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بسم الله الرحمن الرحيم

GENETICS & Molecular Biology

Number: 15

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Subject: Translation of mRNA

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Date:

Translation

(Please refer to the pictures in the slides; they might really help you understand)

What is the general meaning of translation?

- ❖ It's the conversion of mRNA to Protein.

What are the **main** components needed for translation?

- ❖ mRNA, tRNA, rRNA (ribosomal) & some proteins.

Some information about **tRNA**:

- Small RNA molecule that can be attached to amino acids.
- These amino acids are attached to the 3' end of the tRNA.
- A charged tRNA molecule is a tRNA molecule that is attached to amino acids.
- Their sequence ends with CCA.
- Each tRNA molecule has only one anticodon. (sequences that are complementary to the mRNA sequence) and that's the base of how translation and peptide formation is done.
- Just like 2 complementary DNA strands, tRNA with mRNA are read anti-parallel. i.e.) if the mRNA strand is read from 5' to 3' from left to right, the tRNA strand above it will be 3' to 5' from left to right and vice versa.

  (Example) AUG is the codon on mRNA, the anticodons on tRNA is CAU.

  Translation depends on something called the genetic codon table.

Genetic codon table (DON'T memorize the table)

Second letter

First letter	U	C	A	G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Trp UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Met AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Stop AGG Stop	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

Features of the table:

✓ It's not universal; That means that there are exceptions. e.g.) AUA in mitochondria is Methionine, while in cytosol it's Isoleucine!

✓ Each codon contains three letters; **first** & **second** letters are universal, while the **third** letter is variable.

- The third base is called "degenerate base" **or** "wobble base".

So, the interaction of the third base on the mRNA with its anticodon on tRNA is called *Wobble base pairing*. (It doesn't follow Watson-Crick base pairing) → e.g. If the third base on a specific codon on the mRNA is "G", it would interact with a base that is NOT "C" on the tRNA anticodon. We conclude that in most of the cases, the third base is not important to determine the type of amino acid that will be generated. (Weakest base to interact with tRNA is the third).

- On the other hand, the second base is very important to determine the type of amino acid!

The second base determines the type or "class" of the amino acid. For example, if the second base of a codon codes for a hydrophobic amino acid and that base is mutated, another hydrophobic amino acid will replace it (or a partially charged amino acid to another partially charged amino acid, etc..)

SO, is a mutation in the third **or** second bases in the codon very damaging?

Answer is NO. Why? Because a mutated third base will rarely lead to an amino acid change, and a mutation in the second base will lead to a change of the amino acid but it will still be from the same class.

Ribosomes (factory of protein formation)

- Ribosomes in eukaryotes & prokaryotes are almost the same in structure; They are both composed of 2 ribosomal subunits (large & small)
- Each one of the ribosomal subunits is composed of rRNA molecules & some proteins.
- The formation of peptide bonds is catalyzed by rRNA.. NOT the proteins.

It was discovered that life started with **RNA**. It represents a genetic material that is able to catalyse chemical reactions, thus, from it, we can make proteins **&** genetic material (DNA). This theory was supported by the discovery of Retroviruses and their Reverse Transcriptase.

General Mechanism of Translation

- Three stages: Initiation → Elongation → Termination
- mRNA is read from 5' to 3'
- Protein synthesis starts at the N-terminus (amino) going to the C-terminus (carboxyl)

Differences between prokaryotes & eukaryotes in Translation:

- In prokaryotes, both transcription & translation are coupled in space and time. That means that at the same time that mRNA is being formed, Ribosomes works on it producing polypeptides (proteins) and all that happens in the same space.
- In eukaryotes, transcription occurs in the nucleus while translation occurs in the cytoplasm, both separated by nuclear membrane. Also translation starts after transcriptions is finished and the whole mRNA molecule is been synthesized.
- Prokaryotic mRNA is Polycistronic (one mRNA can be translated to many proteins)
- Eukaryotic mRNA is Monocistronic (one mRNA can be translated to only one protein)

Note: we said eukaryotic mRNA is monocistronic, NOT eukaryotic gene. A eukaryotic gene can be transcribed and translated to many proteins. e.g.) Calcitonin.

In both prokaryotes & eukaryotes, translation does NOT start from the beginning of the mRNA strand, it rather starts from a starting codon "AUG" and the sequence that is not translated upstream of AUG is called **"5' untranslated region" (5' UTR)**.

Translation also doesn't stop at the end of the mRNA strand; it rather stops once it reads one of the stop codons (UAA, UAG, UGA). The sequence that is not translated downstream of the last stop codon is called the **"3' untranslated region" (3' UTR)**.

The codon AUG codes for the amino acid Methionine.

- In eukaryotes, the first amino acid added is Methionine.
- In most prokaryotes, the first amino acid added is N-Formylmethionine. (modified methionine)

But does translating start from the first AUG? **No**. we'll talk about this now.

- **In prokaryotes,** there's another condition in order for translation to start. It's a consensus sequences called "Shine-Dalgarno sequence" –that is complementary to the sequence on the 16S rRNA of the small ribosomal subunit– and it must come before each AUG. So after the rRNA strand finds Shine-Dalgarno sequence it will start looking for AUG in order to start translating and when it finds a stop codon it will look for another Shine-Dalgarno sequence and then an AUG in order to translate another part of the mRNA to another protein, all this will continue until the mRNA strand is read completely.

- **In eukaryotes,** there are two conditions other than the AUG in order to start. The ribosome must first hit the mRNA 7-methylguanosine (m7G) cap and then a consensus sequence called "Kozak sequence" before it starts searching for an AUG.

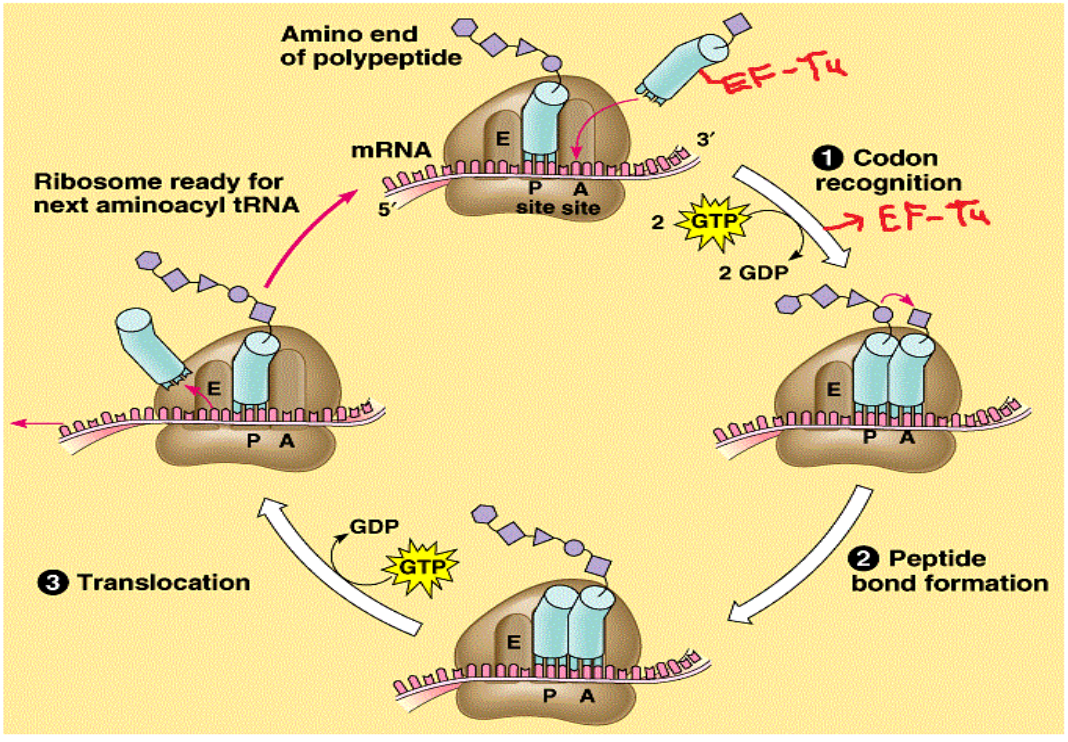
  Concluding the conditions for translation initiation for each type of cell:
  Prokaryotes: Shine-Dalgarno sequence → AUG.
  Eukaryotes: Cap → Kozak sequence → AUG.

Step 1 (initiation)

- ✓ Formation of the 30S initiation complex
  (30S **small** ribosomal subunit**,** mRNA**,** tRNA carrying Met or Fmet **&** initiation factor proteins IF1 & IF2).

- ✓ Formation of the 70S initiation complex
  After the binding of all these components, the 50S **large** ribosomal subunit is added to the 30S initiation complex to complete the formation of the full complex.

Step 2 (elongation) look at the picture in order to understand the steps



Steps of elongation:

1) Ribosome has 3 sites in where tRNA can bind. **P-site** (peptide), **A-site** (aminoacyl-tRNA) & **E-site** (exit).
2) The tRNA that carries Met or Fmet is already in the P-site from initiation.
3) When the ribosome reaches the second codon on the mRNA strand, another tRNA (aminoacyl-tRNA) binds to the A-site of the ribosome. That tRNA is:

    a. Carrying an amino acid (coded by that mRNA codon & complementary to the tRNA anticodon)

    b. Bound to GTP & elongation factor Tu (EF-Tu).

    When tRNA binds, GTP will become GDP and will be released with EF-Tu. The hydrolysis of GTP into GDP catalyses the binding of the aminoacyl-tRNA by inducing **conformational changes** in the Ribosomes and thus, increasing their affinity to the binding of aminoacyl-tRNA. → The EF-TU_GDP is then recycled to EF-TU_GTP again.

4) A peptide bond between the amino acids on the two tRNA molecules is formed. (The formation of the peptide bond is catalysed by enzymes in the **large ribosomal subunit**).
5) The (older) tRNA molecule that was on the P-site becomes uncharged (free of amino acid) and will move to the E-site by elongation factor 2 (EF-2) and get released & the newly added tRNA (now bound to 2 amino acids) will move to the P-site and the A-site will become free.
6) Now another tRNA will bind to the free A-site and the cycle keeps repeating until translation of the mRNA is completed and a new whole polypeptide is formed.

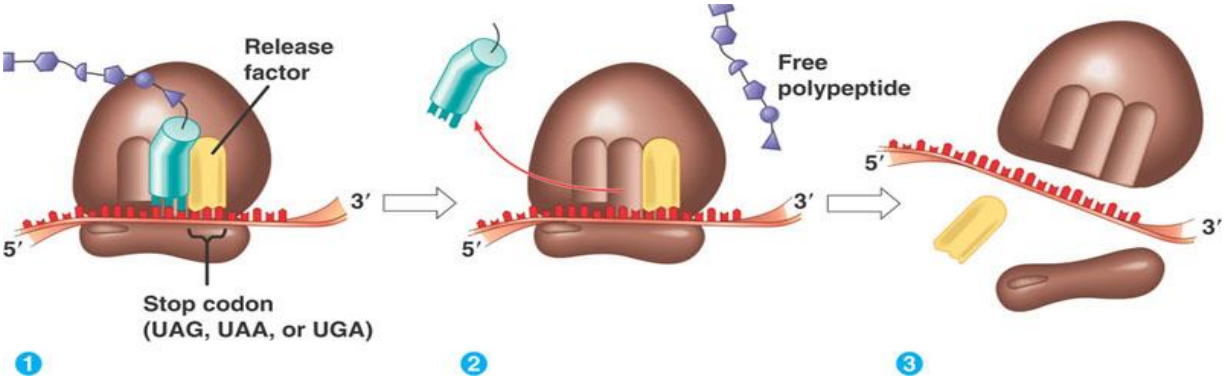
Now the strong question remains! Where do these tRNA molecules that carry amino acids come from?!

- ✓ They are pre-synthesized and stored in the cell. From this we conclude that in translation, we are synthesizing proteins NOT amino acids.

Note: Elongation is regulated by GTP, which implies to the general rule. (ATP is mostly used in energy degenerating pathways while GTP, CTP & UTP are used to regulate energy consuming pathways)

Step 3 (termination)

- When the ribosome hits a stop codon, no tRNA molecule will recognize it and bind to the A-site, instead, a protein called "release factor" binds, dissociating the whole complex and the polypeptide chain will be released by hydrolyzing the bond between it and the tRNA.



Selenocystein

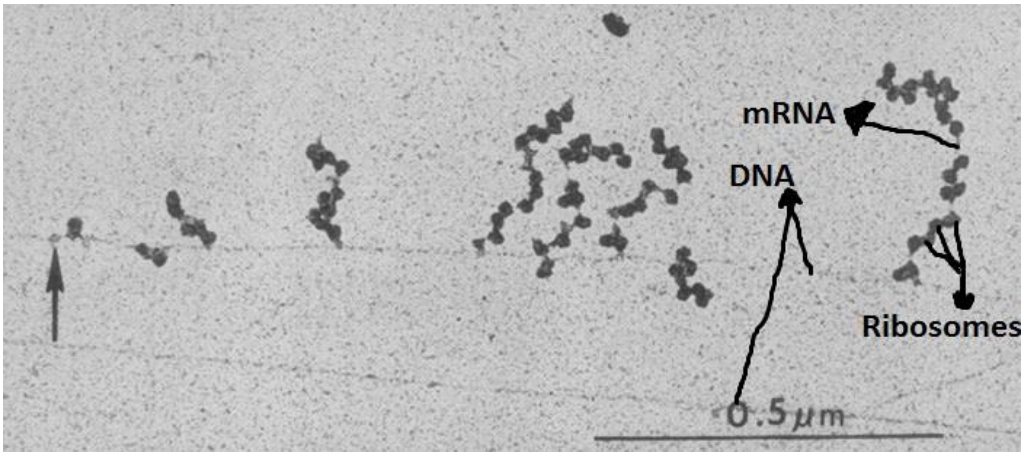
Sometimes, the stop codon "**UGA**" is not read as a stop codon, it's rather read by a special tRNA molecule called **$tRNA^{sec}$**; a tRNA molecule that has Selenocystein bound to it. Here, the translation will resume until the ribosome hits a real stop codon.

- Selenocystein is a modified Serine. (Oxygen of Serine replaced by Selenium) and it's considered the amino acid #21.

The case in where UGA is not read as a stop codon is when it's followed by a stem-loop structure (mRNA signal) that is recognized by the protein "Sel B".

Polyribosomes

It's the structure of a DNA strand generating many mRNA strands and on each mRNA strand there are many Ribosomes (the black dots on the figure) → Many ribosomes translating each one mRNA strand. **They are mainly present in prokaryotic cells.**



Inhibitors of translation

Some antibiotics are used to prevent translation mechanisms in prokaryotic cells.

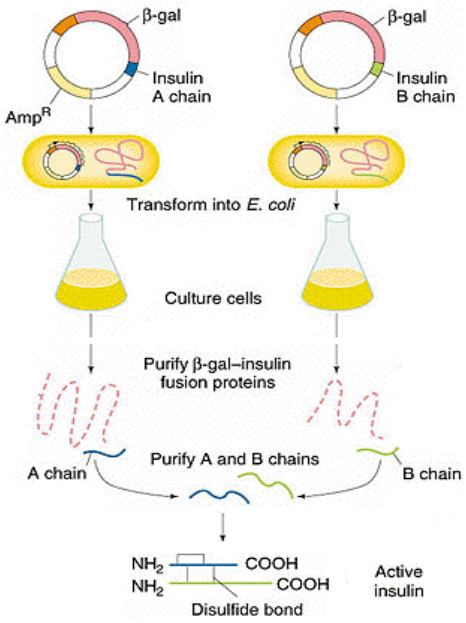
- ✓ **Tetracycline** – inhibits binding of tRNA to A-site of the ribosome.
- ✓ **Streptomycin** – induces binding of the wrong tRNA, resulting in defective proteins.
- ✓ **Chloramphenicol** – blocks enzymes that catalyze peptide formation on the ribosome.
- ✓ **Erythromycin** – blocks the movement (translocation) of the large ribosomal subunit.

In eukaryotes, a bacterial toxic protein called "**Diphtheria toxin**" interferes with protein synthesis by inhibiting the activity of elongation factor 2 (eEF2).

Why do we use cloning?

One of the benefits of cloning is production of eukaryotic proteins in prokaryotes! (e.g.) **Insulin**.

Insulin is a dimer that has a disulfide bond connecting both of its polypeptide subunits (**α** and **β**). Production of disulfide bonds in prokaryotes is difficult, so a DNA strand from each subunit is inserted into a separate bacterial chromosome (cell) and are left to replicate then cells are cultured and the produced insulin polypeptides are purified and mixed to form the complete insulin protein.



Regulation mechanisms:-

1) Heme & protein synthesis

Hemoglobin is produced by reticulocytes.

It would be a waste for those cells to produce globin if there is no heme, so they are regulated to synthesize globin only when there is heme. (hence globin is a protein and it's produced by translation process)

We mentioned that one of the most important proteins required for Step 1 (initiation) is "initiation factor 2" (IF-2) or (eIF-2) → 'e' stands for eukaryotic.

The regulation of heme synthesis is done by stimulating or inhibiting this protein.

- eIF-2 is active when it's **dephosphorelated** & vice versa.
- eIF-2 is active when it's bound to **GTP** & inactive when it's bound to GDP. (They are both related to each other)

So, normally, when there is adequate supply of heme, GTP will replace GDP on eIF-2, making it active → Translation continues (globin is synthesized).

When there is heme deficiency, a protein kinase phosphorelates eIF-2 so that GDP will remain bound to the protein and GTP won't be able to replace it and the protein will remain inhibited → Translation is inhibited.

Apo proteins & protein synthesis

Holo protein: a protein that is bound to a non-protein part. (e.g.) In Hemoglobin, globin is a protein that is bound to a non-protein part which is heme.

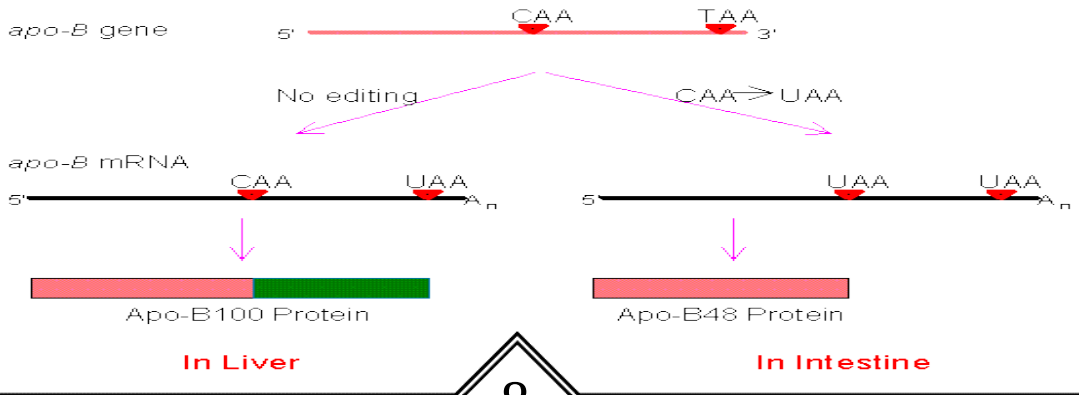
- Apo protein: a protein that is not bound to a non-protein part. (ONLY protein)

For example, Lipoprotein: a protein that is bound to a lipid.

- Apo lipoprotein: it's that same Lipoprotein but without the lipid part.
  - **ApoB-48** (48 kDa of protein): It's part of chylomicrons & synthesizes lipoproteins that transport lipids from intestinal cells to liver.
  - **ApoB-100** (100 kDa of protein): It's part of a low-density lipoprotein (LDL) that's responsible for transporting lipids from liver to other parts of the body.

ApoB-48 and ApoB-100 are both synthesized from the same gene (apo-B gene) by a process called *Gene Editing*;

- The gene normally has a CAA sequence in its DNA.
- The DNA is transcribed into mRNA and the latter it will have a CAA as well.
- AFTER transcription, the CAA might remain unedited on the mRNA and will be translated into an **ApoB-100**.
- If the CAA is edited to a UAA (stop codon) on the mRNA, the strand will be translated into an **ApoB-48**.



2) "silent" SNPs & protein synthesis

To recap, SNPs is of many types; (*silent*, causative & linked SNPs)

Silent SNPs are genetic variations on mRNA strands that do not result in a change of amino acid sequence of a protein. But how do they affect protein synthesis?

➔ They slow down the movement of ribosomes during translation.
This will affect the exit of the polypeptide chain from the ribosome leading to proteins with different structures & activities but with identical amino acid sequences. (The doctor considered them misfolded proteins).

3) miRNA & protein synthesis

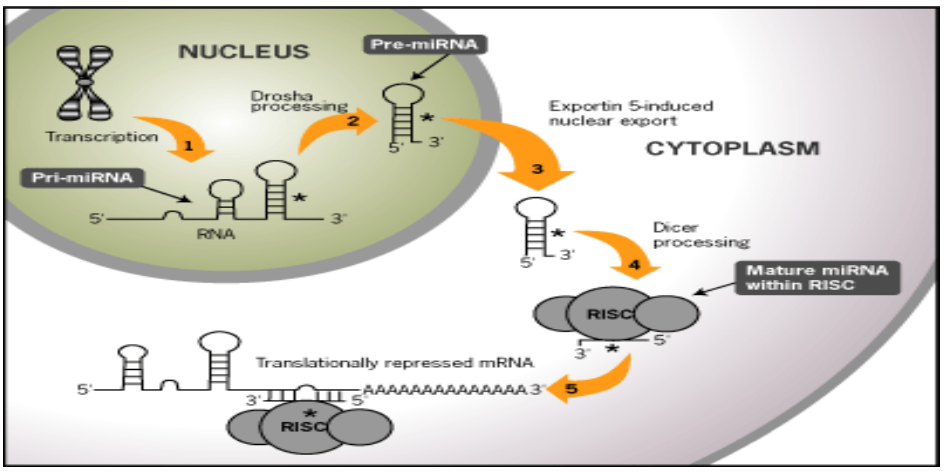
- Small molecules, complementary to part of the mRNA and can affect translation by **either** blocking translation **or** degrading mRNA → Less protein production.
- Synthesized from their own genes. (miRNA genes)

In the nucleus:

- Transcription by "RNA polymerase II" forming a single-stranded RNA which has a part that is complementary to another part in it, leading to the formation of a stem-loop structure called primary miRNA **(pri-miRNA)**
- Modified to **pre-mRNA** by a protein called "Drosha"
- Exported to the cytosol by a protein called "Exportin 5"

In the cytoplasm:

- pre-mRNA is modified into a mature double-stranded **miRNA duplex** by breaking the stem-loop structure by "Dicer" (part of an endonuclease complex).
- The miRNA is then carried by a protein called "RISC" to target part of the mRNA strand, inhibiting its translation.



Level of miRNA differs in different diseases, for example: **Cancers**:

- If there is miRNA acting on **oncogenes**, they will be inhibited and this will lead for less cell proliferation, thus, it will be a useful method to treat cancer.
- If there are miRNA acting on **tumor suppressors**, they will be inhibited, leading for more cell proliferation, thus, the cancer will be more dangerous.

Fate of mis-folded or un-folded proteins

Two choices that a cell can make:

Either Refolding the protein (fixing) **or** Degrading it if it can't be fixed.

Degradation can happen by one of these two mechanisms:

- Lysosomal degradation (by lysosomal enzymes)
- Ubiquitinylation (by macromolecular proteasomes)

In ubiquitilation; Proteins are tagged by ubiquitin → Protein-ubiquitin complex is sent to proteasome to get degraded → The protein is degraded & ubiquitin is recycled to be used again.

The sheet is now over.
Special dedication la Abdelkarim hyari
Never underestimate yourself, each one of you has one specialty that makes him different from others… Just never let go of your dreams w tafaa2alu ^_^
Mwafageen :D
Omar Mahafza.