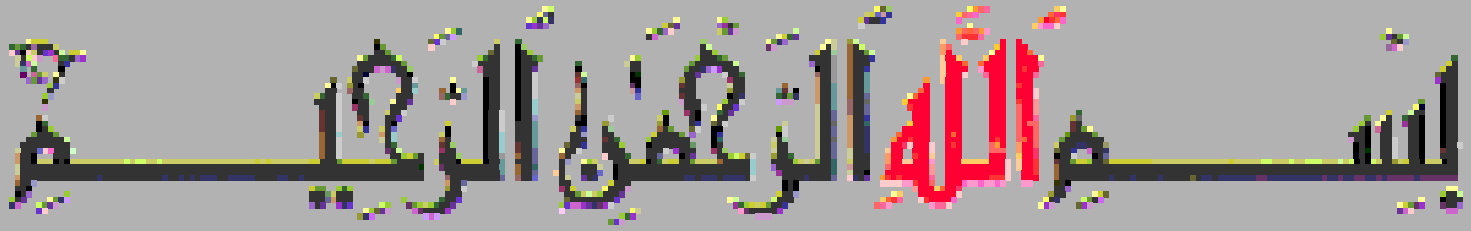

In the Name of Allah, Most Compassionate, Most Merciful

Coarse Control of Metabolism

Prof. Arjumand S. Warsy
Prof. Arjumand S. Warsy

Expression of Genes of enzymes

There are estimated 25,000 – 30,000 genes in the human genome in each cell. Many for enzymes

- Only a fraction are expressed/ tissue.
- Different genes are expressed in different tissues
- Route of expression of genes differs significantly where some gene products are produced in large amounts, others in very small amounts& some are not produced at all in some cells

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- This gives each tissue its distinctive character and function.
- This is the basic mechanism underlying cell differentiation

CONTROL OF GENE EXPRESSION

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Types of Genes in Cells

House-keeping genes (Constitutive E)

Genes for products that are required at all times and hence the gene is expressed at almost a constant rate.

Inducible Genes

Genes for some gene products that rise and fall in response to molecular signals.

These genes can be induced and repressed.

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How is the Expression of an Enzyme Gene regulated?

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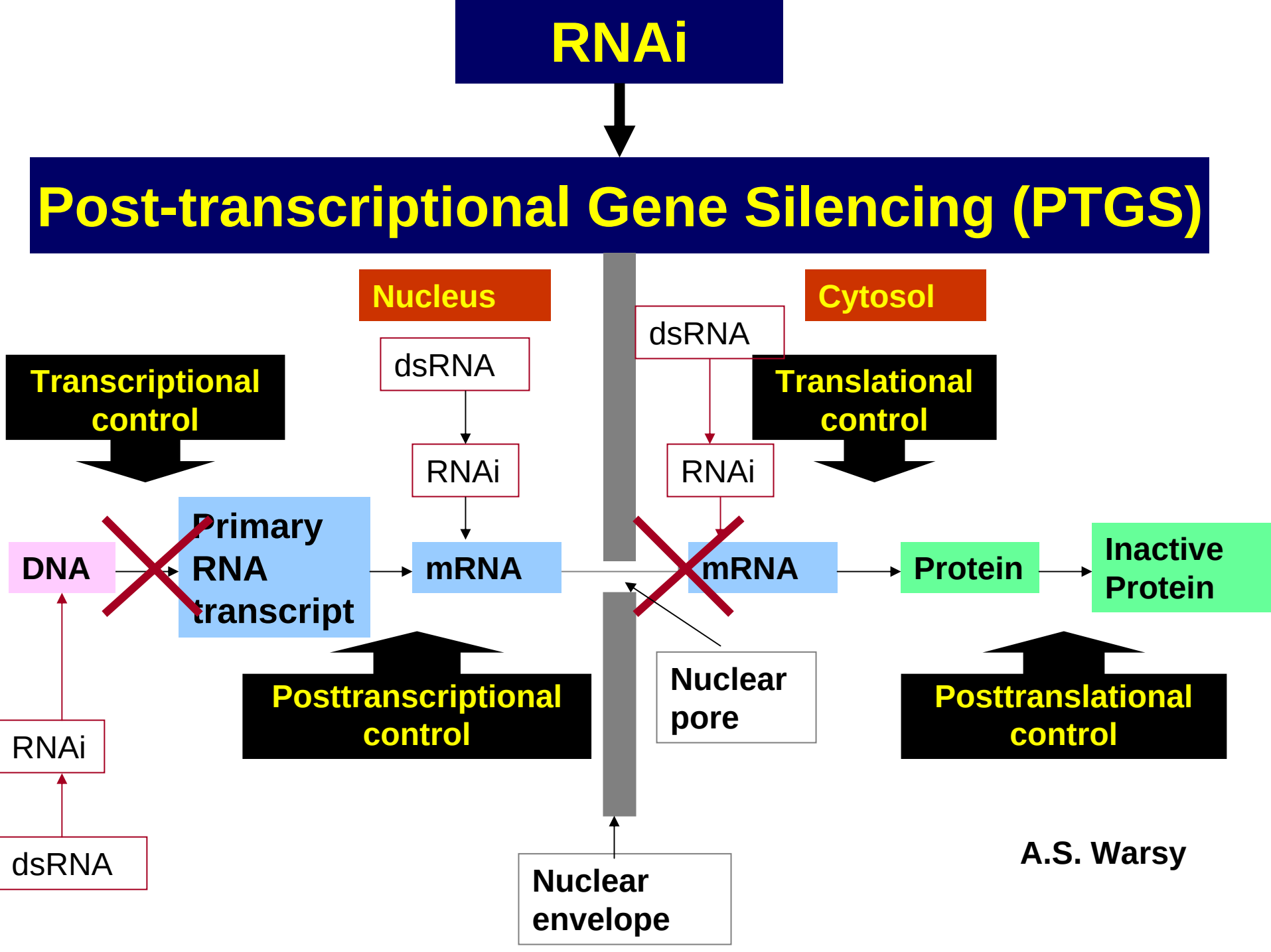
RNAi
Post-transcriptional Gene Silencing (PTGS)
Nucleus
Cytosol
dsRNA
dsRNA
Transcriptional control
Translational control
RNAi
RNAi
DNA
Primary RNA transcript
mRNA
mRNA
Protein
Inactive Protein
Posttranscriptional control
Nuclear pore
Posttranslational control
RNAi
dsRNA
Nuclear envelope
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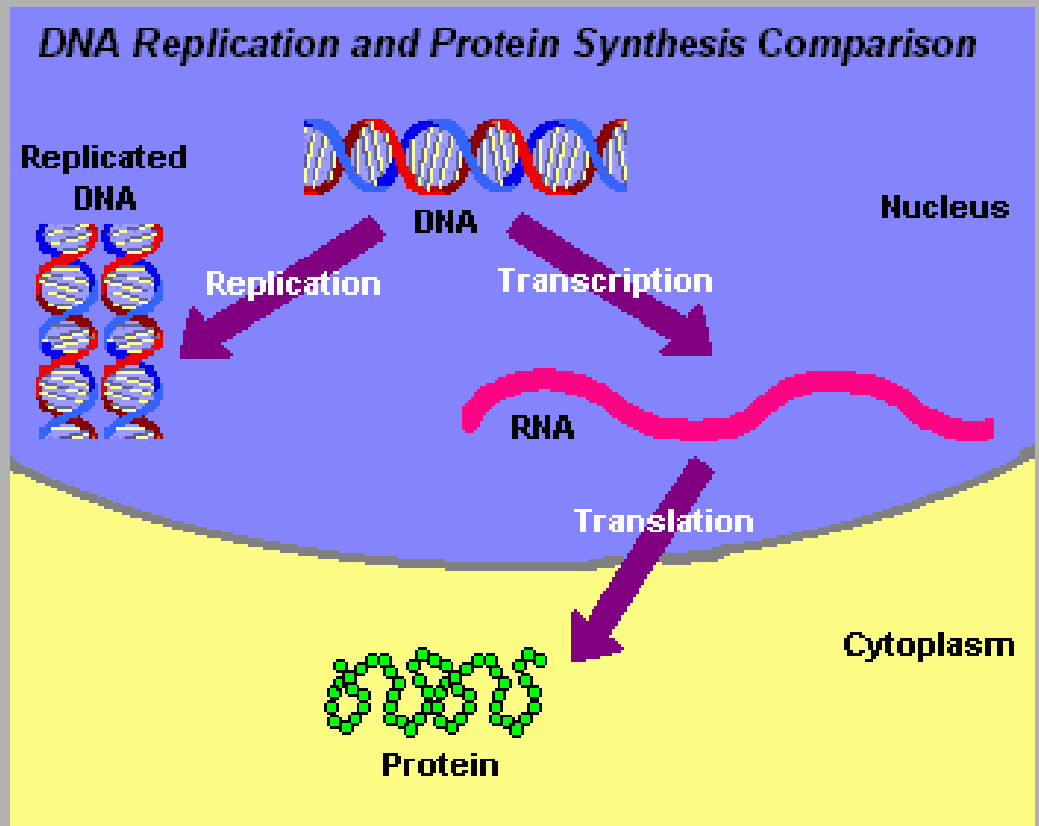
Coarse Control

Regulation of Gene Expression

Regulation of Transcription



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GENE EXPRESSION

Gene expression depends on:

- Chromatin structure.
- Activity of RNA polymerase.
- Sequence in the upstream activator and enhancer.
- Regulatory proteins.

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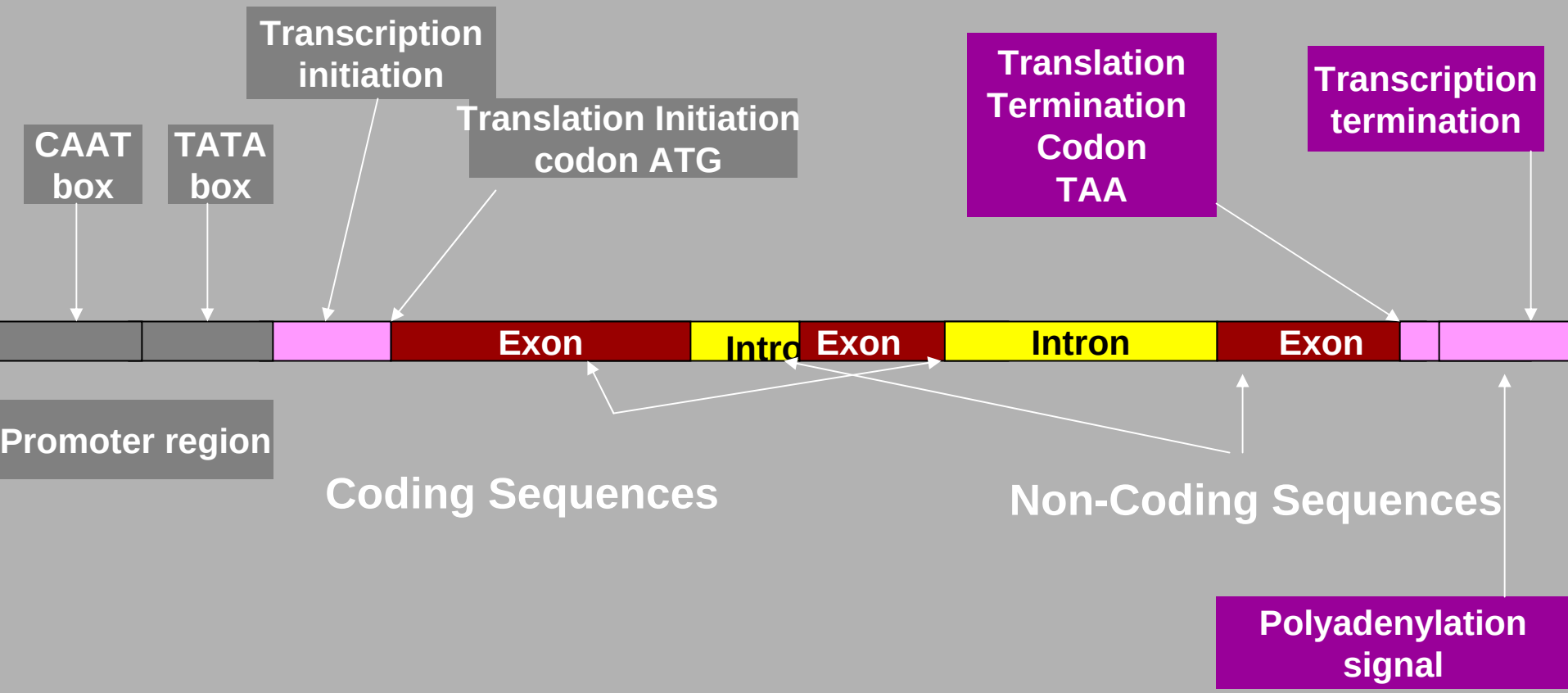
Genes-exons and introns and promoter regions



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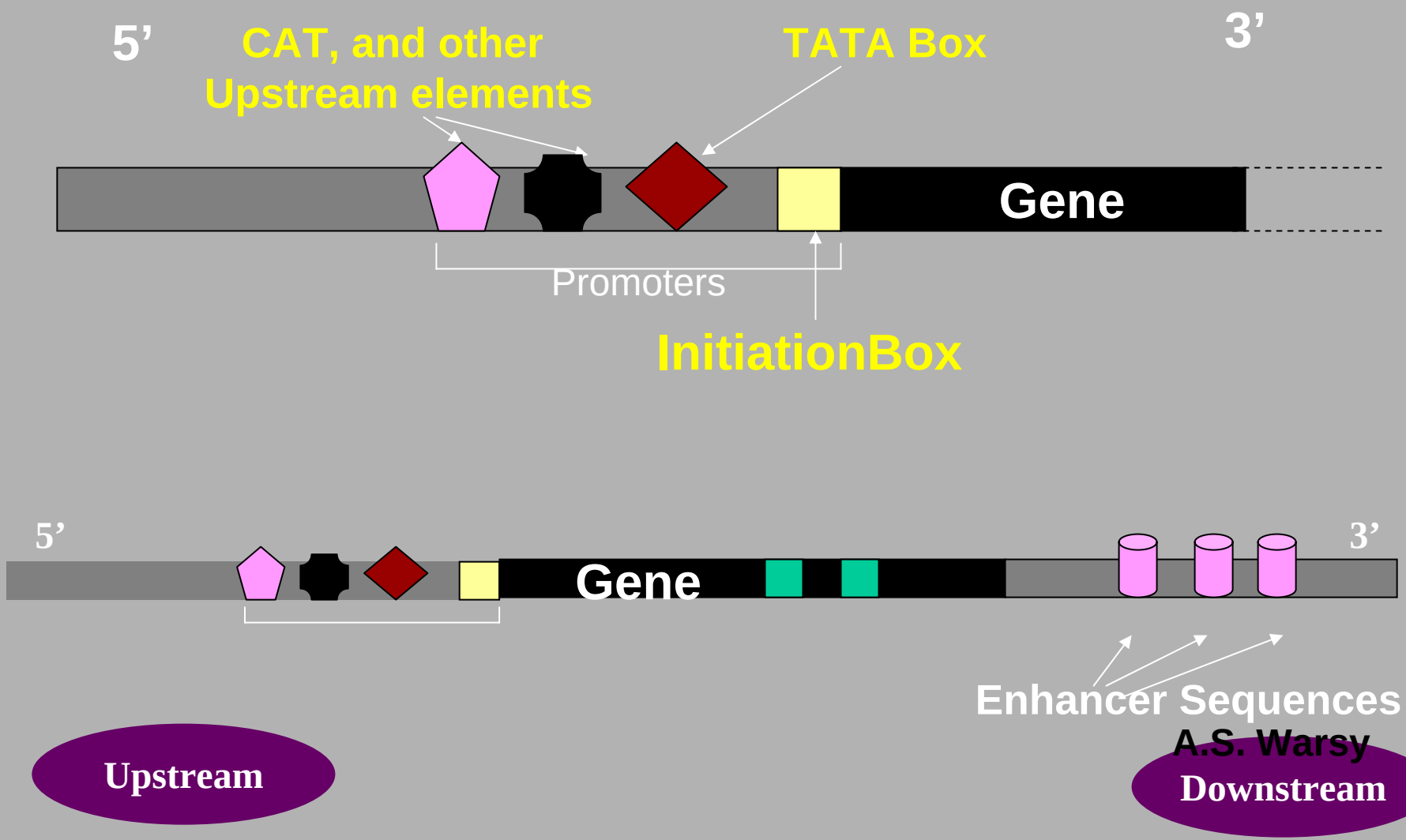
Promoter and Enhancer layouts in Eucaryotes



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REGULATION OF PROMOTERS

- Most eucaryotic promoters are *positively regulated*,i.e.
  - RNA polymerase has no or very little affinity for their promoter.
  - One or more activator proteins bind to promoter.
  - This influences binding of RNA polymerase.

Contd…….

REGULATION OF PROMOTERS ….Contd

- Some promoters are *negatively regulated*, i.e.
  - RNA polymerase binds promoter and initiates transcription.
  - Repressor proteins bind to promoter and inhibits transcription.

Transcription Factors

- Specialized proteins that **regulate gene expression**.
- Have four **Domains**:
  - for **binding** to a specific sequence on the **DNA**
  - for **binding** to the **RNA polymerase II** complex
  - for **entering the nucleus**
  - for **responding to stimuli** which signal switching on or off a gene.
- Transcription factors for a specific gene **appear** mainly **in cells** where that **gene has to be expressed**.

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TRANSCRIPTION FACTORS

- Proteins
- May be:
  - inducers - increase transcription, or
  - repressors – decrease transcription.
- Recognizes and bind to:
  - Consensus sequences i.e.
    - TATA box (TATAAA)
    - GC Box (GGGCGG)
    - CCAAT box (GCCAAT)
  - Enhancers

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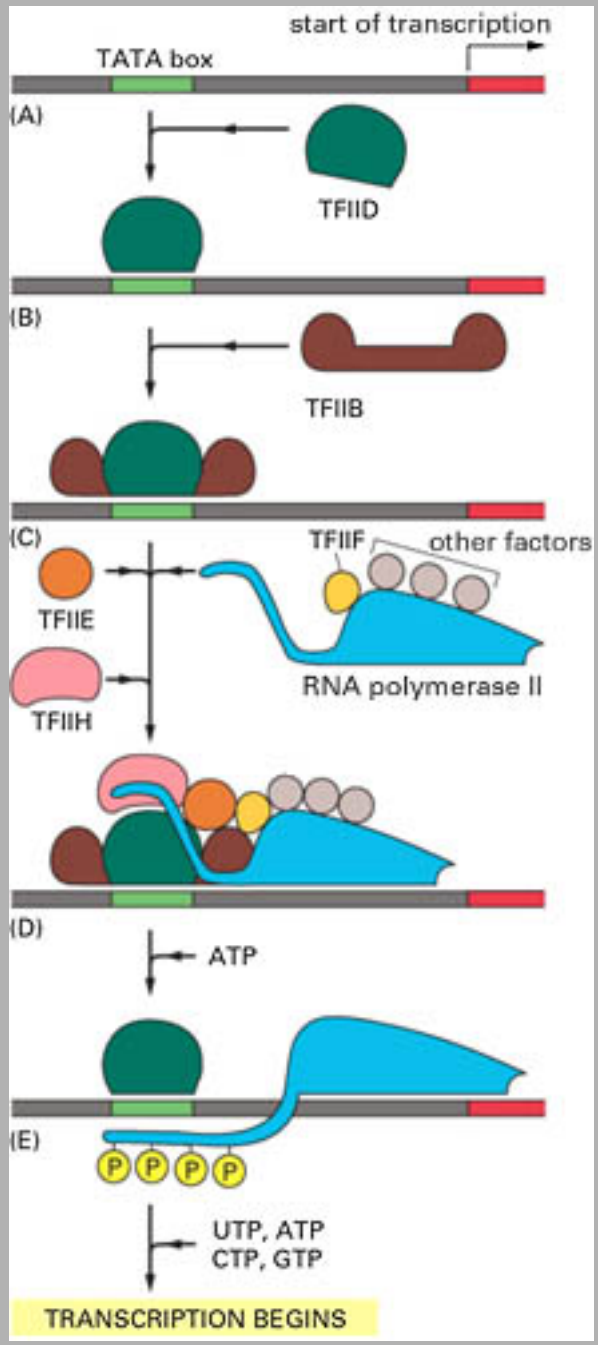
start of transcription
TATA box
(A)
TFIID
(B)
TFIIB
(C)
TFIIF
other factors
TFIIE
TFIIH
RNA polymerase II
(D)
ATP
(E)
P
P
P
P
UTP, ATP
CTP, GTP
TRANSCRIPTION BEGINS

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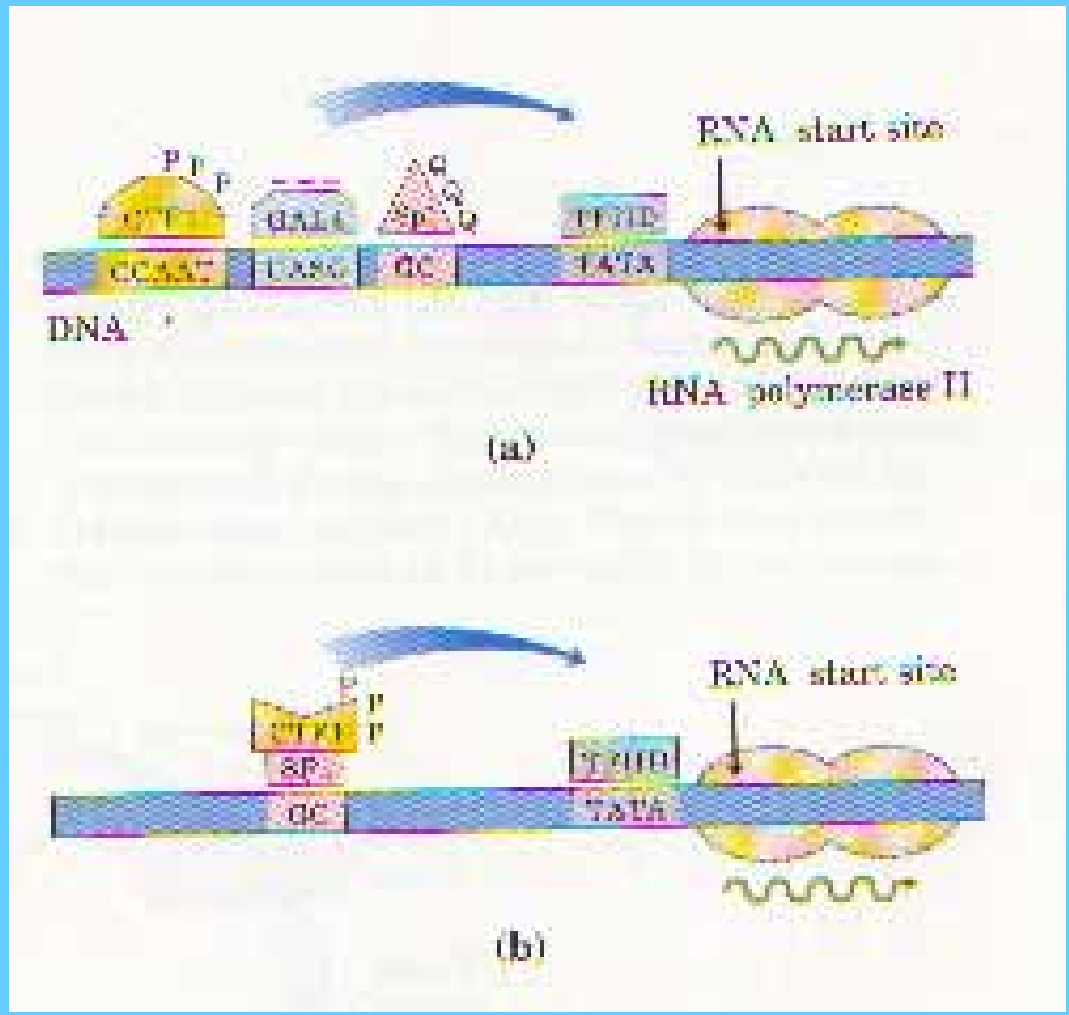
Positive Control of Gene Expression



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EXAMPLES OF TRANSCRIPTION FACTORS

- Sp1 (Has Gln-rich domain; Zn finger protein).
- CTF1 (Has pro-rich domain)
- C/EBP (Has pro-rich domain, Lencine Zipper)
- GALA (Zn finger, Acidic activation domain)

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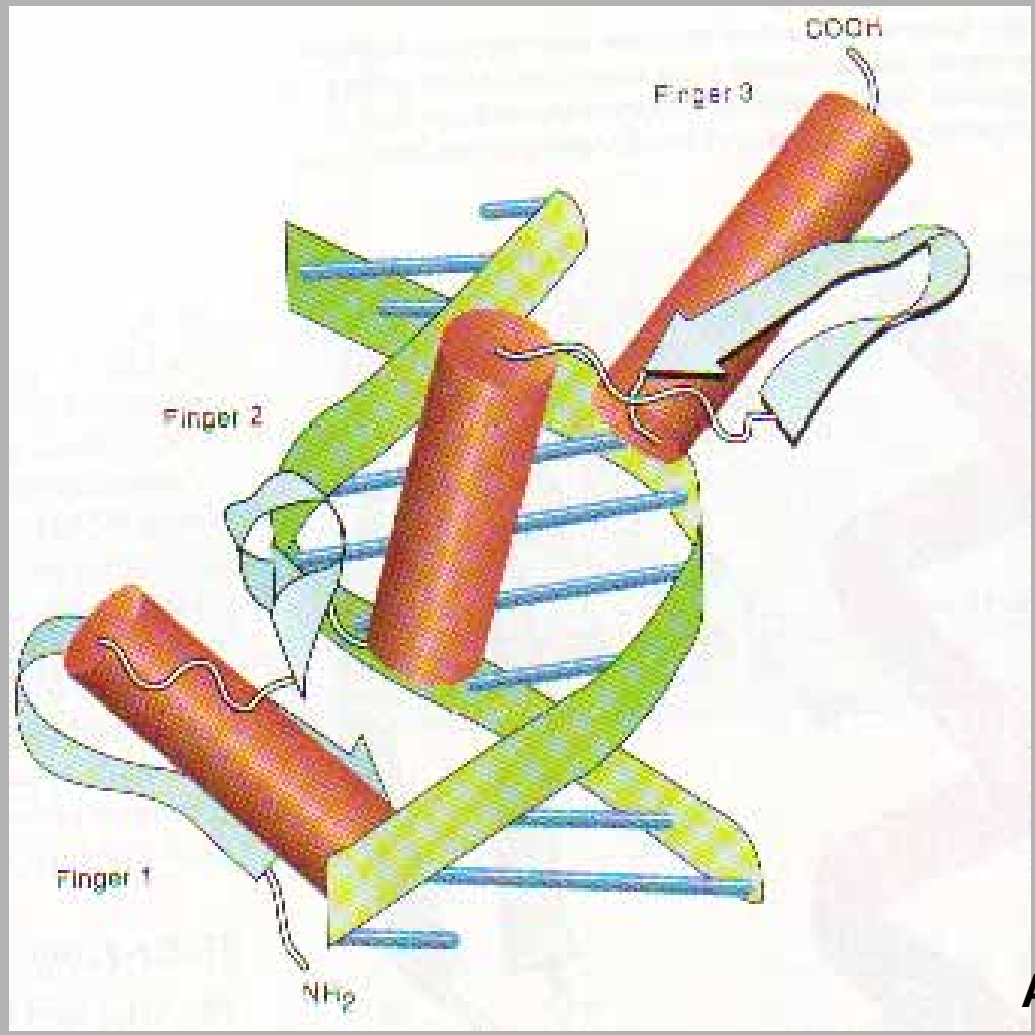
DNA BINDING DOMAIN OF REGULATORY PROTEINS

- Zinc finger: About 30 a.a residues,

  Four are either Cys or 2Cys and 2 His, coordinated to single Zn^{++}

Contd….

Zinc Finger Motif & DNA Interaction



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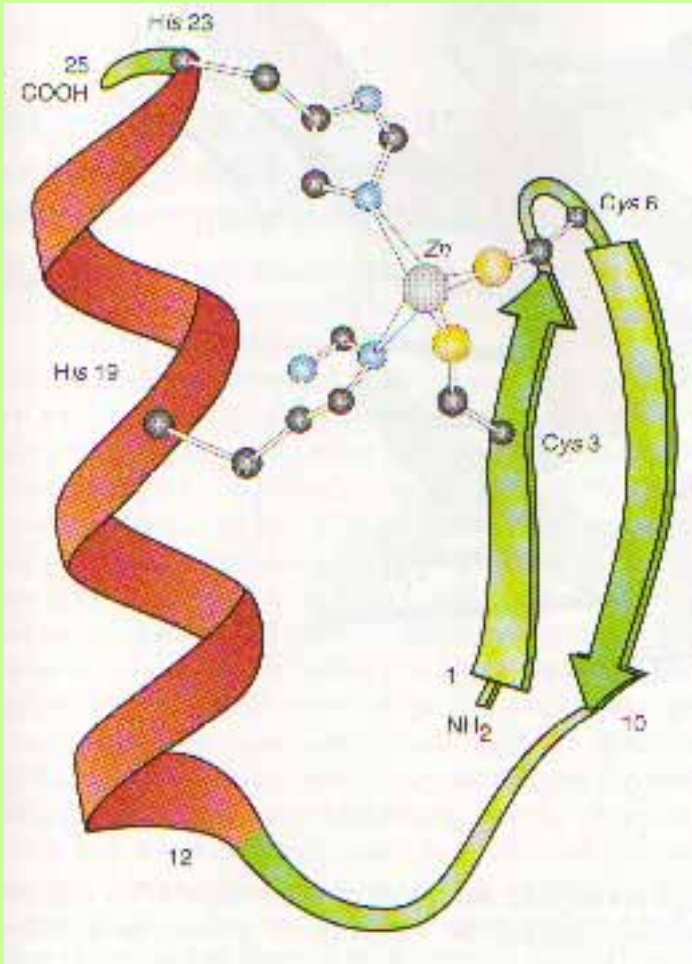
A Zinc Finger Motif



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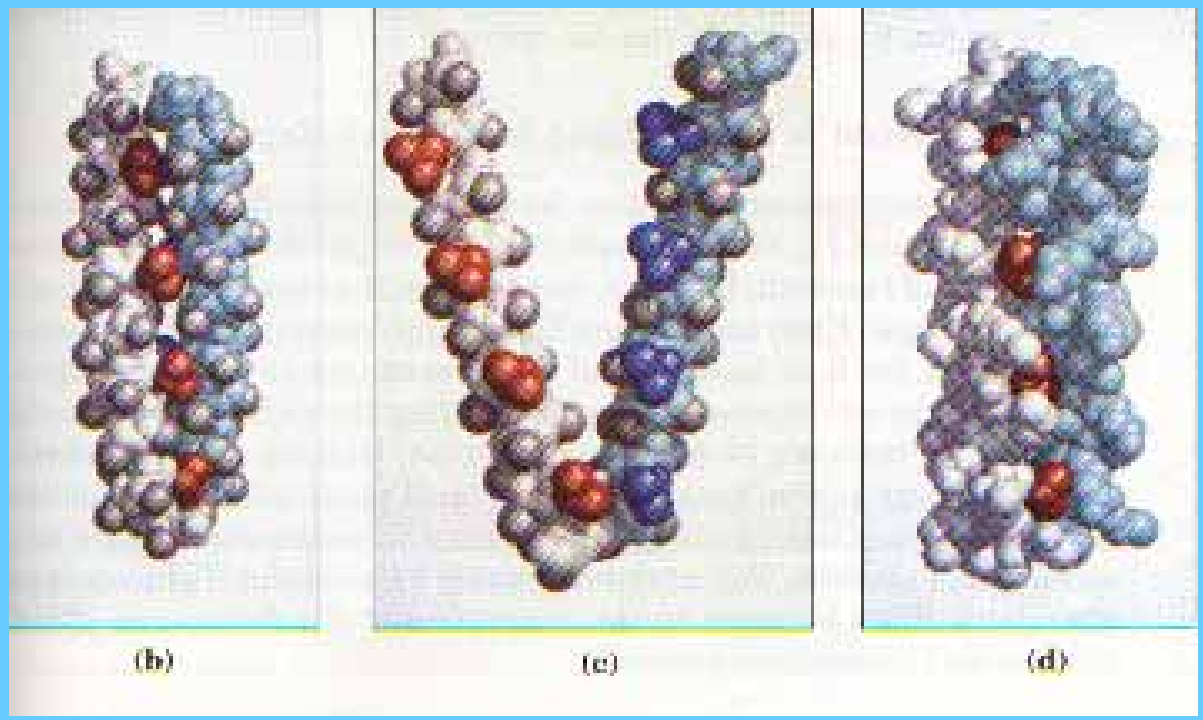
DNA BINDING DOMAIN OF REGULATORY PROTEINS …. Contd

- The Helix – turn Helix: Two short α-helical sequences (7-9 a.a. long) separated by a β turn (~ 20 a.a). One of the α-helices is the recognition helix and binds DNA major groove.

DNA BINDING DOMAIN OF REGULATORY PROTEINS …. Contd

- The leucine zipper: Has amphipathic α-helix, with hydrophobic a.a. on one size. Every 7^{th} a.a. is a leucine.

Leucine Zipper Motif



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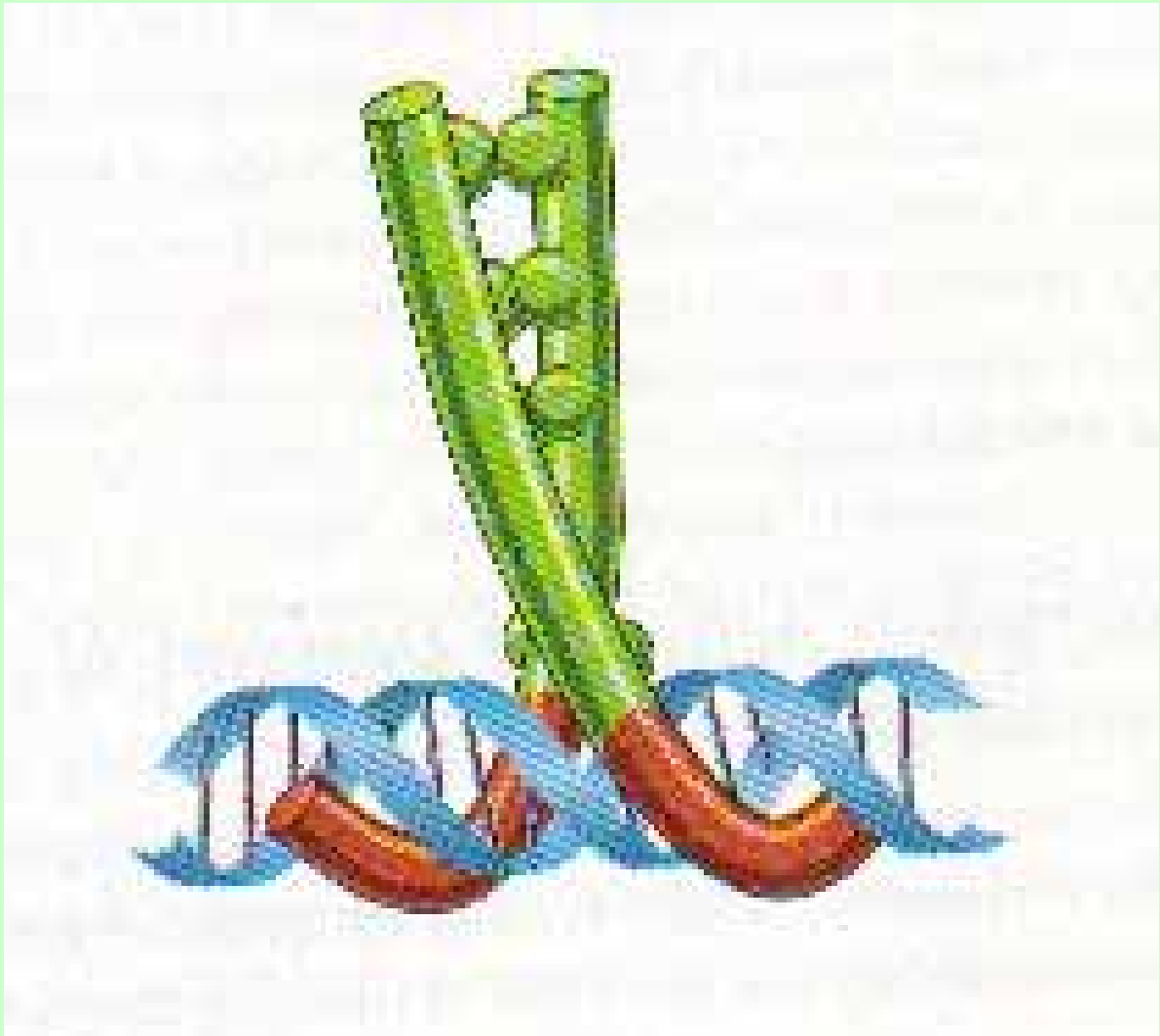
A Leucine Zipper Motif



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Control of gene expression by RNA processing

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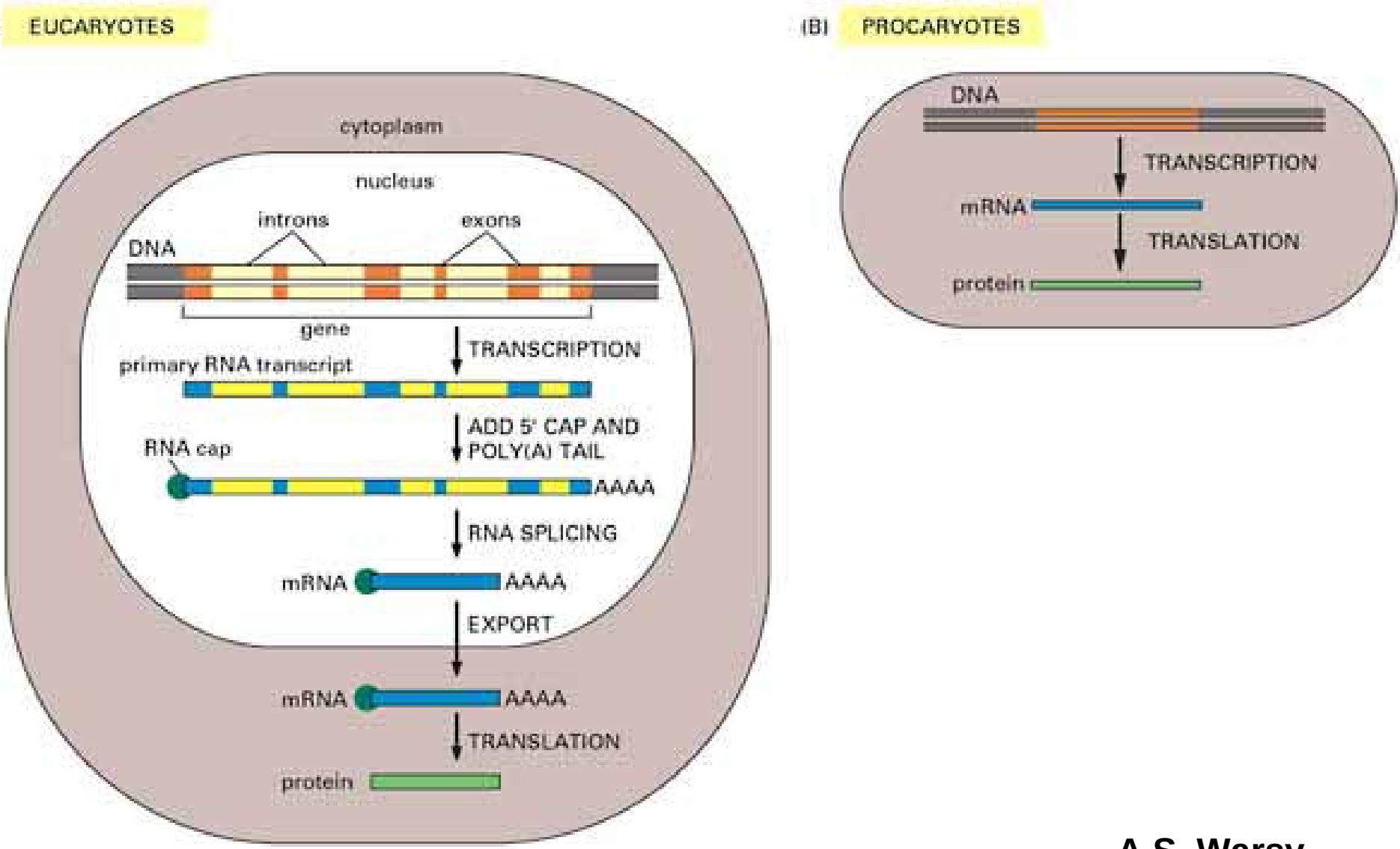
(A) EUCARYOTES
cytoplasm
nucleus
introns
exons
DNA
gene
TRANSCRIPTION
primary RNA transcript
ADD 5' CAP AND POLY(A) TAIL
RNA cap
AAAA
RNA SPLICING
mRNA
AAAA
EXPORT
mRNA
AAAA
TRANSLATION
protein
(B) PROCARYOTES
DNA
TRANSCRIPTION
mRNA
TRANSLATION
protein

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RNA interference (RNAi)- a mechanism for controlling gene expression

- "The ability of double-stranded RNA (dsRNA) to induce sequence-specific gene silencing" is known as RNA interference.
  - It is a form of post-transcriptional gene silencing (PTGS)
  - An important mechanism in eucaryotes to shut-off genes
  - Originally believed to shut off only in presence of foreign genes (e.g.viruses), but now shown to naturally shut-off cellular genes, e.g. transposons and control genes involved during development and other functions.

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RNAi Pathways

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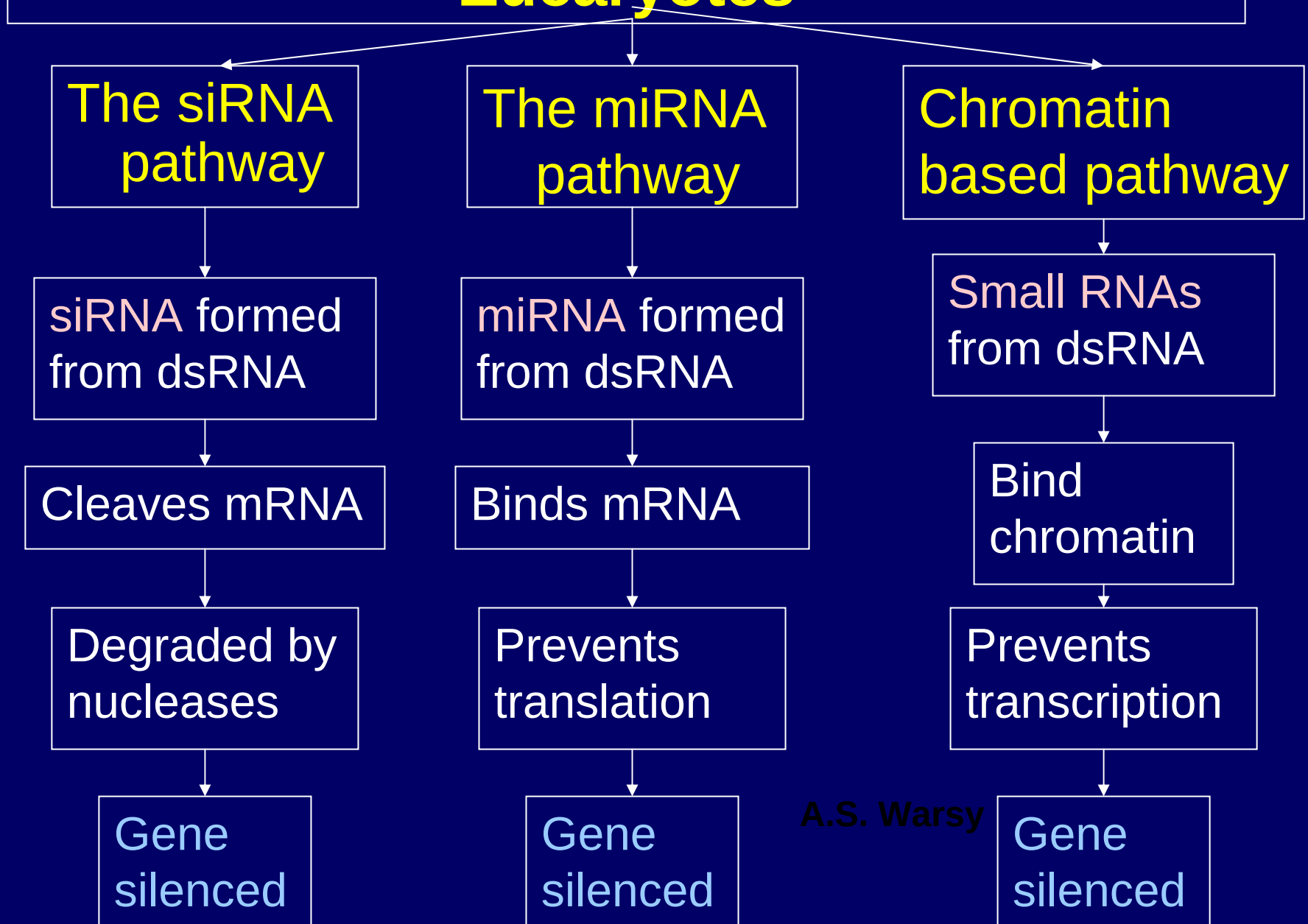
RNAi pathways for gene silencing in Eucaryotes



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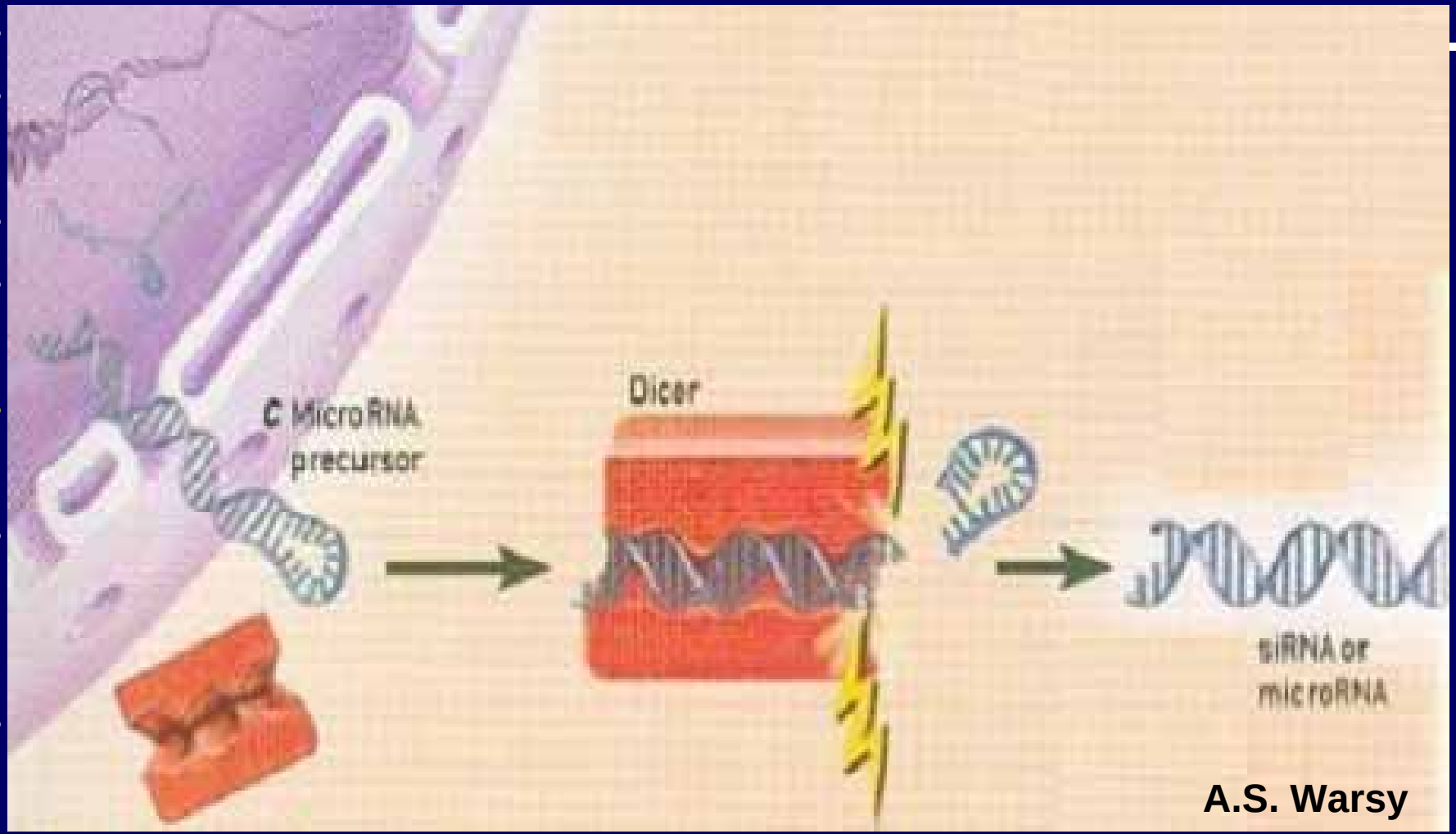
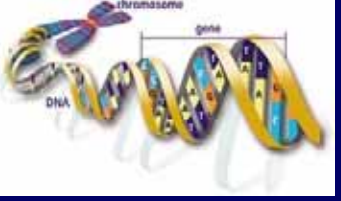
A new class of short RNAs --microRNAs (miRNA)





miRNA Genes

- **Unusual** genes. Arranged in tandem repeats.
- First genes identified were ***lin-4*** and ***let-7*** in C.elegans
- First make a **multiply bulged, partially duplexed precursors** (such as stRNA) which are cleaved to miRNA.
- **Final product** is an **RNA**, not a protein.
- To-date, **hundreds** of different miRNA genes discovered in worms, flies, plants and humans.
- In different organisms they make up from **0.5-1.0%** of the total genome.
- **Humans** are estimated to have **200-250 miRNA genes (~ 1%** of total human genes). Over 175 confirmed by biochemical analysis.

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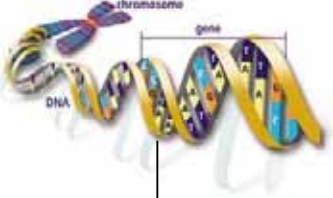
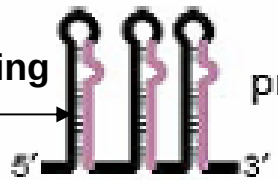
Formation of miRNA

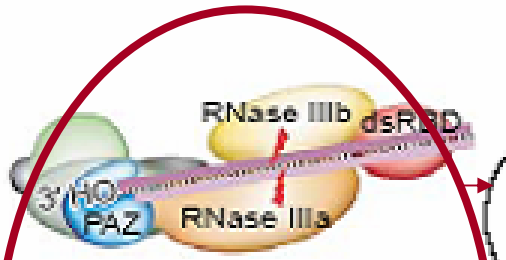


ssRNA

Folding

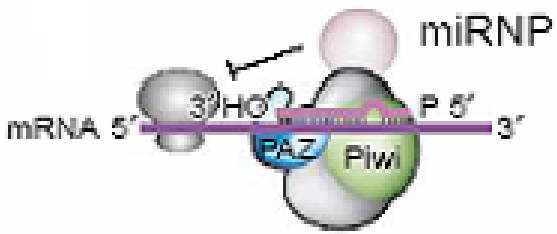


Drosha



Dicer complex

Argonaute complex



Translational repression

Genome region

e.g. Lin-4, lit-7

miRNA precursor, pri-miRNA

Folds back forming hairpin loops

Drosha

Pre-miRNA

Dicer

Cut into smaller piece (miRNA)

Dicer complex

Regulate gene expression by silencing different genes

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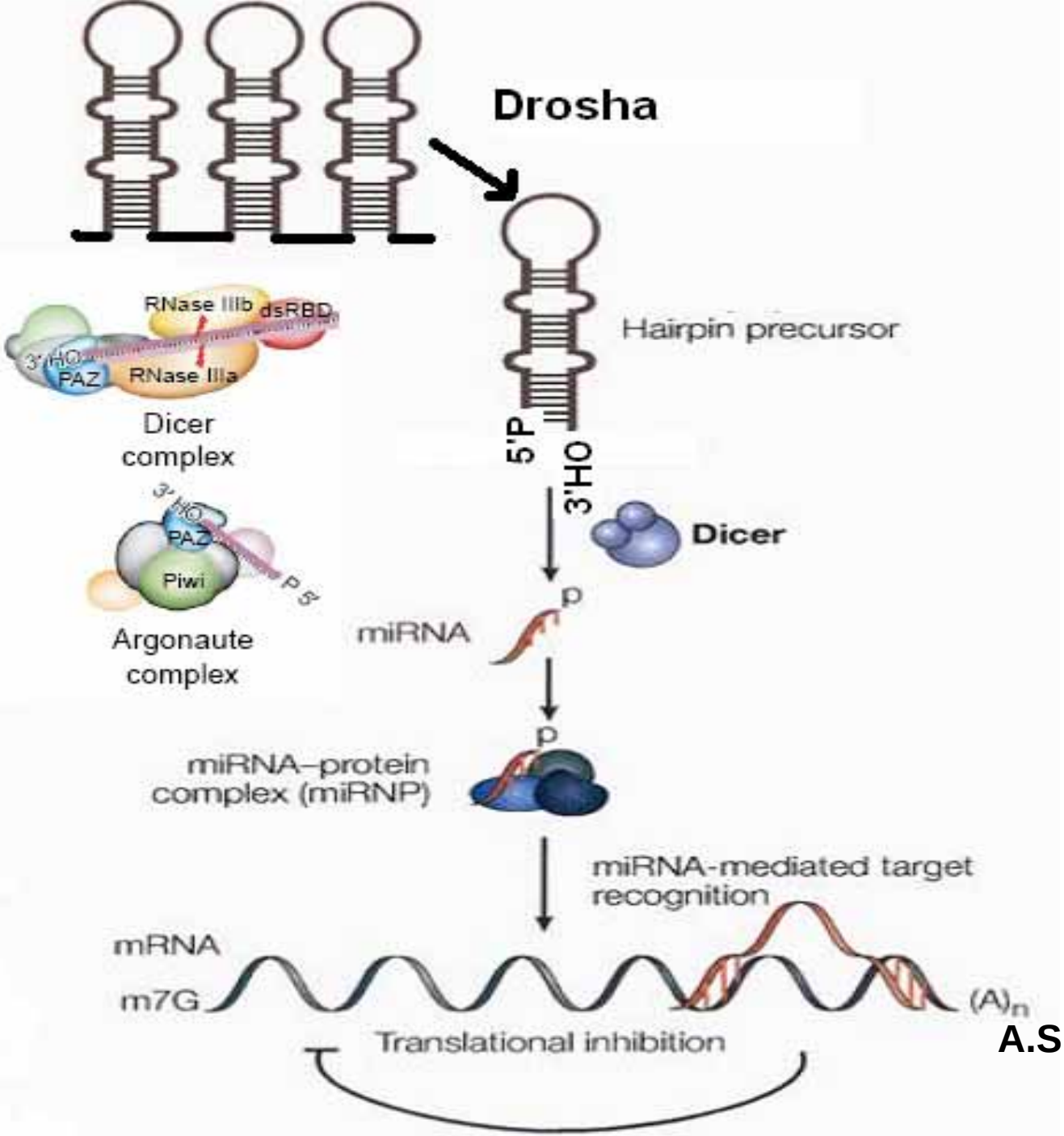
Drosha
Hairpin precursor
5'P
3'HO
RNase IIIb
dsRBD
3'HO
PAZ
RNase IIIa
Dicer complex
Dicer
3'HO
PAZ
Piwi
P 5'
Argonaute complex
miRNA
p
p
miRNA–protein complex (miRNP)
miRNA-mediated target recognition
mRNA
m7G
(A)n
Translational inhibition

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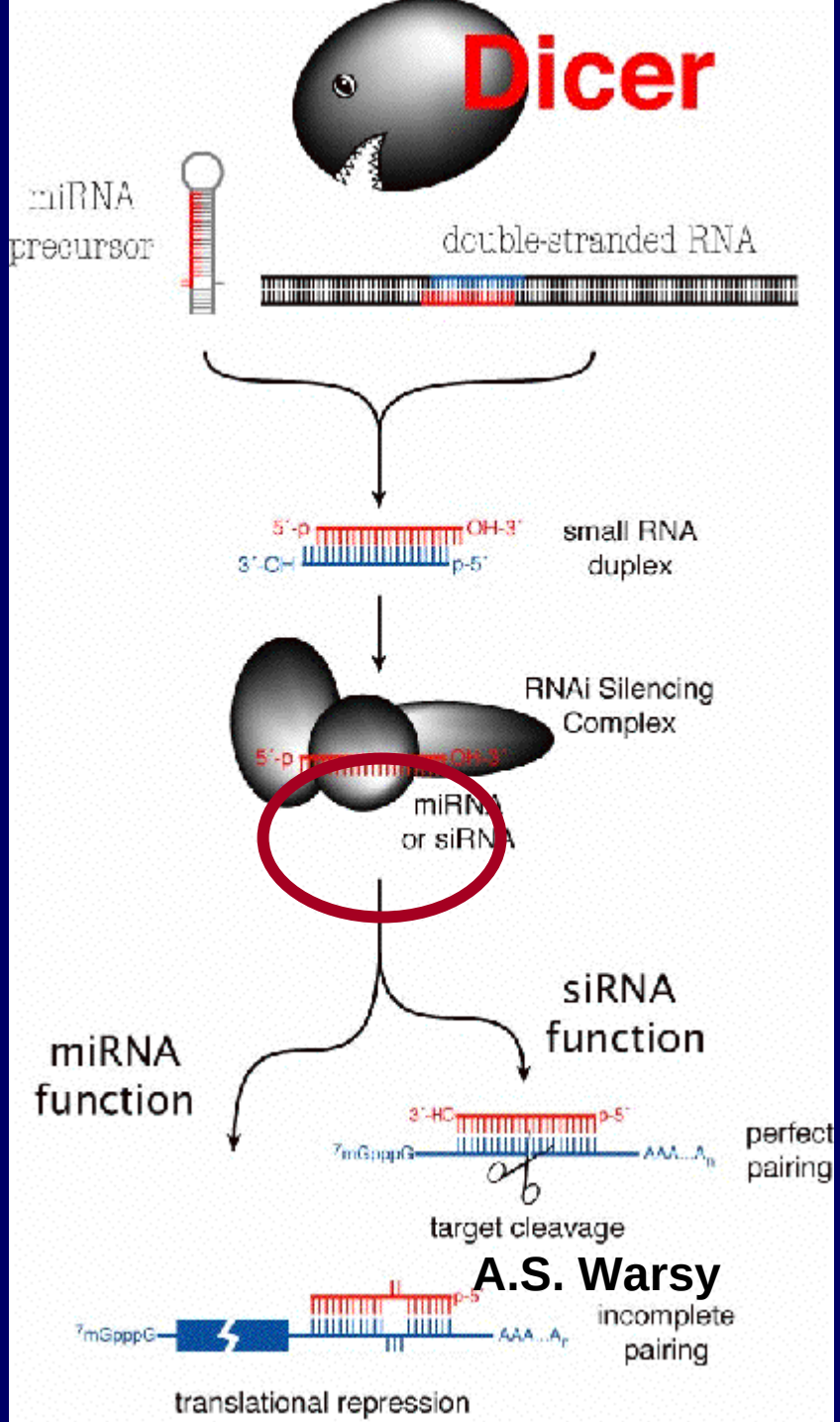
Difference between miRNAi and siRNA action

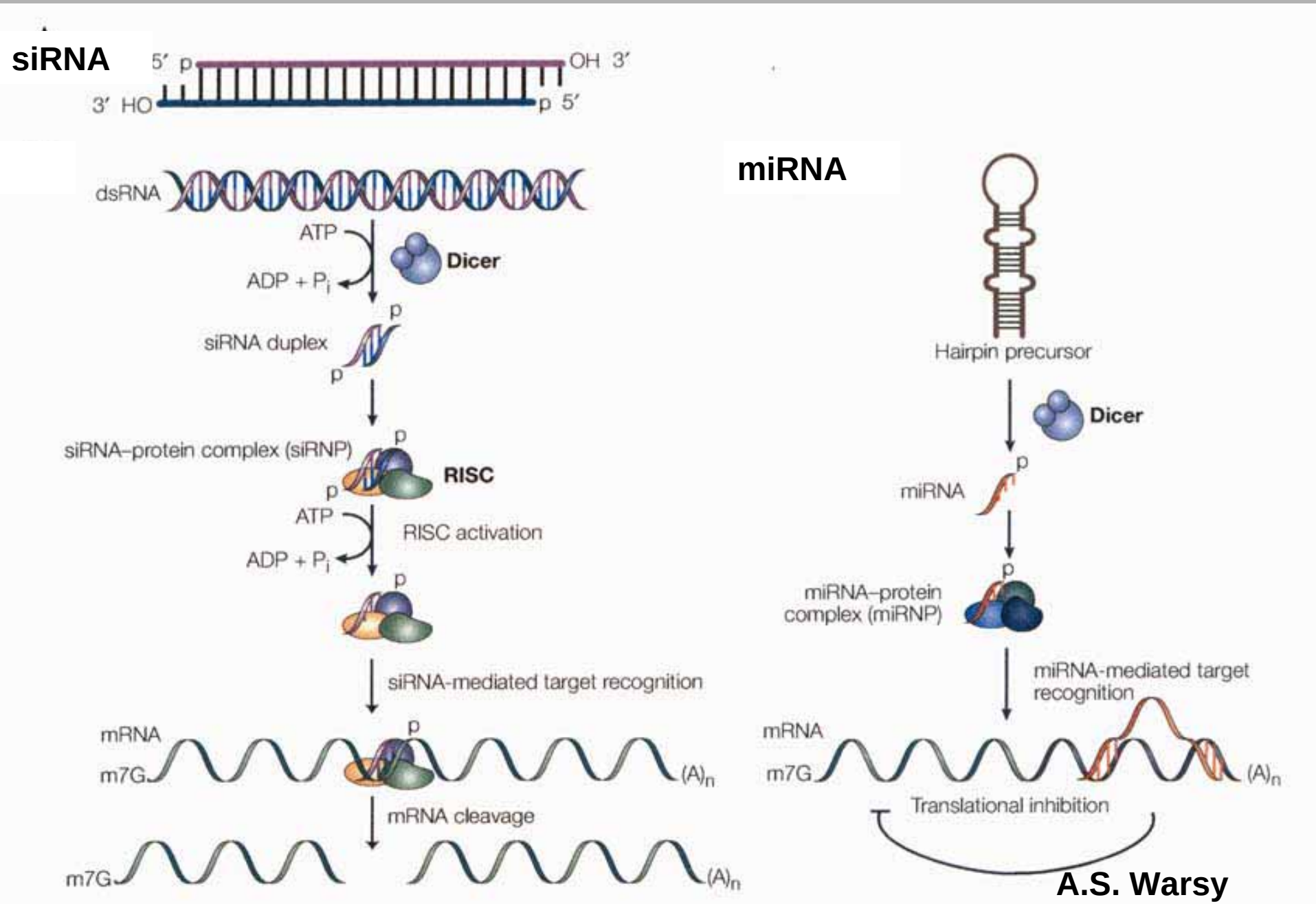
siRNA

Inhibits protein synthesis by cleaving mRNA

miRNA

Inhibits protein synthesis by binding to mRNA




siRNA
5′ p
OH 3′
3′ HO
p 5′
dsRNA
ATP
ADP + Pi
Dicer
siRNA duplex
siRNA–protein complex (siRNP)
RISC
ATP
ADP + Pi
RISC activation
siRNA-mediated target recognition
mRNA
m7G
(A)n
mRNA cleavage
m7G
(A)n
miRNA
Hairpin precursor
Dicer
miRNA
miRNA–protein complex (miRNP)
miRNA-mediated target recognition
mRNA
m7G
(A)n
Translational inhibition
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miRNA induced RNAi

Imperfect sequence complementarity with mRNA

Perfect sequence complementarity with mRNA

Binding of miRNA to mRNA at the complementary region

Binding of miRNA to mRNA and its degradation

Translational inhibition

Translational inhibition

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miRNA functions

In plants and animals miRNA genes play a very important role in development. (By clearing certain messages from cells during development, RNAi help cells to mature into correct type and proper structure)

To specify temporal progression of cell fate

Developmental Control

General role in gene regulation of other genes

Control stability and translation of mRNA of other genes

May amplify and spread throughout the source

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Function of RNAi

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Function of RNAi

Gene-censoring mechanisms

To protect plants, animals, fungi against viruses and mobile genetic element

Help silence normal cellular genes:
•during developmental transitions, required for cell differentation (e.g. into brain, heart, nerve cells, muscle cells, skin, etc.)
• Transposons

Genes are turned ‘ON’ or ‘OFF’ during development and as the organ makers

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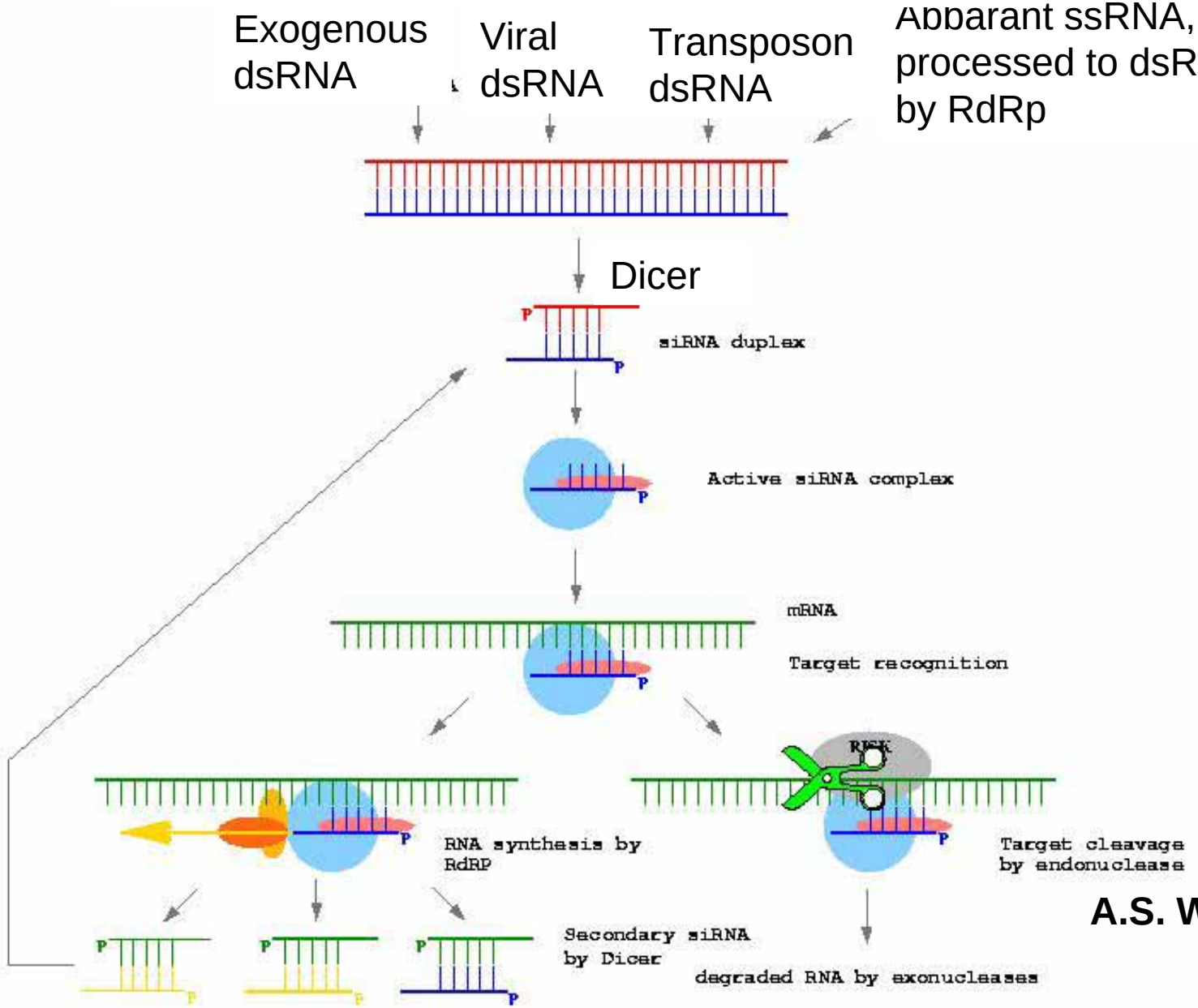
RNA Interference



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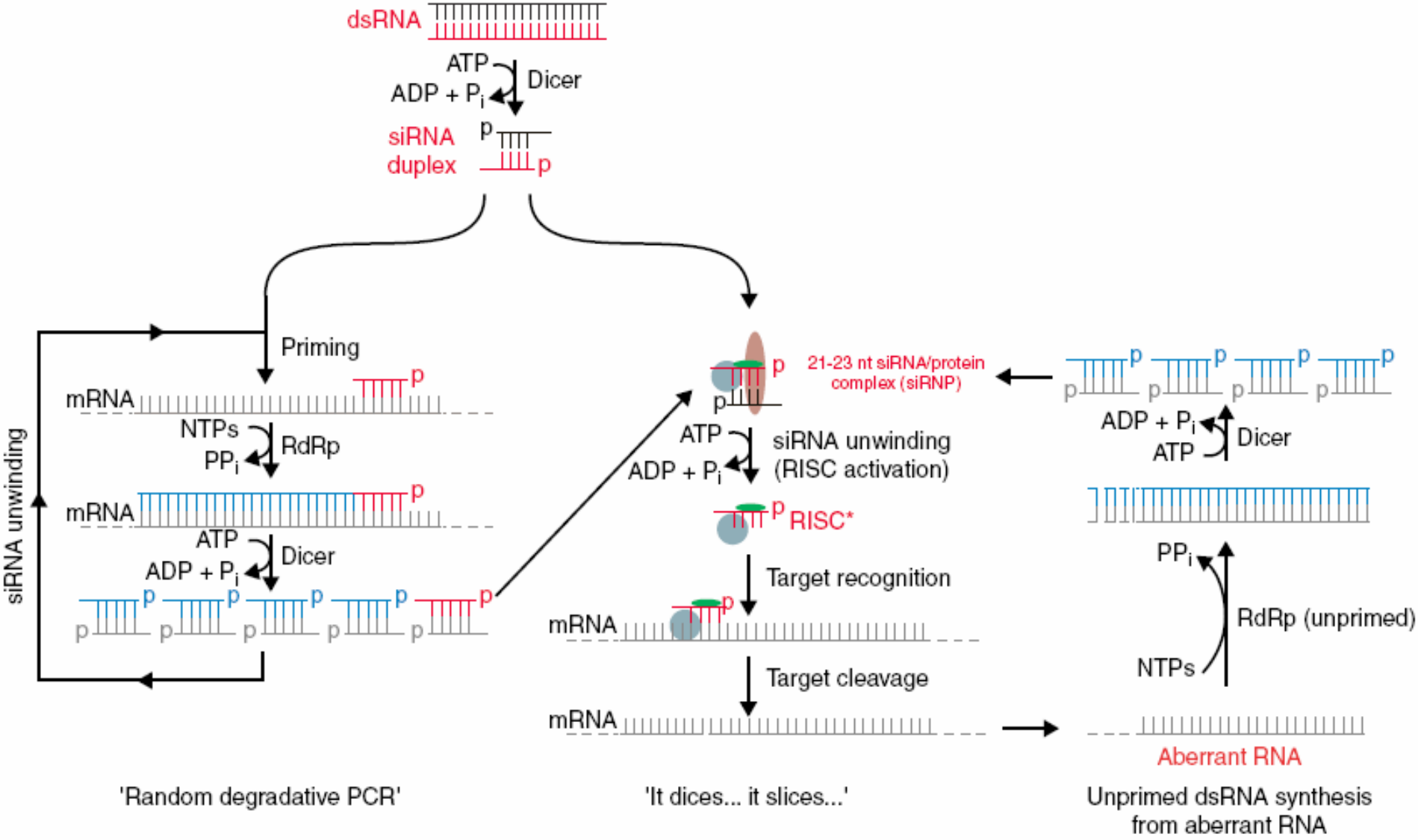
dsRNA
ATP
ADP + Pi
Dicer
siRNA
duplex
p
Priming
mRNA
NTPs
PPi
RdRp
ATP
ADP + Pi
Dicer
siRNA unwinding
21-23 nt siRNA/protein complex (siRNP)
ATP
ADP + Pi
siRNA unwinding (RISC activation)
RISC*
Target recognition
Target cleavage
mRNA
ADP + Pi
ATP
Dicer
PPi
RdRp (unprimed)
NTPs
Aberrant RNA
'Random degradative PCR'
'It dices... it slices...'
Unprimed dsRNA synthesis from aberrant RNA

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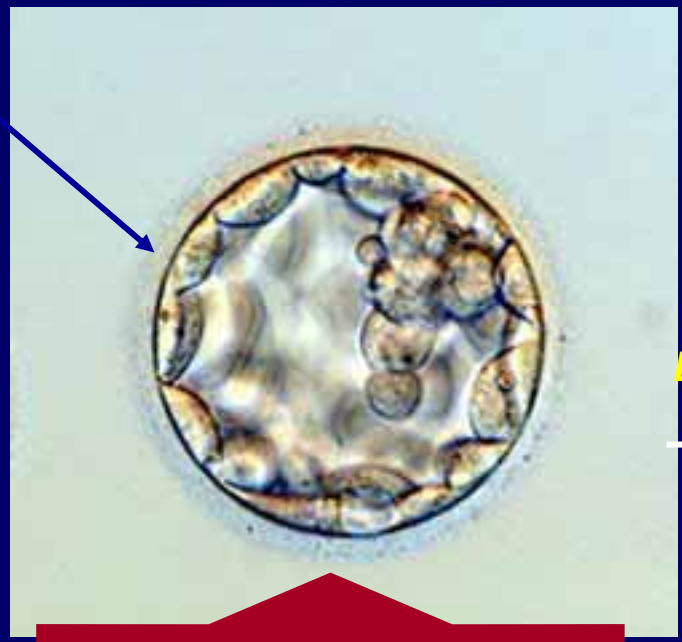
Differentiation and Development

Fertilized cell

Blastocyte

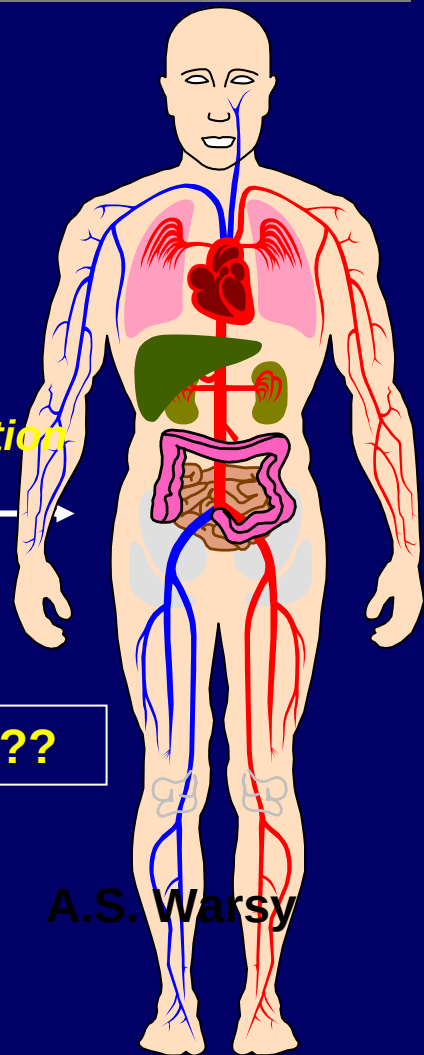


Note: Every cell in the body has the same genetic material

Differentiation

RNAi ???

Human body



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GENES AND THEIR EXPRESSION

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