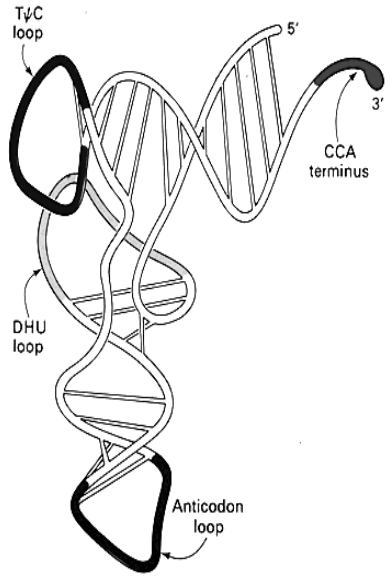
بسم الله الرحمن الرحيم



ليه التعقيد .. ركز معنا ☺

☑ الطريقة أصبحت معروفة للجميع ☺

☑ هذه المذكرة اهداء الى جدي الغالي – رحمه الله فلا تنسوه من دعائكم ،،،

RNA Structure & synthesis

Team leader : مجهول

والشكر لجميع من ساهم في اخراجها بالصورة التي هي عليه وأخص بالشكر :

أبو يسرا

عبدالعزيز التركي

Ocean

Blue eye

وجنودنا المجهولين ☺

◙ **Note :** N.B : eukaryotic cell produce only monocistronic messages . that is , each eukaryotic mRNA moleculeencodes just 1 protein (MCQ)
Doctor said that we have to change 1 protein into 1 polypeptide . (خطأ في الكتاب)

V. POSTTRANSCRPTIONAL MODIFICATION OF RNA :

A primary transcript :

- **is a linear copy of a transcriptional unit–the segment of DNA between specific initiation & termination sequences.**

	prokaryotes	eukaryotes
rRNA	are post-transcriptionally modified by cleavage of the original transcripts by ribonucleases	are post-transcriptionally modified by cleavage of the original transcripts by ribonucleases
tRNA	are post-transcriptionally modified by cleavage of the original transcripts by ribonucleases . tRNAs are then further modified to help give each species its unique identity	are post-transcriptionally modified by cleavage of the original transcripts by ribonucleases . tRNAs are then further modified to help give each species its unique identity
mRNA دائما تأتي بالاختبار	generally identical to its primary transcript(no posttranscription)	extensively modified posttranscriptonally

A. Ribosomal RNA:

prokaryotes	eukaryotes
in the nucleolus	in the nucleolus
are synthesized from long precursor molecules called preribosomal RNAs.	are synthesized from long precursor molecules called preribosomal RNAs.
DNA -----> rRNA gene by (RNA polymerase 1) -----> pre rRNA by (riboncleases) ----> intermediate – sized piece of rRNA by (trimmed) -----> rRNA species .	DNA -----> rRNA gene by (RNA polymerase 1) -----> pre rRNA by (riboncleases) ----> intermediate – sized piece of rRNA by (trimmed) -----> rRNA species .
The 23S,16S & 5S ribosomal RNAs of prokaryotes are produced from a single RNA precursor molecule	28S,18S & 5.8S rRNAs of eukaryotes are produced from a single RNA precursor molecule
Other notes : (MCQ) some of the protein destined to become components of the ribosome associate with rRNA precursor prior to & during its posttranscriptional modification in the nucleolus	Other notes : (MCQ) Eukaryotes 5S rRNA is synthesized by *RNA polymerase III (not 1 like others)* & modified separately

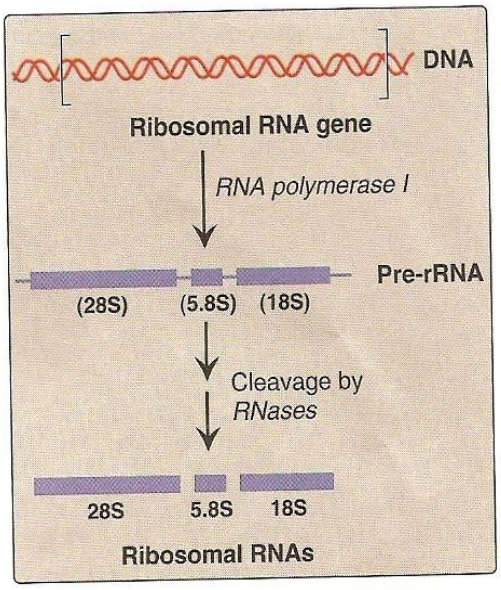


Figure 30.15
Posttranscriptional processing of eukaryotic ribosomal RNA by ribonucleases.

B. Transfer RNA : (exactly like what the doctor said)

- Both eukaryotic & prokaryotic transfer RNAs are also made from longer precursor molecules that must be modified .

- **14-nucleotide intron** must be removed from the **anticodon loop** by **splicing** , & sequences at both 5'-&3'- ends of the molecule must be terminated .

- **16 – nucleotide sequence at the 5' –end is cleaved by RNase P .**

- Other posttranscriptional modifications include replacement of uracil by addition of a–CCA sequence by *(nucleotidyltransferase*) to the 3'-terminal end of tRNA **(found on all mature tRNA) . (MCQ)(always come in final exams)**

- modification of bases at a specific positions to produce "**unusual bases**". **then**

  e.g : dihydrouracil .

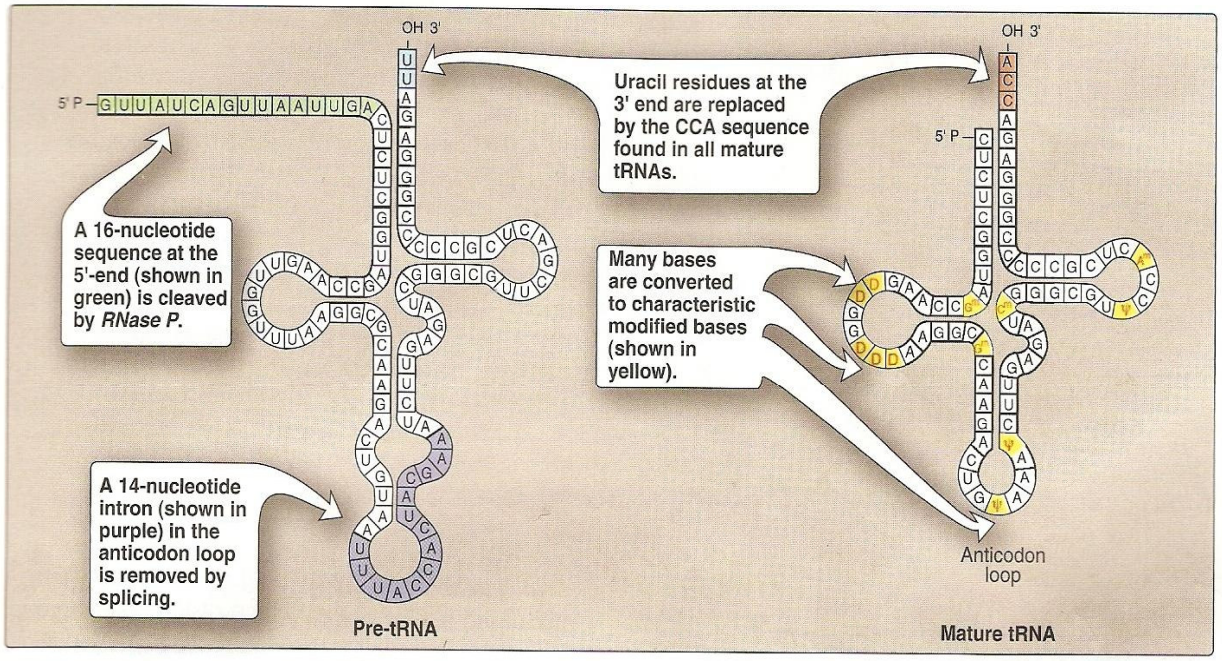


Figure 30.16
A. Primary tRNA transcript. B. Functional tRNA after posttranscriptional modification. Modified bases include D (dihydrouridine), ψ (pseudouridine), and ^{m}, which means that the base has been methylated.

C. Eukaryotic messenger RNA :

- The RNA molecule synthesized by *RNA polymerase II* **(primary transcript) (Not polymerase 1 always come in exam so RNA polymerase 1 we find it In rRNA)** (MCQ)
- contain the sequences that are found in cytosolic mRNA .
- The collection of all the precursor molecules for mRNA is known heterogeneous nuclear RNA (hnRNA) .
- The primary transcript are extensively modified in the nucleus after transcription , *these modifications include* :

1. 5' "capping":

- This is the first processing reactions for hnRNA .

✓ The cap is 7-methyl-guanosine attached **backward**(MCQ) to the terminal end of the mRNA forming an unusual 5' ➔ 5' triphosphate linkage .

✓ The addition of the guanosine triphosphate part of the cap is catalyzed by the **nuclear** *guanylyltransferase* (note not cytoplasmis its nuclear) .

✓ Methylation of terminal guanine is catalyzed by the cytosolic *guanine -7-methyltransferase (note not nuclear its cytoplasmic)*

✓ , methyl donor is SAM (S-adenosylmethionie) .

✓ Additional methylation steps may occur .

✓ Functions of the 7-methyl-guanosine cap are :

a. Initiation of translation (eukaryotic mRNAs lacking the cap are not efficiently translated) .
b. Stabilizing mRNA .

Are you still confusing turn the page to see how we change the ☹

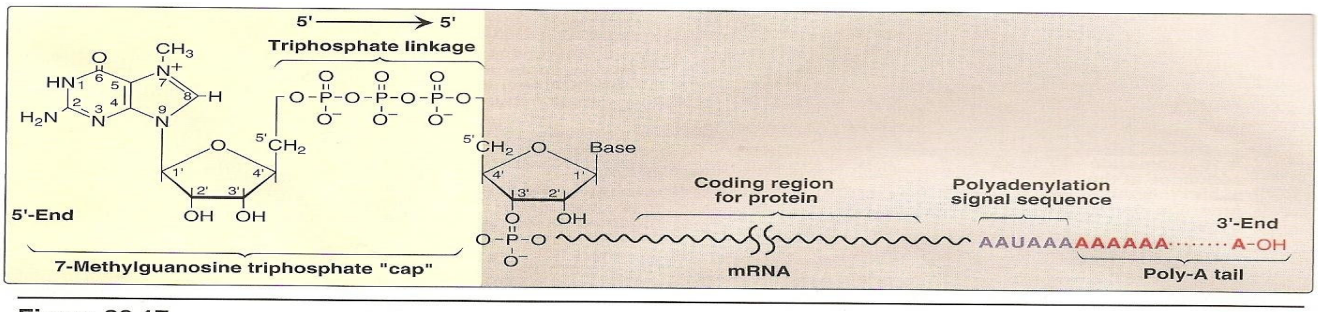


Figure 30.17
Posttranscriptional modification of mRNA showing the 7-methylguanosine cap and poly-A tail.

So,

According to **5' "capping": (☹ ---- > ☺) (MCQ)**

Cap : 7-methyl-guanosine .

Bond : unusual 5' ➔ 5' triphosphate linkage .

Site : nucleus & cytosol .

Enzymes : 1- **nuclear** *guanylyltransferase* .
2- **cytosolic** *guanine -7-methyltransferase* ,
3 - methyl donor is SAM (S-adenosylmethionie) .

Functions :

a. Initiation of translation (eukaryotic mRNAs lacking the cap are not efficiently translated).
b. Stabilizing mRNA .

2. Addition of a poly-A tail

- Most eukaryotic mRNAs have a chain of 40-200 adenine (MCQ) nucleotides attatched to the 3'-end (poly-A tail) . (MCQ)

  ✓ Note that mRNAs those coding for the histones & some interferons doesn't have poly-A tail. (T) (always come in final exams)

✓ The poly-A tail is not transcribed from DNA هاااااااام , but is added after transcription by *nuclear polyadenylate polymerase* .

✓ A consensus sequence called the polyadenylation signal sequence (AAUAAA) found near the 3'-end of the RNA molecule , signal the adding of poly-A tail to the mRNA .

✓ Functions of poly-A tail :
a. Stabilizing mRNA .
b. Facilitate the exit of mRNA from the nucleus to the cytosol .

✓ After mRNA enters the cytosol , the poly-A tail is gradually shortened .

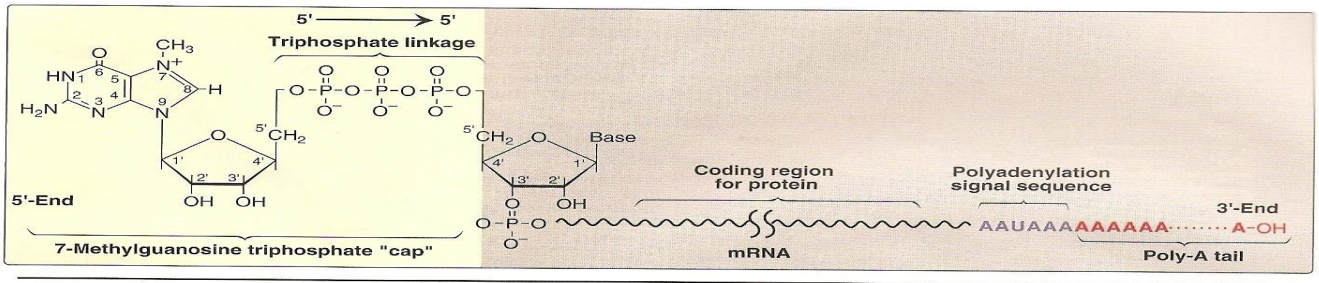


Figure 30.17
Posttranscriptional modification of mRNA showing the 7-methylguanosine cap and poly-A tail.

So,

According to **poly-A tail:** (☹ ----- > ☺)

- **Nature** : a chain of 40-200 adenine nucleotides attatched to the 3'-end of mRNA.

  Remember :
  - Not found in mRNAs of the histones & some interferons ☺ .
  - is not transcribed from DNA , but is added after transcription .

- **Site** : nucleus & cytosol .

- **Enzyme of adding** : *nuclear polyadenylate polymerase* (MCQ)(not cytoplasm) .

- **signal of adding** : polyadenylation signal sequence (AAUAAA) found near the 3'-end of the RNA molecule . **(MCQ)**

- **Functions** :
  a. Stabilizing mRNA .
  b. Facilitate the exit of mRNA from the nucleus to the cytosol .

Don't forget :After mRNA enters the cytosol , the poly-A tail is gradually shortened ☺ .

3.Removal of introns :

◙ In the maturation of EUARYOTIC mRNA … usually RNA sequences are removed (*don't code for protein(MCQ) (introns or intervening sequences) from the primary transcripts)*

◙ The remaining coding sequence (exon) are spliced together to form the mature mRNA

◙ So mature mRNA is smaller than primary transcripts (MCQ).

◙ This process is accomplished by (spliceosome) (MCQ)

Intron	exon
- Sequence not coding for protein(MCQ) - No , few or many . -some contain more than 50% intervening sequences(such as the primary transcripts for the α-chains of collagen) that must be removed before mature mRNA is ready for translation	-Sequence coding protein

a. role of small nuclear RNAs (snRNAs):

- (snRNPs or " snurps ") = snRNAs + proteins . (MCQ)
- spliceosome = snRNPs + primary transcripts . (MCQ)
- Function : these facilities the splicing of exon segments by foming base pair with the consensus sequences at each end of the intron .

◙ Note : Removal of intron is an energy dependent mechanism .

◙ systemic lupus erythematosus : (MCQ)

- Fatal inflammatory disease
- autoimmune response .
- antibodies against host protein including snRNPs

a. role of small nuclear RNAs (snRNAs):

- (snRNPs or " snurps ") = snRNAs + proteins . (MCQ)
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◙ Note : Removal of intron is an energy dependent mechanism .

◙ systemic lupus erythematosus : (MCQ)

- **Fatal inflammatory disease**
- **autoimmune response .**
- **antibodies against host protein including snRNPs**

b. Mechanism of excision of introns :

دائما ما يأتي نوع الرابطة والتعداد الرابع ،،،

1- binding of snRNPs .

Function : brings the sequences of the neighboring exons into the correct alignment for splicing .

2- The 2'-OH group of an adenosine (A) residue (known as the branch site)

- **Function** : for intron attacks (IMP)(always come in exams) .
- **Type of bond** : forms a phosphodiester bond with the phosphate at the 5'-end of intron 1 .

Note : the GU & AG sequences at the branch site are invariant

3- The newly freed 3'- OH of the upstream exon 1 then forms a phosphodiester bond with the 5'-end of the downstream exon 2 .

4- The excised intron is released as a **"lariat" structure** , which is degraded.

◙ After removal of all introns, the mature mRNA molecules leave the nucleus by passing into the cytosol through pores in the nuclear membrane.

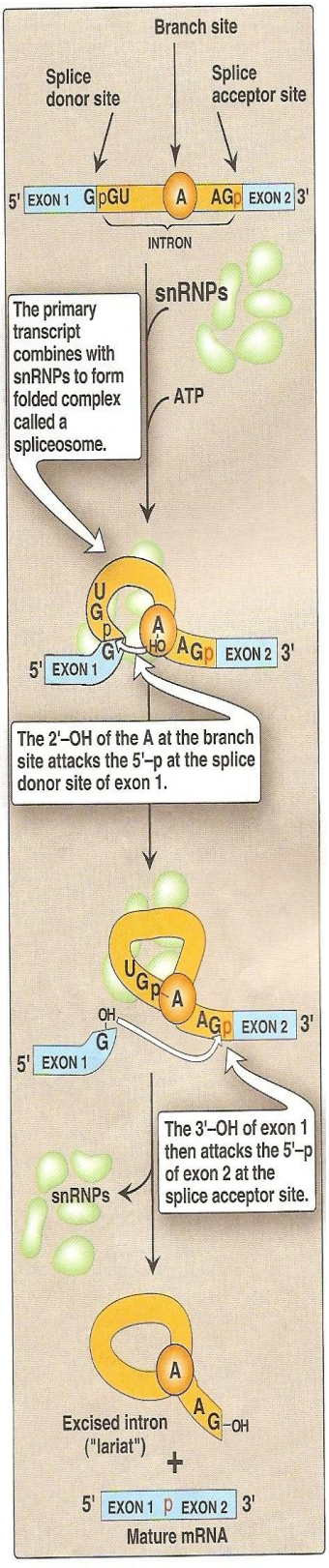


Figure 30.18
Removal of introns. snRNP = small nuclear ribonucleoprotein particle.

C . effect of splice site mutation :-

* mutation at splices site can lead to :

- improper splicing
- production of aberrant proteins

* 15% of genetic desease result from mutation affect RNA splicing

* EX : β thalassemia : (you will have a question on it)

- from mutation affect the splicing of RNA
- disease in which the production of the β-globin protien is defective

*Alternative splicing of mRNA:

◙ the pre-mRNA molecules from some genes can be spliced in **two or more** alternative pathway→ produce multiple variation of the mRNA and therefore of its protein product

◙ this mechanism for producing a divers sets of protein from limited set of genes (I see it in all exams)

e.g. different type of muscle cell all produce the same primary transcript from tropomyosin gene

however different patterns of splicing in the different cell types produce a family of tissue –specific tropomyosin protein molecules

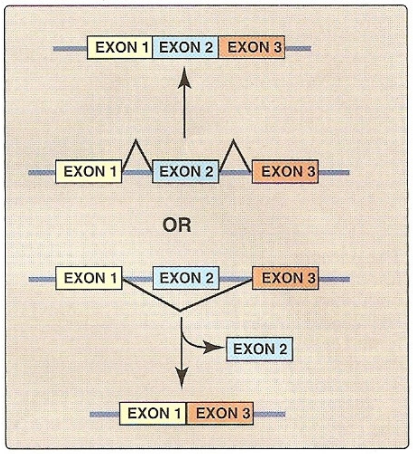


Figure 30.19
Alternative splicing patterns in eukaryotic mRNA.

Study Questions

Choose the ONE correct answer

30.1 A one-year-old male with chronic anemia is found to have β-thalassemia. Genetic analysis shows that one of his β-globin genes has a G to A mutation that creates a new splice acceptor site nineteen nucleotides upstream from the normal splice acceptor site of the first intron. Which of the following best describes the new messenger RNA molecule that can be produced from this mutant gene?

A. Exon 1 will be too short.
B. Exon 1 will be too long.
C. Exon 2 will be too short.
D. Exon 2 will be too long.
E. Exon 2 will be missing.

Correct answer = D. Because the mutation adds an additional splice acceptor site (the 3'-end) of intron 1 upstream, the nineteen nucleotides that are usually found at the 3'-end of the excised intron 1 lariat can remain behind as part of exon 2 as a result of aberrant splicing. Exon 2 can therefore, have these extra nineteen nucleotides at its 5'-end. The presence of these extra nucleotides in the coding region of the mutant messenger RNA molecule will prevent the ribosome from translating the message into a normal β-globin protein molecule. Those mRNAs for which the normal splice site is used to remove the first intron, will be normal, and their translation will produce normal β-globin protein.

30.2 A culture of E. coli that has been growing in medium containing lactose as its only source of energy is suddenly supplemented by the addition of a large amount of glucose. What change occurs in these bacteria to cause the rate of β-galactosidase synthesis to dramatically decrease?

A. The CAP protein dissociates from its DNA binding site.
B. The CAP protein becomes bound to its DNA binding site.
C. The inducer dissociates from the repressor.
D. The repressor dissocates from the operator.
E. The repressor becomes bound to the operator.

Correct answer = A. The addition of glucose causes cyclic AMP production to decrease. In the absence of cyclic AMP, the CAP protein cannot remain bound to its DNA binding site. An empty CAP binding site is not able to help RNA polymerase initiate transcription, so the rate of transcription decreases. Lower mRNA production results in decreased β-galactosidase synthesis. Because lactose is still present, the inducer (allolactose) remains bound to the repressor, which continues to be unable to bind to the operator.

30.3 The base sequence of the strand of DNA used as the template for transcription has the base sequence GATCTAC. What is the base sequence of the RNA product? (All sequences are written according to standard convention.)

A. CTAGATG
B. GTAGATC
C. GAUCUAC
D. CUAGAUG
E. GUAGAUC

Correct answer = E. All sequences are written in the standard convention (5'→3'). The RNA product has a sequence that is complementary to the sequence of the template strand of DNA. Uracil (U) is found in RNA in place of the thymine (T) in DNA. Thus, the DNA template 5'-GATCTAC-3' would produce the RNA product 3'-CUAGAUG-5' or, written correctly in the standard direction, 5'-GUAGAUC-3'.

30.4 A four-year-old child who becomes easily tired and has trouble walking is diagnosed with Duchenne muscular dystrophy, an X-linked recessive disorder. Genetic analysis shows that the patient's gene for the muscle protein dystrophin contains a mutation in its promoter region. What would be the most likely effect of this mutation?

A. Initiation of dystrophin transcription will be deficient.
B. Termination of dystrophin transcription will be deficient.
C. Capping of dystrophin mRNA will be defective.
D. Splicing of dystrophin mRNA will be defective.
E. Tailing of dystrophin mRNA will be defective.

Correct answer = A. Mutations in the promoter prevent formation of the RNA polymerase II transcription complex, and the initiation of mRNA synthesis will be greatly decreased. A deficiency of dystrophin mRNA will result in a deficiency in the production of the dystrophin protein.

الحمد لله ،،، انتهينا من آخر محاضرات هذا الباب ،،

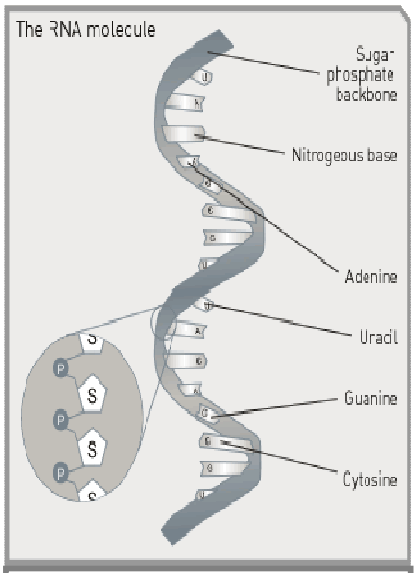
وفي نفس الوقت انتهينا من آخر مذكرات هذا الفصل الدراسي ،،

هذه المذكرات الثلاث في هذا الباب فصلنا فيها وذلك لكثرة الأسئلة عليها في اختبار منتصف السنة ،،

بالنسبة للكويز لكم منا هديتين :
الأولى : مني أنا مجهول .
الثانية : من أعضاء البيو تيم .
سوف تعلن يوم الجمعة باذن الله تعالى على KSUMS

الى ذاك الحين
دمتم بخير ،،

أخوكم : أحمد العقيل



نأمل أن نكون قد فككنا التعقيد :☺

Team leader :
مجهول

والشكر لجميع من ساهم في اخراجها بالصورة التي هي عليها وأخص بالشكر :

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نلقاكم في الفصل القادم لكم منا كل المحبة والتقدير ،،،