The semi-synthetic "Minimal Cell" : a model for early living cells

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Centro Enrico Fermi

" KITP 2007 "

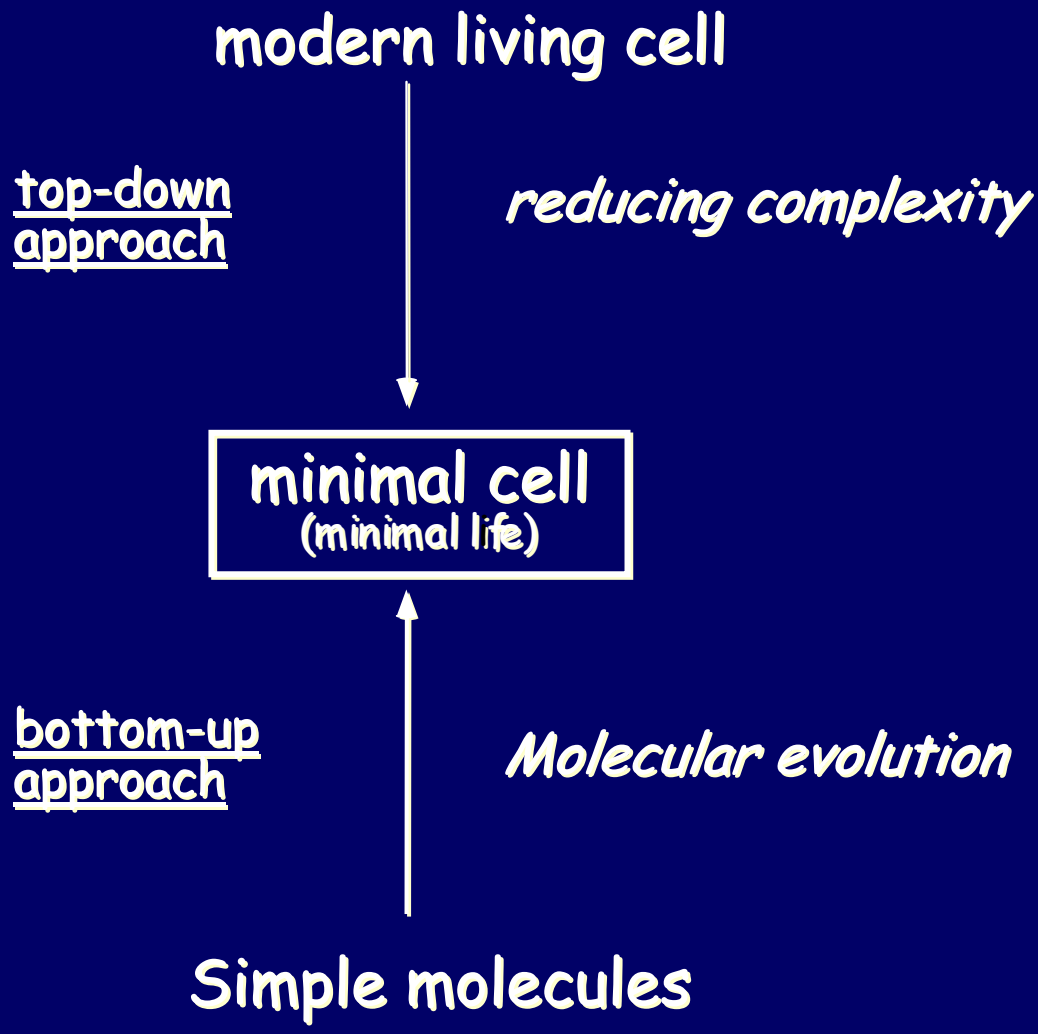
A modern prokaryotic Cell

- Genes ~ 400-500 (*Mycoplasma genitalium, Buchnera aphydicola)*
- Proteins ~ 400-500 "These are bacterial parasites"

  - Recently new small genome: (*Carsonella rudii* 182 genes)
- "Parasite of phloem feeding insects"

  - Complexity ↓

two working directions



Theoretical minimal genome

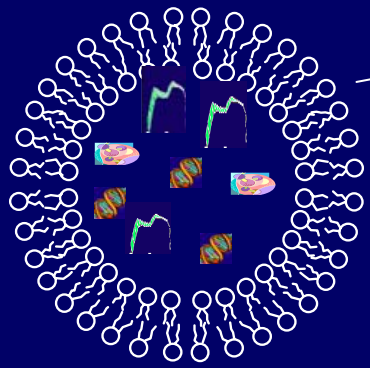
~ 200 genes or less?

Andres Moya has reconstructed the core of an hypothetical minimal bacterial gene set.

Computational comparative analysis of eight bacterial genomes

Core of minimal gene set: 206 genes |

The notion of the minimal cell:



Lipidic bilayer

Containing the minimal and sufficient number of components to be alive

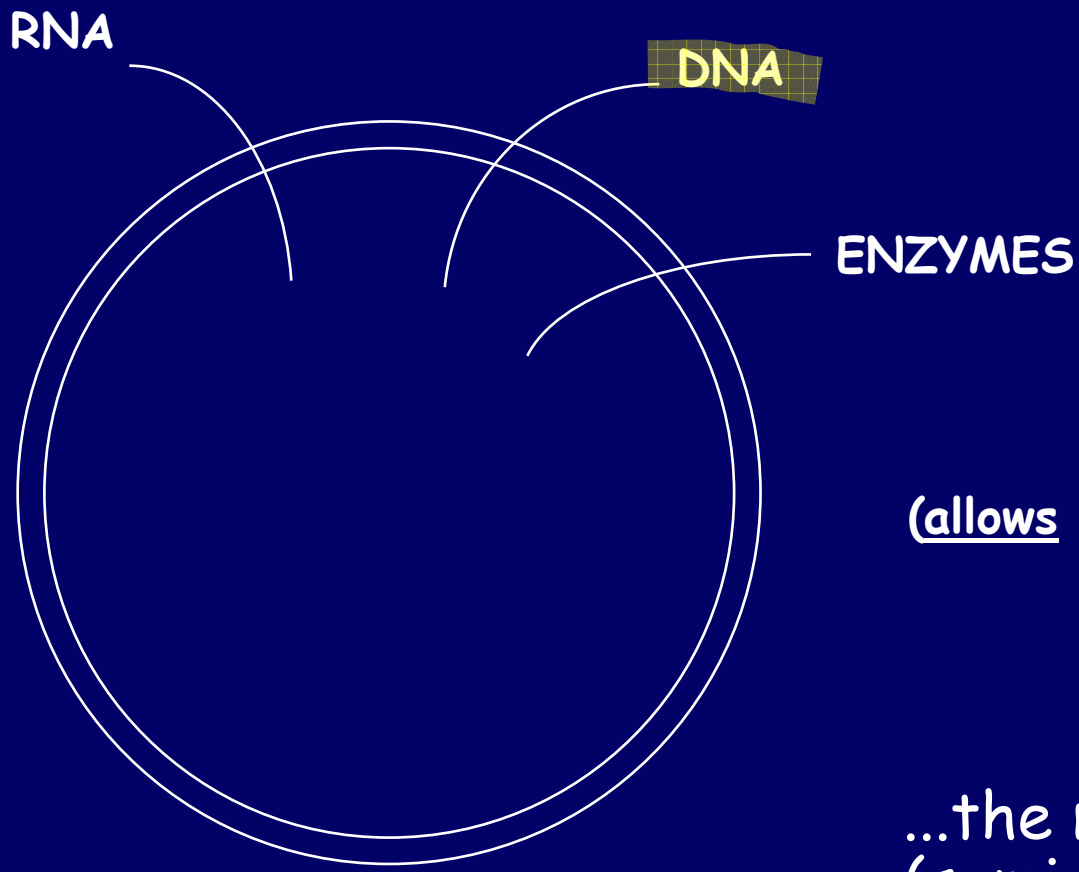
alive

- self-maintenance
- reproduction
  - Shell reproduction
  - Shell and core reproduction
- mutation → evolution

A minimal Cell should be able to perform some basic functions……..

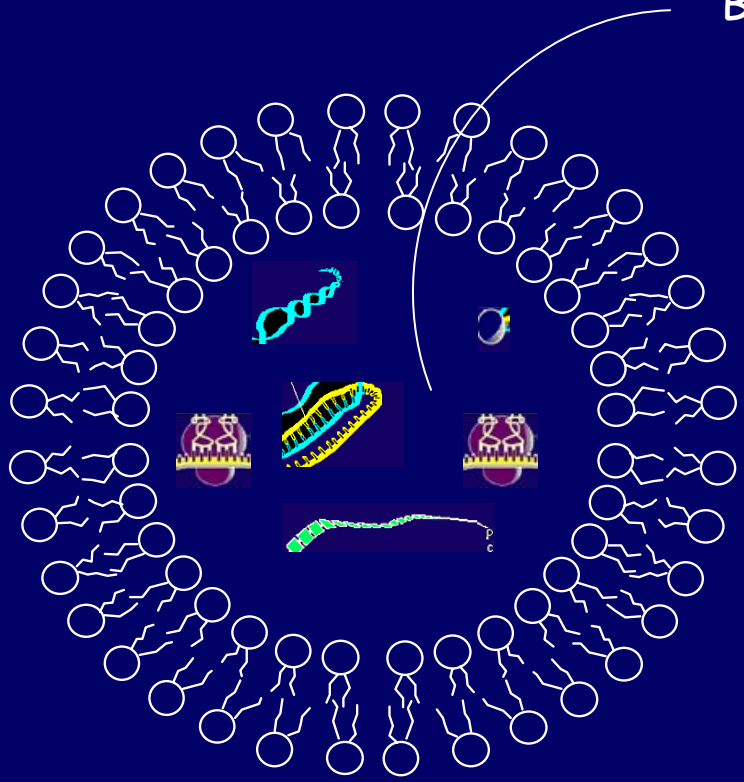
- With the lowest possible degree of complexity.
- Therefore with the minimal number of genes and proteins *(enzymes etc….)*
- This number can be even lower if nutrients and energy is provided from the outside (*ATP, Glucose, Amminoacids etc..)*

Construction of a semi-artificial minimal Cell



(allows ...)

...the minimal artificial (semi-artificial?) form of life.



Liposomes, as closed spherical bilayers, are considered the most likely precursors of early living cells

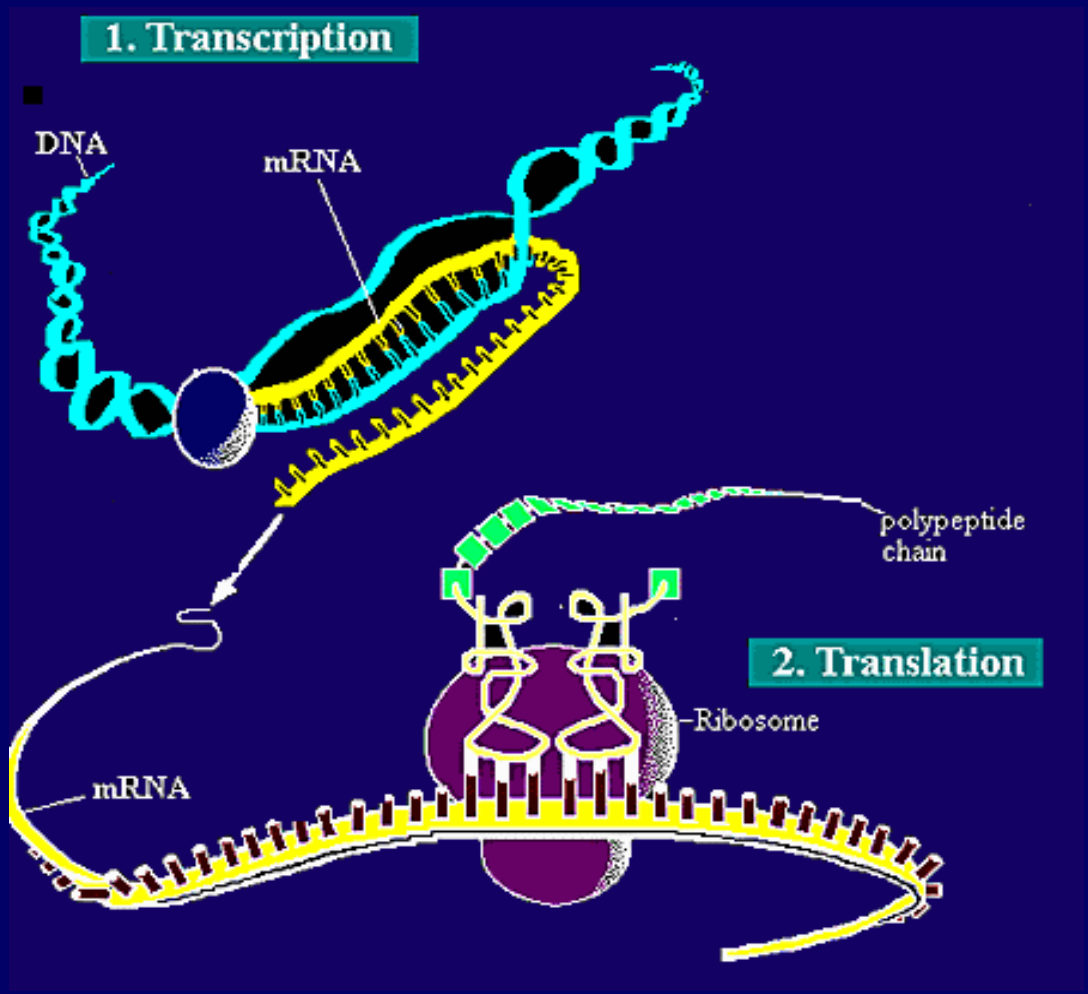
Questions

1 Self-maintenance: Can we reconstruct a minimal protein synthesis system within liposomes?

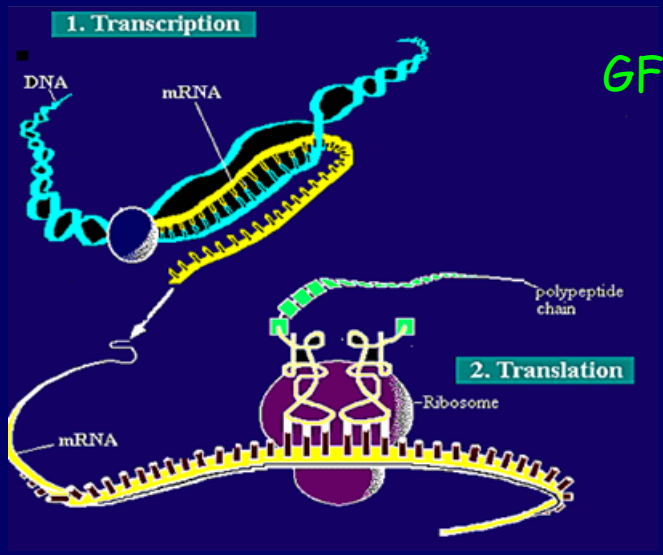
2 Shell and core reproduction: Can we induce Vesicles reproduction?

.....core reproduction ?

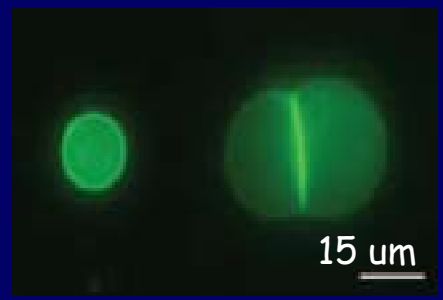
Self-maintenance: Protein synthesis



Protein expression inside the lipidic compartments

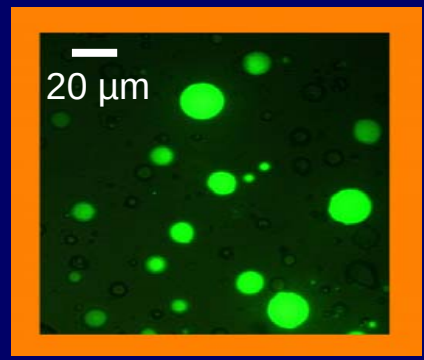


GFP expressed in Giant-vesicles



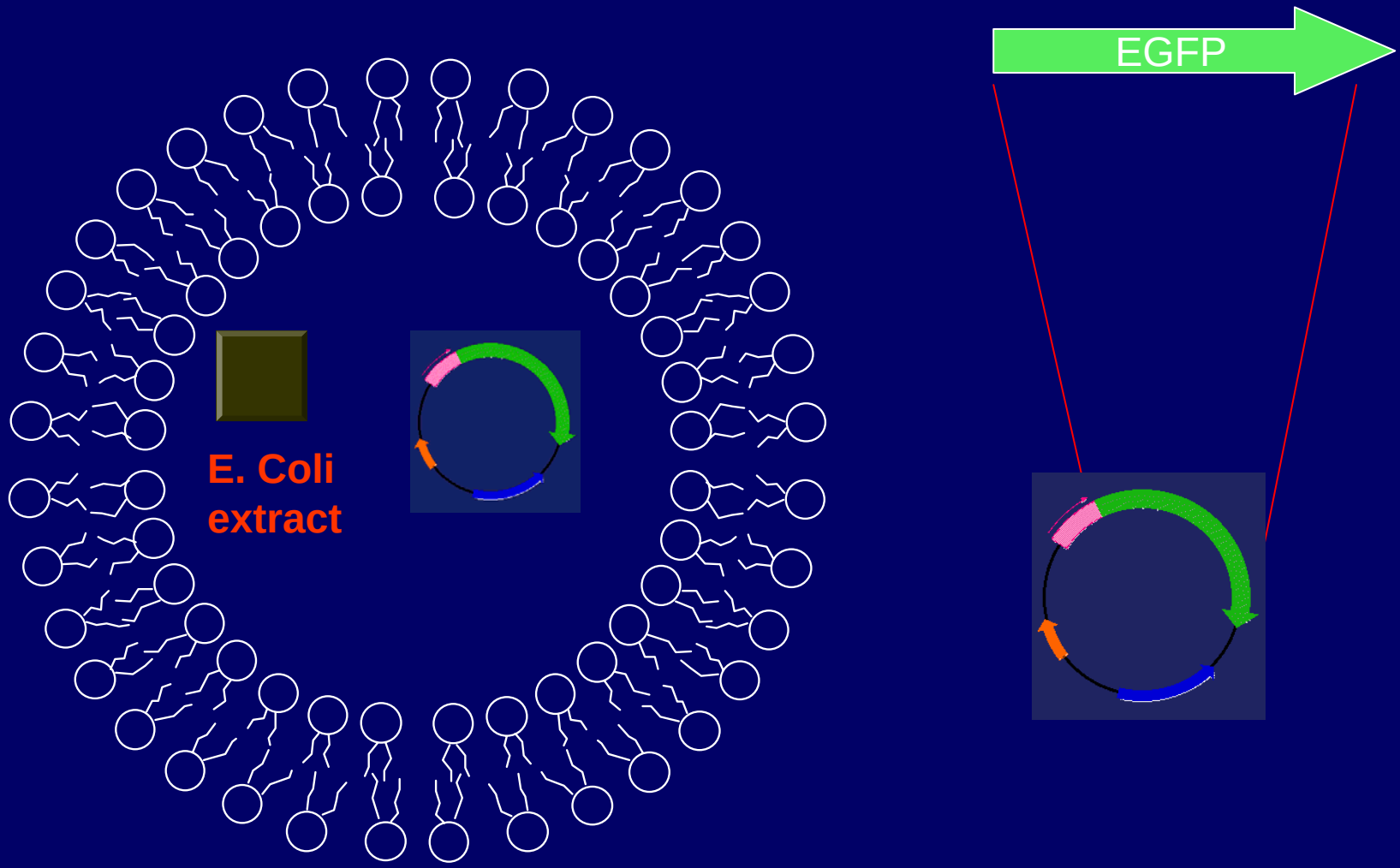
V. Noireaux and A. Libchaber

GFP expressed in water in oil emulsion



PL. Luisi et al.

A cell-free E. Coli extract for protein synthesis drives the EGFP expression

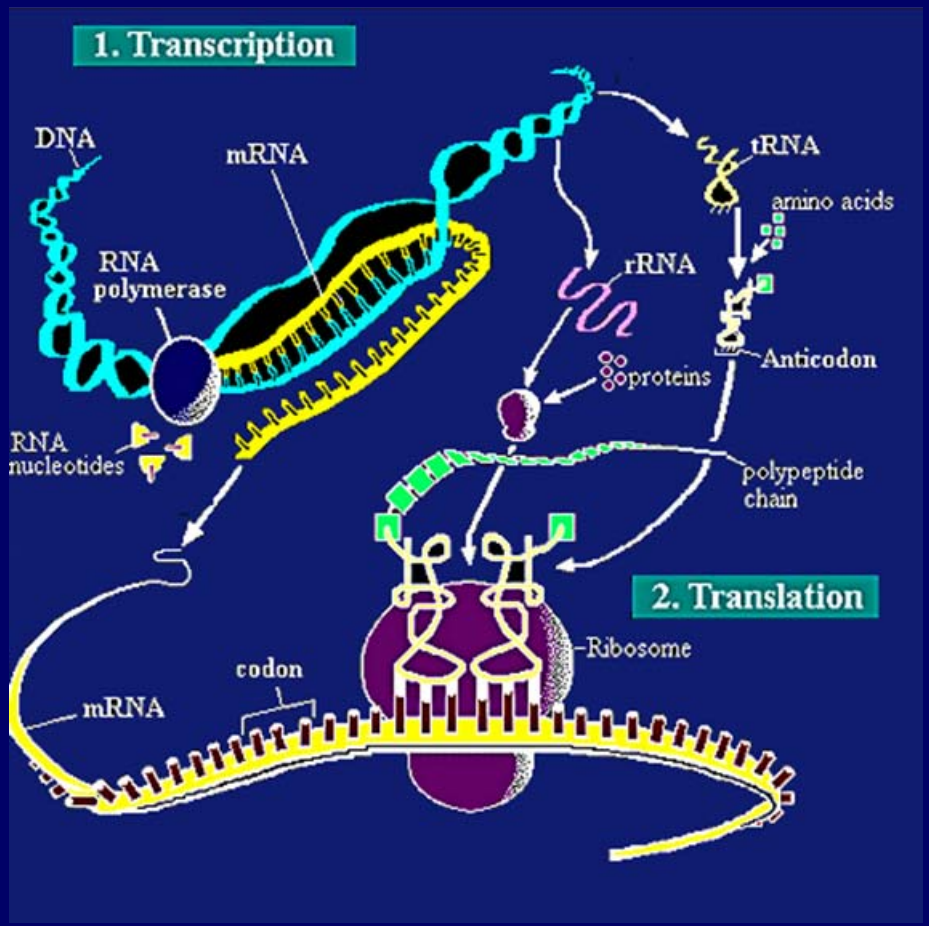


Can we replace "the black box" with a minimal set of enzymes for protein synthesis ?

- Ueda's lab has cloned all the E.coli component required for transcription and translation

- The PURESYSTEM replaces  = the commercial Cell-free
- E.coli extract within the minimal cell

Minimal set of enzymes x protein synthesis: The "PURESYSTEM"



37 Enzymes

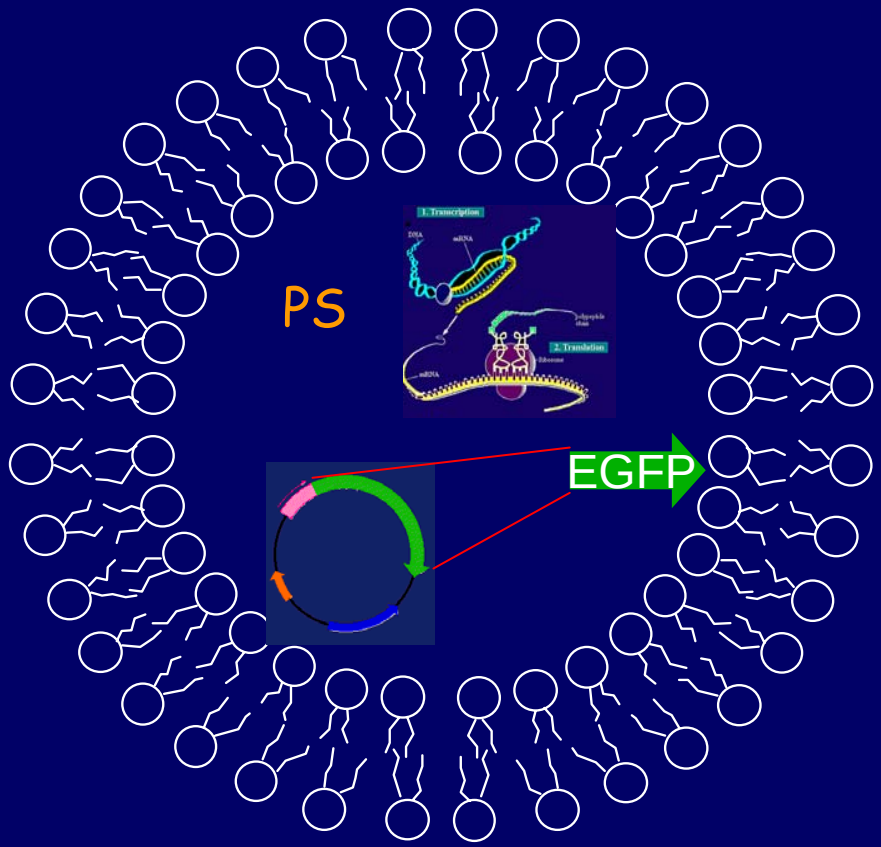
Iniziation factors : IF1, IF2, IF3.
Elongation factors : EF1, EF2, EF3.
Release factors : RF1, RF2, RF3.

- Ribosome recycling factor (RRF).,
- 20 amnoacyl-tRNA synthetases
- Methionyl-tRNA formyltransferase (MTF)
- T7RNA polymerase
- Creatine phosphate,Creatine kinase, myokinase, nucleoside diphosphate kinase, pyrophosphatase,

- + Ribosomes

and Low MW molecules

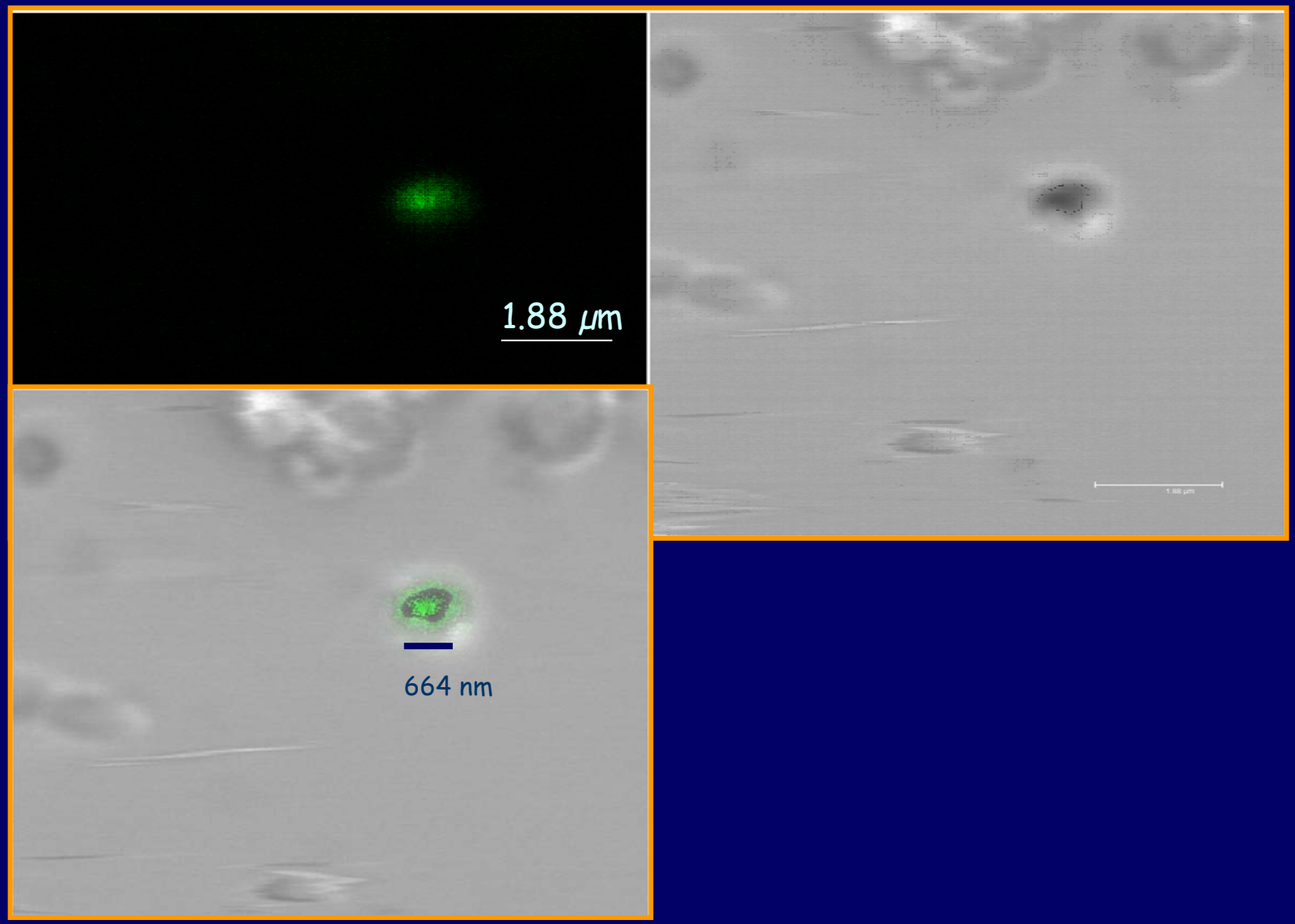
Does PURESYSTEM (PS) work in liposomes?



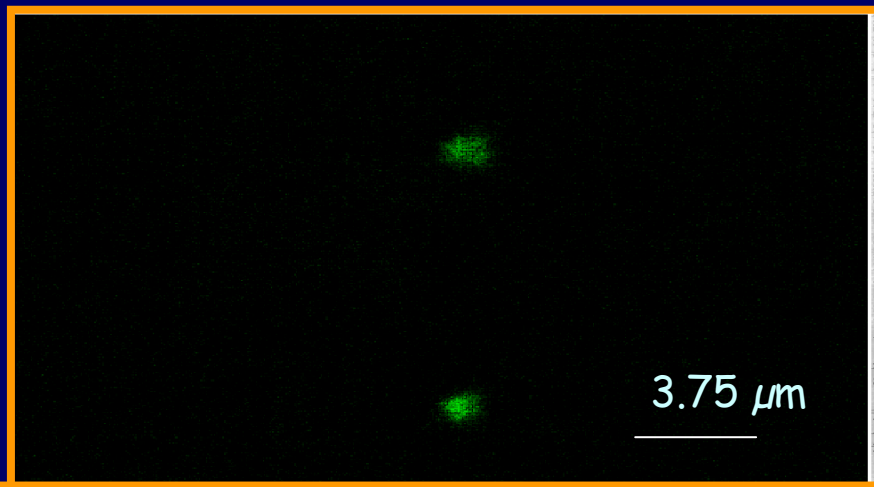
To monitor EGFP fluorescence

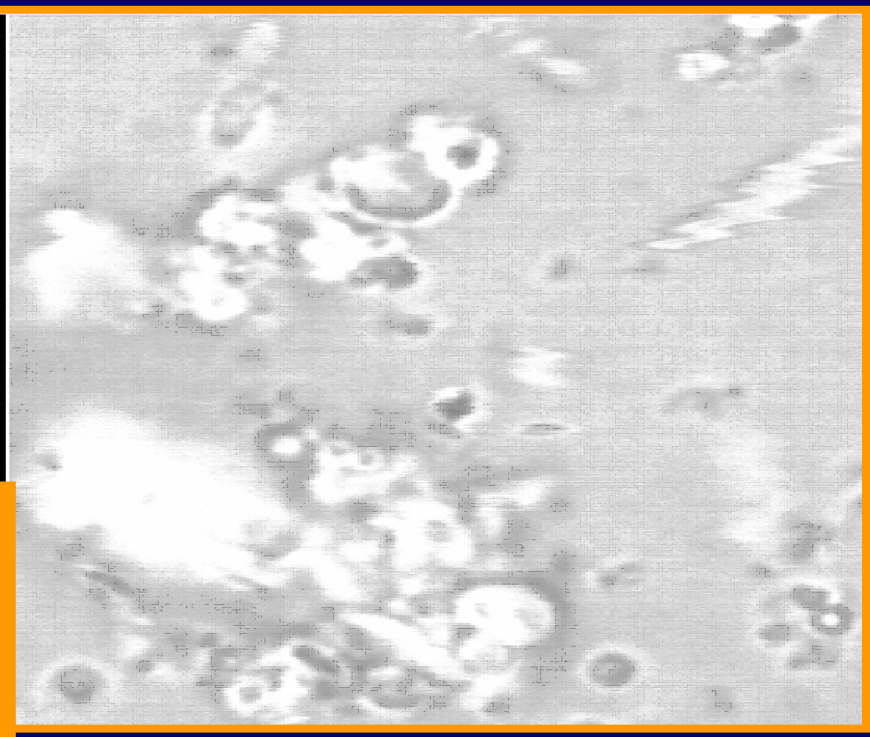
Confocal microscopy

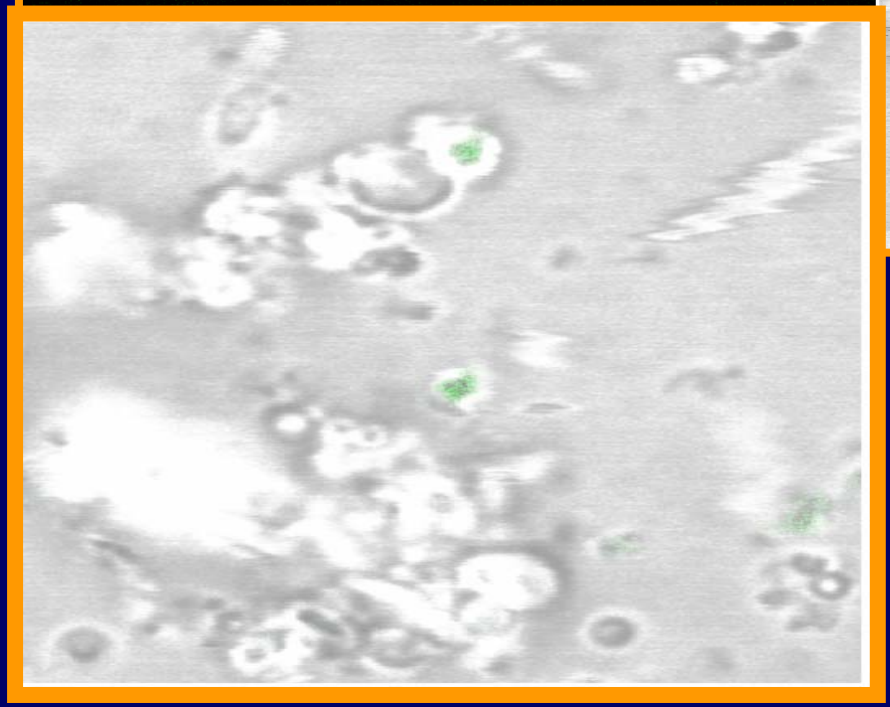
Confocal microscopy



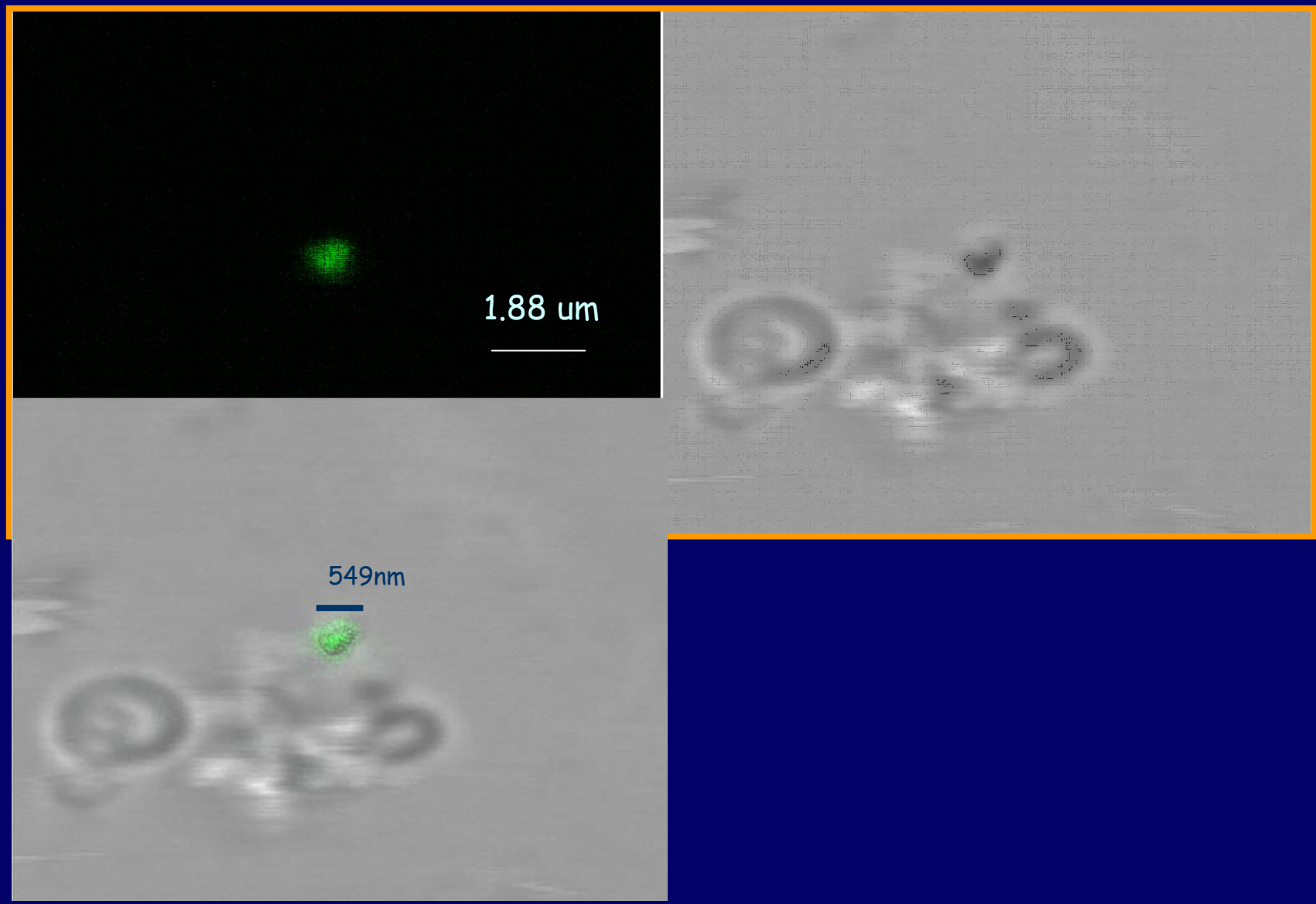
Confocal microscopy







Confocal microscopy



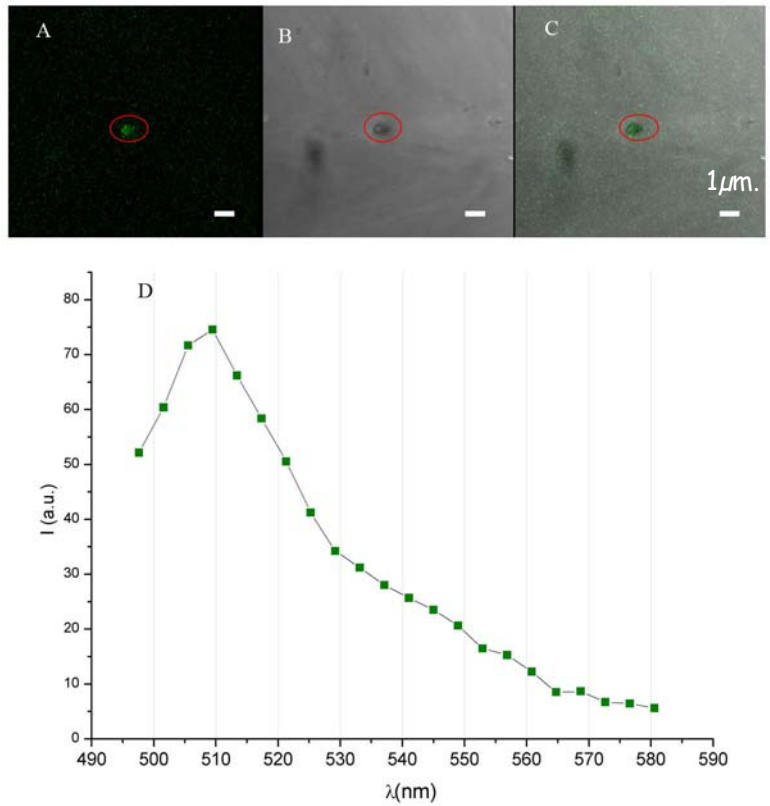
Confocal images of EGFP fluorescent liposomes.

A) Fluorescence acquired in the range 500-560nm (GREEN)

B) Transmission image (GREY)

C) Overlay of A and B

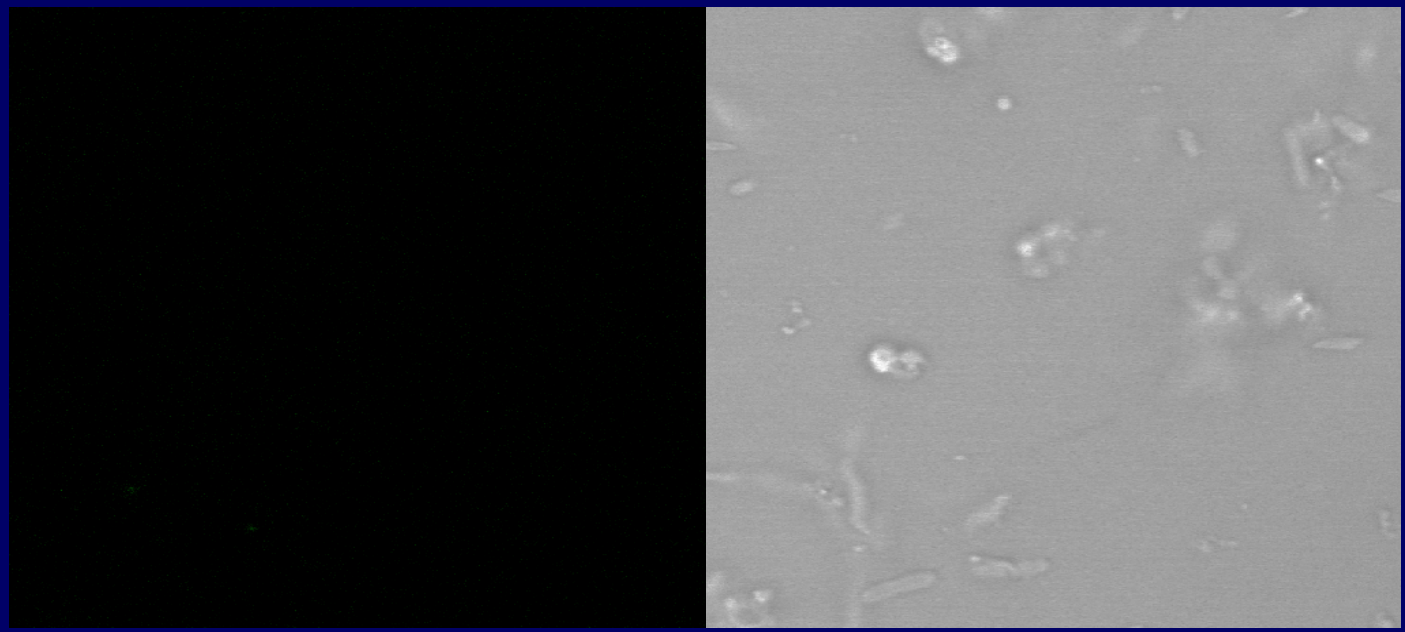
D) Graph extracted from the spectral series done on the same liposome



Excitation λ 488 nm.

Confocal microscopy

Monitoring the DHFR sample (-control)



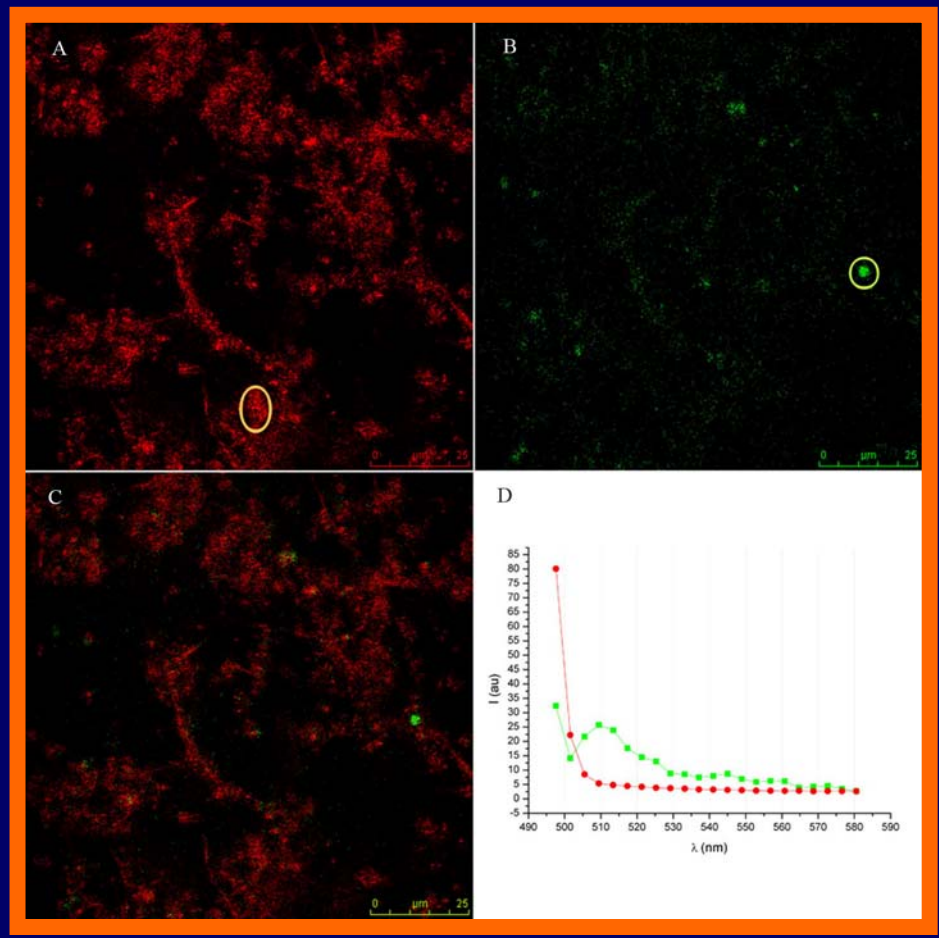
Confocal images of EGFP fluorescent liposomes

A) image extracted from the spectra of the reflection (RED)

B) image extracted from the Fluorescence spectra (GREEN)

C) Overlay of A and B.

D) Graphs extracted from the spectral series done on the liposome and reflection :



1. Self-maintenance:

Can we reconstruct a minimal protein synthesis system within liposomes?

We can reconstruct protein synthesis within liposomes with a minimal set of 38 genes + ribosomes and low MW components

Shell and core reproduction

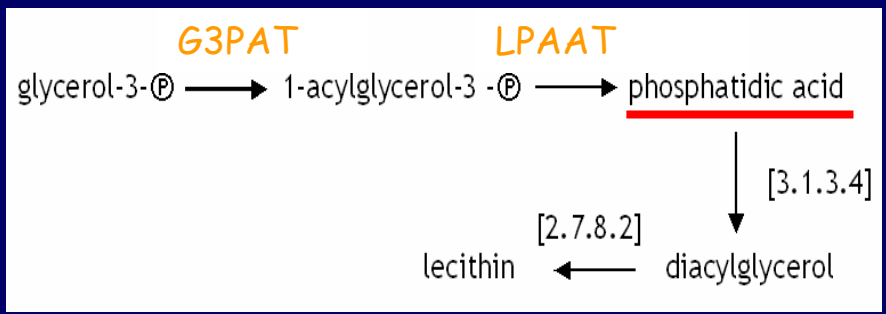
- Shell reproduction: Vesicles reproduction
- Core reproduction: Reproduction of a minimal genome, including gene expression system: PS

Can we induce Vesicles reproduction?

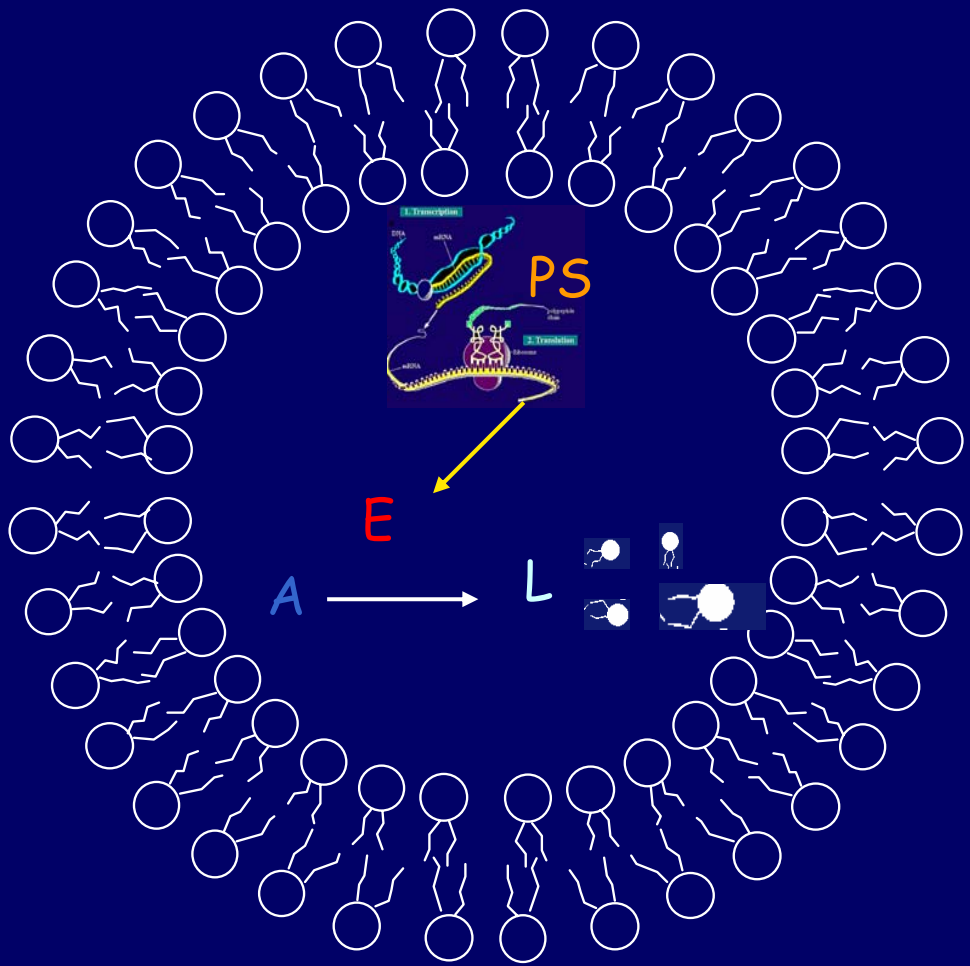
- Enzymes to make lipidic membrane:
- Enzymes that produce phosholipids (lecithin)

  The " Lipid Salvage pathway"……..

- ……..obtaining phosphatidic acid, a molecule that forms *per se* lipid bilayers and vesicles



Introducing the Enzymes for lipid synthesis:

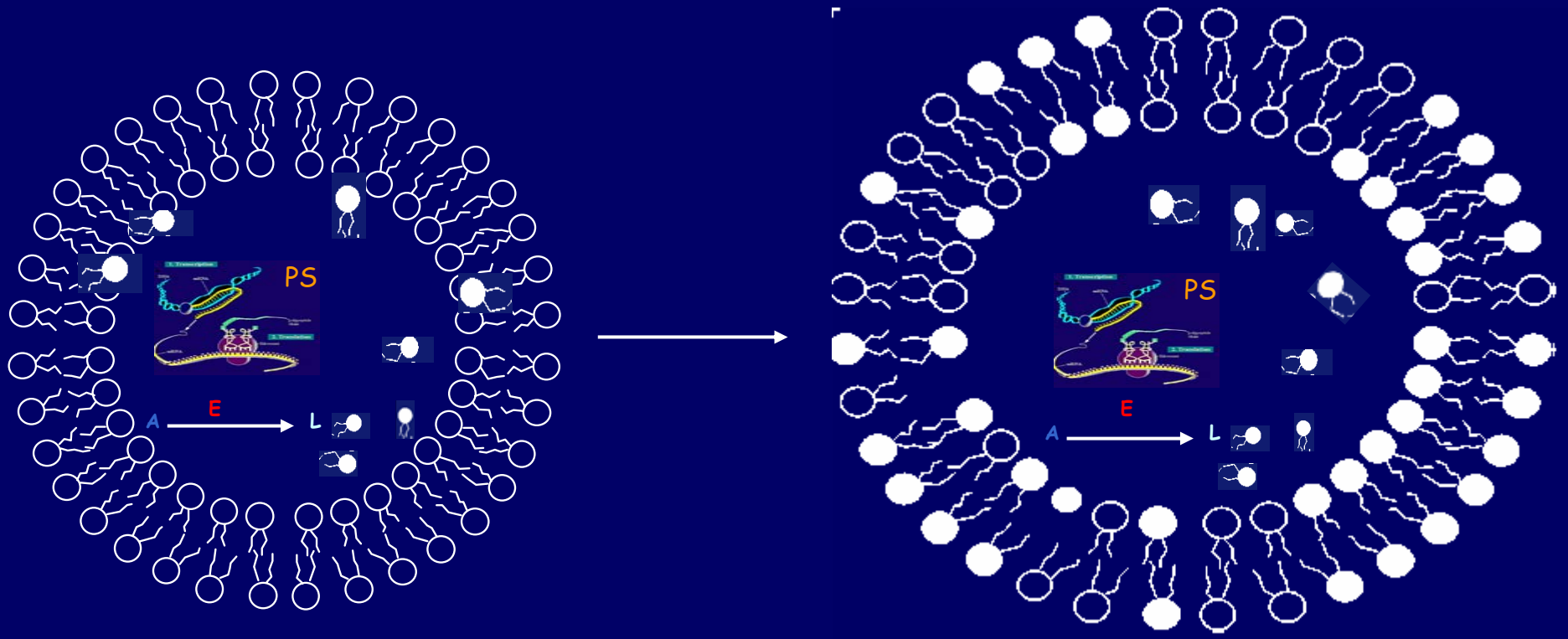


E= Enzymes (genes)

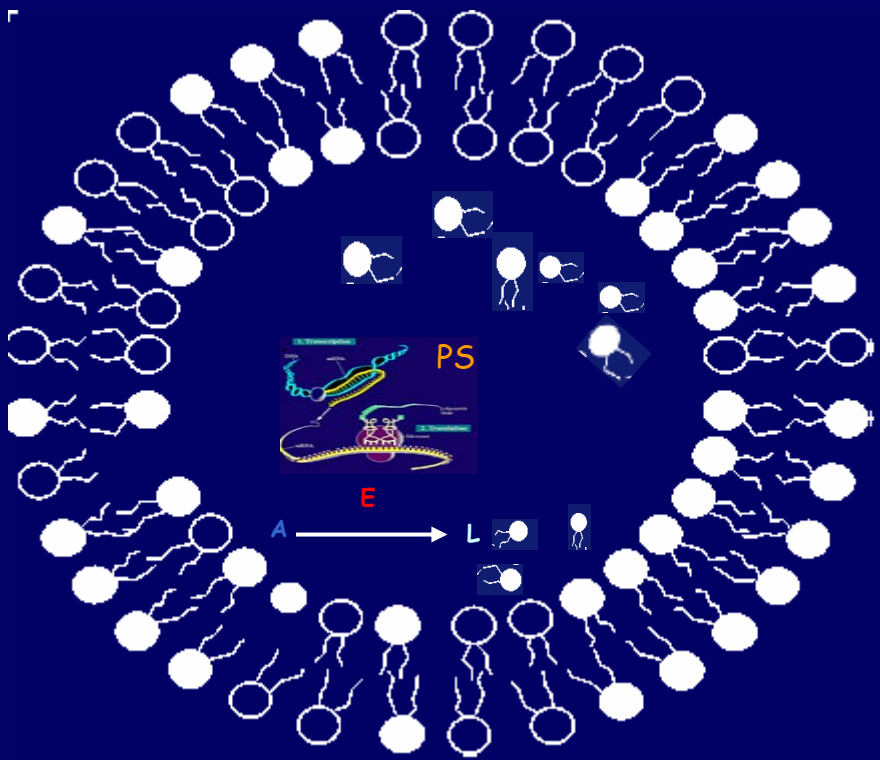
A= set of precursors

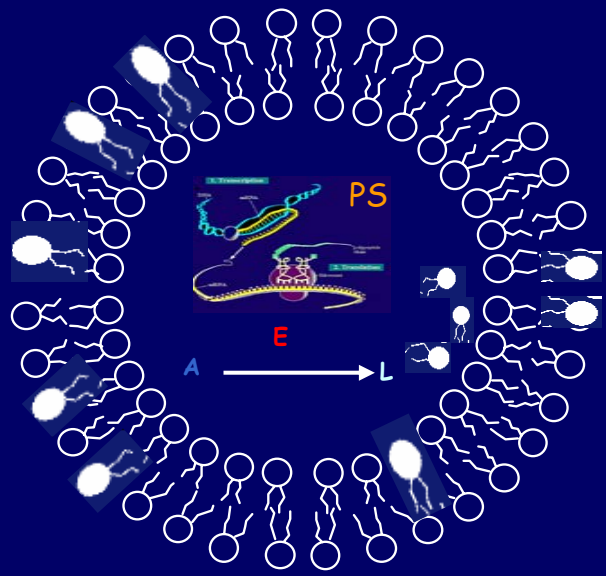
L= lipids

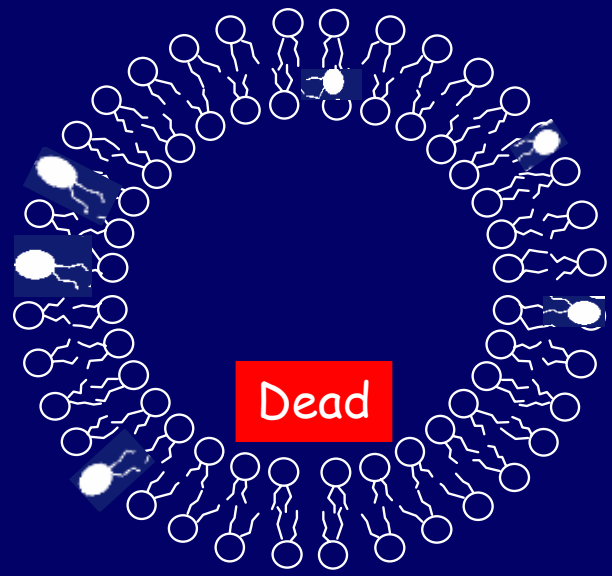
Synthesis of membrane lipids



Vescicles reproduction







Shell and core reproduction

- Shell reproduction: Vesicles reproduction (Work in progress)

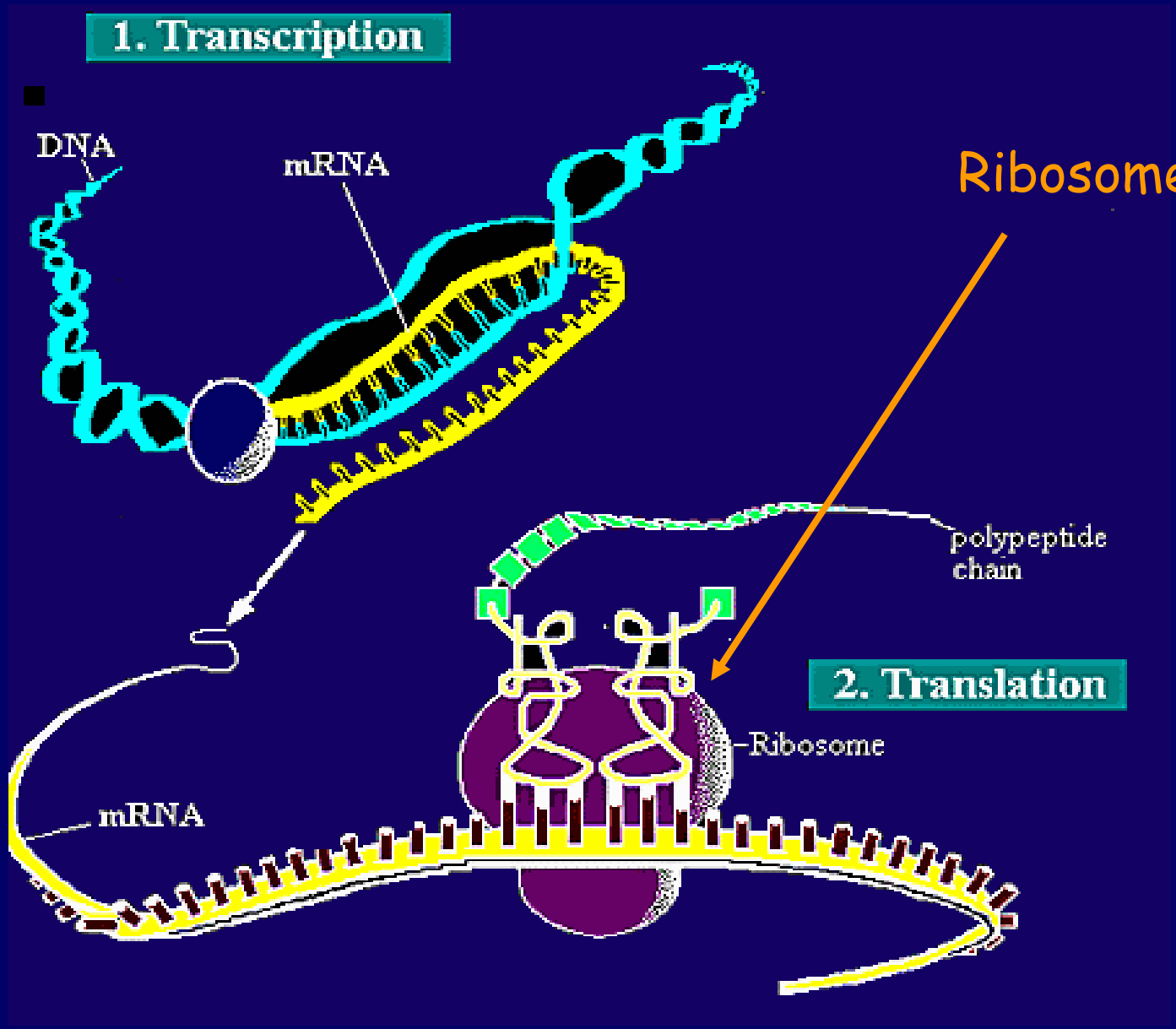
- Core reproduction:
  - Reproduction of a minimal genome
  - Including Self-reproduction of PS

Core reproduction: future work

Reproduction of a minimal genome:

7 genes for DNA replication

54 genes for Self-replication of PS
Including 16 genes for t-RNAs molecules
Excluding ribosomes


1. Transcription
DNA
mRNA
Ribosome
polypeptide chain
2. Translation
Ribosome
mRNA

The reconstruction of a minimal ribosome

- rRNAs genes and genes for ribosomal proteins have been cloned from E. coli, 54 genes in M *genitalium*
- Based on comparative sequence analysis: 33 ribosomal proteins correspond to functional domains evolutionarily conserved (Jason A. Harvey SC Team, JMB 2002)
- rRNA is the catalytic molecule and ribosomal proteins stabilize and orientate the otherwise floppy RNA into an active structure (Noller HF, CSHL press 2006)

Minimal although limping Cell

- Shell reproduction: Vesicles reproduction, 2 genes required

- Core reproduction:
  - Reproduction of a minimal genome, including Self-reproduction of PS, 60 genes required
  - 33 – (?) r-protein genes and 2-3 rRNA genes required for minimal ribosome

A Minimal Cell of 98-(?) genes

Minimal set of genes for a "Minimal Cell"

- Using extant biological molecules I find hard to believe that an early simple cell, although limping minimal cell (alive) can ever existed with only 30-40 genes.
- Unless we think of low-specific enzymes assisting more then one reaction (ancient molecules)
- We may have had independent minimal steps controlling independent functions within compartments ……
- …… merging later on in a more complex structure and function such as

  ribosome

Acknowledgments

- Prof. Pier Luigi Luisi — Coordinator "Minimal Cell" project

- Prof. Alberto Diaspro — Confocal Microscopy
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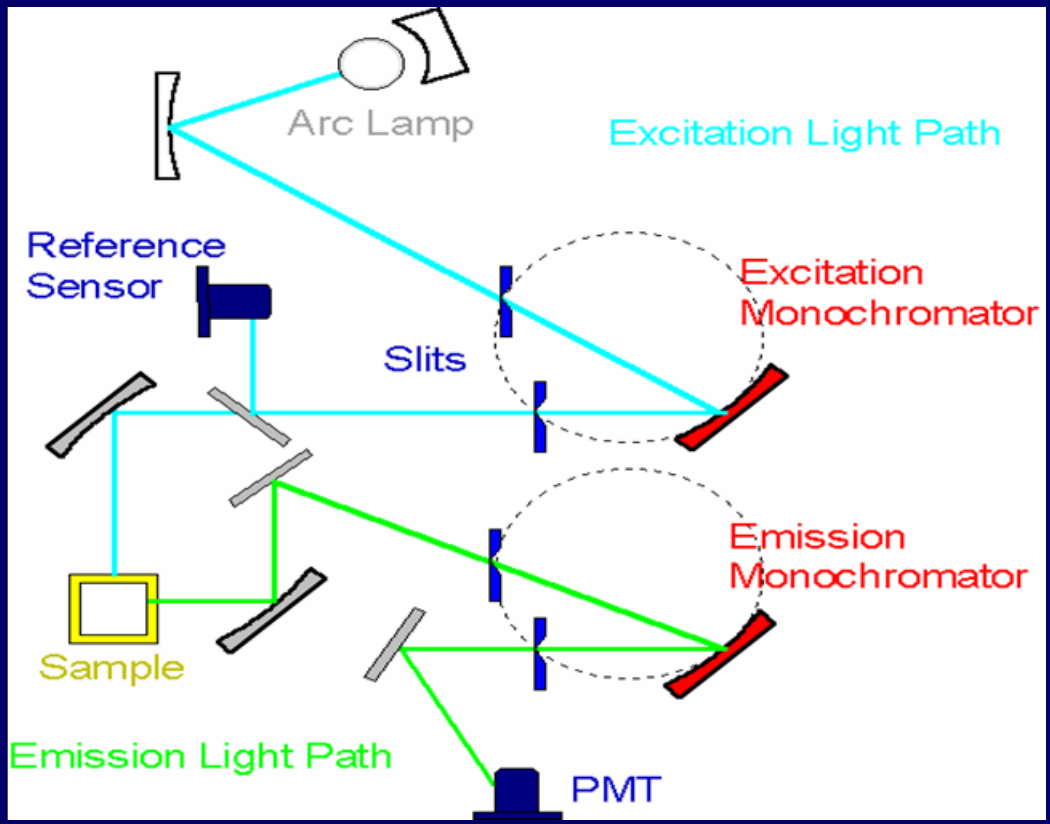
- Dr. Yutetsu Kuruma — "Salvage pathway" work
- "Enrico Fermi" Centre, Rome
- Biology Department, University Rome 3

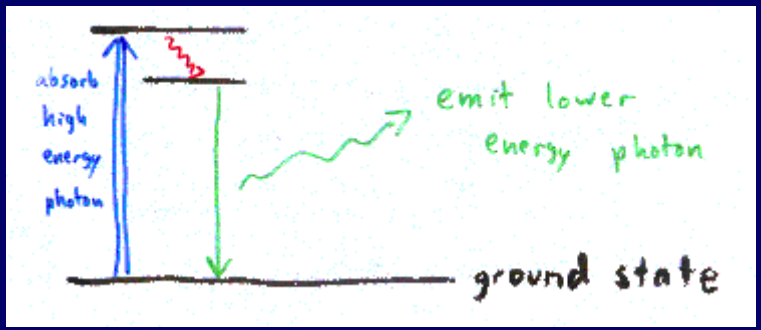
- Funding: "E. Fermi" Rome, Centre

Fluoromotric analysis

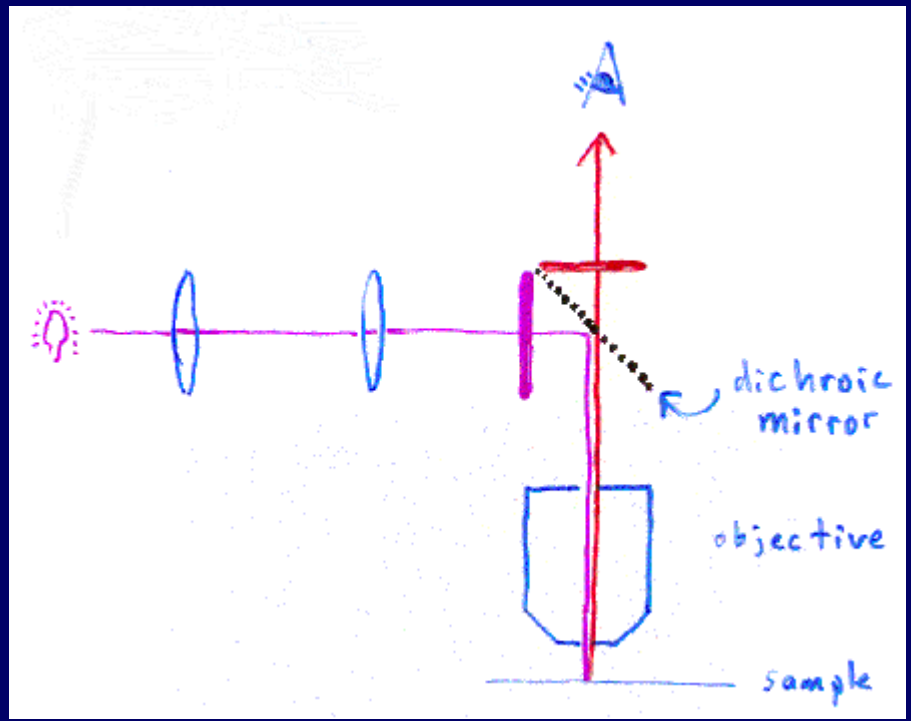


Fluorescence Microscopy

What is fluorescence?

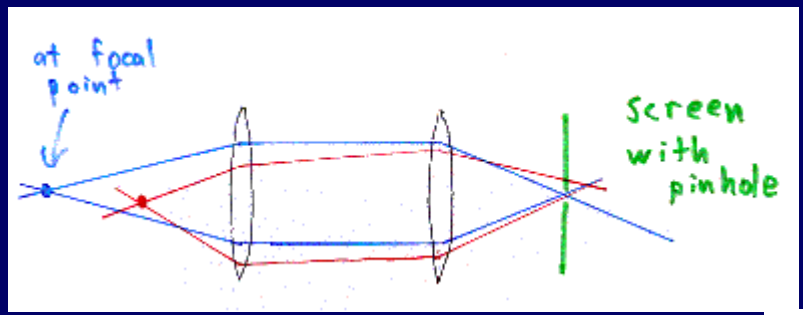


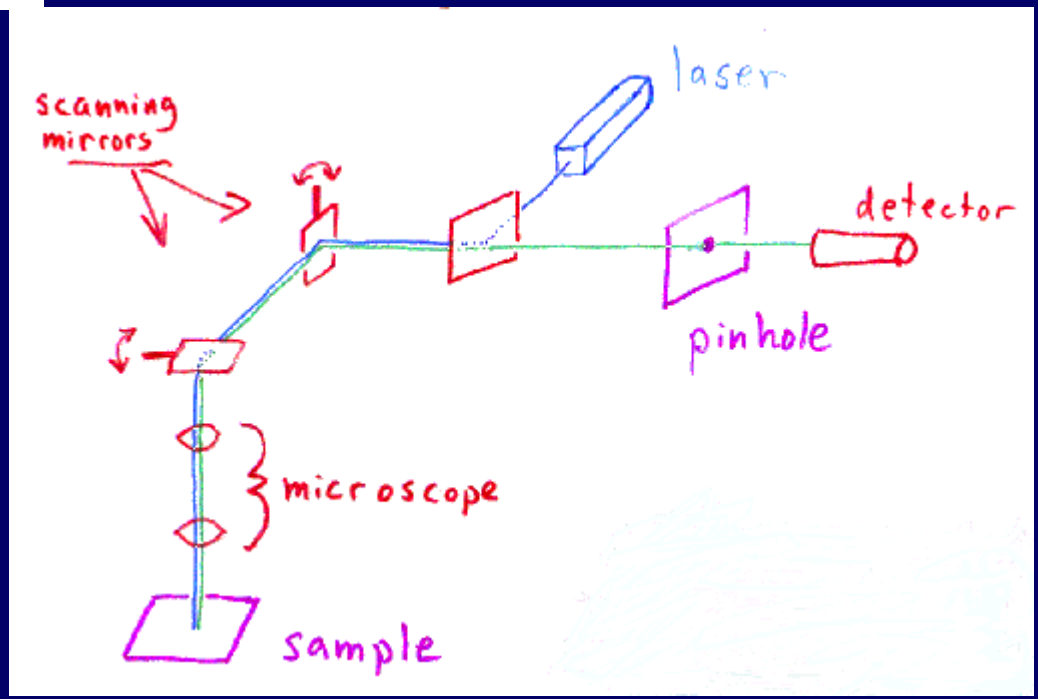
How does a fluorescence microscope work?

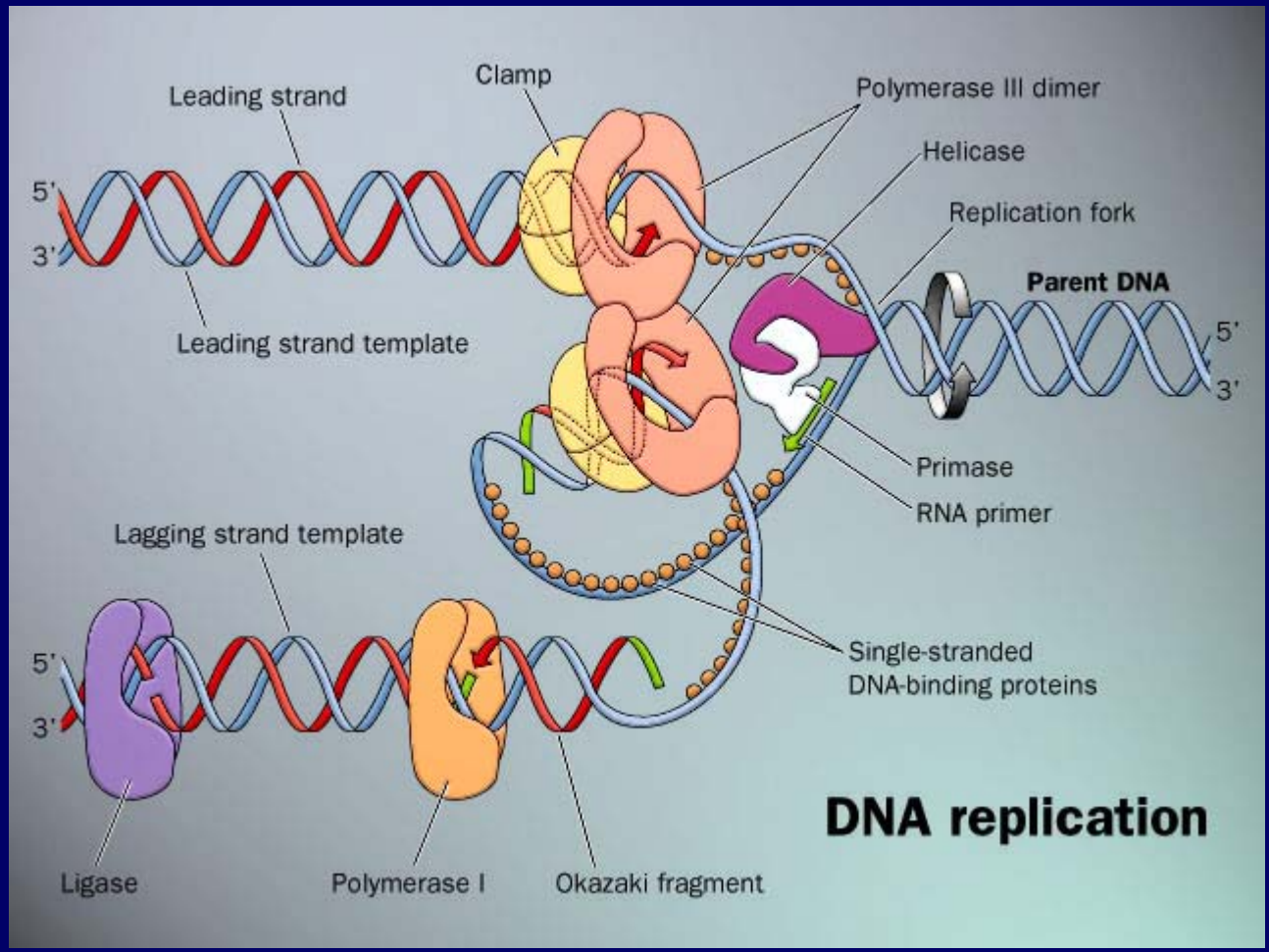


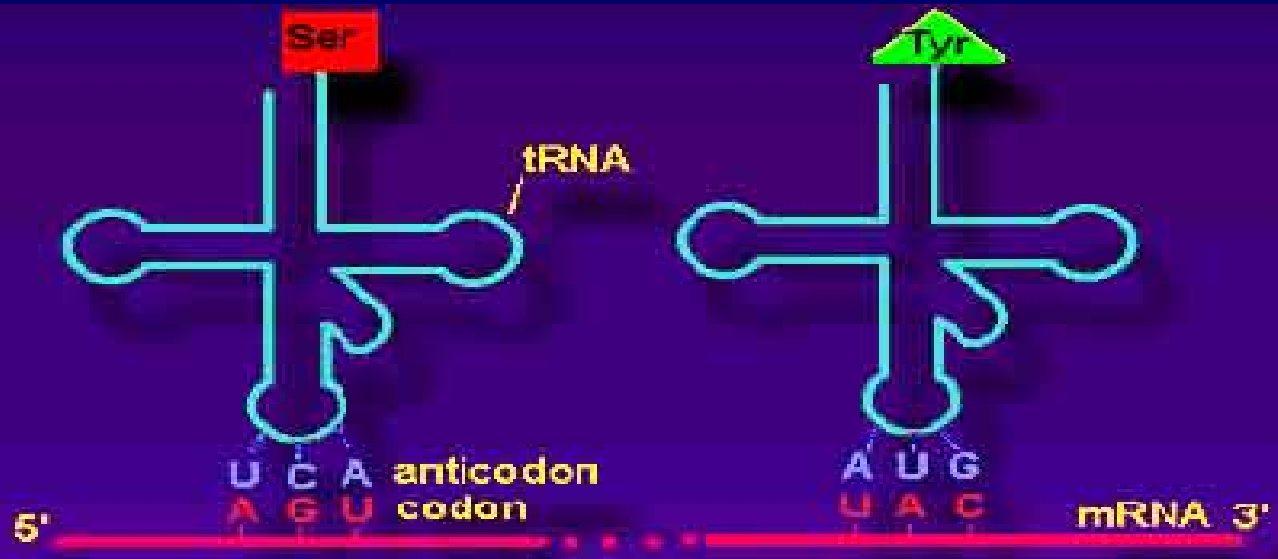
Confocal Microscopy

How does a confocal microscope work?



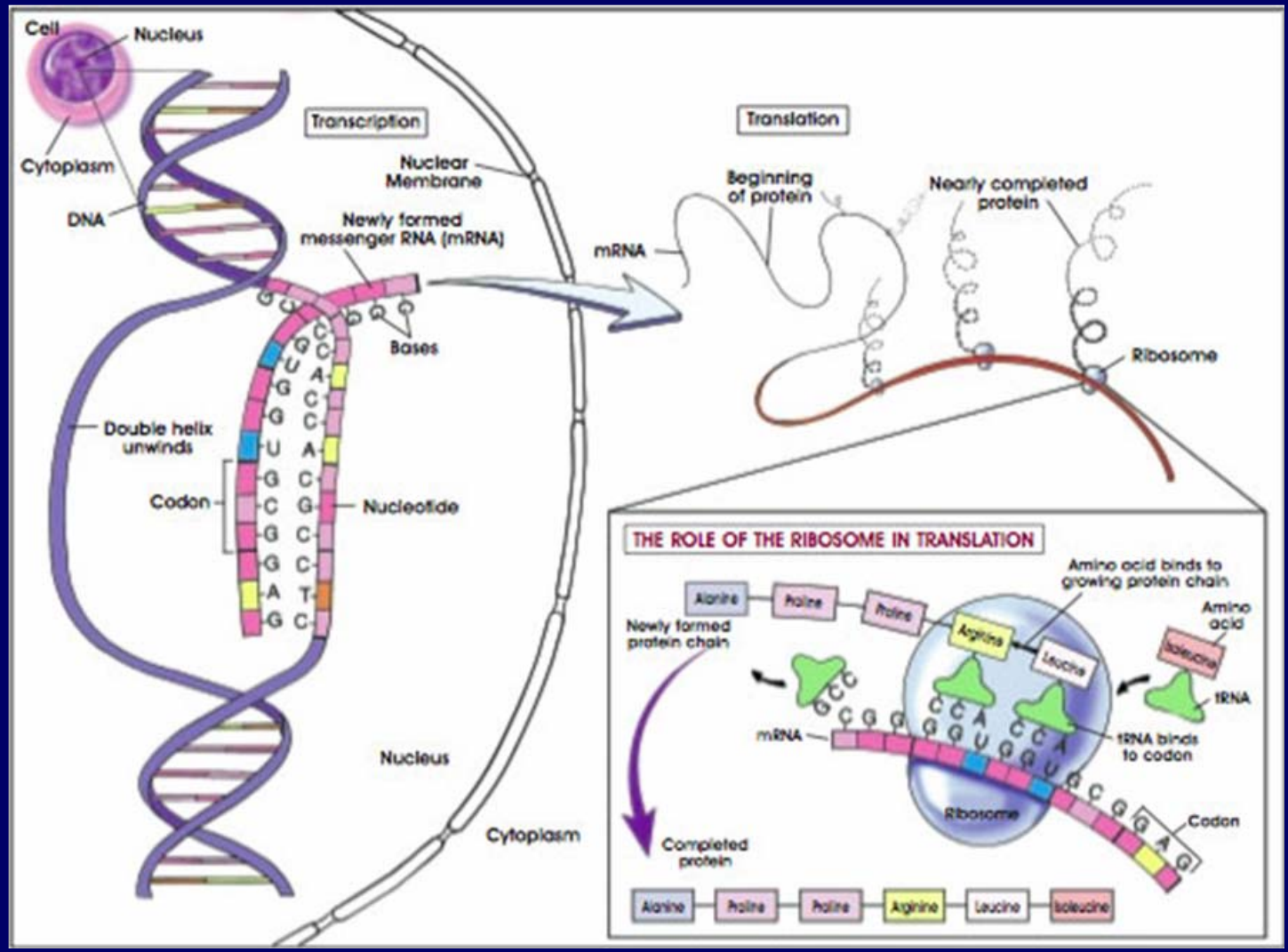


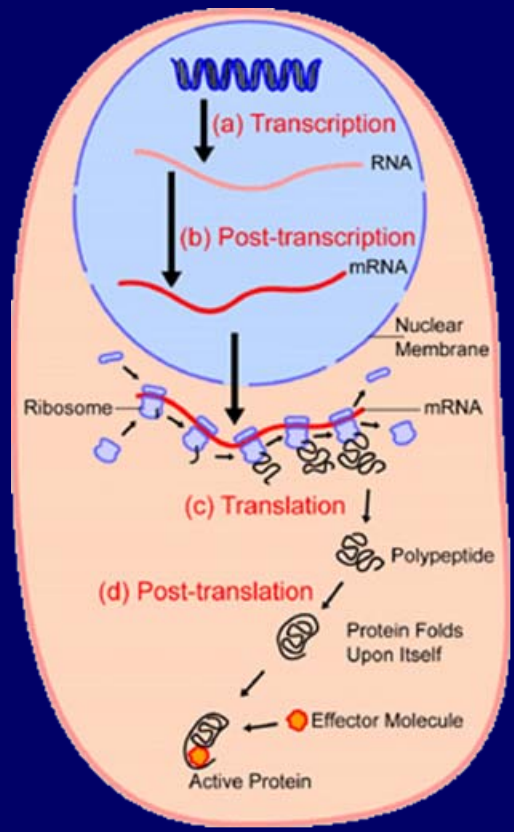

Leading strand
Clamp
Polymerase III dimer
Helicase
Replication fork
5'
3'
Parent DNA
Leading strand template
5'
3'
Primase
RNA primer
Lagging strand template
Single-stranded
DNA-binding proteins
5'
3'
DNA replication
Ligase
Polymerase I
Okazaki fragment



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	Ilis	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


Cell
Nucleus
Cytoplasm
DNA
Transcription
Nuclear Membrane
Newly formed messenger RNA (mRNA)
Bases
Double helix unwinds
Codon
Nucleotide
Nucleus
Cytoplasm
mRNA
Translation
Beginning of protein
Nearly completed protein
Ribosome
THE ROLE OF THE RIBOSOME IN TRANSLATION
Amino acid binds to growing protein chain
Alanine
Proline
Proline
Arginine
Leucine
Isoleucine
Amino acid
Newly formed protein chain
tRNA
mRNA
tRNA binds to codon
Ribosome
Codon
Completed protein
Alanine
Proline
Proline
Arginine
Leucine
Isoleucine


(a) Transcription
RNA
(b) Post-transcription
mRNA
Nuclear Membrane
Ribosome
mRNA
(c) Translation
Polypeptide
(d) Post-translation
Protein Folds Upon Itself
Effector Molecule
Active Protein

FAS Assay

FAS activity:

- rate of NADPH oxidation
- incorporation of radiolabeled acetyl-CoA or malonyl-CoA into **palmitate**

The fatty acids are analysed as phenacyl esters by HPLC