GEM4 - AUGUST 2006 8/8/06

I INTRODUCTION TO MOLECULAR CELL BIOLOGY

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REFERENCE TEXT BOOK ⇒ MOLECULAR CELL BIOLOGY, LODISH et al

HUMANS → $> 10^{13}$ CELLS
- # OF TYPES ~ 200
- DIV/SEC ~ 10^7
- DIV/LIFETIME ~ 10^{16}

} ALL FROM ONE SINGLE CELL!!

DIVISION → MULTIPLY CELLS
↳ TRANSMIT INFORMATION (DNA)

CELL CYCLE
- RESTING CELL (G1-G0)
- DNA REPLICATION (S-PHASE)
- RNA & PROTEIN SYNTHESIS (G2)
- CELL DIVISION (MITOSIS)

THERE ARE CHECKPOINTS THAT ALLOW PROGRESS THROUGH THE CELL CYCLE

DNA STRUCTURE & TEMPLATE DRIVEN REPLICATION

INFORMATION ENCODED IN LINEAR ORDER OF BASES (C, G, T, A)

DNA → HELICAL SHAPE ⇒ TWO COMPLIMENTARY STRANDS

REPLICATION ⇒ SEMI-CONSERVATIVE

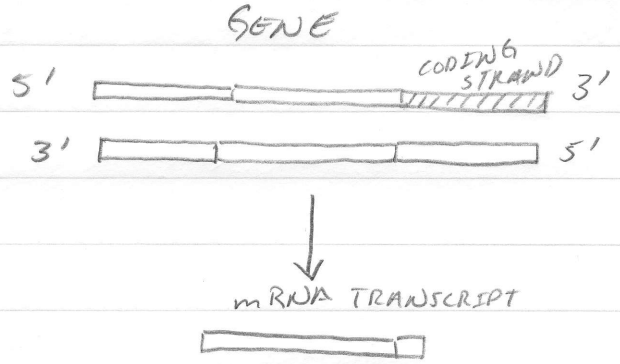
FROM INFORMATION TO MACHINERY

GENE —(COPY, EDIT; mRNA, tRNA, INTERPRETER)→ PROTEIN —(SELF-ASSEMBLY INSTRUCTIONS; PROTEIN FOLDING)→ EXECUTE MACHINERY

GENE: 4 LETTER CODE (BASES)

PROTEIN: 20 LETTER CODE (AMINO ACIDS)

INFORMATION TRANSFER: TRANSCRIPTION



TRANSCRIPTION MACHINERY:

RNA POLYMERASE ⇒ GENERATES mRNA

↳ OPTICAL TRAPPING EXPERIMENTS MEASURE PROCESSIVITY OF POLYMERASE

ORGANIZATION OF EUKARYOTIC GENES

IN A CHROMOSOME THERE ARE ACTIVE AND INACTIVE GENES

DIFFERENT SITES HAVE DIFFENT ENCODINGS.

INFORMATION TRANSFER: TRANSLATION ⇒ RIBOSOME

mRNA transcript ⟶ POLYPEPTIDE

AMINO ACIDS ARE ENCODED IN A SEQUENCE OF THREE BASES.

ERROR RATE/CODON $\sim 5 \times 10^{-4}$

PROTEIN ARCHITECTURE

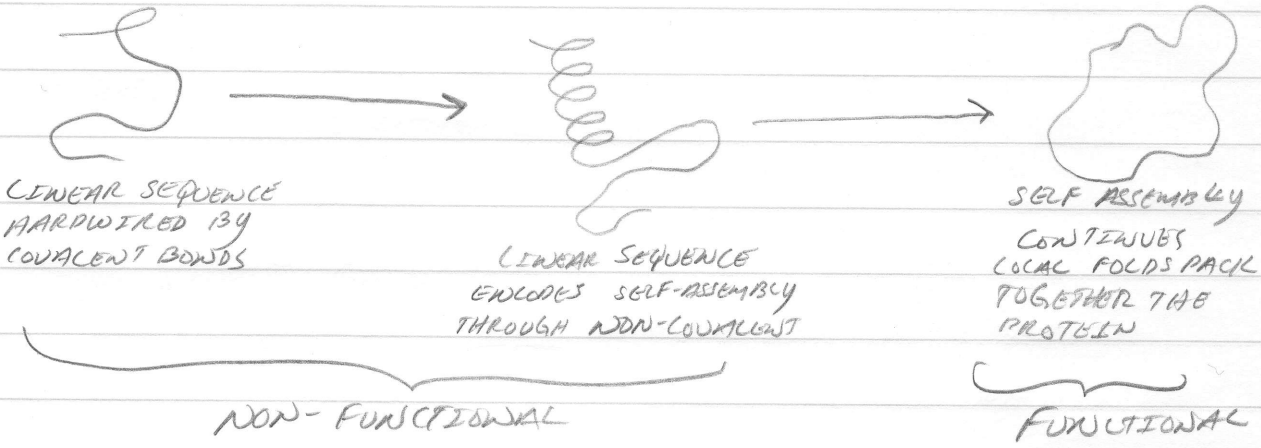
LINEAR ORGANIZATION DEFINES FOLDING PROPERTIES DUE TO CHARGES AND HYDROPHOBICITY OF EACH A.A

ONCE FOLDED, THE PROTEINS IS READY TO PERFORM ITS FUNCTION.

FOLDING PROBLEM : MANY ($\sim 10^{67}$) CONFORMATIONS POSSIBLE FOR A 150 AA PROTEIN !!

$\sim 10^{48}$ YEARS TO FOLD !!

IN VIVO FOLDING TIME ~ 0.1 SEC



IN VIVO SELF-ASSEMBLY : ACTIN FILAMENTS

F-ACTIN HAS DIRECTIONALITY → POLYMERIZES FASTER FROM ONE END (BARBED OR ⊕ END)

CLOSE TO THE MEMBRANE THE FILAMENT'S END ARE FREE SO MONOMERS CAN BE ADDED AND CELL CAN MOVE.

CHAPERONES HELP PROTEINS TO FOLD RIGHT AFTER THE RIBOSOMES

CROWDING OF MIS-FOLDED PROTEIN CAN LEAD TO AMYLOID FORMATION WHICH CAN LEAD TO UNWANTED BEHAVIOR / FUNCTION

GENOMES: SEQUENCING STARTED EARLY 80's

DNA ORGANIZATION: PACKAGING

dsDNA → NUCLEOSOME → CHROMATIN → EXTENDED SCAFFOLD
CHROMOSOME ← CONDENSED SCAFFOLD ←

INFORMATION STORAGE

FIDELITY	ERROR RATE
CHEMICAL COMPLEMENTARITY	10^{-1}
PROOFREADING & SELECTION	10^{-7}
REPAIR	10^{-10}

II TRANSCRIPTIONAL REGULATION

DR. KEVIN TAN (NATIONAL UNIVERSITY OF SINGAPORE)

CELLS ARE: - HIGHLY DIFFERENTIATED
- DIFFER IN STRUCTURE & FUNCTION
- YET IDENTICAL GENETIC CODE !!!

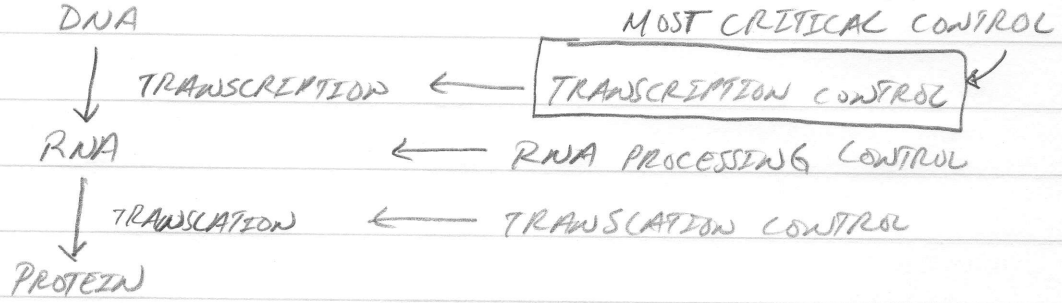
CLONING FROM EPITHELIAL CELL (UDDER) ⇒ HAS ALL GENETIC INFORMATION ⇒ DOLLY THE SHEEP!

DIFFERENT CELL TYPES SYNTHESIZE DIFFERENT PROTEINS

MICROARRAYS ⇒ GLOBAL VIEW OF UPREGULATED AND DOWNREGULATED GENES

CENTRAL DOGMA ⇒ REPLICATION → TRANSCRIPTION → TRANSLATION

GENE EXPRESSION → REGULATED AT THE STEPS IN THE PATHWAY FROM DNA TO RNA TO PROTEIN



TRANSCRIPTION CONTROL ⇒ CONTROL AT START IS MORE EFFICIENT THAN AT THE END, LESS MATERIAL IS "WASTED".

GENETIC SWITCHES ⇒ TRANSCRIPTION FACTORS (REGULATORY PROTEINS)

REGULATORY PROTEINS BIND TO MAJOR GROOVE OF DNA

- ↳ HELIX-TURN-HELIX
- ↳ ZINC FINGERS
- ↳ LEUCINE ZIPPER
- ↳ OTHERS

WHY BIND TO MAJOR GROOVE? IT CONTAINS MORE INFORMATION AND VARIETY OF BINDING SITES, MORE EXPOSED BASES.

REPRESSORS AND ACTIVATORS

- TRYPTOPHAN REPRESSOR ⇒ W/O TRYPTOPHAN, REPRESSOR CANNOT BIND
  ⇒ W TRYPTOPHAN, IT BINDS TO REPRESSOR, REPRESSOR BINDS TO SITE AND BLOCKS RNA POLYMERASE

EUKARYOTES IS VERY COMPLEX

- REGULATORY PROTEINS CAN BIND 1000bp AWAY FROM PROMOTER
- RNA Pol II REQUIRES TRANSCRIPTION FACTORS
- PACKAGING IN CHROMATIN ALLOW FOR REGULATION NOT AVAILABLE OTHERWISE

ENHANCERS ⇒ ENHANCE TRANSCRIPTION
ACTIVATORS BIND TO ENHANCER TO ACTIVATE TRANSCRIPTION

REGULATION OF EUKARYOTIC TRANSCRIPTION

- ATTRACT, POSITION AND ACTIVATE Pol II
- MORE EFFICIENT

CHROMATIN REMODELING CAN PROMOTE OR INHIBIT TRANSCRIPTION

- ACETYLATION OF HISTONES → PROMOTES TRANSCRIPTION
- DE-ACETYLATION → REPRESSES TRANSCRIPTION
- METHYLATION → ASSOCIATED WITH INACTIVATION
- PHOSPHORYLATION → CONDENSING AND LOOSENING CHROMATIN

INSULATORS ⇒ DNA SEQUENCES THAT PREVENT GENE REGULATORY PROTEINS FROM INFLUENCING DISTANT GENES

- THEY BLOCK ENHANCERS
- ARE LOCATED BETWEEN ENHANCERS AND PROMOTERS

REGULATORY SYSTEM IS VERY COMPLEX WHICH IS RELATED TO THE COMPLEXITY OF EUKARYOTE ORGANISMS.