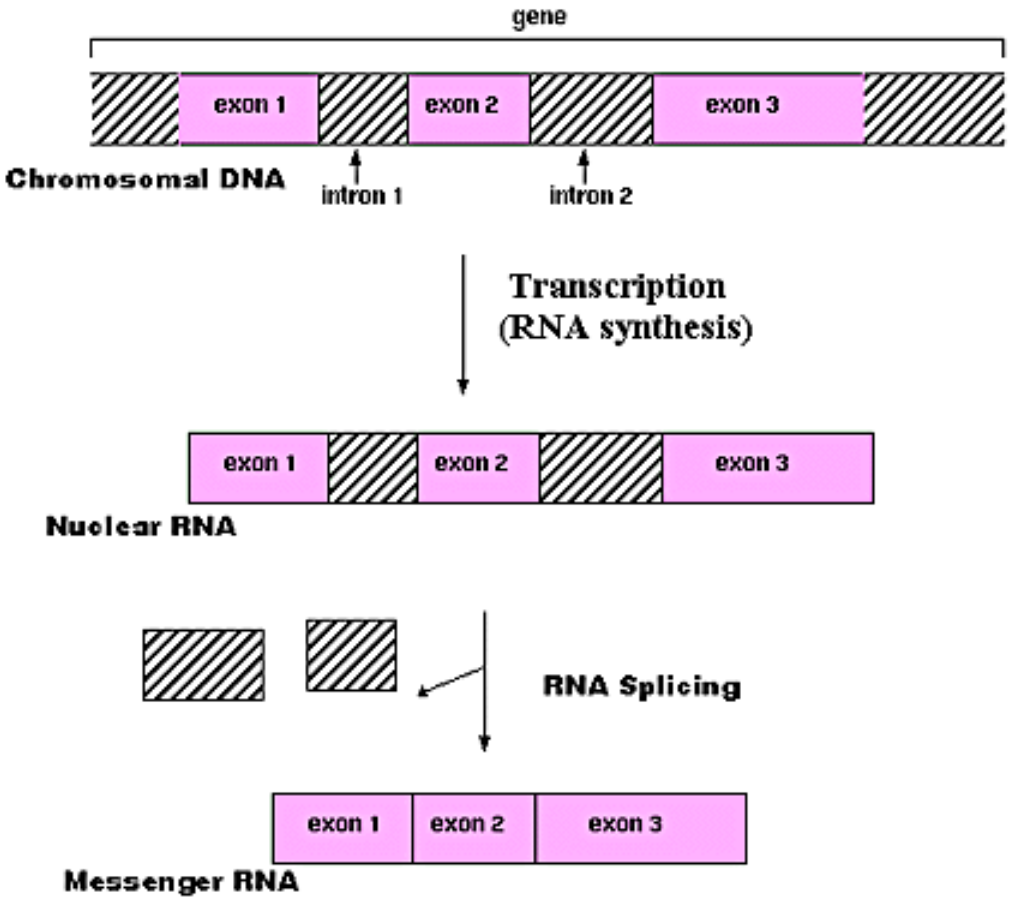

Chromosome
Intron 1
Intron 2
DNA
(Interrupted
Gene)
Exon 1
Exon 2
Exon 3
TRANSCRIPTION
Precursor
mRNA
SPLICING
mRNA
TRANSLATION
Protein

Transcription Regulation



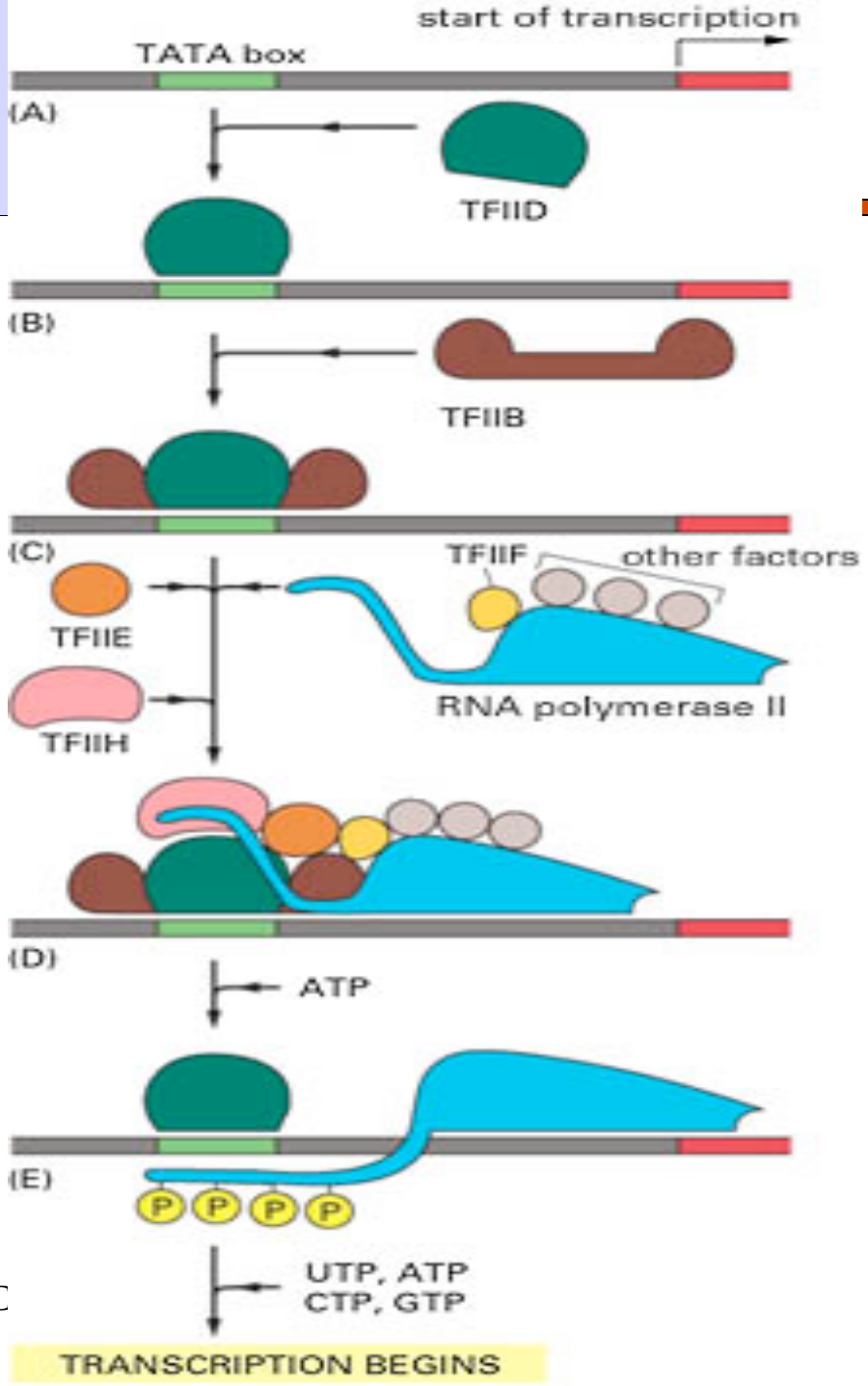
Courtesy: Kalai Mathee

DNA Transcription



RNA synthesis and processing

Transcription Initiation



Transcription

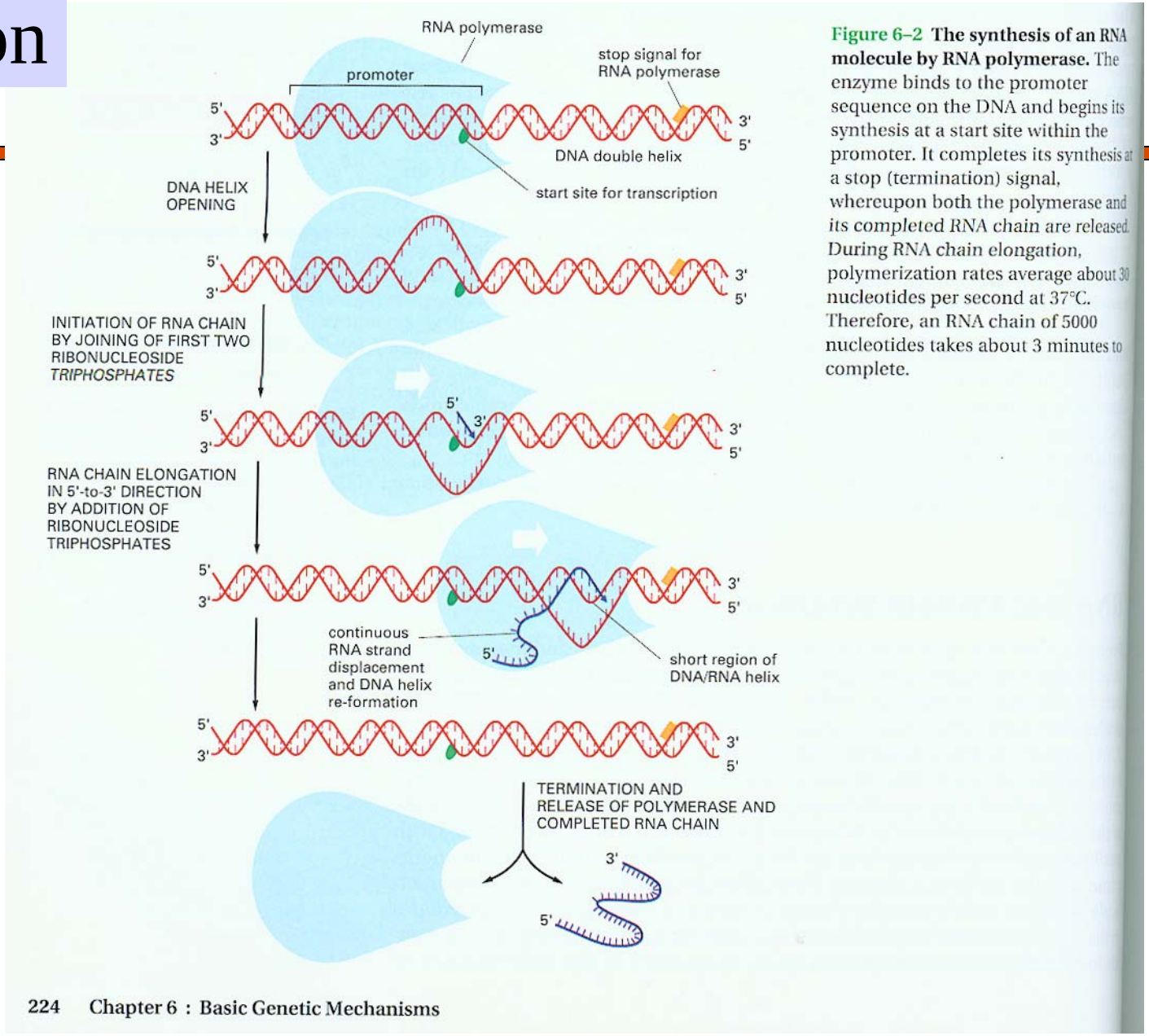


Figure 6–2 The synthesis of an RNA molecule by RNA polymerase. The enzyme binds to the promoter sequence on the DNA and begins its synthesis at a start site within the promoter. It completes its synthesis at a stop (termination) signal, whereupon both the polymerase and its completed RNA chain are released. During RNA chain elongation, polymerization rates average about 30 nucleotides per second at 37°C. Therefore, an RNA chain of 5000 nucleotides takes about 3 minutes to complete.

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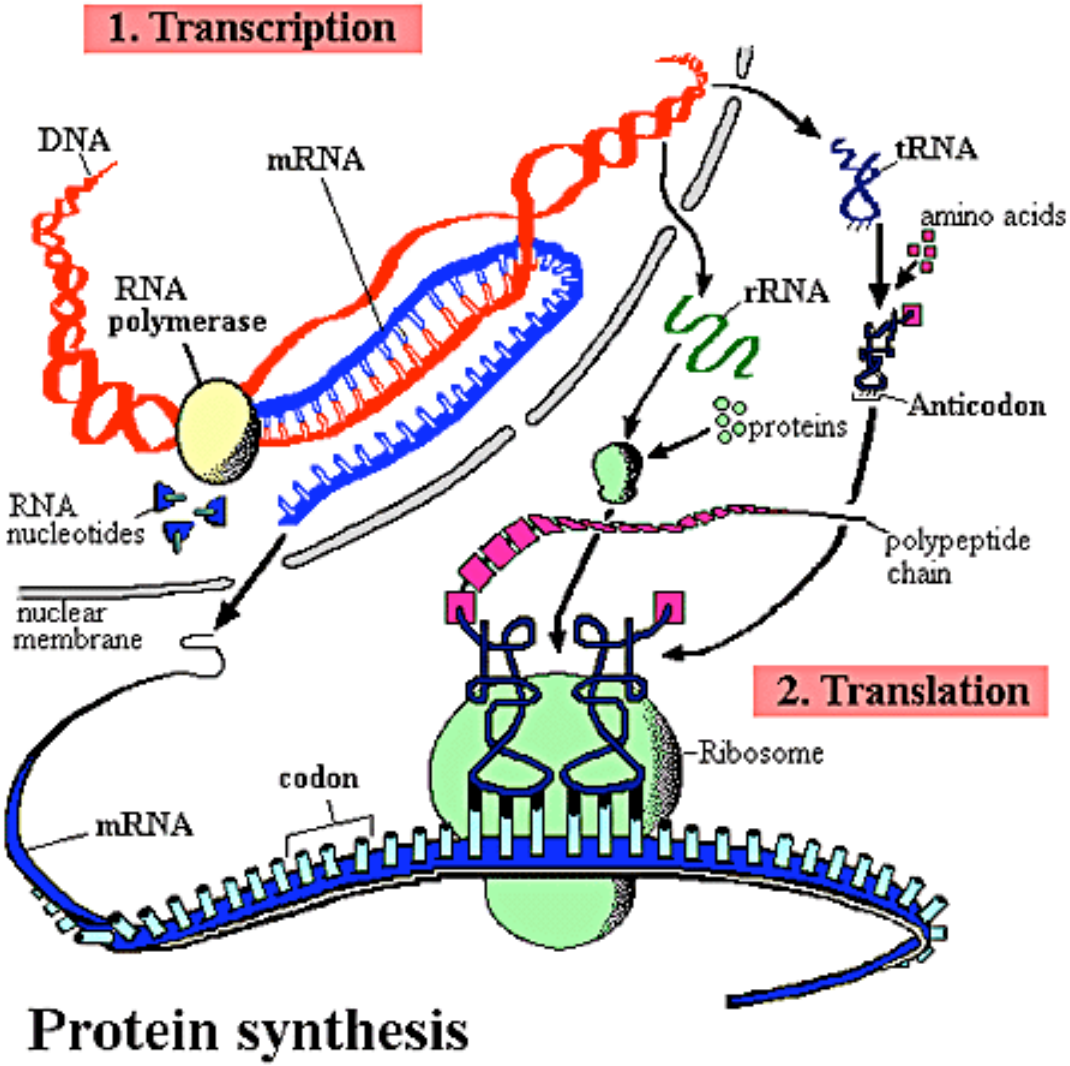
Transcription Factors

- The general transcription factors have been highly conserved in evolution; some of those from human cells can be replaced in biochemical experiments by the corresponding factors from simple yeasts.

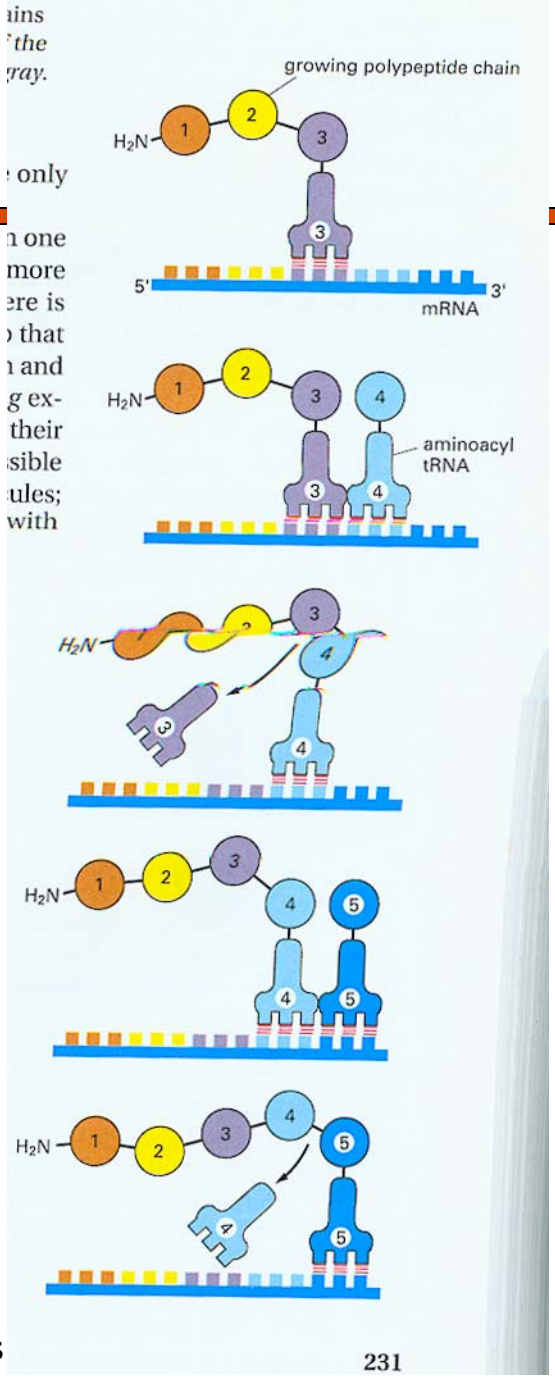
Protein Synthesis

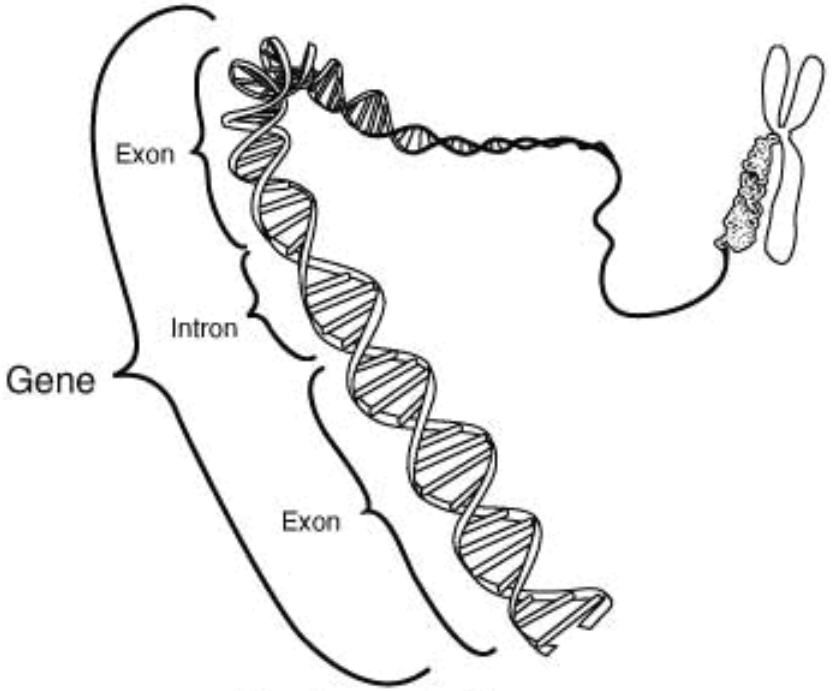


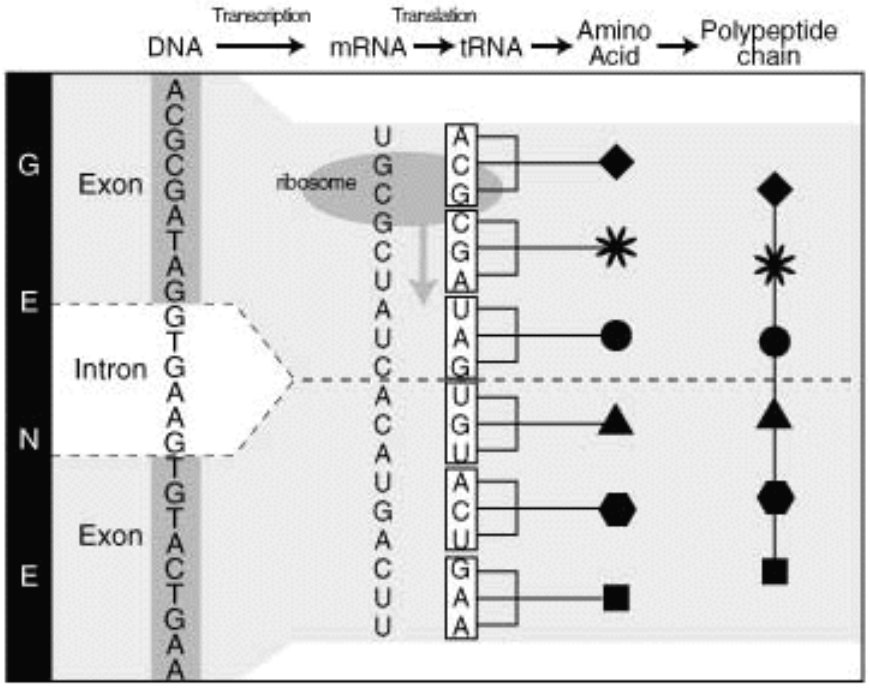
Protein synthesis

Protein Synthesis:

Incorporation of amino acid into protein




Exon
Intron
Gene
Exon


Transcription
Translation
DNA
mRNA
tRNA
Amino Acid
Polypeptide chain
G
E
N
E
Exon
Intron
Exon
ACGCGATAGGTGAAGTGTACTGAA
ribosome
UGCGCUAUCCACAUGACUU
ACG
CGA
UAG
UGU
ACU
GAA

Genomic Databases

- Entrez Portal at National Center for Biotechnology Information (NCBI) gives access to:
  - Nucleotide (GenBank, EMBL, DDBJ)
  - Protein (PIR, SwissPROT, PRF, and Protein Data Bank or PDB)
  - Genome
  - Structure
  - 3D Domains
  - Conserved Domains
  - Gene; UniGene; HomoloGene; SNP
  - GEO Profiles & Datasets
  - Cancer Chromosomes
  - PubMed Central; Journals; Books
  - OMIM
  - Database Neighbors and Interlinking

Sequence Alignment – Why?

>gi|12643549|sp|O18381|PAX6_DROME Paired box protein Pax-6 (Eyeless protein)
MRNLPCLGTAGGSGLGGIAGKPSPTMEAVEASTASHRHSTSSYFATTYYHLTDDECHSGVNQLGGVFVGG
RPLPDSTRQKIVELAHSGARPCDISRILQVSNGCVSKILGRYYETGSIRPRAIGGSKPRVATAEVVSKIS
QYKRECPSIFAWEIRDRLLQENVCTNDNIPSVSSINRVLRNLAAQKEQQSTGSGSSSTSAGNSISAKVSV
SIGGNVSNVASGSRGTLSSSTDLMQTATPLNSSESGGASNSGEGSEQEAIYEKLRLLNTQHAAGPGPLEP
ARAAPLVGQSPNHLGTRSSHPQLVHGNHQALQQHQQQSWPPRHYSGSWYPTSLSEIPISSAPNIASVTAY
ASGPSLAHSLSPPNDIESLASIGHQRNCPVATEDIHLKKELDGHQSDETGSGEGENSNGGASNIGNTEDD
QARLILKRKLQRNRTSFTNDQIDSLEKEFERTHYPDVFARERLAGKIGLPEARIQVWFSNRRAKWRREEK
LRNQRRTPNSTGASATSSSTSATASLTDSPNSLSACSSLLSGSAGGPSVSTINGLSSPSTLSTNVNAPTL
GAGIDSSESPTPIPHIRPSCTSDNDNGRQSEDCRRVCSPCPLGVGGHQNTHHIQSNGHAQGHALVPAISP
RLNFNSGSFGAMYSNMHHTALSMSDSYGAVTPIPSFNHSAVGPLAPPSPIPQQGDLTPSSLYPCHMTLRP
PPMAPAHHHIVPGDGGRPAGVGLGSGQSANLGASCSGSGYEVLSAYALPPPPMASSSAADSSFSAASSAS
ANVTPHHTIAQESCPSPCSSASHFGVAHSSGFSSDPISPAVSSYAHMSYNYASSANTMTPSSASGTSAHV

>gi|6174889|PAX6_HUMAN Paired box protein (Oculorhombin) (Aniridia, type II protein)
MQNSHSGVNQLGGVFVNGRPLPDSTRQKIVELAHSGARPCDISRILQVSNGCVSKILGRYYETGSIRPRA
IGGSKPRVATPEVVSKIAQYKRECPSIFAWEIRDRLLSEGVCTNDNIPSVSSINRVLRNLASEKQQMGAD
GMYDKLRMLNGQTGSWGTRPGWYPGTSVPGQPTQDGCQQQEGGGENTNSISSNGEDSDEAQMRLQLKRKL
QRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQVWFSNRRAKWRREEKLRNQRRQASN
TPSHIPISSSFSTSVYQPIPQPTTPVSSFTSGSMLGRTDTALTNTYSALPPMPSFTMANNLPMQPPVPSQ
TSSYSCMLPTSPSVNGRSYDTYTPPHMQTHMNSQPMGTSGTTSTGLISPGVSVPVQVPGSEPDMSQYWPR
LQ

Drosophila Eyeless vs. Human Aniridia

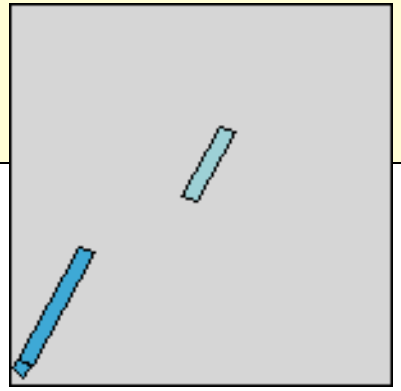
```
Query: 57   HSGVNQLGGVFVGGRPLPDSTRQKIVELAHSGARPCDISRILQVSNGCVSKILGRYYETG 116
            HSGVNQLGGVFV GRPLPDSTRQKIVELAHSGARPCDISRILQVSNGCVSKILGRYYETG
Sbjct: 5    HSGVNQLGGVFVNGRPLPDSTRQKIVELAHSGARPCDISRILQVSNGCVSKILGRYYETG 64

Query: 117 SIRPRAIGGSKPRVATAEVVSKISQYKRECPSIFAWEIRDRLLQENVCTNDNIPSVSSIN 176
            SIRPRAIGGSKPRVAT EVVSKI+QYKRECPSIFAWEIRDRLL E VCTNDNIPSVSSIN
Sbjct: 65   SIRPRAIGGSKPRVATPEVVSKIAQYKRECPSIFAWEIRDRLLSEGVCTNDNIPSVSSIN 124

Query: 177 RVLRNLAAQKEQ 188
            RVLRNLA++K+Q
Sbjct: 125 RVLRNLASEKQQ 136
```

```
Query: 417 TEDDQARLILKRKLQRNRTSFTNDQIDSLEKEFERTHYPDVFARERLAGKIGLPEARIQV 476
           +++ Q RL LKRKLQRNRTSFT +QI++LEKEFERTHYPDVFARERLA KI LPEARIQV
Sbjct: 197 SDEAQMRLQLKRKLQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQV 256

Query: 477 WFSNRRAKWRREEKLRNQRR 496
           WFSNRRAKWRREEKLRNQRR
Sbjct: 257 WFSNRRAKWRREEKLRNQRR 276
```



E-Value = 2e-31

Implications of Sequence Alignment

- Mutation in DNA is a natural evolutionary process. Thus sequence similarity may indicate common ancestry.
- In biomolecular sequences (DNA, RNA, protein), high sequence similarity implies significant structural and/or functional similarity.

Discovery based on alignments

- Early 1970s: Simian sarcoma virus causes cancer in some species of monkeys.
- 1970s: infection by certain viruses cause some cells in culture (in vitro) to grow without bounds.
  - Hypothesis: Certain genes (oncogenes) in viruses encode cellular growth factors, which are proteins needed to stimulate growth of a cell colony. Thus uncontrolled quantities of growth factors produced by the infected cells cause cancer-like behavior.
- 1983:
  - The oncogene from SSV called v-sis was isolated and sequenced.
  - The partial amino-acid sequence for platelet-derived growth factor (PDGF) was sequenced and published. It stimulates the proliferation of normal cells.
  - R.F. Doolittle was maintaining one of the earliest home-grown databases of published amino-acid sequences.
  - Sequence Alignment of v-sis and PDGF showed something surprising.

PDGF and v-sis

- One region of 31 amino acids had 26 exact matches
- Another region of 39 residues had 35 exact matches.
- Conclusion:
  - The previously harmless virus incorporates the normal growth-related gene (proto-oncogene) of its host into its genome.
  - The gene gets mutated in the virus, or moves closer to a strong enhancer, or moves away from a repressor.
  - This causes an uncontrolled amount of the product (the growth factor, for example) when the virus infects a cell.
- Several other oncogenes known to be similar to growth-regulating proteins in normal cells.

V-sis Oncogene - Homologies

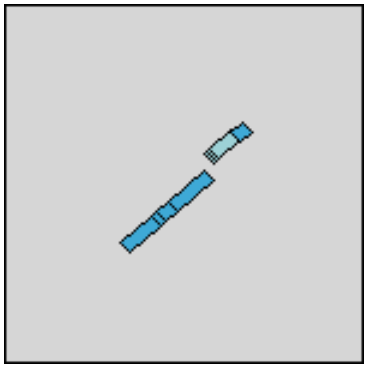
Sequences producing significant alignments:	Score (bits)	E Value
gi|332623|gb|J02396.1|SEG_SSVPCS2 Simian sarcoma virus v-si...	4591	0.0
gi|61774|emb|V01201.1|RESSV1 Simian sarcoma virus proviral ...	4504	0.0
gi|332622|gb|J02395.1|SEG_SSVPCS1 Simian sarcoma virus LTR ...	1283	0.0
gi|885929|gb|U20589.1|GLU20589 Gibbon leukemia virus envelo...	1140	0.0
gi|4505680|ref|NM_002608.1| Homo sapiens platelet-derived g...	954	0.0
gi|20987438|gb|BC029822.1| Homo sapiens, platelet-derived g...	954	0.0
gi|338210|gb|M12783.1|HUMSISPDG Human c-sis/platelet-derive...	954	0.0

Sequence Alignment

```
>gi|4505680|ref|NM_002608.1|    Homo sapiens platelet-derived growth
   factor beta polypeptide (simian sarcoma viral (v-sis) oncogene
   homolog) (PDGFB), transcript variant 1, mRNA Length = 3373 Score = 954
   bits (481), Expect = 0.0 Identities = 634/681 (93%), Gaps = 3/681 (0%)
   Strand = Plus / Plus
Query: 1015 agggggaccccattcctgaggagctctataagatgctgagtggccactcgattcgctcct 1074
           ||||||||||||||| |||||||| ||| |||||||||||| ||||||||| |||||||
Sbjct: 1084 agggggaccccattcccgaggagctttatgagatgctgagtgaccactcgatccgctcct 1143
      > 21 E  G  D  P  I  P  E  E  L  Y  E  M  L  S  D  H  S  I  R  S
Query: 1075 tcgatgacctccagcgcctgctgcagggagactccggaaaagaagatggggctgagctgg 1134
           | ||||| ||||| |||||||||| |||||| ||||| | |||||||||| ||| |||
Sbjct: 1144 ttgatgatctccaacgcctgctgcacggagaccccggagaggaagatggggccgagttgg 1203
     > 61 D  L  N  M  T  R  S  H  S  G  G  E  L  E  S  L  A  R  G  R
```

Sequence Alignment

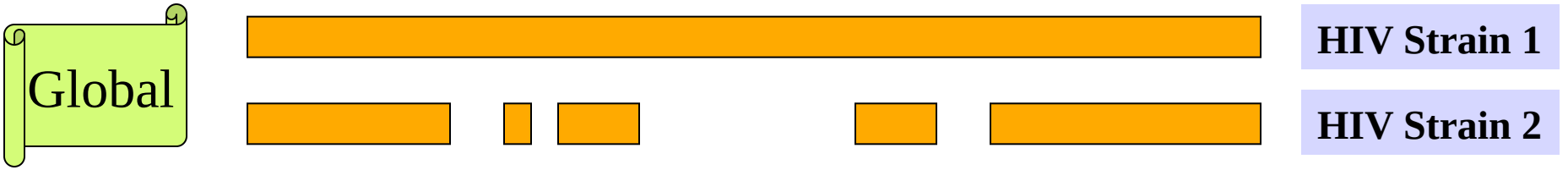
Sequence 1 gi 332624 Simian sarcoma virus v-sis transforming protein p28 gene, complete cds; and 3' LTR long terminal repeat, complete sequence. Length 2984 (1 .. 2984)
Sequence 2 gi 4505680 Homo sapiens platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog) (PDGFB), transcript variant 1, mRNA Length 3373 (1 .. 3373)



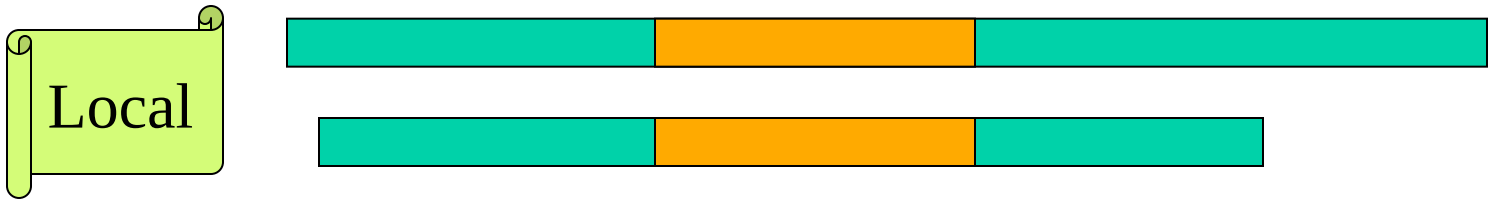
Similarity vs. Homology

- Homologous sequences share common ancestry.
- Similar sequences are "near" to each other by some appropriately defined measurable criteria.

Types of Sequence Alignments - 1



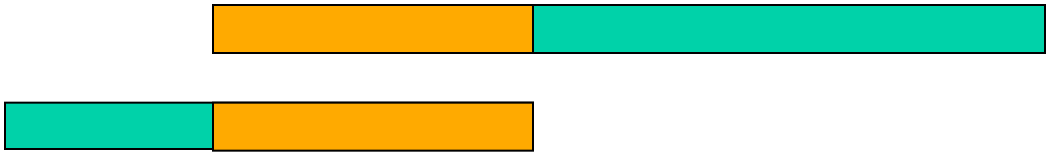
- Global Alignment: similarity over entire length



- Local Alignment: no overall similarity, but some segment(s) is/are similar

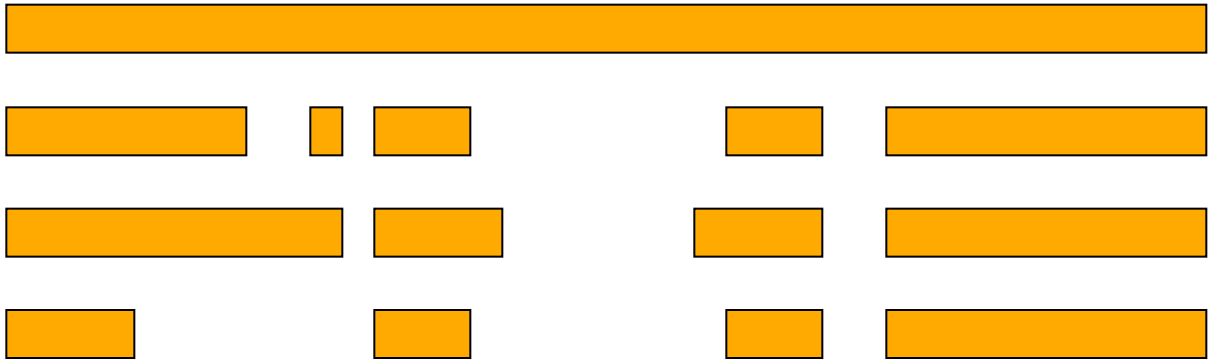
Types of Sequence Alignments - 2

Semi-Global



- Semi-global Alignment: end segments may not be similar

Multiple



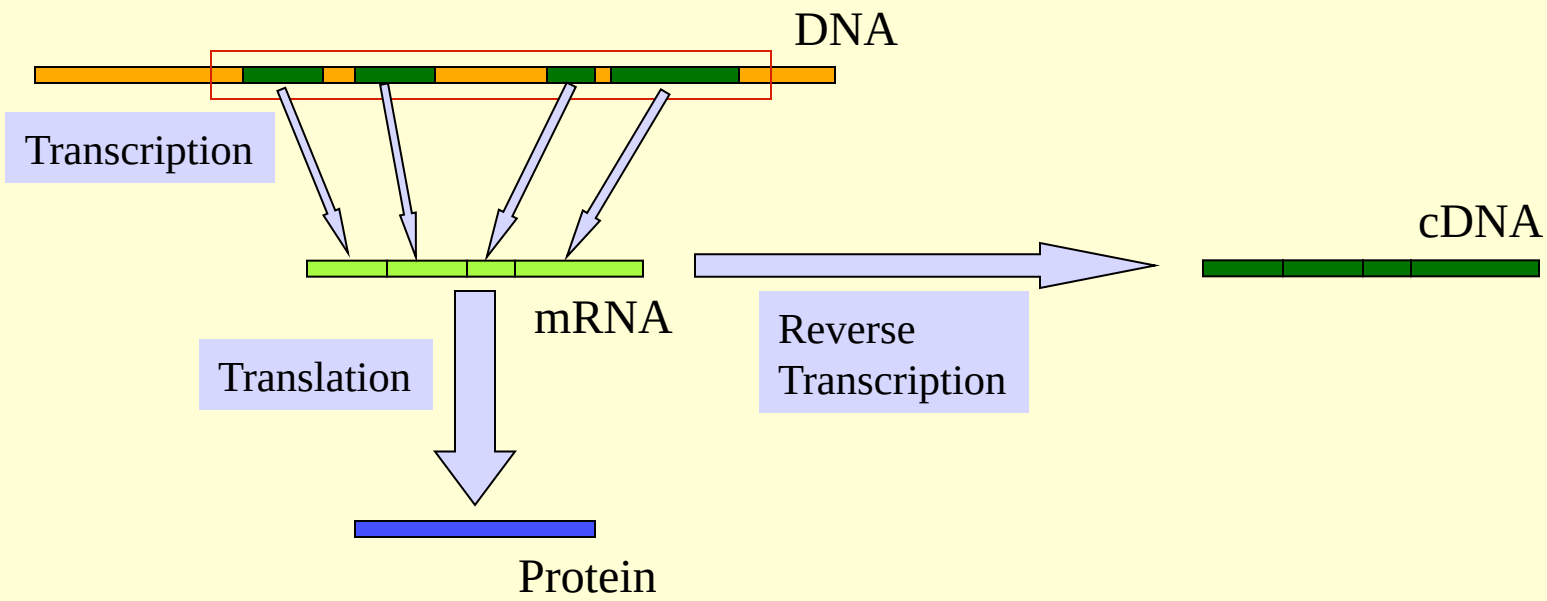
- Multiple Alignment: similarity between sets of sequences

Sequence Alignment

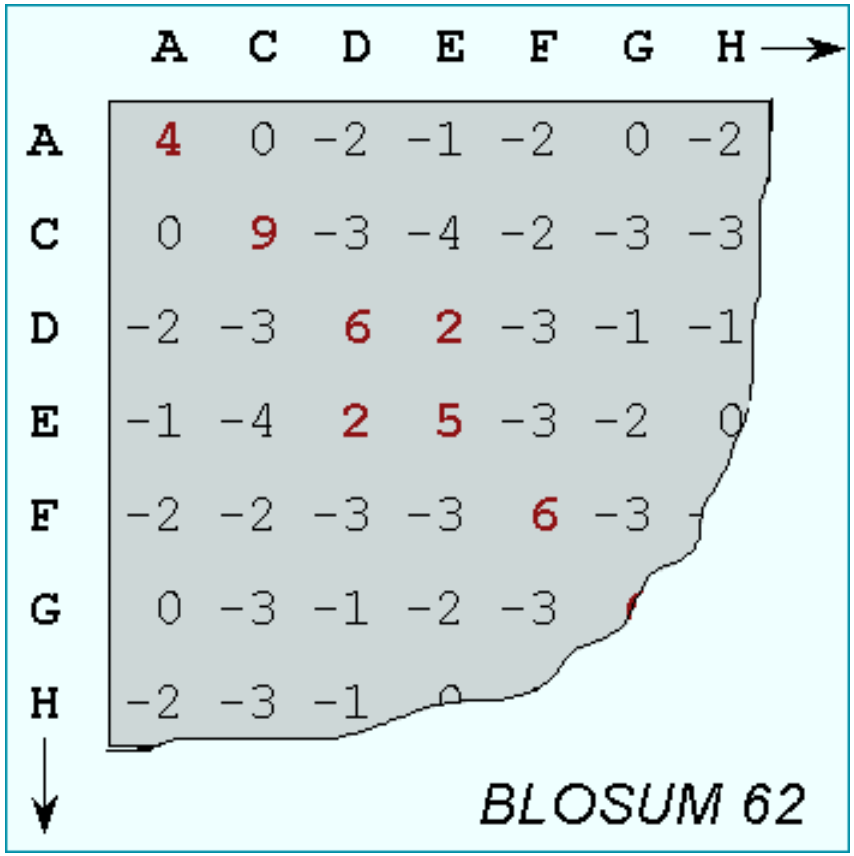
- Global:
  - Needleman-Wunsch-Sellers (1970).
- Local:
  - Smith-Waterman (1981)
  - Useful when commonality is small and global alignment is meaningless. Often unaligned portions "mask" short stretches of aligned portions. Example: comparing long stretches of anonymous DNA; aligning proteins that share only some motifs or domains.
- Dynamic Programming (DP) based.

Why gaps?

- Example: Finding the gene site for a given (eukaryotic) cDNA requires "gaps".
- What is cDNA? cDNA = Copy DNA



How to score mismatches?



	A	C	D	E	F	G	H →
A	4	0	-2	-1	-2	0	-2
C	0	9	-3	-4	-2	-3	-3
D	-2	-3	6	2	-3	-1	-1
E	-1	-4	2	5	-3	-2	0
F	-2	-2	-3	-3	6	-3	
G	0	-3	-1	-2	-3		
H ↓	-2	-3	-1	0			

BLOSUM 62

BLAST & FASTA

- FASTA

  [Lipman, Pearson '85, '88]

- Basic Local Alignment Search Tool

  [Altschul, Gish, Miller, Myers, Lipman '90]

BLAST Overview

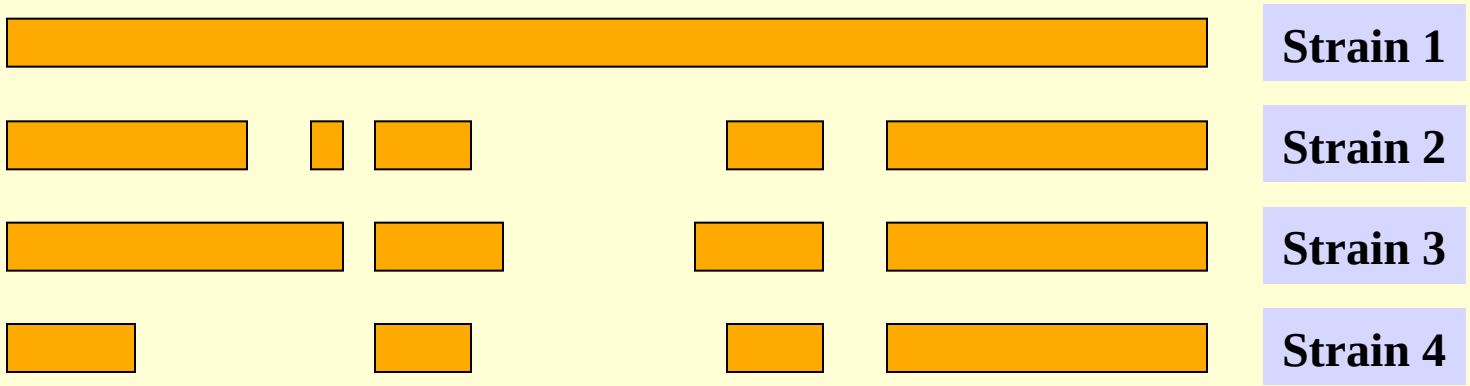
- Program(s) to search all sequence databases
- Tremendous Speed/Less Sensitive
- Statistical Significance reported
- WWWBLAST, QBLAST (send now, retrieve results later), Standalone BLAST, BLASTcl3 (Client version, TCP/IP connection to NCBI server), BLAST URLAPI (to access QBLAST, no local client)

BLAST Strategy & Improvements

- Lipman et al.: speeded up finding "runs" of "hot spots".
- Eugene Myers '94: "Sublinear algorithm for approximate keyword matching".
- Karlin, Altschul, Dembo '90, '91: "Statistical Significance of Matches"

Why Gaps?

- Example: Aligning HIV sequences.



BLAST Variants

- **Nucleotide BLAST**
  - **Standard blastn**
  - **MEGABLAST** (Compare large sets, Near-exact searches)
  - **Short Sequences** (higher E-value threshold, smaller word size, no low-complexity filtering)
- **Protein BLAST**
  - **Standard blastp**
  - **PSI-BLAST** (Position Specific Iterated BLAST)
  - **PHI-BLAST** (Pattern Hit Initiated BLAST; reg expr. Or Motif search)
  - **Short Sequences** (higher E-value threshold, smaller word size, no low-complexity filtering, PAM-30)
- **Translating BLAST**
  - **Blastx**: Search nucleotide sequence in protein database (6 reading frames)
  - **Tblastn**: Search protein sequence in nucleotide dB
  - **Tblastx**: Search nucleotide seq (6 frames) in nucleotide DB (6 frames)

BLAST Cont'd

- **RPS BLAST**
  - Compare protein sequence against Conserved Domain DB; Helps in predicting rough structure and function
- **Pairwise BLAST**
  - blastp (2 Proteins), blastn (2 nucleotides), tblastn (protein-nucleotide w/ 6 frames), blastx (nucleotide-protein), tblastx (nucleotide w/6 frames-nucleotide w/ 6 frames)
- **Specialized BLAST**
  - Human & Other finished/unfinished genomes
  - *P. falciparum*: Search ESTs, STSs, GSSs, HTGs
  - VecScreen: screen for contamination while sequencing
  - IgBLAST: Immunoglobin sequence database

BLAST Credits

- Stephen Altschul
- Jonathan Epstein
- David Lipman
- Tom Madden
- Scott McGinnis
- Jim Ostell
- Alex Schaffer
- Sergei Shavirin
- Heidi Sofia
- Jinghui Zhang

Databases used by BLAST

- **Protein**
  - nr (everything), swissprot, pdb, alu, individual genomes
- **Nucleotide**
  - nr, dbest, dbsts, htgs (unfinished genomic sequences), gss, pdb, vector, mito, alu, epd
- **Misc**

Rules of Thumb

- Most sequences with significant similarity over their entire lengths are homologous.
- Matches that are > 50% identical in a 20-40 aa region occur frequently by chance.
- Distantly related homologs may lack significant similarity. Homologous sequences may have few absolutely conserved residues.
- A homologous to B & B to C ⇒ A homologous to C.
- Low complexity regions, transmembrane regions and coiled-coil regions frequently display significant similarity without homology.
- Greater evolutionary distance implies that length of a local alignment required to achieve a statistically significant score also increases.

Rules of Thumb

- Results of searches using different scoring systems may be compared directly using normalized scores.
- If S is the (raw) score for a local alignment, the **normalized** score S' (in bits) is given by

$$S' = \frac{\lambda - \ln(K)}{\ln(2)}$$

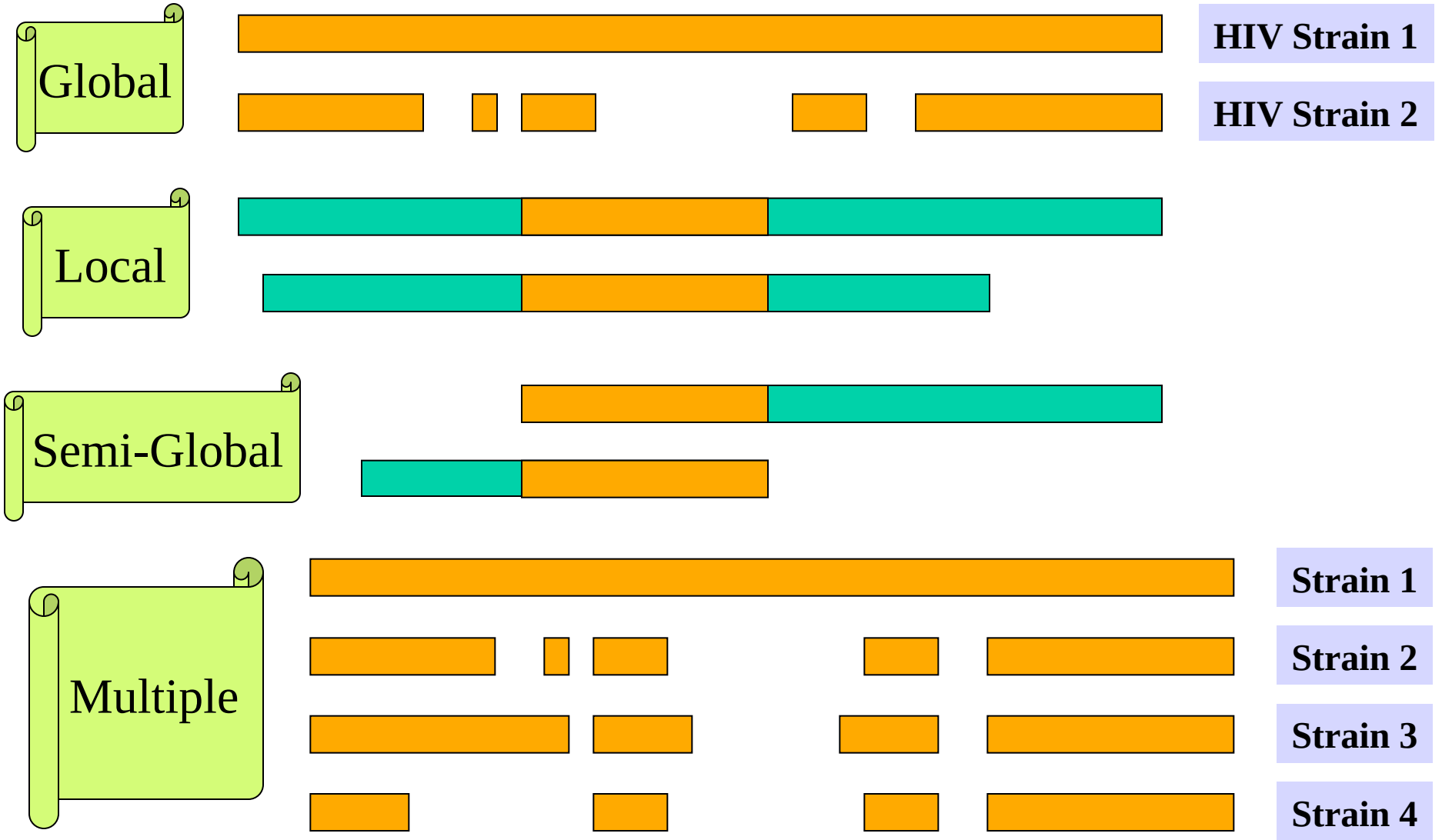
The parameters depend on the scoring system.

- **Statistically significant normalized score**,

$$S' > \log\left(\frac{N}{E}\right)$$

where E-value = E, and N = size of search space.

Types of Sequence Alignments



Global Alignment: An example

```
V: G A A T T C A G T T A
W: G G A T C G A
```

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0											
G	0											
A	0											
T	0											
C	0											
G	0											
A	0											

Given

$\delta[I, J]$ = Score of Matching
the I^{th} character of sequence V &
the J^{th} character of sequence W

Compute

$S[I, J]$ = Score of Matching
First I characters of sequence V &
First J characters of sequence W

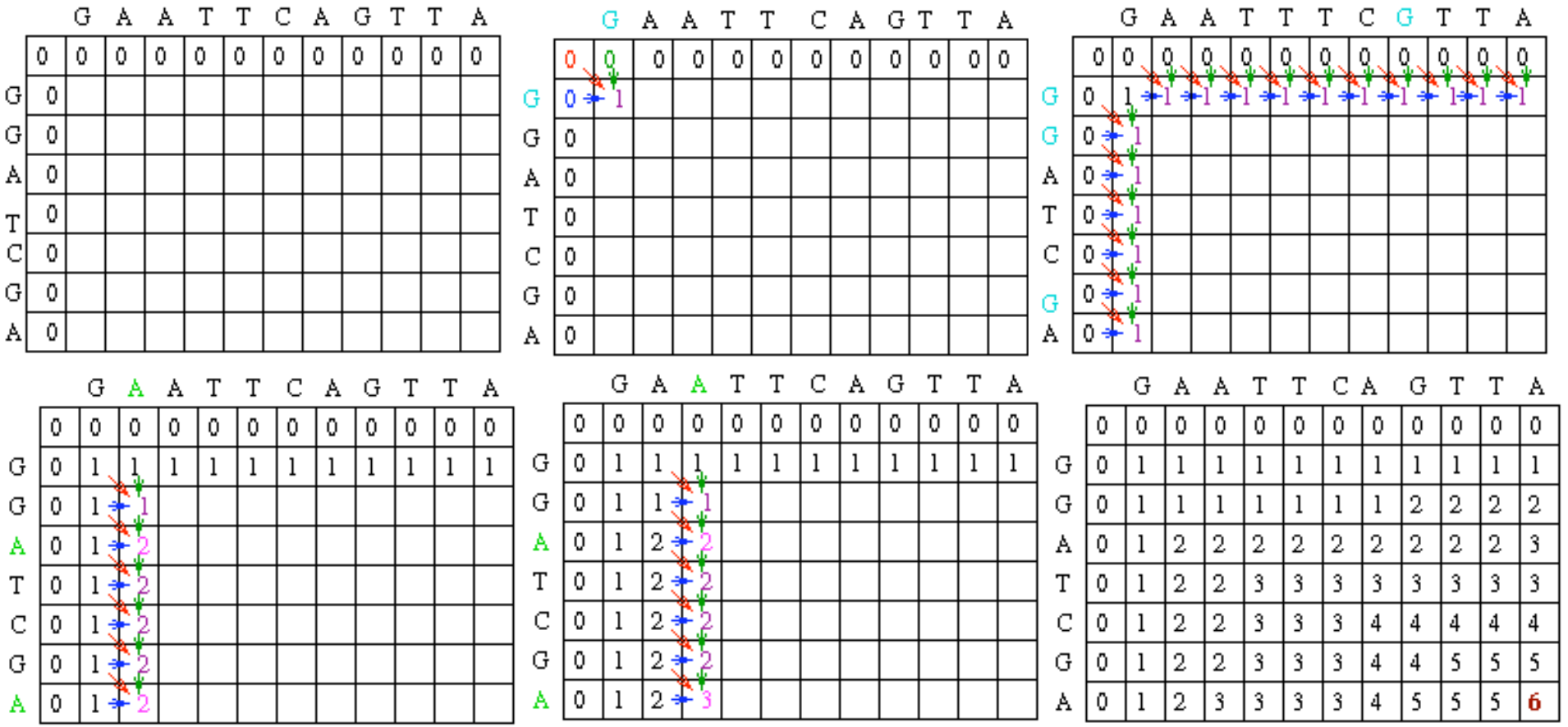
Recurrence Relation

$S[I, J] = MAXIMUM \{$
$S[I-1, J-1] + \delta(V[I], W[J])$,
$S[I-1, J] + \delta(V[I], —)$,
$S[I, J-1] + \delta(— , W[J]) \}$

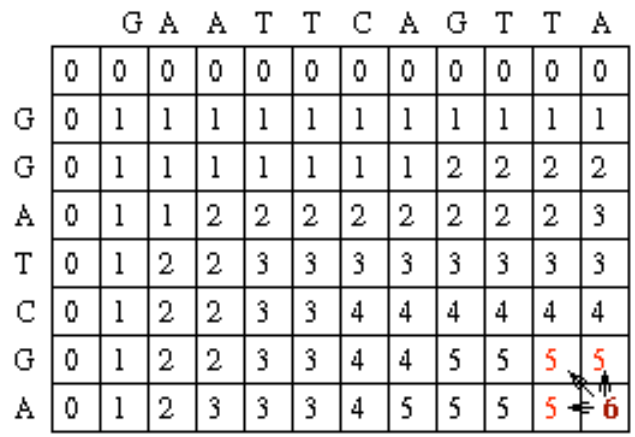
Global Alignment: An example

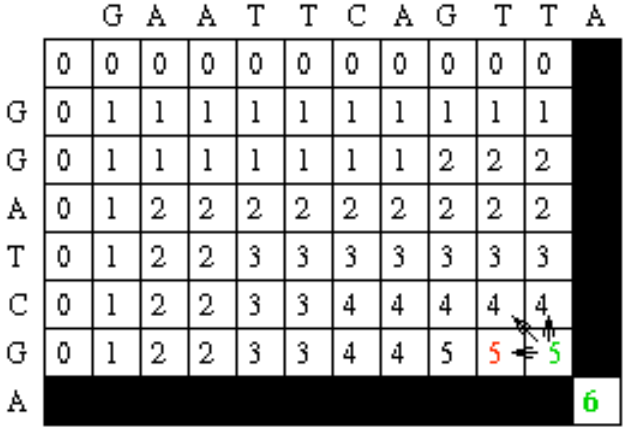
$$S[I, J] = \text{MAXIMUM}\{ S[I-1, J-1] + \delta(V[I], W[J]),\ S[I-1, J] + \delta(V[I], —),\ S[I, J-1] + \delta(— , W[J]) \}$$

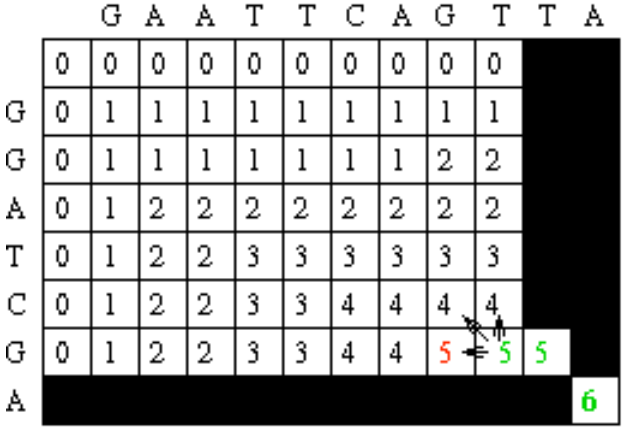
```
V: G A A T T C A G T T A
W: G G A T C G A
```

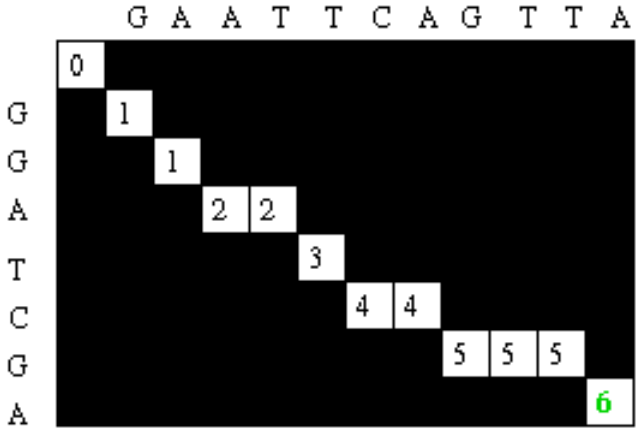


Traceback



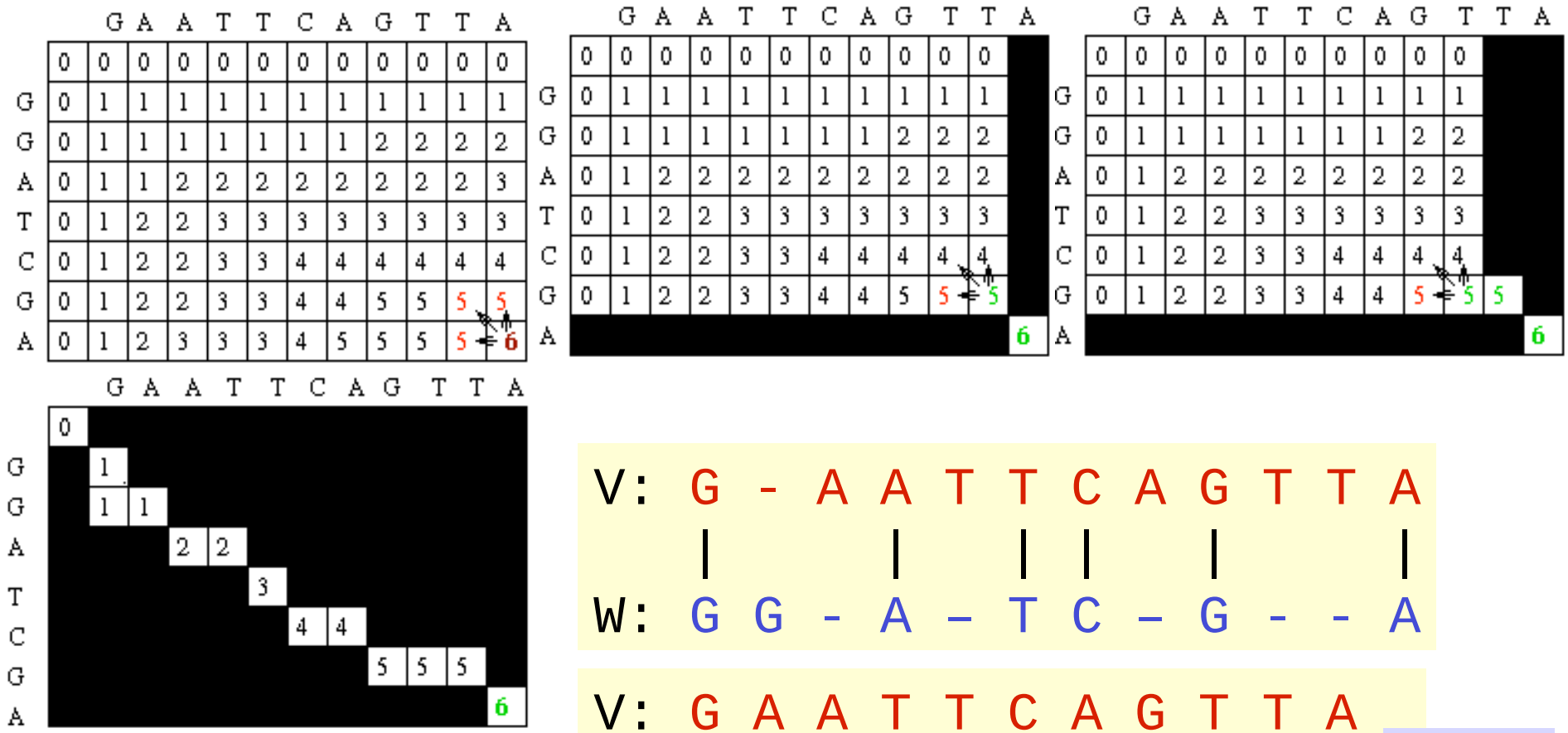







```
V: G A A T T C A G T T A
   |   |   | |   |     |
W: G G A - T C - G - - A
```

Alternative Traceback




```
V: G - A A T T C A G T T A
   |   |   | |   |     |
W: G G - A – T C – G - - A
```

```
V: G A A T T C A G T T A
   |   |   | |   |     |
W: G G A – T C – G - - A
```

Previous

Improved Traceback

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	×1	←1	← 1	← 1	← 1	← 1	← 1	×1	← 1	← 1	← 1
G	0	×1	↑1	↑1	↑1	↑1	↑1	↑1	×2	← 2	← 2	← 2
A	0	↑1	↑1	×2	← 2	← 2	← 2	×2	↑2	↑2	↑2	×3
T	0	↑1	← 2	↑2	×3	×3	← 3	← 3	← 3	×3	×3	↑3
C	0	↑1	↑2	↑2	↑3	↑3	×4	← 4	← 4	← 4	← 4	← 4
G	0	↑1	↑2	↑2	↑3	↑3	↑4	↑4	×5	← 5	← 5	← 5
A	0	↑1	↑2	×3	↑3	↑3	↑4	×5	↑5	↑5	↑5	×6

Improved Traceback

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	×1	←1	← 1	← 1	← 1	← 1	← 1	×1	← 1	← 1	← 1
G	0	×1	↑1	↑1	↑1	↑1	↑1	↑1	×2	← 2	← 2	← 2
A	0	↑1	↑1	×2	← 2	← 2	← 2	×2	↑2	↑2	↑2	×3
T	0	↑1	← 2	↑2	×3	×3	← 3	← 3	← 3	×3	×3	↑3
C	0	↑1	↑2	↑2	↑3	↑3	×4	← 4	← 4	← 4	← 4	← 4
G	0	↑1	↑2	↑2	↑3	↑3	↑4	↑4	×5	← 5	← 5	← 5
A	0	↑1	↑2	×3	↑3	↑3	↑4	×5	↑5	↑5	↑5	×6

Improved Traceback

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	×1	←1	← 1	← 1	← 1	← 1	← 1	×1	← 1	← 1	← 1
G	0	×1	↑1	↑1	↑1	↑1	↑1	↑1	×2	← 2	← 2	← 2
A	0	↑1	↑1	×2	← 2	← 2	← 2	×2	↑2	↑2	↑2	×3
T	0	↑1	← 2	↑2	×3	×3	← 3	← 3	← 3	×3	×3	↑3
C	0	↑1	↑2	↑2	↑3	↑3	×4	← 4	← 4	← 4	← 4	← 4
G	0	↑1	↑2	↑2	↑3	↑3	↑4	↑4	×5	← 5	← 5	← 5
A	0	↑1	↑2	×3	↑3	↑3	↑4	×5	↑5	↑5	↑5	×6

```
V: G A - A T T C A G T T A
   |   |   |   |   |     |
W: G - G A – T C – G - - A
```

Subproblems

- Optimally align V[1..I] and W[1..J] for every possible values of I and J.
- Having optimally aligned
  - V[1..I-1] and W[1..J-1]
  - V[1..I] and W[1..J-1]
  - V[1..I-1] and W[1, J]

  it is possible to optimally align V[1..I] and W[1..J]

- $O(mn)$,
  where m = length of V,
  and n = length of W.

Generalizations of Similarity Function

- Mismatch Penalty = α
- Spaces (Insertions/Deletions, InDels) = β
- Affine Gap Penalties:
  (Gap open, Gap extension) = (γ,δ)
- Weighted Mismatch = $\Phi(a,b)$
- Weighted Matches = $\Omega(a)$

Alternative Scoring Schemes

		G	A	A	T	T	C	A	G	T	T	A
	0	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
G	-2	× 1	← -1	← -2	← -3	← -4	← -5	← -6	← -7	← -8	← -9	← -10
G	-3	↑-1	× -1	← -3	← -4	← -5	← -6	← -7	× -5	← -7	← -8	← -9
A	-4	↑-2	× 0	× 0	← -2	← -3	← -4	← -5	← -6	← -7	← -8	× -7
T	-5	↑-3	↑ -2	↑-2	× 1	← -1	← -2	← -3	← -4	← -5	← -6	← -7
C	-6	↑-4	↑ -3	↑-3	↑-1	× -1	× 0	← -2	← -3	← -4	← -5	← -6
G	-7	↑-5	↑-4	↑-4	↑-2	↑-3	↑-2	× -2	× -1	← -3	← -4	← -5
A	-8	↑-6	↑-5	↑-5	↑-3	↑-4	↑-3	× -1	↑-3	× -3	× -5	× -3

Match +1
Mismatch –2
Gap (-2, -1)

```
V: G A A T T C A G T T A
   |   | |   |   |     |
W: G G A T - C – G - - A
```

Local Sequence Alignment

- Example: comparing long stretches of anonymous DNA; aligning proteins that share only some motifs or domains.
- Smith-Waterman Algorithm

Recurrence Relations (Global vs Local Alignments)

- S[I, J] = MAXIMUM {
  S[I-1, J-1] + δ(V[I], W[J]),
  S[I-1, J] + δ(V[I], —),
  S[I, J-1] + δ(— , W[J]) }

Global Alignment

- S[I, J] = MAXIMUM { 0,
  S[I-1, J-1] + δ(V[I], W[J]),
  S[I-1, J] + δ(V[I], —),
  S[I, J-1] + δ(— , W[J]) }

Local Alignment

Local Alignment: Example

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	× 1	0	0	0	0	0	0	0	0	0	0
G	0	× 1	← 0	0	0	0	0	0	× 1	0	0	0
A	0	0	× 2	× 1	0	0	0	× 1	0	0	0	× 1
T	0	0	↑ 0	× 1	× 2	← 1	0	0	0	× 1	× 1	0
C	0	0	0	0	↑ 0	× 0	× 2	0	0	0	0	0
G	0	0	0	0	0	0	0	0	× 1	0	0	0
A	0	0	× 1	× 1	0	0	0	× 1	0	0	0	× 1

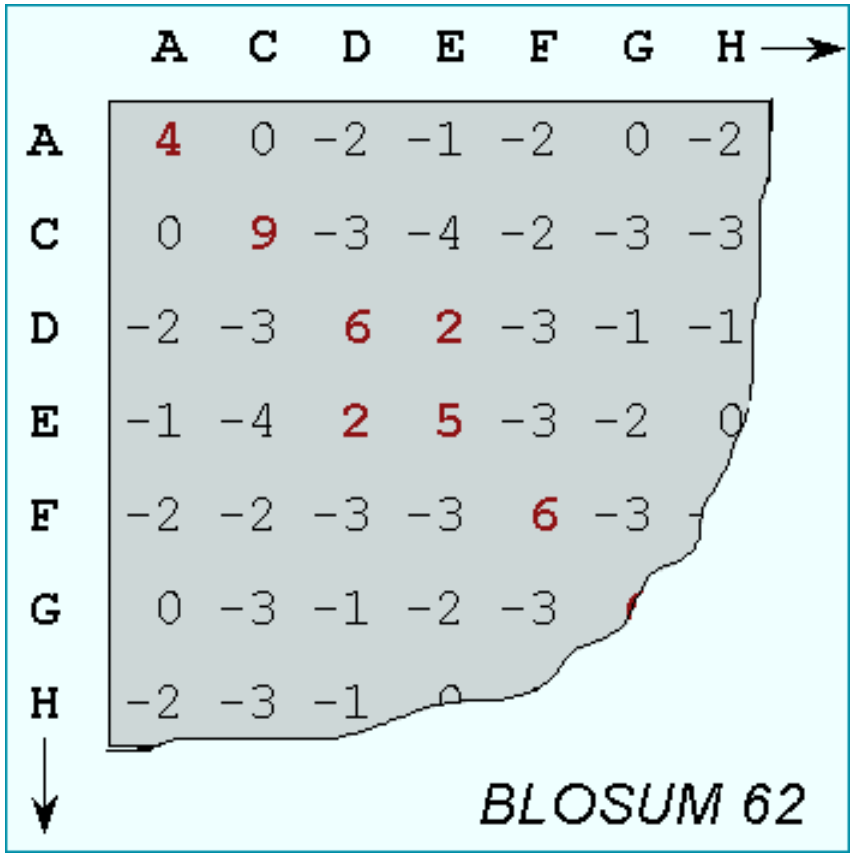
Match +1
Mismatch –1
Gap (-1, -1)

```
V: - G A A T T C A G T T A
     |   | |   |
W: G G - A T - C – G - - A
```

Properties of Smith-Waterman Algorithm

- How to find all regions of "high similarity"?
  - Find all entries above a threshold score and traceback.
- What if: Matches = 1 & Mismatches/spaces = 0?
  - Longest Common Subsequence Problem
- What if: Matches = 1 & Mismatches/spaces = $-\infty$?
  - Longest Common Substring Problem
- What if the average entry is positive?
  - Global Alignment

How to score mismatches?



BLOSUM n Substitution Matrices

- For each amino acid pair a, b
  - For each BLOCK
    - Align all proteins in the BLOCK
    - Eliminate proteins that are more than n% identical
    - Count F(a), F(b), F(a,b)
    - Compute Log-odds Ratio

$$\log\left(\frac{F(a,b)}{F(a)F(b)}\right)$$