
النادي الطبي
Genetics
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Transcription in Eukaryotes

By

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- عند حصول عملية transcription وتكوّن RNA transcript فإنه لن يبقى متصل بـ DNA template :
١) يجب على RNA transcript أن ينفصل ويخرج من nucleus ليقوم بوظيفته سواء أكان mRNA, tRNA, rRNA

٢ " " " " " " " " ليتم عمل نسخ جديدة من RNA transcript.

❖ Remember:

- **RNA polymerase** (the enzyme that catalyzes RNA synthesis) does not need a primer; rather, it can initiate transcription de novo.

- The RNA product **does not remain base-paired to the template DNA strand**. This displacement is critical for the RNA to perform its functions (e.g., as is most often the case, to be translated to produce its protein product). **Furthermore**, because of this release, multiple RNA polymerase molecules can transcribe the same gene at the same time. Thus, a cell can synthesize large numbers of transcripts from a single gene in a short time.

يمتلكان DNA polymerase و RNA polymerase - وكلا proofreading activity أقل كفاءة من DNAP.

RNAP: في كل ١٠٠٠٠ نيوكليوتايد يوجد واحد خطأ.

DNAP: في كل ١٥m نيوكليوتايد يوجد واحد خطأ.

- Transcription, although very accurate, is **less accurate than replication** (one mistake occurs in 10,000 nucleotides added, compared with one in 10 million for replication). This difference reflects the lack of extensive proofreading mechanisms for transcription, although proofreading for RNA synthesis do exist.

- It makes sense for the cell to worry more about the accuracy of replication than of transcription. DNA is the molecule in which the genetic material is stored, and DNA replication is the process by which that genetic material is passed on. Any mistake that arises during replication can therefore easily be catastrophic: it becomes permanent in the genome of that individual and gets passed on to subsequent generations. **Transcription, in contrast, produces only transient copies and normally several from each transcribed region. Thus, a mistake during transcription will rarely do more harm than render one out of many transient transcripts defective.**

DNA replication هي العملية التي تنتقل عن طريقها genetic material إلى daughter cells، فبالتالي أي خطأ في DNA replication يكون permenant mistake in genom of the cell وبالتالي فإنه ينتقل إلى daughter cells (كارثي catastrophic) حيث أن أي خطأ يكون

الخطأ في عملية transcription أقل خطورة من الخطأ في replication حيث أن RNA copies تتكسر ولا تبقى داخل الخلية ويتم اتباع الكثير منها -

- **The choice of which regions to transcribe is not random:** there are specific DNA sequences that direct the initiation of transcription at the start of each region and others at the end that terminate transcription.

in different cells

- In different cells, or in the same cell at different times, different sets of genes might be transcribed. Therefore, for example, two genetically identical cells in a human will, in many cases, transcribe different sets of genes, leading to differences in the character and function of those two cells (e.g., one might be a muscle cell and the other a neuron).

- تختلف الجينات التي يحدث لها transcription من خلية لأخرى حيث انتاج insulin

turned off يكون في خلايا الدماغ

turned on يكون في خلايا البنكرياس

في خلايا البنكرياس يحدث expression لجينات وفي خلايا القلب يحدث expression لجينات اخرى على الرغم من انهم يحتوون على نفس الجينات.

in the same cells at different time

- تختلف الجينات التي يحدث لها transcription في نفس الخلية.

عندما تكون الخلية في cell division فإن البروتينات التي تدخل في عملية DNA replication يتم انتاجها

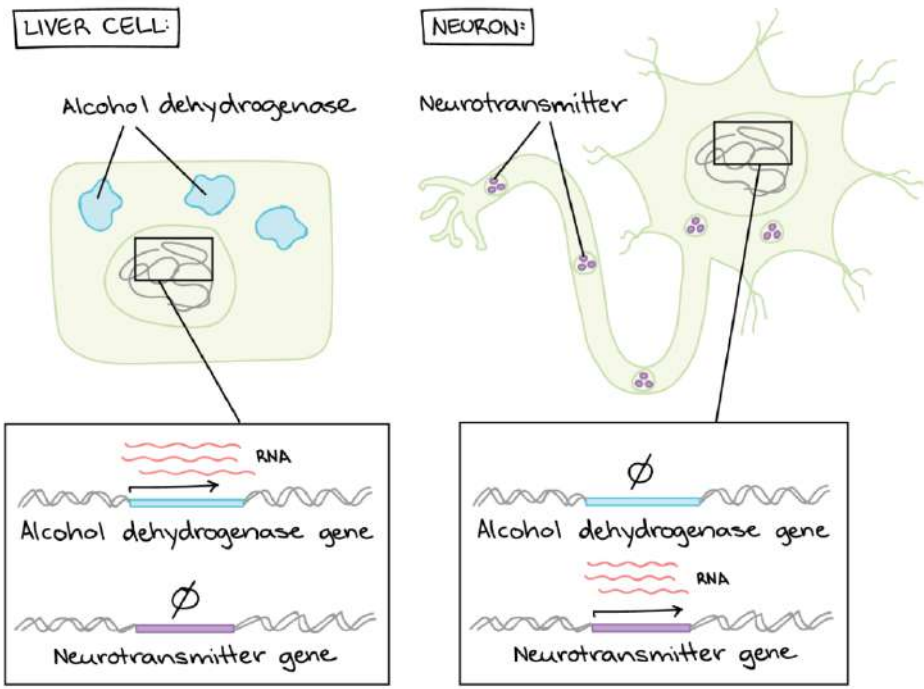
ولكن عندما لا تكون في // // فإنها ليست حاجة الى انتاج هذه البروتينات والجينات المسؤولة عن انتاج هذه البروتينات لن تكون في نفس الكفاءة

لتوضيح السلايد إلى فوق

Gene regulation makes cells different

Gene regulation is how a cell controls which genes, out of the many genes in its genome, are "turned on" (expressed). Thanks to gene regulation, each cell type in your body has a different set of active genes – despite the fact that almost all the cells of your body contain the exact same DNA. These different patterns of gene expression cause your various cell types to have different sets of proteins, making each cell type uniquely specialized to do its job.

For example, one of the jobs of the liver is to remove toxic substances like alcohol from the bloodstream. To do this, liver cells express genes encoding subunits (pieces) of an enzyme called alcohol dehydrogenase. This enzyme breaks alcohol down into a non-toxic molecule. The neurons in a person's brain don't remove toxins from the body, so they keep these genes unexpressed, or "turned off." Similarly, the cells of the liver don't send signals using neurotransmitters, so they keep neurotransmitter genes turned off.



There are many other genes that are expressed differently between liver cells and neurons (or any two cell types in a multicellular organism like yourself).

- في prokaryotic يوجد أنزيم واحد يقوم بـ transcription ← RNA polymerase
- في eukaryotic يوجد ٣ أنزيمات تقوم بـ transcription (لكل واحد لنوع معين من RNA) ← RNA polymerase I, II, III

- **Bacteria have only one RNA polymerase, all eukaryotes have three different ones (Pol I, II, and III)**. **In addition,** whereas bacteria require only one additional initiation factor (**σ**), several initiation factors are required for efficient and promoter-specific initiation in eukaryotes. **These are called the general transcription factors (GTFs).**

initiation factor ← هو اللي يساعد على التعرف على promoter .

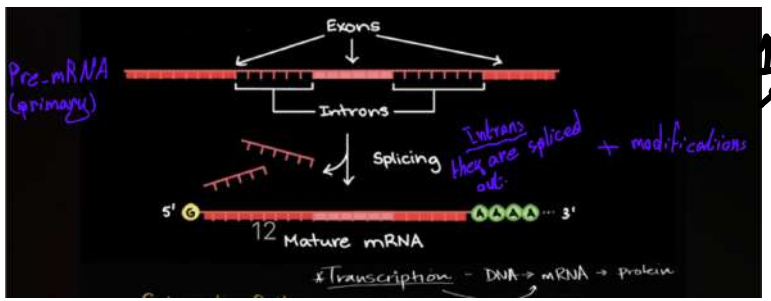
- في prokaryotic يوجد initiation factor واحد وهو sigma factor
- في eukaryotic يوجد العديد من initiation factors (اسمهم general transcription factor)

- في prokaryotic ← RNA الى بنتج. بيكون جاهز لايحتاج اي تعديلات
- في eukaryotic ← RNA الى بنتج. بيحتاج modifications ← عملية اضافة cap, tail
ثم عملية splicing التي يتم فيها ازالة introns وجمع exons ببعضهم

- Once transcribed, eukaryotic RNA has to be processed in various ways before being exported from the nucleus where it can be translated.
- These processing events include capping of the 5’ end of the RNA, splicing, and polyadenylation of the 3’ end of the RNA. The most complicated of these is splicing—the process whereby non-coding introns are removed from RNA to generate the mature mRNA.

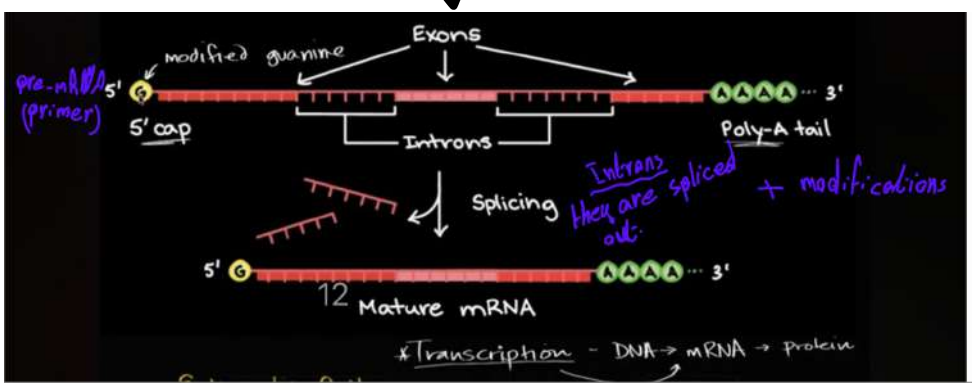
ملاحظة: عملية إضافة cap, tail تكون قبل عملية splicing وليس خلالها.

أنا اخطأت في محاضرة 2023 lecrure 6 RNA Structure في سلايد رقم 10



من سلايد 10

التصحيح



Synthesis of RNA in eukaryotes

- Nuclear RNA polymerases of eukaryotic cells:

-There are three types of nuclear polymerases:

1- **RNA pol I:** Transcribes 18S ,5.8 S ,28S ribosomal RNA genes

2- **RNA pol II:** It transcribes mRNA ,and most small nuclear RNAs (snRNA)

3- **RNA pol III:** It transcribes , tRNA and 5S rRNA and some small nuclear RNAs (snRNA)

- **Mitochondrial RNA pol:** Resembles bacterial RNA pol than eukaryotic enzyme. Responsible for mitochondrial gene expression **<u>as well as</u>** for providing RNA primer for initiation of replication of the mitochondrial genome.

(18s,5.8s,28s) ribosome الداخلة في تركيب rRNA ← transcription يعمل ← RNA polymerase I

small nuclear RNAs + mRNA ← transcription يعمل ← RNA polymerase II

some snRNA + 5S rRNA + t.RNA ← transcription يعمل ← RNA polymerase III

(prokaryotes في RNA P مثل) mitochondria في الخلايا transcription عن مسؤول ← Mitochondrial RNA polymerase

❖Transcription phases:

- Similar to prokaryotes, eukaryotic RNA synthesis include three main phases:

1- **Initiation** : involves the binding of RNA polymerase to a region on the DNA which is specific and is known as the promoter region.

2- **Elongation** : after the promoter region is recognized by the RNA polymerase, it starts to synthesize a complementary transcript to the template DNA strand. The RNA polymerase utilizes ribonucleotide triphosphate (ATP, GTP, CTP, UTP) and releases pyrophosphate each time a nucleotide is added to the growing chain.

3- **Termination** : elongation of the RNA chain continues until a termination signal is reached.

Synthesis of mRNA

RNA Polymerase II Core Promoters Are Made Up of Combinations of Different Classes of Sequence Element:

- The eukaryotic core promoter refers to the minimal set of sequence elements required for accurate transcription initiation by the Pol II machinery.
- A core promoter is typically about 40–60 nucleotides long, extending either upstream or downstream from the transcription start site.

RNAPII المساعدين هي transcription factors II
كيلاتهم بيساعدوا (RNAPII)

- The Figure shows the location, relative to the transcription start site, of elements found in Pol II core promoters. These are the TFIIB recognition element (BRE), the TATA element (or box), the initiator (Inr), and the downstream promoter elements (known as DPE, DCE, and MTE).

غالباً promoter إذا كان بيحتوي على TATA ما بيكون بيحتوي على DPE (والعكس صحيح).

Typically, a promoter includes some subset of these elements. Thus, for example, promoters typically have either a TATA element or a DPE element, not both. Often, a TATA-containing promoter also contains a DCE.

غالباً promoter إذا كان بيحتوي على TATA بيكون بيحتوي على DCE

The Inr is the most common element, found in combination with both TATA and DPEs.

سواء كان بيحتوي TATA أو DPE بيكون فيه Inr.

- The core promoter serves as a binding platform for the transcription machinery, which comprises Pol II and its associated **general transcription factors** (GTFs)

RNA polymerase II transcription factors > transcription machinery بيعمل كمنصة يتجمع عليها core promoter

start point ل بالنسبة upstream بيكون. prokaryotes في promoter -
downstream و upstream جزء بيكون eukaryotes في core promoter -
بيكون من 40-60 نيوكليوتايد

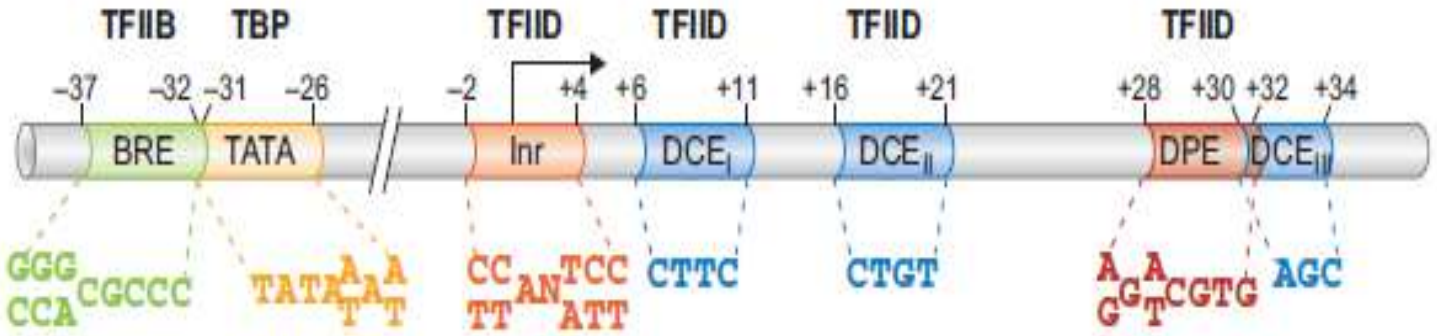


FIGURE 13-15 **Pol II core promoter.** The figure shows the positions of various DNA elements relative to the transcription start site (indicated by the arrow above the DNA). These elements, described in the text, are as follows: (BRE) TFIIB recognition element; (TATA) TATA box; (Inr) initiator element; (DPE) downstream promoter element; and (DCE) downstream core element. Another element, MTE (motif ten element), described in the text, is not shown in this figure but is located just upstream of the DPE. Also shown are the consensus sequences for each element (determined in the same way as described for the bacterial promoter elements; see Box 13-1) and (above) the name of the general transcription factor that recognizes each element.

transcription factors + RNA polymerase II + core promoter → preinitiation complex

❖ RNA Polymerase II Forms a Preinitiation Complex with General Transcription Factors at the Promoter:

- The general transcription factors help polymerase bind to the promoter and melt the DNA. They also help polymerase escape from the promoter and embark on the elongation phase. The complete set of general transcription factors and polymerase, bound together at the promoter and poised for initiation, is called the **preinitiation complex.**
- As we described above, many Pol II promoters contain a so-called TATA element (some 30 bp upstream of the transcription start site). This is where preinitiation complex formation begins. **The TATA element is recognized by the general transcription factor called TFIID.** (The nomenclature "TFII" denotes a transcription factor for Pol II, with individual factors distinguished as A, B, and so on.).

- transcription factors ← بيساعدوا
  - بيساعدوا ← RNAPII على انه يمسك بـ DNA double strands ويعمل melting (يفتح جزء من DNA double strands) عشان يكشوف ويقدر يعمل transcription
  - بيساعدوا ← RNAPII حتى يخرج من promoter يبدء عملية elongation

- اغلب promoters تحتوي على TATA (بالتالي فإنها لاتحتوي على DPE)
- TFIID هو اللي دوره يعمل recognition لـ TATA

السلايد القادم

- TFIID يتكون من subunits واحدة منهم هي التي ترتبط بـ TATA (TATA binding protein (TBP)) وباقي subunits أسمها (TBP associated factor) ← recognise of other elements. ex: DPE, DCE, Inr

- Like many of the general transcription factors, TFIID is, in fact, a multi-subunit complex. The component of TFIID that binds to the TATA DNA sequence is called TBP (TATA-binding protein).
- The other subunits in this complex are called **TAFs**, for TBP-associated factors. Some TAFs recognize other core promoter elements such as the Inr, DPE, and DCE, although the strongest binding is between TBP and TATA. Thus, **TFIID is a critical factor in promoter recognition and preinitiation complex establishment.**

TFIID ← transcription factors من factor أي

- The resulting TBP–DNA complex provides a platform to recruit other general transcription factors and polymerase itself to the promoter. These proteins assemble at the promoter in the following order: TFIIA, TFIIB, TFIIF together with polymerase, and then TFIIE and TFIIH.
- Formation of the preinitiation complex containing these components is followed by promoter melting. **In contrast to the situation in bacteria, promoter melting in eukaryotes requires hydrolysis of ATP and is mediated by TFIIH.**

- عملية ارتباط TFIID مع TATA بيودي إلى تتابع معين من factors في تتابع باقي TFIIA ← TFIIB ← TFIIF مابيكون معه RNAP II

TFIIH ← TFIIE ←

preinitiation complex حيث بيكون تكون
وبالتالي تكون جاهزين لعمل DNA melting

- في proKaryotis عند ارتباط RNAP يحدث melting مباشرة".
- في euKaryotis يجب حدوث hydrolysis لـ ATP ← عن طريق TFIIH الذي يعمل ATPase activity
وتحصل على طاقة لتتكسر من خيوط double strands

- لحتى يترك RNAP II الـ promoter ويبدأ يعمل elongation ← يجب انه يحدث phosphorylation لـ tail of polymerase

ATP hydrolysis ونحتاج ←

السلايد القادم

- tail (domain terminal-carboxy) ← عبارة عن sequence of a.a (Tyr, Ser, pro, Thr, ser, Pro, Ser) ← هذول السبعة a.a بيتكرروا 52 مرة.

- phosphorylation of tail يكون عن طريق TFIIH (عندها Kinase activity)

TFIIH ← ATPase activity عندها
Kinase activity عندها

❖ Promoter Escape Requires Phosphorylation of the Polymerase "Tail"

- In eukaryotes, promoter escape involves two steps not seen in bacteria: **one** is ATP hydrolysis (in addition to the earlier ATP hydrolysis needed for DNA melting), and **the other** is phosphorylation of the polymerase.

- The large subunit of Pol II has a carboxy-terminal domain (CTD), which is referred to as the "tail". The CTD contains a series of repeats of the heptapeptide sequence: Tyr-Ser-Pro-Thr-Ser-Pro-Ser. There are 52 of these repeats in humans. Each repeat contains sites for phosphorylation by **specific kinases, including one that is a subunit of TFIIH.**

TBP عن طريق TATA لـ recognition يعمل TFIID ←

TFIIB ثم TFIIA ← TFIID يتبع ←

TFIIE ثم RNAP II مع باقي وعامل TFIIF ثم
TFIID ثم ←

preinitiation complex ←

melting by ATPase activity ←
of TFIIH

phosphorylation of tail ← عن طريق RNAP II يخرج ←
ATP hydrolysis

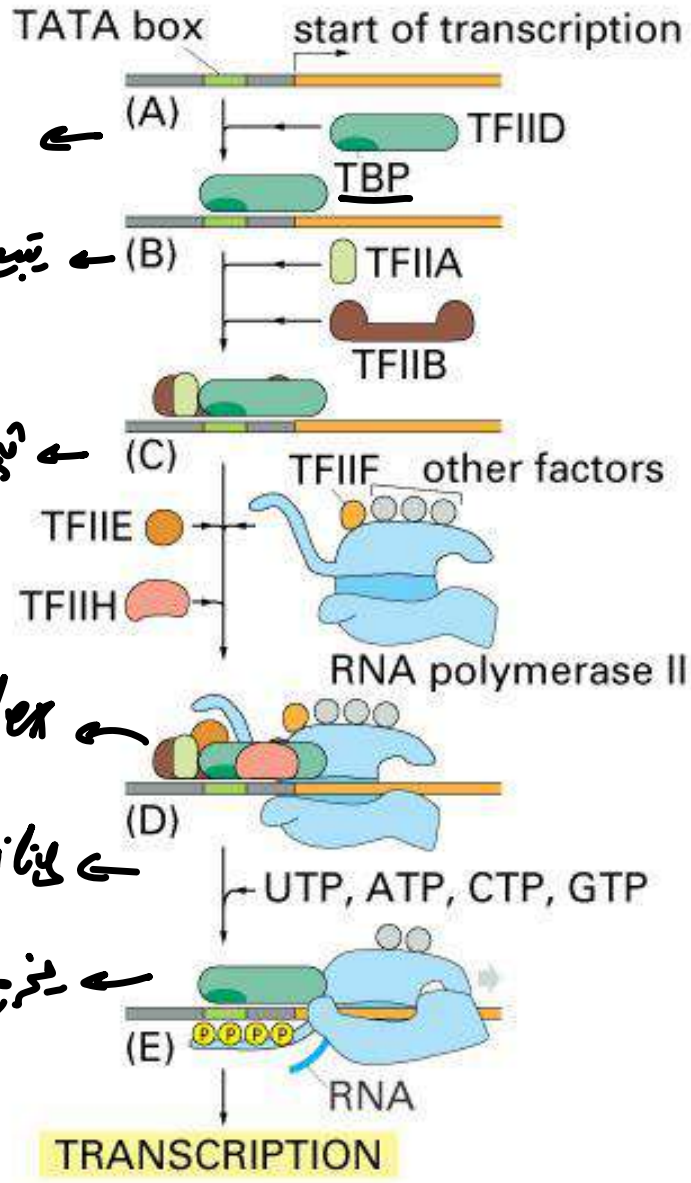


Figure 8-10 Essential Cell Biology, 2/e. (© 2004 Garland Science)

1-The first step is binding of the **TFII D** (contains TATA binding protein, TBP)to the TATA box.

2- Binding of **TFII A&B**, followed by binding of **RNA polymerase II-TFII F complex** (TFII F brings the RNAP II to the promoter site).

3- Binding of **TFII E&H** to form **preinitiation complex (PIC).**

4- Phosphorylation by a kinase produces activation of the polymerase II.

✓ *TFII F brings the RNAP II to the promoter site, while TFIIH activates it by phosphorylation.*

- For pol II-transcribed genes, and unlike bacterial RNA polymerase, **promoter melting requires hydrolysis of ATP and is mediated by TFIIH.**

- ***TFIIH is a ten-subunit protein, including both ATPase and protein kinase activities.***

5- Release of TFII A, B, E,&H

6- Pol II-TF IIF complex leaves the promoter, and starts transcription.

7- Transcription proceeds till the termination signal is reached.

8- Pol II-TF IIF complex is dissociated.

9- Pol II-TF IIF complex is dephosphorylated by a phosphatase.

10- A new cycle of transcription may start again.