**Bacterial Transcription**

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**Aim:**
- Understand the general process of bacterial transcription
- Reference: Schaefer et al, Microbes, p141-8

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**Main topics:**
- Overall scheme of information processing in cell
  - DNA → RNA → Protein (‘central dogma’)
  - Transcription and Translation
- Components of the transcription system in bacteria
  - RNA polymerase
  - DNA template, nucleotides, addition of new bases
- Stages of the transcription process
  - RNAP binding to promoter, DNA unwinding, Initiation, elongation, termination
  - Consensus promoters, Terminators

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**Bacterial Transcription**

- **Main Points:**
  - a) Overall scheme of information processing in cell
  - DNA → RNA → Protein (‘central dogma’)

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**Bacterial Transcription**

- Transcription is the synthesis of an RNA molecule, called a transcript, from a DNA template.
- Bacteria have only one RNA-P (eukarya have 3)
- The bacterial RNA-P enzyme synthesises all the RNA species in the cell
  - Stable RNAs are tRNA, rRNA
  - Unstable RNA is mRNA, < 1 min 1/2-life

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**Analysing transcripts by Northern Blot hybridisation**

- Size of RNA
- Viral transcripts (RNA) separated by agarose gel electrophoresis.
  - Time post infection
**Bacterial Transcription**

**RNA polymerase (RNA-P):**
- Links ribonucleoside triphosphates (ATP, GTP, CTP and UTP) in 5' - 3' direction
- Copies the DNA coding strand using the template strand
- Can be modified to selectively transcribe genes by associating with sigma factors

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**Phosphodiester bond formation**

![Image of Phosphodiester bond formation](image)

**E. coli** RNA polymerase

RNA polymerase on virus promoters

3D structure (from EM)

![E. coli RNAP](image)

*E. coli* RNAP, –100 x 100 x 160 Å

Darst et al., 1989

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**Bacterial Transcription**

- Note deoxythymidine in DNA is replaced by uridine in RNA

![Image of DNA base replacement](image)

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**E. coli** RNA polymerase

Core enzyme - will bind to any DNA at low affinity. Selective binding requires the activity of sigma factor.

![Image of E. coli RNA polymerase](image)

Darst et al., 1989
**Terms**

1. **TRANSCRIPTION**: synthesis of RNA using a DNA template
2. **CODING STRAND**: the DNA strand that is copied by RNA polymerase
3. **TEMPLATE STRAND**: the DNA strand used by RNA polymerase as the template. It is complementary to the coding strand, and the transcript.
4. **TRANSCRIPT**: the product of transcription.
**Terms**

1. **PROMOTER**: The sequence of DNA needed for RNA polymerase to bind and to initiate transcription.
2. **START POINT**: First base pair transcribed into RNA
3. **UPSTREAM**: sequence before the start point
4. **DOWNSTREAM**: sequence after the start point.
5. **TERMINATOR**: a DNA sequence that causes RNA pol to terminate transcription

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**Typical Bacterial Promoter Sequence**

Three main parts, the -35, -10 consensus sequences, and the start point.

Promoter for σ70 sigma factor of *E. coli*

**E. coli** Sigma Factors

**A Transcription Unit**

- **Binding**: DNA sequence transcribed into an RNA from promoter to terminator
- **Initiation**: Transcription
- **Elongation**: RNA synthesis continues in the transcription bubble
- **Termination**: RNA being synthesised

**PROMOTER - RNAP**

One face of the DNA contacts the polymerase

**Fig 14.14, Genes V (Lewin)**
**RNA pol activities**

**Diagram**

From www.ergito.com website

**Model**

From www.ergito.com website

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**RNA Pol: Core and Holo enzyme are mainly found on DNA.**

500-1000 cRNAP at loose complexes

500-1000 hRNAP at loose complexes

Small % free hRNAP

500-1000 hRNAP in closed (or open) complexes at promoters

~ 2500 cRNAP actively transcribing genes.

Fig 14.11, Genes V (Lewin)

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**RNA Pol: finding promoters quickly**

3 models

1. Random diffusion to target

2. Random diffusion to any DNA, followed by random displacement to any DNA

3. Sliding along DNA

Fig 14.12, Genes V (Lewin)
RNA Pol: finding promoters quickly

3 models
1. Random diffusion to target
2. Random diffusion to any DNA, followed by random displacement to any DNA
3. Sliding along DNA

Too slow

Favoured

Unknown

Footprint Analysis

Transcription occurs inside a region of opened DNA, a ‘bubble’.
The DNA duplex is unwound ahead of transcription, and reforms afterwards, displacing the RNA

RNA Pol covers less DNA as it progresses from initiation to elongation. Partly because of sigma factor release, and partly from conformational changes of the core enzyme itself.

Footprint Analysis

RNAP+  RNAP-

Initial contact -55 to +20 = ~ 75 bp

RNA Pol binding to a promoter
Transcription Unit

DNA sequencetranscribed into an RNA, from promoter to terminator.

Fig 14.14, Genes V  (Lewin)

Transcription termination:
**Intrinsic terminator**

- stem-loop structures, 7-20 bp.
- GC-rich region followed by a poly-U region
- Structure forms within transcription bubble, making RNA-P pause
- A-U base pairs easily broken, leading to release of transcript

Fig 16.3, Genes V  (Lewin)

Transcription termination:
**Rho dependent terminator**

Rho protein binds to RNA

C-rich, G-poor region in RNA preceding termination

Fig 16.4, Genes V  (Lewin)

Commercial transcription systems

Phage RNA polymerases (T3, T7, SP6)

Fig 16.4, Genes V  (Lewin)

**Transcription - the movie**

DNA (10kb) is attached at one end to a plastic bead, and tethered to a glass capillary.

Watching single RNA polymerase enzymes move along a DNA template
Summary of Bacterial Transcription

- Know the main terms in this process
- Understand the process:
  - Template recognition: RNAP binds dsDNA
  - DNA unwinding at promoter
  - Initiation (short chains, 2-9nt, made)
  - Elongation (RNA made)
  - Termination (RNAP and RNA released)

Next lecture on regulation of gene expression